**APPENDIX B–1:  Dose-Response Modeling for Derivation of an RfD for Nitrobenzene**

**B–1.1  METHODS**

The models in U.S. EPA's Benchmark Dose (BMD) software (version 1.3.2) were fit to multiple data sets presented in a 90-day study of gavage exposure to nitrobenzene in F344 rats and B6C3F$_1$ mice (NTP, 1983).  The endpoints designated for full modeling were splenic lesions (congestion, lymphoid depletion) and methemoglobin in male and female rats.  Additional endpoints designated for limited modeling were hemoglobin, hematocrit, red blood cell (RBC) count, reticulocytes, and absolute and relative liver weight in male and female rats, with the understanding that the full modeling regimen would be employed for any of these endpoints that proved to be potentially critical.

The dose levels used were those reported in the study.  In accordance with the U.S. EPA (2000) BMD methodology, default benchmark responses (BMRs) of 10% increase in extra risk and a change in the mean equal to 1 standard deviation (SD) from the control mean were used for dichotomous and continuous data, respectively.   For continuous data, the first model run was conducted to evaluate the assumption of constant variance across dose groups.  If variance was statistically homogenous (p > 0.05), then fit of the various models to the means was evaluated while assuming a constant variance.  If variance was non-constant, then variance was modeled as a power function of the mean (the only option currently available in the BMDS for non-homogenous variance).  For data sets where the fit of the power function to the variance was adequate (p > 0.05), the fit to the means of the various models was evaluated while using the power function to model the variance.

Models were run using the default restrictions on parameters built into the BMDS.  For many of the continuous data sets, the higher degree polynomial models defaulted to linear models.  These models were run again, relaxing the restriction on model parameters to allow non-monotonic dose-response curves.  In some cases, a statistically significant fit was achieved in this way.  For these cases, the shape of the dose-response curve was inspected visually for evaluation of reasonableness, with primary focus on the low-dose region, as unrestricted polynomial models can produce a wide variety of shapes that may have no plausible biological explanation.

## B–1.2  RESULTS

The BMD modeling results are summarized in Table B-1.1.  This table shows the BMDs and BMDLs derived from each endpoint modeled in male and female rats.  The most suitable endpoints for use as potential points of departure for derivation of the RfD appear to be spleen congestion, reticulocyte count, and metHb concentration.  The remainder of this section shows detailed summaries of the modeling results for splenic congestion, reticulocyte count, and metHb concentration, presented sequentially.

| Table B-1.1.  Summary of BMD Modeling Results Based on NTP (1983) | | | | | | |
|---|---|---|---|---|---|---|
| Endpoint | Type | BMR | Modeling Regimen | Sex | BMD (mg/kg) | BMDL (mg/kg) |
| **Spleen congestion** | **dichotomous** | **10% ER** | **full** | **M** | **2.73** | **1.81** |
| **Spleen congestion** | **dichotomous** | **10% ER** | **full** | **F** | **7.07** | **2.70** |
| spleen lymphoid depletion | dichotomous | 10% ER | full | M | 35.24 | 24.51 |
| spleen lymphoid depletion | dichotomous | 10% ER | full | F | 17.79 | 14.22 |
| **Methemoglobin** | **continuous** | **1 SD** | **full** | **M** | **3.08** | **2.17** |
| Methemoglobin | continuous | 1 SD | full | F | ND | ND |
| Hemoglobin | continuous | 1 SD | partial | M | 6.51-8.99 | 5.09-5.60 |
| Hemoglobin | continuous | 1 SD | partial | F | 22.79-35.99 | 16.11-18.70 |
| Hematocrit | continuous | 1 SD | partial | M | ND | ND |
| Hematocrit | continuous | 1 SD | partial | F | ND | ND |
| Rbc | continuous | 1 SD | partial | M | 10.62-18.58 | 7.69-11.59 |
| Rbc | continuous | 1 SD | partial | F | 21.08-30.48 | 11.81-21.48 |
| **Reticulocytes** | **continuous** | **1 SD** | **partial** | **M** | **7.49-9.43** | **5.80-7.94** |
| **Reticulocytes** | **continuous** | **1 SD** | **full** | **F** | **2.37-2.81** | **1.77-1.91** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| absolute liver weight | continuous | 1 SD | partial | M | 7.57-9.51 | 4.73-7.24 |
| absolute liver weight | continuous | 1 SD | partial | F | 8.52-12.13 | 6.19-9.70 |
| relative liver weight | continuous | 1 SD | partial | M | 3.01-4.18 | 2.32-3.42 |
| relative liver weight | continuous | 1 SD | partial | F | 5.07-5.37 | 3.54-4.09 |
| ER = extra risk; SD = standard deviation; M = male; F = female; ND = not determined | | | | | | | |

