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Provisional Peer-Reviewed Toxicity Values for

Phenyl isothiocyanate
(CASRN 103-72-0)

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

COMMONLY USED ABBREVIATIONS

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
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
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
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR PHENYL ISOTHIOCYANATE (CASRN 103-72-0)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.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. U.S. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. 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 U.S. EPA's 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 U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. 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 documents 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 Resource Conservation and Recovery Act (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 document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. 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 U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Some isothiocyanates, such as sulforaphane, are natural products that have been shown to inhibit carcinogenesis and tumorigenesis (for example, Talalay et al., 2007). However, other isothiocyanates have less desirable properties. A chronic reference dose (RfD) for phenyl isothiocyanate is not available in the U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS; U.S. EPA, 2009), the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006), or the Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 1997). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) includes no documents for phenyl isothiocyanate. A toxicological review of phenyl isothiocyanate is not available from the Agency for Toxic Substances and Disease Registry (ATSDR, 2007) or the World Health Organization (WHO, 2007).

No chronic inhalation reference concentration (RfC) is available for phenyl isothiocyanate on IRIS (U.S. EPA, 2009) or in the HEAST (U.S. EPA, 1997). The American Conference of Governmental Industrial Hygienists (ACGIH, 2006), Occupational Safety and Health Administration (OSHA, 2007), and the National Institute for Occupational Safety and Health (NIOSH, 2007) have not established occupational health standards for phenyl isothiocyanate.

A carcinogenicity assessment for phenyl isothiocyanate is not available on IRIS (U.S. EPA, 2009) or in the HEAST (U.S. EPA, 1997). Phenyl isothiocyanate has not been evaluated by the International Agency for Research on Cancer (IARC, 2007) nor is it included in the National Toxicology Program's (NTP) 11th Report on Carcinogens (NTP, 2005).

Literature searches were conducted for studies relevant to the derivation of provisional toxicity values for phenyl isothiocyanate in July 2007 in MEDLINE, TOXLINE special, and DART/ETIC (1960s−July 2007); BIOSIS (2000−June 2007); TSCATS/TSCATS2, RTECS, CCRIS, HSDB, and GENETOX (not date limited); and Current Contents (September 2008−May 2009). The literature review was subsequently updated to May 2009.

REVIEW OF PERTINENT DATA

Human Studies

No studies were located regarding the effects of subchronic or chronic exposure of humans to phenyl isothiocyanate by oral or inhaled routes.

Animal Studies

Oral Exposure

No studies were located regarding the effects of long-term oral exposure to phenyl isothiocyanate in animals.

In a 4-week study using SPF Wistar rats of the Riv:Tox [M] strain, Speijers et al. (1985) administered phenyl isothiocyanate (99% pure) in sunflower seed oil by gavage to groups of 6 male rats on 5 days/week. Doses of 0, 2.5, 10, or 40 mg/kg-day were administered using a constant intubation volume of 1 ml sunflower seed oil per 100 g body weight. The rats were housed two to a cage and allowed free access to food (semi-purified diet with an iodine content of 30 mg/kg diet) and water. Rat body weights were measured at the beginning of the experiment and weekly thereafter. Initial body weights ranged from 30 to 50 g. Blood was collected for hematology (erythrocyte count, leukocyte count [total and differential], packed cell volume, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]) and clinical chemistry (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and thyroxine [T_4, total and free]). The heart, liver, spleen, kidneys, thyroid, adrenals, and mesenteric lymph nodes were weighed and examined histopathologically.

Terminal body weights of the rats in the highest-dose group (40 mg/kg-day) were reduced approximately 11% compared to controls; however, these results are not statistically significant. Food and water consumption data are not reported. Relative heart, liver, kidney, and adrenal weights were significantly increased in the highest-dose group compared to control values. The study authors suggested that the increases in these relative weights were probably due to the lower body weights of the rats in this group. No changes in absolute weights of these organs were noted, and no histopathological changes were observed. Hematological analysis found that rats in the 10 mg/kg-day exposure group showed an increase in packed cell volume ($p < 0.05$), while rats in the 40 mg/kg-day exposure group showed a decrease in packed cell volume ($p < 0.05$). Erythrocyte and leukocyte counts and MCV among treated rats did not differ significantly from controls, although there were significant increases in MCH ($p < 0.05$) and MCHC ($p < 0.01$) in the highest-dose group. ALT and AST analyses demonstrated no statistically significant changes in the treated groups. There were statistically significant decreases in total serum T_4 levels (at 40 mg/kg-day, $p < 0.01$) and free serum T_4 levels (at 10 mg/kg-day, $p < 0.05$; at 40 mg/kg-day, $p < 0.001$) following phenyl isothiocyanate exposure (data presented in Table 1). The study authors suggested that the dose-related decreases in T_4 values indicate a potential hypothyroid effect. In general, because the free T_4 assay is not affected by serum protein levels, it is considered a more accurate reflection of thyroid hormone function. While the decreased thyroid function was not found to be associated with an increase in thyroid weight or with morphological changes in the thyroid, the study authors stated that the change in the T_4 concentration was the most sensitive indicator of an effect in this experiment.