**PART I.  Male F344 rat spleen congestion**

adequate fit (p>0.01) with all of the models

weibull, gamma, 1-degree polynomial, and quantal linear all converged on the same model, which gave best fit (no others with AIC within 0.5)

BMD= 2.73 mg/kg
BMDL= 1.81 mg/kg

| Model fit to means | $\chi^2$ | df | p-value for model fit | AIC for fitted model | BMD (mg/kg) | BMDL (mg/kg) |
|---|---|---|---|---|---|---|
| Gamma (power >=1) | 3.48 | 4 | 0.48 | 53.33 | 2.73 | 1.81 |
| logistic | 4.57 | 4 | 0.33 | 54.47 | 6.21 | 4.24 |
| log logistic  (slope>=1) | 4.41 | 3 | 0.22 | 56.53 | 3.95 | 0.85 |
| 4 degree polynomial (pos betas) | 3.06 | 3 | 0.38 | 54.68 | 3.29 | 1.91 |
| 3 degree polynomial (pos betas) | 3.32 | 3 | 0.34 | 54.99 | 3.22 | 1.86 |
| 2 degree polynomial (pos betas) | 3.49 | 3 | 0.32 | 55.24 | 3.07 | 1.83 |
| 1 degree polynomial (pos betas) | 3.48 | 4 | 0.48 | 53.33 | 2.73 | 1.81 |
| probit | 4.35 | 4 | 0.36 | 54.18 | 6.16 | 4.37 |
| log probit (slope>=1) | 4.28 | 4 | 0.37 | 54.11 | 4.65 | 3.02 |
| quantal linear | 3.48 | 4 | 0.48 | 53.33 | 2.73 | 1.81 |
| quantal quadratic | 5.77 | 4 | 0.22 | 55.73 | 11.77 | 8.39 |
| weibull (power >=1) | 3.48 | 4 | 0.48 | 53.33 | 2.73 | 1.81 |

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**PART II.  Female F344 rat spleen congestion**

adequate fit (p>0.1) with all of the models

log logistic, gamma, and 3 degree polynomial gave best fit (AIC within 0.5)

BMD= avg (8.54, 7.32,5.36) = 7.07 mg/kg
BMDL= avg (4.00, 2.57, 1.52) = 2.70 mg/kg

| model fit to means | $\chi^2$ | df | p-value for model fit | AIC for fitted model | BMD (mg/kg) | BMDL (mg/kg) |
|---|---|---|---|---|---|---|
| gamma (power >=1) | 0.02 | 4 | 1.00 | 27.90 | 7.32 | 2.57 |
| logistic | 1.52 | 4 | 0.82 | 29.94 | 2.38 | 1.41 |
| log logistic (slope>=1) | 0.00 | 4 | 1.00 | 27.87 | 8.54 | 4.00 |
| 4 degree polynomial (pos betas) | 0.01 | 3 | 0.9997 | 29.89 | 6.22 | 1.42 |
| 3 degree polynomial (pos betas) | 0.16 | 4 | 0.9970 | 28.12 | 5.36 | 1.52 |
| 2 degree polynomial (pos betas) | 0.97 | 4 | 0.91 | 29.25 | 3.54 | 0.93 |
| 1 degree polynomial (pos betas) | 3.39 | 4 | 0.50 | 32.26 | 0.96 | 0.59 |
| Probit | 1.38 | 4 | 0.85 | 29.66 | 2.19 | 1.39 |
| log probit (slope>=1) | 0.00 | 3 | 1.00 | 29.87 | 8.38 | 3.74 |
| quantal linear | 3.39 | 4 | 0.50 | 32.26 | 0.96 | 0.59 |
| quantal quadratic | 0.97 | 4 | 0.91 | 29.25 | 3.54 | 2.61 |
| weibull (power >=1) | 0.00 | 3 | 1.00 | 29.87 | 6.88 | 2.08 |

**PART III.  Male F344 rat reticulocytes**

homogenous variance (p=0.43)

adequate fit (p>0.1) to means with linear and unrestricted 2-degree polynomial model (plausible curve at all dose levels)

marginal fit (p>0.05) with unrestricted 3-degree polynomial; plausible curve at all dose levels

with BMR of 1 SD:
BMD = 7.49 – 9.43 mg/kg
BMDL = 4.97 – 7.94 mg/kg

| model fit to means | df | p-value for model fit | AIC for fitted model | BMD (mg/kg) | BMDL (mg/kg) |
|---|---|---|---|---|---|
| Linear | 3 | 0.16 | 0.52 | 9.43 | 7.94 |
| 3 degree polynomial (unrestricted betas) | 1 | 0.08 | 2.50 | 7.79 | 4.97 |
| 2 degree polynomial (unrestricted betas) | 2 | 0.21 | 0.52 | 7.49 | 5.80 |

restricted higher degree polynomials same as linear

**PART VI.  Female F344 rat reticulocytes**

non-homogenous variance

BMDS variance model had adequate fit (p = 0.29)

adequate fit (p>0.1) to means with unrestricted 3- and 4-degree polynomial
models; plausible dose-response curve, esp at lower doses

with BMR of 1 sd:
BMD= 2.37-2.81 mg/kg
BMDL= 1.77-1.91 mg/kg

| model fit to means | df | p-value for model fit | AIC for fitted model | BMD (mg/kg) | BMDL (mg/kg) |
|---|---|---|---|---|---|
| Linear | 4 | <0.01 | 77.30 | 2.36 | 1.86 |
| 4 degree polynomial (betas >= 0) | 1 | <0.01 | 55.59 | 4.68 | 3.15 |
| 4 degree polynomial (betas unrestricted) | 1 | 0.64 | 43.06 | 2.81 | 1.91 |
| 3 degree polynomial (betas >= 0) | 2 | <0.01 | 62.99 | 4.23 | 2.79 |
| 3 degree polynomial (betas unrestricted) | 2 | 0.46 | 42.38 | 2.37 | 1.77 |
| 2 degree polynomial (betas >= 0) | 3 | <0.01 | 71.15 | 3.62 | 2.45 |
| 2 degree polynomial (betas unrestricted) | 3 | <0.01 | 71.15 | 3.62 | 2.45 |
| power model (power >= 1) | 3 | <0.01 | 76.82 | 3.50 | 2.17 |
| power model (power unrestricted) | 3 | <0.01 | 76.82 | 3.50 | 2.17 |

hill model crashes with or without restriction

**PART V.  Male F344 rat methemoglobin**

non-homogenous variance

BMDS variance model had adequate fit (p = 0.27)

adequate fit (p>0.1) to means with hill model (although BMDL calculation failed) and with unrestricted power and polynomial models

best fit with unrestricted 2-degree polynomial(no others with AIC within 0.5): plausible dose-response curve, esp at lower doses

with BMR of 1 sd:
BMD= 3.08 mg/kg
BMDL= 2.17 mg/kg

with BMR of 1.96 sd (95% CI [assuming unlimited df; actually some lower CI]):
BMD= 6.17 mg/kg
BMDL= 4.33 mg/kg

| model fit to means | df | p-value for model fit | AIC for fitted model | BMD (mg/kg) | BMDL (mg/kg) |
|---|---|---|---|---|---|
| Linear | 3 | <0.01 | 65.92 | 7.96 | 4.70 |
| 3 degree polynomial (pos betas) | 1 | <0.01 | 65.92 | 7.96 | 4.70 |
| 3 degree polynomial (unrestricted betas) | 1 | 0.42 | 46.20 | 2.52 | 1.70 |
| 2 degree polynomial (pos betas) | 2 | <0.01 | 65.92 | 7.96 | 4.70 |
| 2 degree polynomial (unrestricted betas) | 2 | 0.36 | 45.58 | 3.08 | 2.17 |
| power (power >=1) | 2 | <0.01 | 67.92 | 7.96 | 4.70 |
| power (power unrestricted) | 2 | 0.20 | 46.75 | 1.02 | 0.38 |
| hill (power >=1) | 1 | 0.41 | 46.23 | 3.03 | ND |
| hill (power unrestricted) | 1 | 0.41 | 46.23 | 3.03 | ND |

**PART VI.  Female F344 rat methemoglobin**

non-homogenous variance

BMDS variance model does not fit

**APPENDIX B–2:**

**Dose-Response Modeling and Derivation of an RfC for Nitrobenzene based on a Methemoglobin Levels and Olfactory Degeneration**

### B-2.1 Study Selection - Methemoglobinemia

In the case of methemoglobinemia, all data sets from the chronic study (terminal sacrifice) proved to be less sensitive than the data for F344 and CD rats as well as B6C3F1 mice from the subchronic study (CIIT, 1984, 1993). As shown in Table B-2.1.1, the methemoglobin data from the subchronic study exhibited a clear dose-dependent response.