Table 1. Total and Free T_4 Levels in Serum from Male Rats Given Phenyl Isothiocyanate by Gavage 5 Days/Week for 4 Weeks[a]

Dose (mg/kg-day)	No. of rats	Total T_4 (nmol/liter)		Free T_4 (pmol/liter)	
		Mean	SD	Mean	SD
0	6	124	21	27	4
2.5	6	123	33	25	5
10	6	113	14	22[b]	2
40	6	72[c]	25	16[d]	4

[a]Speijers et al. (1985)
[b]$p < 0.05$
[c]$p < 0.01$
[d]$p < 0.001$

Other isothiocyanates have also been shown to affect thyroid function. In a short-term gavage study in rats, allyl isothiocyanate affected thyroid function and produced antithyroid effects in vitro (Langer and Greer, 1968, 1977). Methyl isothiocyanate has also been reported to have antithyroid effects in vivo and in vitro (Langer and Greer, 1977).

Eastman Kodak Co. (1979) administered 0, 10, or 100 mg/kg-day phenyl isothiocyanate (purity not reported) in corn oil by gavage to groups of five male rats (strain not reported) for up to 15 days. As the rats in the high-dose group demonstrated weakness and poor general condition, they either died or were terminated after 4 days. Blood was collected for hematology (hemoglobin, hematocrit, white blood cell counts, and differential cell counts) and clinical chemistry (glutamic oxaloacetic transaminase [SGOT = AST], glutamic pyruvic transaminase [SGPT = ALT], lactic dehydrogenase [LDH], alkaline phosphatase, urea nitrogen, and glucose). The liver and kidneys from rats in the low-dose group were weighed. A list of specific tissues examined microscopically was not provided; however, it is apparent that the liver, kidneys, and thymus were examined. No statistical analysis of the results was conducted.

Rats in the high-dose group demonstrated a severe depression in both feed intake and body weight gain. Clinical signs included rough coats, pallor, ataxia, tremors, hypothermia, and bloody urine. Differential blood counts showed relative neutrophilia and red blood cell poikilocytosis. Serum LDH was "moderately" elevated and ALT was "greatly" elevated (no quantitative data provided). Other hematological and clinical chemistry tests were not performed, and relative organ weights were not determined for the 100 mg/kg-day group. Histopathology revealed thymic necrosis in 3/5 rats in this group. No significant changes from controls were reported in rats administered 10 mg/kg-day phenyl isothiocyanate.

In additional single-dose testing, groups of five male rats each received 100, 500, or 1000 mg/kg-day phenyl isothiocyanate by gavage for 1 day (Eastman Kodak Co., 1979). Controls received 1000 mg/kg distilled water. Within 24 hours, animals in the high-dose group developed rough hair coats, weakness, and tremors; all but one rat died. Animals in the low-dose

group showed weakness and rough coats. The study authors reported the approximate oral LD_{50} (observation period not reported) for phenyl isothiocyanate as 141 mg/kg-day in male rats and 83 mg/kg-day in male mice; however, details of the mouse experiments were not provided.

Chung et al. (1984) fed groups of male F344 rats (3 to 4 per treatment group), initially weighing 200 to 300 g each, NIH-07 diets for 2 weeks. Treated rats were maintained on the NIH-07 diet containing 0.003 mmol phenyl isothiocyanate/g of diet. This concentration was determined following initial range-finding experiments. Exposure to diets containing 0.03 mmol/g of diet demonstrated considerable weight loss (5 to 50 g). Control rats were fed with the NIH-07 diet only. On average, the rats consumed about 15 g of diet per day. The study authors reported that the total dose of phenyl isothiocyanate at which decreased body weight (0.03 mmol/g-diet) was seen was about 21 mmol/kg of body weight. Based on the molecular weight of phenyl isothiocyanate (135.19 g), this is equivalent to 2830 mg/kg body weight. The animals were sacrificed on Day 15, and the esophagus and liver were removed and examined for effects on nitrosamine metabolism. Pretreatment with phenyl isothiocyanate inhibited the formation of alpha-hydroxylation products of N-nitrosopyrrolidine (NPYR) and N'-nitrosonornicotine (NNN). In a follow-up study, Chung et al. (1985) fed groups of male F344 rats (5 rats) NIH-07 diets for 2 weeks containing 0.003-mmol phenyl isothiocyanate/g of diet. Following the 2-week feeding, the rats were administered N-nitrosodimethylamine (NDMA) by i.p. injection (25 mg/kg in 0.9% NaCl w/v) or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (85 mg/kg in 0.9% NaCl w/v) by tail-vein injection. The rats were sacrificed 4 hours after injection, and their livers were removed. Pretreatment with phenyl isothiocyanate caused a marked decrease in NDMA and NNK demethylation. The study authors suggested that these results are indicative that phenyl isothiocyanate may help inhibit the carcinogenic activity of NDMA and NNK, but further analysis is needed.