**Table B-2.1.1. Methemoglobinemia in F344 rats following subchronic inhalation exposure to nitrobenzene**

|  | Sex | Exposure level (ppm) | | | |
|---|---|---|---|---|---|
|  |  | 0 | 5 | 16 | 50 |
| **Methemoglobin (%)** | M | $1.2 \pm 0.4$ | $3.0 \pm 1.0$[a] | $4.4 \pm 1.3$[a] | $10.1 \pm 1.2$[a] |
|  | F | $1.6 \pm 0.8$ | $3.2 \pm 0.9$ | $3.9 \pm 1.3$[a] | $10.5 \pm 1.5$[a] |

[a] Significantly different from controls.

Source: CIIT, 1984.

In contrast, increases occurred mostly at high doses in the terminal sacrifice animals from the chronic study. Furthermore, there appears to be a compensatory mechanism as to methemoglobin formation. The increased methemoglobin levels were more obvious in the interim sacrifices (15 months) compared to the terminal sacrifices (24 months) among all species tested. For example, at 25-ppm exposures, interim F344 rats exhibited 163% (males) and 251% (females) increases in methemoglobin levels relative to the corresponding controls. In contrast, the levels were only 136% (males) and 187% (females) versus controls at terminal sacrifice. This compensatory response was even more evident with male CD rats. In short, interim sacrificed animals exhibited statistically significant increases in methemoglobin levels at 1 ppm (346% ↑), 5 ppm (527% ↑), and 25 ppm (496% ↑) versus controls. In contrast, animals at terminal sacrifice showed 104%, 85%, and 167% at the same exposure levels relative to the controls, with only the highest dose (25 ppm) being statistically significant from controls. Lastly, the methemoglobin data from the chronic studies showed inconsistent dose responses. Therefore, methemoglobinemia in the subchronic inhalation study (CIIT, 1984) was selected as a minimally adverse effect because it displayed the most sensitive response (Table B-2.1.2).

**Table B-2.1.2. Methemoglobin Levels in Animals Exposed to Nitrobenzene for 90 Days**

| Species/Strain/Sex | Concentration of Nitrobenzene (ppm) | | | |
|---|---|---|---|---|
| | **0** | **5** | **16** | **50** |
| | Percentage of Methemoglobin in Plasma (mean ± std dev) | | | |
| **F344 rats** males | 1.2 ± 0.4 (5)[a] | 3.0 ± 1.0* (5) | 4.4 ± 1.3* (5) | 10.1 ± 1.2* (5) |
| females | 1.6 ± 0.8 (5) | 3.2 ± 0.9 (5) | 3.9 ± 1.3* (4) | 10.5 ± 1.4* (5) |
| **CD rats** males | 0.6 ± 0.2 (5) | 0.9 ± 0.6 (5) | 3.2 ± 0.7* (5) | 10.1 ± 2.0* (5) |
| females | 2.1 ± 1.2 (5) | 2.3 ± 0.6 (5) | 3.7 ± 0.2 (5) | 9.6 ± 2.5* (5) |
| **B6C3F1 mice** males | 0.7 ± 0.6 (5) | 1.6 ± 0.4 (5) | 2.1 ± 1.3 (5) | 5.8 ± 1.7* (5) |
| females | 1.3 ± 0.9 (5) | 0.8 ± 0.5 (5) | 2.0 ± 0.6 (5) | 5.1 ± 0.8* (5) |

[a] Values in parentheses are the number of animals evaluated in each group.
**\*** Statistically significant difference from controls (p < 0.05).

Source: CIIT, 1984.

## B-2.2 Method of Analysis — Benchmark Dose Modeling (Methemoglobinemia)

The most discriminating and clearly dose-dependent results for methemoglobinemia are compiled in Table B-2.2.1. All results originate from the 90-day subchronic study (CIIT, 1984); since these results were presented in continuous form, a BMR of 1 SD was applied.

**Table B-2.2.1. Benchmark concentrations for methemoglobinemia in mice and rats following subchronic nitrobenzene inhalation**

| Species/strain | Sex | Model used | $p$-Value | EC$_{1SD}$ (ppm) | LEC$_{1SD}$ (ppm) |
|---|---|---|---|---|---|
| Mouse, B6C3F1 | M | Linear [a] | 0.77 | 5.14 | 3.21 |
| | F | Linear [b] | 0.09 | 8.64 | 6.57 |
| Rat, F344 | M | Linear [b] | 0.29 | 5.80 | 4.49 |
| | F | Linear [b] | 0.20 | 6.25 | 4.81 |
| Rat, CD | M | Power (≥ 1) [a] | 0.36 | 2.63 | 1.44 |
| | F | NF [c] | -- | -- | -- |

[a] Non-homogenous variance.
[b] Homogenous variance.
[c] Data cannot be fitted with BMD software.

It should be noted that BMD modeling for the data from the chronic study (Cattley et al., 1994; CIIT, 1993) resulted in higher $EC_{1SD}/LEC_{1SD}$ values (Table B-2.2.2).

The lowest effective concentrations obtained were for metHb levels in male CD rats:

$$EC_{1SD} = 2.63 \text{ ppm}$$
$$LEC_{1SD} = 1.44 \text{ ppm} \qquad \text{(methemoglobinemia)}$$

The complete data set including all doses could be used in this case for BMD modeling.

| Table B-2.2.2.  Summary of BMD Modeling Results for Nitrobenzene Inhalation Data | | | | | | |
|---|---|---|---|---|---|---|
| Endpoint | Type | BMR | Species | Sex | BMC (ppm) | BMCL (ppm) |
| methemoglobin (term chronic) | continuous | 1 SD | mouse | M | 29.07 | 19.48 |
| methemoglobin (term chronic) | continuous | 1 SD | mouse | F | 23.34 | 18.65 |
| methemoglobin (interim chronic) | continuous | 1 SD | F344 rat | M | 13.15[b] | 7.83[b] |
| methemoglobin (term chronic) | continuous | 1 SD | F344 rat | M | ND | ND |
| methemoglobin (interim chronic) | continuous | 1 SD | F344 rat | F | 8.84 | 5.49 |
| methemoglobin (term chronic) | continuous | 1 SD | F344 rat | F | ND | ND |
| methemoglobin (interim chronic) | continuous | 1 SD | CD rat | M | ND | ND |
| methemoglobin (term chronic) | continuous | 1 SD | CD rat | M | 23.87[c] | 18.73[c] |
| methemoglobin (subchronic) | continuous | 1 SD | F344 rat | M | 5.80 | 4.49 |
| methemoglobin (subchronic) | continuous | 1 SD | F344 rat | F | 6.25 | 4.81 |
| **methemoglobin (subchronic)** | **continuous** | **1 SD** | **CD rat** | **M** | **2.63** | **1.44** |

| methemoglobin (subchronic) | continuous | 1 SD | CD rat | F | ND | ND |
|---|---|---|---|---|---|---|
| methemoglobin (subchronic) | continuous | 1 SD | mouse | M | 5.14 | 3.21 |
| methemoglobin (subchronic) | continuous | 1 SD | mouse | F | 8.64[c] | 6.57[c] |

[a]high dose group dropped to achieve adequate fit
[b]BMDS variance model provided only marginally adequate fit for this endpoint
[c]BMD/L based on model with only marginally adequate fit to the means
ER = extra risk; SD = standard deviation; M = male; F = female; ND = not determined

The BMD model outputs for metHb in male CD rats are provided below.