Becker and Plaa (1965) conducted an acute study of the lethality and hepatotoxicity of single oral doses of phenyl isothiocyanate and other organic isothiocyanates in male Swiss Webster mice. Only phenyl isothiocyanate was studied in any detail. Groups of 10 mice each were treated with a single dose of phenyl isothiocyanate dissolved in corn oil and administered (0.01 ml/g) by gavage. Most deaths from phenyl isothiocyanate occurred during the first 48 hours, although some mice died on the 3rd and 4th days. The total duration of observation was not reported. The acute lethality study resulted in an LD_{50} value for 24 hours of 400 mg/kg (95% confidence limits: 282−570 mg/kg) and an LD_{50} value for 48 hours of 350 mg/kg (95% confidence limits: 246−497 mg/kg). These values were not statistically different. In addition, phenyl isothiocyanate caused increases in plasma bilirubin and sulfobromophthalein retention. Histopathology of livers from mice dosed with 300 mg/kg phenyl isothiocyanate demonstrated irregularly distributed necrosis of the larger intrahepatic bile duct epithelia. The study authors also noted some necrosis of the bile ductile epithelium and perivascular inflammation.

Rothkopf-Ischebeck (1978) exposed male and female Wistar rats orally to phenyl isothiocyanate dissolved in Neobee-O[6] oil (5 ml/kg volume) to investigate the effect of phenyl isothiocyanate shown to induce profuse pleural cavity exudation (pleurisy) in a prior metabolism study. A number of experiments were performed to determine an ED_{50}, an LD_{50}, a time course of phenyl isothiocyanate, the age and weight dependency, and any antagonizing effects, each of which was based on varying durations of exposure. The number of rats in each treatment group was not reported. The ED_{50} values for pleurisy in male and female Wistar rats aged 88 and

98 days, respectively, were 73.8 mg/kg (65.6−82.4) for males and 63.9 mg/kg (54.1−74.4) for females. The LD_{50} values (14-day observation) for male and female rats aged 81 and 72 days, respectively, were 110 mg/kg (99.9−121) for males and 86.9 mg/kg (78.2−96) for females. Both male and female rats died between the 48th and 56th hours after provocation during the maximum exudation period. The exudate volume produced by the same mg/kg dose of phenyl isothiocyanate progressively increased with the age of the rats. Gross examination revealed no changes in the kidneys, liver, heart, and spleen, but the examination did show tissue irritation of the intestinal region at doses >100 mg/kg. Sporadic maculae were seen on the lungs of rats that received lethal doses, and the frequency of thymus swelling increased with increased exudate volumes. Histopathological examinations were not performed. Pleurisy was not induced in mice or guinea pigs by oral phenyl isothiocyanate.

Inhalation Exposure

No data regarding the toxicity of phenyl isothiocyanate in animals following inhalation exposure were located.

Other Studies

Dermal Studies

The results of the dermal studies indicate that phenyl isothiocyanate is readily absorbed, highly irritating, and a sensitizer, although there is some inconsistency in results. Application of phenyl isothiocyanate to the abdomen of guinea pigs under an impervious cuff for 24 hours resulted in severe irritation. Death occurred within 24 hours in guinea pigs receiving doses of 5 ml/kg or higher on the skin. A single uncovered application of 0.5 ml to the clipped backs of guinea pigs resulted in the death of five guinea pigs tested within 24 hours. Repeated application of 0.1 ml/day in the same manner severely irritated the skin and resulted in either weight loss or no weight gain. A skin sensitization test in guinea pigs gave a moderate response in four of the five test animals and a weak response in one animal (Eastman Kodak Co., 1979).

In other studies, phenyl isothiocyanate was classified as a "moderate sensitizer" in the guinea pig maximization test (Fregert et al., 1983), but, in another sensitization test, it did not elicit a significant swelling response in mouse ears (Schmidt and Chung, 1993).

Parenteral Studies

In a screening developmental toxicity study of 48 compounds, phenyl isothiocyanate was administered subcutaneously, through dissolution in dimethyl sulfoxide, on Gestation Days (GD) 6−14 at 48 mg/kg-day to C3H mice (3 litters) and at 25 mg/kg-day to BL6 mice (2 groups of 6 litters) and on GDs 6−15 at 25 mg/kg-day to AKR mice (12 litters) (Bionetics Research Labs, 1968). The numbers of litters in the corresponding control groups were 12 (C3H), 73 and 75 (BL6), and 33 (AKR). The number of dams was not specified. Maternal body weights were unaffected by phenyl isothiocyanate. Fetal mortality was normal in all groups. In one group of BL6 mice, the number of implantations per litter was increased, mean fetal weight was decreased, and the incidence of abnormalities (as percent fetuses) was increased. In the other group of BL6 mice, the number of implantations and live fetuses per litter were increased, but fetal weight and the incidence of abnormalities were unaffected. In the AKR mice, the number of live fetuses per litter and mean fetal weight were increased, but implantations per litter and incidences of abnormalities were unaffected. The study authors stated that they had no resolution for these inconsistencies.

Tumor Inhibition

Many citations identified in the literature searches were studies investigating the potential effects of phenyl isothiocyanate on tumor inhibition. The database demonstrates that phenyl isothiocyanate may be protective in some systems but not in others. For example, phenyl isothiocyanate inhibits melanoma formation (Manesh and Kuttan, 2003), mammary tumor formation (Wattenberg, 1977), and tumor-specific angiogenesis (Thejass and Kuttan, 2007) in mice. In the latter study, the study authors demonstrated that phenyl isothiocyanate down-regulated serum nitrous oxide (NO) and tumor necrosis factor-alpha (TNF-α), both of which are involved in angiogenesis (the process that is critical to the transformation of premalignant lesions to the malignant phenotype). However, other studies with prostate cancer cells (Xiao et al., 2004), HeLa cells (Yu et al., 1998), squamous cell carcinoma (Lui et al., 2003), and lung cancer bioassays with mice (Morse et al., 1989, 1991; Smith et al., 1990; Thomson et al., 2006), demonstrate that phenyl isothiocyanate does not induce apoptosis or inhibit tumor formation processes.