**PART I.  Subchronic study: male CD rat methemoglobin**

non-homogenous variance

BMDS variance model had adequate fit (p = 0.17)

adequate fit (p>0.10) with power and 2-degree polynomial models; best fit with power model

with BMR of 1 sd:
BMD= 2.63 ppm
BMDL= 1.44 ppm

| model fit to means | df | p-value for model fit | AIC for fitted model | BMD (ppm) | BMDL (ppm) |
|---|---|---|---|---|---|
| linear | 2 | 0.09 | 14.06 | 1.49 | 0.98 |
| 2 degree polynomial (pos betas) | 1 | 0.19 | 13.00 | 2.04 | 1.21 |
| power (power >=1) | 1 | 0.36 | 12.16 | 2.63 | 1.44 |

```
========================================================================
       Polynomial Model. Revision: 2.2  Date: 9/12/2002
       Input Data File: C:\BMDS\UNSAVED1.(d)
       Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                   Sat Mar 05 11:42:31 2005
 ========================================================================

 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
The polynomial coefficients are restricted to be positive
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

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 =        1.2225
                                rho =             0
                            beta_0 =      0.221782
                            beta_1 =      0.195956

                              Parameter Estimates

                                            95.0% Wald Confidence Interval
        Variable        Estimate      Std. Err.    Lower Conf. Limit    Upper Conf. Limit
         alpha          0.187388       0.079278        0.0320064            0.342771
          rho           1.29953        0.364616        0.584895             2.01416
        beta_0          0.474179       0.122112        0.234845             0.713514
        beta_1          0.178494       0.016629        0.145902             0.211086

            Asymptotic Correlation Matrix of Parameter Estimates

                    alpha          rho         beta_0        beta_1
       alpha           1          -0.66         -0.32          0.35
         rho         -0.66           1           0.45          -0.5
      beta_0         -0.32         0.45            1          -0.49
      beta_1          0.35         -0.5         -0.49            1

       Table of Data and Estimated Values of Interest

 Dose        N     Obs Mean    Obs Std Dev    Est Mean    Est Std Dev     Chi^2
Res.
------      ---    -------     -----------    -------     -----------    ---------
-

    0        5       0.6          0.2          0.474         0.267          1.06
    5        5       0.9          0.6          1.37          0.53          -1.97
   16        5       3.2          0.7          3.33          0.946        -0.308
   50        5      10.1           2           9.4           1.86          0.845

Model Descriptions for likelihoods calculated

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

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

 Model A3:          Yij = Mu(i) + e(ij)
          Var{e(ij)} = alpha*(Mu(i))^rho

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


                    Likelihoods of Interest

          Model      Log(likelihood)     DF         AIC
           A1          -9.777544          5       29.555088
           A2           1.150392          8       13.699216
           A3          -0.653099          6       13.306198
         fitted        -3.032525          4       14.065049
            R         -38.013168          2       80.026337


                     Explanation of Tests

 Test 1:  Does response 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)

                     Tests of Interest

   Test     -2*log(Likelihood Ratio)   Test df        p-value
   Test 1            78.3271            6             <.0001
   Test 2            21.8559            3             <.0001
   Test 3            3.60698            2             0.1647
   Test 4            4.75885            2             0.0926
```

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 less than .05.  A non-homogeneous variance model
appears to be appropriate

The p-value for Test 3 is greater than .05.  The modeled variance appears to
be appropriate here