Carcinogenicity

Musk and Johnson (1993) demonstrated the genotoxic potential of phenyl isothiocyanate in comparison to three other isothiocyanates (i.e., allyl-, benzyl-, and phenethyl isothiocyanate). Phenyl isothiocyanate induces chromosomal aberrations (chromatid gaps, breaks, and rearrangements) in an SV_{40}-transformed Indian muntjac cell line (SVM) in the absence of an exogenous metabolic activation system. Phenyl isothiocyanate was one order of magnitude less cytotoxic than the other three isothiocyanates.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR PHENYL ISOTHIOCYANATE

Subchronic p-RfD

The longest duration study based on oral exposure to phenyl isothiocyanate is the 4-week study by Speijers et al. (1985). Results of this study indicate that interference with thyroid hormone levels in rats treated orally with ≥10 mg/kg-day phenyl isothiocyanate production is a sensitive effect of this chemical. Eastman Kodak Co. (1979) found no effects in rats treated orally with 10 mg/kg-day phenyl isothiocyanate for 15 days, but this study did not specifically evaluate for thyroid effects.

The Speijers et al. (1985) study was selected as the basis for the subchronic p-RfD. The critical effect in this study is a decrease in thyroid function as measured by significantly decreased serum T_4 (total and free) levels. A LOAEL of 10 mg/kg-day was identified for the most sensitive effect, decreased free T_4 serum levels, with a corresponding NOAEL of 2.5 mg/kg-day. Models for continuous variables in U.S. EPA's Benchmark Dose Software (BMDS) version 2.1 beta were fit to the total and free serum T_4 data (see Table 1) in accordance with U.S. EPA (2000) methodology. It was considered whether data are available to establish a biologically based benchmark response (BMR) for changes in serum T_4. The reference range for T_4 in adult male Wistar rats is approximately 2.5−7.0 μg/dL (32−90 nmol/L) (DePaolo and Masoro, 1989). However, T_4 levels in the control and low-dose rats in the Speijers et al. (1985) study were considerably higher (124 ± 21 nmol/L), suggesting that the reference range presented above is not applicable. In humans, there is considerable variation in serum T_4 measured

depending on methodology and population tested, and, as a result, it is recommended that reference ranges be established by the analytic laboratory (Leise and Sibilia, 1993). Similarly, in animals, serum T_4 levels are known to be influenced by strain, sex, age, circadian rhythms, room temperature, stress, and activity level (Christian and Trenton, 2003). Therefore, in accordance with U.S. EPA (2000) guidelines and in the absence of any cogent basis for selecting a BMR for the total and free T_4 data, a BMR of 1 standard deviation (SD) from the control mean can be used, as recommended as the standardized reporting level for comparisons for continuous data. BMD modeling was performed using the doses administered in the study—not duration-adjusted average daily doses. For total serum T_4 data, the Linear model provides the best model fit, and the estimated BMD_{1SD} and $BMDL_{1SD}$ for total T_4 are 16.66 and 11.68 mg/kg-day, respectively. For free serum T_4 data, the Hill model estimates a BMD_{1SD} and $BMDL_{1SD}$ of 6.46 and 2.33 mg/kg-day, respectively. Details of BMD modeling and plots of the best-fitting models are presented in Appendix B.

Potential points of departure (PODs) for derivation of the subchronic RfD for phenyl isothiocyanate include the $BMDL_{1SD}$ values of 11.68 and 2.33 mg/kg-day for reduced total and free serum T_4, respectively, and the NOAEL value of 2.5 mg/kg-day. Use of BMDL values is preferred over use of the NOAEL because, in contrast to the NOAEL/LOAEL approach, the BMD approach uses all of the data in the study, takes into account the shape of the dose-response curve, and accounts for uncertainty in the observed dose-response due to the experimental design. Thus, the $BMDL_{1SD}$ for the most sensitive endpoint, 2.33 mg/kg-day for free T_4, was selected as the POD for derivation of the subchronic p-RfD.

The 4-week rat study by Speijers et al. (1985) involved phenyl isothiocyanate exposure by oral gavage 5 days/week; therefore, the $BMDL_{1SD}$ for free T_4 was duration adjusted to 1.7 mg/kg-day. This duration-adjusted $BMDL_{1SD}$ ($BMDL_{1SD\,[ADJ]}$) was divided by a composite UF of 1000 to derive a provisional subchronic RfD for phenyl isothiocyanate, as follows:

$$\begin{aligned} \textbf{Subchronic p-RfD} &= BMDL_{1SD\,[ADJ]} \div UF \\ &= 1.7 \text{ mg/kg-day} \div 1000 \\ &= \textbf{0.002 mg/kg-day or } \mathbf{2 \times 10^{-3}} \textbf{ mg/kg-day} \end{aligned}$$

The UF of 1000 is composed of the following:

- A UF_A of 10 is applied for interspecies extrapolation to account for potential pharmacodynamic and pharmacokinetic differences between rodents and humans.
- A UF_H of 10 is applied for extrapolation to potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans.
- A UF_D of 10 is applied to account for database insufficiencies due to the lack of multigenerational reproduction studies and developmental toxicity studies by oral exposure.

Confidence in the principal study (i.e., Speijers et al., 1985) is low because, despite adequate investigation of endpoints at multiple dose levels, only a small number of male rats were tested and the experiment only lasted 4 weeks. Confidence in the database is low because

the data set includes only short-term, subacute, and acute studies. No studies are available on the potential of ingested phenyl isothiocyanate to induce developmental or reproductive effects. Low confidence in the subchronic p-RfD follows.

Chronic p-RfD

There are no chronic oral studies available for use in developing a chronic p-RfD for phenyl isothiocyanate. The $BMDL_{1SD}$ value of 2.33 mg/kg-day (for free serum T_4 levels) identified from the Speijers et al. (1985) study and used to derive the subchronic p-RfD above could also be used to derive a chronic p-RfD for phenyl isothiocyanate. However, in this case, the composite UF would increase to 10,000. Based on current guidelines and standard operating procedures, composite UFs >3000 cannot be considered for reference value derivation. As such, while a chronic p-RfD cannot be derived here, Appendix A of this document contains an oral "screening value" that may be useful in certain instances. Please refer to Appendix A for details.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR PHENYL ISOTHIOCYANATE

There are no inhalation studies available for use in developing subchronic and/or chronic provisional RfCs for phenyl isothiocyanate.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR PHENYL ISOTHIOCYANATE

Weight-of-Evidence Descriptor

Studies evaluating the carcinogenic potential of oral or inhalation exposure to phenyl isothiocyanate in humans are not available in the current literature. A single study on genotoxicity is, however, available for phenyl isothiocyanate. This study (Musk and Johnson, 2003) demonstrates that phenyl isothiocyanate can induce chromosomal aberrations. Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), "*Inadequate Information is Available to Assess* [the] *Carcinogenic Potential*" of phenyl isothiocyanate.

Quantitative Estimates of Carcinogenic Risk

The lack of suitable data precludes derivation of quantitative estimates of cancer risk for phenyl isothiocyanate.

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APPENDIX A. DERIVATION OF A SCREENING VALUE FOR PHENYL ISOTHIOCYANATE

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for phenyl isothiocyanate. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Hazard identification and dose-response information contained in an Appendix receives the same level of internal and external scientific peer review as the main body of PPRTV documents, to ensure their appropriateness within the limitations detailed in the document. In the OSRTI hierarchy, screening values are considered to be *below* Tier 3, "Other (Peer-Reviewed) Toxicity Values."

Screening values are intended for use in limited circumstances when no Tier 1, 2, or 3 values are available. Screening values may be used, for example, to rank relative risks of individual chemicals present at a site to determine if the risk developed from the associated exposure at the specific site is likely to be a significant concern in the overall cleanup decision. Screening values are not defensible as the primary drivers in making cleanup decisions because they are based on limited (e.g., scope, depth, validity, etc.) information. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

Screening Chronic Oral Value

As noted earlier, there are no chronic oral studies available for use in developing a chronic p-RfD for phenyl isothiocyanate. The longest duration study based on oral exposure to phenyl isothiocyanate is the 4-week study by Speijers et al. (1985). Thus, in addition to the aforementioned recommendations on the intended and appropriate use of screening values, it is important to note that due to the less-than-subchronic 4-week exposure duration of the principal study (Speijers et al., 1985), the certitude of the chronic oral screening value derived below is particularly diminished.

The $BMDL_{1SD}$ for the most sensitive endpoint from the Speijers et al. (1985) study, 2.33 mg/kg-day for free serum T_4, was selected as the POD for derivation of the chronic oral screening value. This study involved phenyl isothiocyanate exposure by oral gavage 5 days/week; therefore, the $BMDL_{1SD}$ for free T_4 was duration adjusted to 1.7 mg/kg-day. This duration-adjusted $BMDL_{1SD}$ ($BMDL_{1SD\,[ADJ]}$) was divided by a composite UF of 10,000 to derive a screening chronic oral value for phenyl isothiocyanate, as follows:

Screening Chronic Oral Value = $BMDL_{1SD\,[ADJ]} \div UF$
= 1.7 mg/kg-day ÷ 10,000
= **0.0002 mg/kg-day or 2×10^{-4} mg/kg-day**

The UF of 10,000 was composed of the following UFs:

- A UF_A of 10 is applied for interspecies extrapolation to account for potential pharmacodynamic and pharmacokinetic differences between rodents and humans.
- A UF_H of 10 is applied for extrapolation to potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans.
- A UF_D of 10 is applied to account for database insufficiencies due to the lack of multigenerational reproduction studies and developmental toxicity studies by oral exposure.
- UF_S of 10 is applied for using data from less than lifetime exposure to assess potential effects from chronic exposure.

Confidence in the principal study (i.e., Speijers et al., 1985) is low because, despite adequate investigation of endpoints at multiple dose levels, only a small number of male rats were tested and the experiment only lasted 4 weeks. Confidence in the database is low because the data set includes only short-term, subacute, and acute studies. No studies are available on the potential of ingested phenyl isothiocyanate to induce developmental or reproductive effects. Low confidence in the screening chronic oral value follows.

APPENDIX B. DETAILS OF BENCHMARK DOSE MODELING FOR THE PROVISIONAL RfDs

Model Fitting Procedure for Continuous Data

The BMD modeling for continuous data (i.e., reduced total and free serum T_4 levels) was conducted with the U.S. EPA's BMD software (BMDS version 2.1 beta). The original data were modeled with all the continuous models available within the software employing a BMR of 1 SD. An adequate fit was judged based on three criteria: (1) the goodness-of-fit *p*-value ($p > 0.1$), (2) magnitude of scaled residuals in the vicinity of the BMR, and (3) visual inspection of the model fit. In addition to the three criteria for judging the adequate model fit, whether the variance needed to be modeled, and if so, how it was modeled also determined final use of the model results. If a constant variance model was deemed appropriate based on the statistical test provided in the BMDS (i.e., Test 2), the final BMD results were estimated from a constant variance model. If the test for constant variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the data (i.e., Test 3; *p*-value < 0.1), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied >3-fold; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) was selected as a potential POD from which to derive an RfD.

Model Predictions for Total and Free Serum T_4 Levels in Male Rats Given Phenyl Isothiocyanate by Gavage 5 Days/Week for 4 Weeks

Because total and free serum T_4 levels were identified as sensitive effects in rats administered phenyl isothiocyanate by gavage 5 days/week for 4 weeks (Speijers et al., 1985), all available continuous models in the BMDS (version 2.1 beta) were fit to the total and free serum T_4 data from this study (see Table 1). BMD modeling has been performed using the doses administered in the study before duration adjustment. A default BMR of 1SD from the control mean was used in the BMD modeling because no specific criteria on the magnitude of change of total and free serum T_4 levels that would be considered biologically significant could be identified.

For total T_4, the Linear, Polynomial, Power, and Hill models in the BMDS provide an adequate fit to the data, and Test 2 ($p = 0.2363$) also indicates that using a constant variance model is appropriate for modeling the data. Thus, all of the BMD modeling results for total T_4 shown in Table B-1 were obtained from constant variance models. The Hill model failed to estimate a goodness-of-fit *p*-value. Based on the goodness-of-fit *p*-values and AICs from all models, the Linear model provided the best to the data (see Table B-1 and Figure B-1). The estimated BMD_{1SD} and $BMDL_{1SD}$ for total T_4 was 16.66 and 11.68 mg/kg-day, respectively.

For free T_4, the Linear, Polynomial, Power, and Hill models in the BMDS provided adequate fit to the data, and Test 2 ($p = 0.2134$) also indicates that using a constant variance model is appropriate for modeling the data. Thus, all of the BMD modeling results shown in Table B-1 for free T_4 were obtained from constant variance models. Based on the goodness-of-fit *p*-values and AICs from all models, the Linear, Polynomial, and Power models provided identical fits to the data (see Table B-1). However, because the $BMDL_{1SD}$s estimated

from the four different models varied >3-fold, the lowest $BMDL_{1SD}$ calculated by the Hill model was selected as the POD (see Table B-1 and Figure B-2). Thus, the estimated BMD_{1SD} and $BMDL_{1SD}$ for free T_4 was 6.46 and 2.33 mg/kg-day, respectively.

Table B-1 BMD Modeling Results Based on Total and Free Serum T_4 Levels in Rats Given Phenyl Isothiocyanate by Gavage 5 Days/Week for 4 Weeks[a]

Model	Test 2	Test 3	Goodness-of-fit *p*-value	AIC	BMD_{1SD}	$BMDL_{1SD}$
Total T_4						
Linear[b,c]	**0.2363**	**0.2363**	**0.9779**	**178.70**	**16.66**	**11.68**
Polynomial[b,c]	0.2363	0.2363	0.8867	180.68	18.59	11.70
Power[c,d]	0.2363	0.2363	0.9066	180.67	18.45	11.70
Hill[c,d]	0.2363	0.2363	NA	182.66	16.22	5.88
Free T_4						
Linear[b,c]	0.2134	0.2134	0.4828	92.47	14.45	10.40
Polynomial[b,c]	0.2134	0.2134	0.4828	92.47	14.45	10.40
Power[c,d]	0.2134	0.2134	0.4828	92.47	14.45	10.40
Hill[c,d]	**0.2134**	**0.2134**	**0.8159**	**93.07**	**6.46**	**2.33**

[a]Speijers et al., 1985.
[b]Restrict betas ≤0.
[c]Constant variance.
[d]Restrict power ≥1.

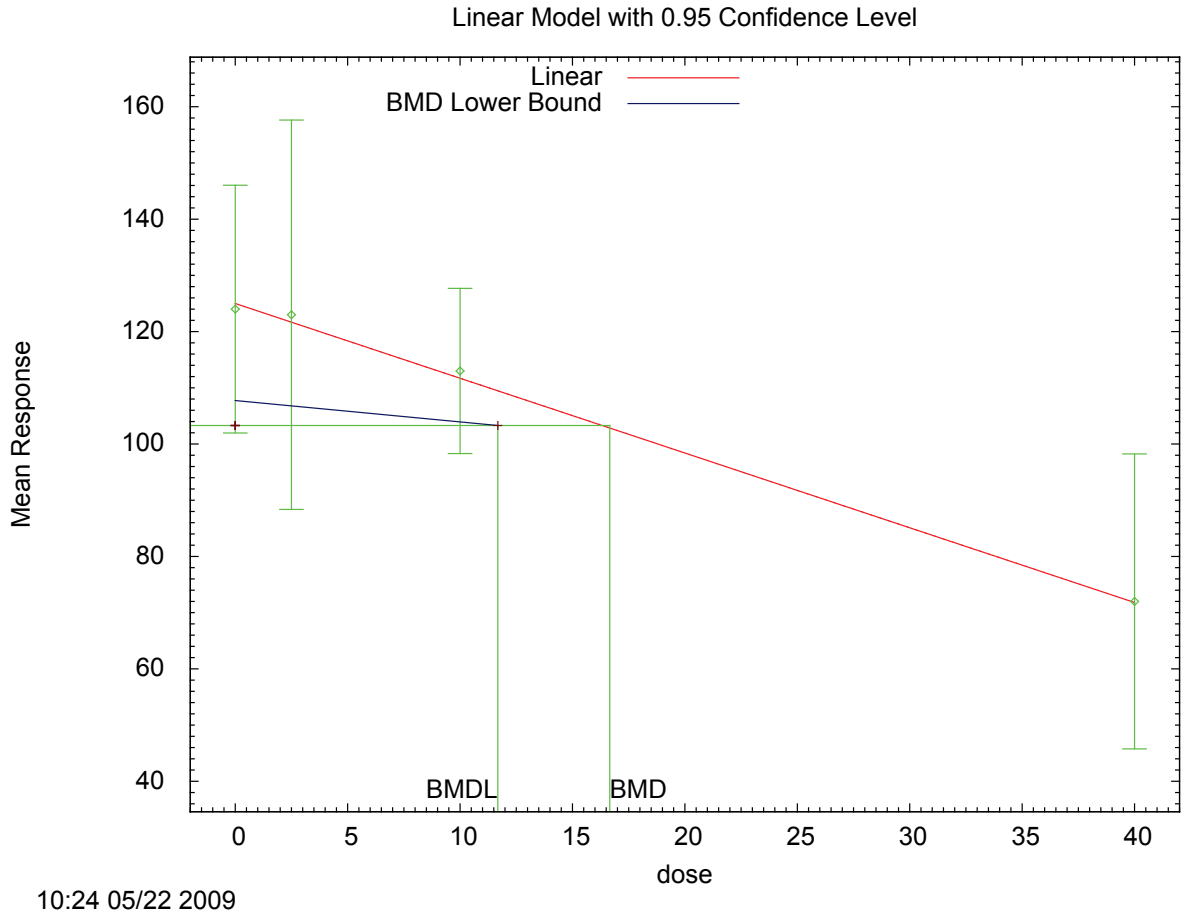

Figure B-1. Dose-Response Modeling of Total Serum T_4 in Rats Given Phenyl Isothiocyanate by Gavage 5 Days/Week for 4 Weeks (Speijers et al., 1985).

The BMDs and BMDLs are associated with a change of 1 SD from the control and are in units of mg/kg-day.

```
=================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\BMDS21Beta\Data\2LinPITLin.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21Beta\Data\2LinPITLin.plt
                                          Tue May 26 11:34:03 2009
 =================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =       587.75
                            rho =            0   Specified
                         beta_0 =      125.452
                         beta_1 =     -1.32965


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                  alpha       beta_0       beta_1

     alpha            1    -3.6e-009     8.2e-010

    beta_0    -3.6e-009            1        -0.64

    beta_1     8.2e-010        -0.64            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha          490.705          141.654             213.068
768.342
```

```
        beta_0          125.452          5.85633             113.973
136.93
        beta_1         -1.32965         0.283553             -1.8854          -
0.773893


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     6        124          125           21         22.2         -0.161
  2.5     6        123          122           33         22.2          0.0965
   10     6        113          112           14         22.2          0.0934
   40     6         72         72.3           25         22.2         -0.0294


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -86.327762            5     182.655523
              A2          -84.205920            8     184.411840
              A3          -86.327762            5     182.655523
          fitted          -86.350115            3     178.700229
               R          -94.154281            2     192.308563


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              19.8967          6        0.002889
   Test 2              4.24368          3          0.2363
   Test 3              4.24368          3          0.2363
   Test 4             0.044706          2          0.9779
```

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 16.66

BMDL = 11.6792

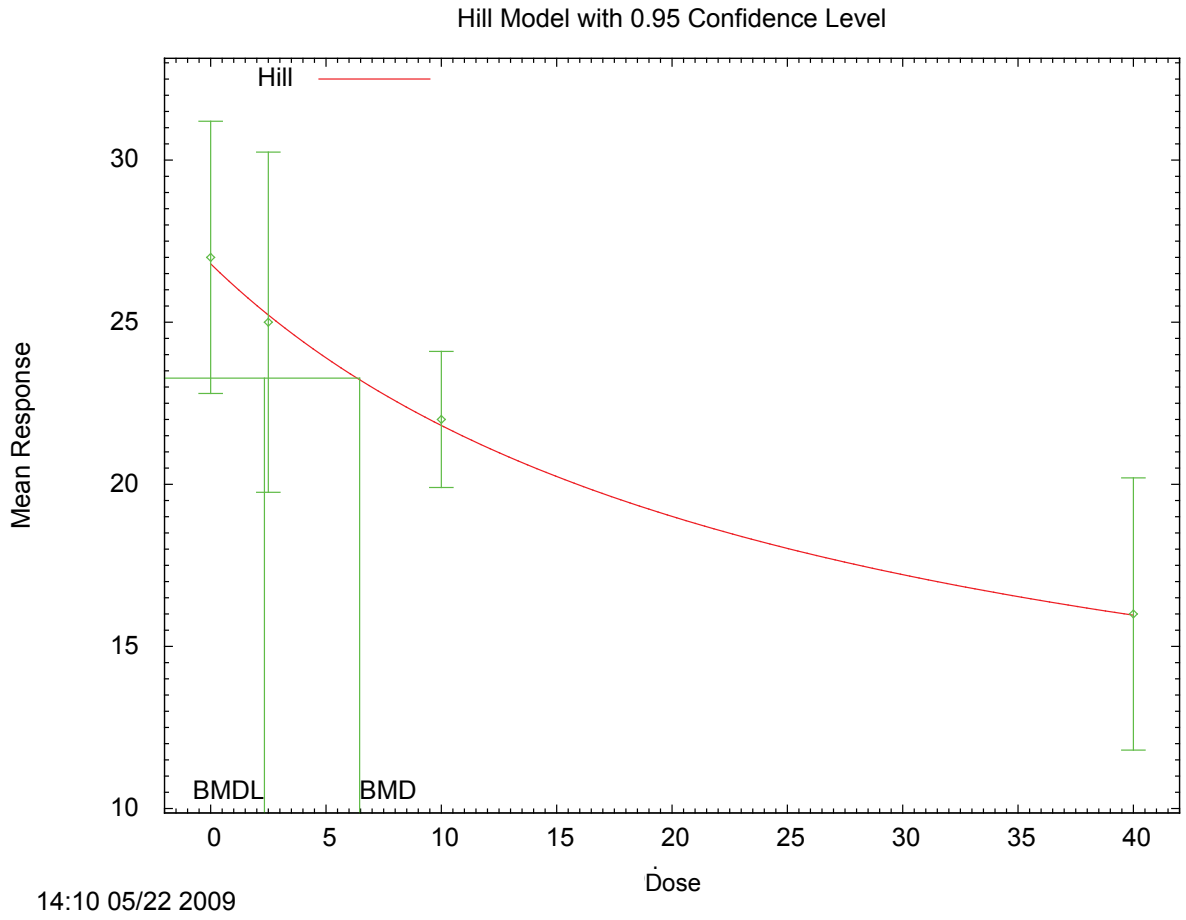

Figure B-2. Dose-Response Modeling of Free Serum T_4 in Rats Given Phenyl Isothiocyanate by Gavage 5 Days/Week for 4 Weeks (Speijers et al., 1985).

The BMDs and BMDLs are associated with a change of 1 SD from the control and are in units of mg/kg-day

```
================================================================
        Hill Model. (Version: 2.14;  Date: 06/26/2008)
        Input Data File: C:\USEPA\BMDS21Beta\Data\1HilPITHil.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21Beta\Data\1HilPITHil.plt
                                          Tue May 26 11:35:53 2009
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =        15.25
                            rho =            0   Specified
                      intercept =           27
                              v =          -11
                              n =     0.875798
                              k =         12.5


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                   alpha    intercept            v            k

     alpha             1     2.4e-008     3.4e-008    -2.9e-008

 intercept     2.4e-008            1         0.31        -0.59

         v     3.4e-008         0.31            1        -0.92

         k    -2.9e-008        -0.59        -0.92            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
```

```
          alpha          12.7371          3.67688             5.53053
19.9436
      intercept          26.8434          1.30271             24.2901
29.3967
              v         -17.7761          7.97842            -33.4135            -
2.1387
              n                1               NA
              k          25.7117          28.4418            -30.0332
81.4567

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	6	27	26.8	4	3.57	0.107
2.5	6	25	25.3	5	3.57	-0.184
10	6	22	21.9	2	3.57	0.0922
40	6	16	16	4	3.57	-0.0156

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model A3: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$
Model A3 uses any fixed variance parameters that were specified by the user

Model R: $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-42.507095	5	95.014191
A2	-40.263184	8	96.526368
A3	-42.507095	5	95.014191
fitted	-42.534207	4	93.068414
R	-52.797690	2	109.595381

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.069	6	0.0003316
Test 2	4.48782	3	0.2134
Test 3	4.48782	3	0.2134
Test 4	0.0542231	1	0.8159

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 6.45889

BMDL = 2.33191