The p-value for Test 4 is greater than .05.  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 =         1.49344
```

## Linear Model with 0.95 Confidence Level
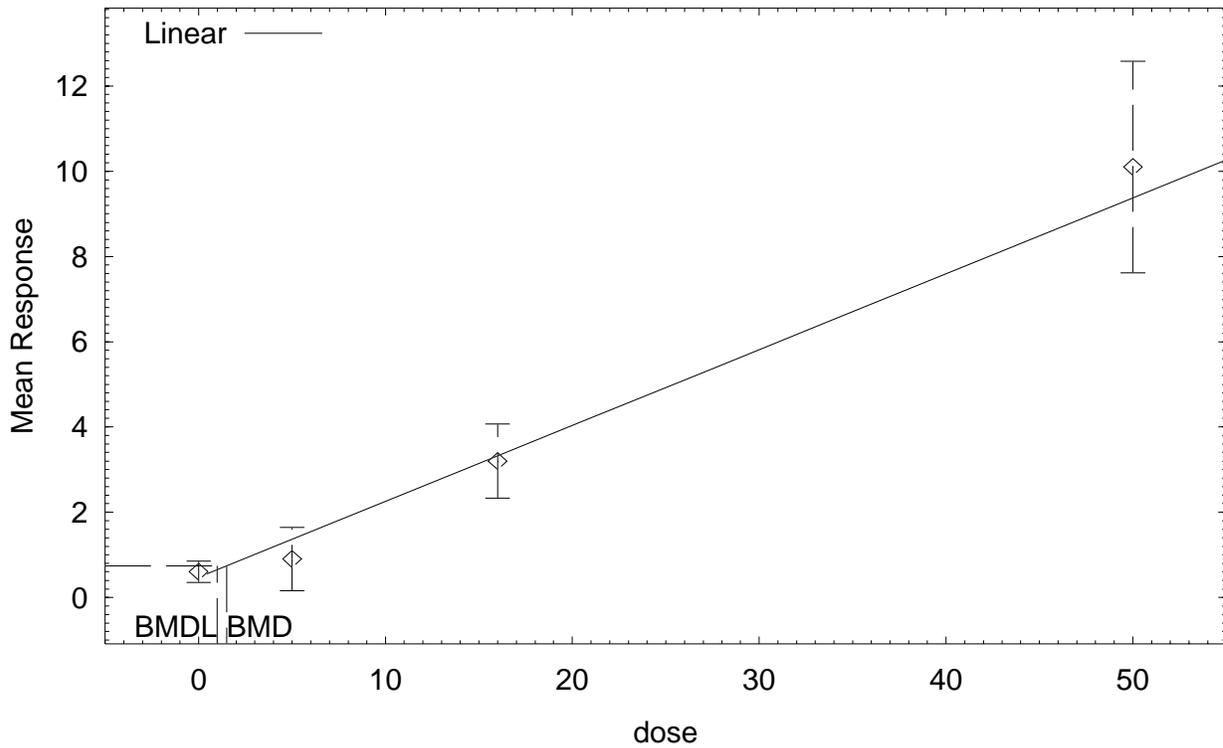


11:42 03/05 2005

BMDL =        0.982284

```
=======================================================================
      Polynomial Model. Revision: 2.2  Date: 9/12/2002
      Input Data File: C:\BMDS\UNSAVED1.(d)
      Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                    Sat Mar 05 11:47:26 2005
=======================================================================

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
  The polynomial coefficients are restricted to be positive
  The variance is to be modeled as Var(i) = alpha*mean(i)^rho

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
```

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```
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                    Default Initial Parameter Values
                          alpha =        1.2225
                            rho =             0
                         beta_0 =      0.413768
                         beta_1 =       0.15339
                         beta_2 =   0.000810597



                           Parameter Estimates


                                         95.0% Wald Confidence Interval
        Variable      Estimate      Std. Err.    Lower Conf. Limit   Upper Conf. Limit
          alpha       0.166137      0.0673099        0.0342118           0.298062
            rho       1.30293       0.333349         0.649576            1.95628
         beta_0       0.518161      0.118364         0.286172            0.750151
         beta_1       0.127539      0.033286         0.0623002           0.192779
         beta_2       0.00131891    0.00078269      -0.00021513          0.00285296


           Asymptotic Correlation Matrix of Parameter Estimates


                    alpha         rho       beta_0       beta_1       beta_2
        alpha           1       -0.62        -0.21         0.26        -0.24
          rho       -0.62           1         0.26        -0.32         0.29
       beta_0       -0.21        0.26            1        -0.53         0.41
       beta_1        0.26       -0.32        -0.53            1        -0.91
       beta_2       -0.24        0.29         0.41        -0.91            1


       Table of Data and Estimated Values of Interest

 Dose        N     Obs Mean    Obs Std Dev   Est Mean    Est Std Dev    Chi^2
Res.
 ------      ---   --------    -----------   --------    -----------    ---------
-

    0        5       0.6          0.2         0.518        0.266          0.689
    5        5       0.9          0.6         1.19         0.456         -1.42
   16        5       3.2          0.7         2.9          0.815          0.833
   50        5      10.1          2          10.2          1.85          -0.112



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)} = alpha*(Mu(i))^rho
```

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


                      Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
             A1          -9.777544        5      29.555088
             A2           1.150392        8      13.699216
             A3          -0.653099        6      13.306198
           fitted       -1.498830        5      12.997661
             R          -38.013168        2      80.026337


                      Explanation of Tests

 Test 1:  Does response 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)
                      Tests of Interest

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

    Test 1            78.3271            6            <.0001
    Test 2            21.8559            3            <.0001
    Test 3            3.60698            2            0.1647
    Test 4            1.69146            1            0.1934
```

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 less than .05.  A non-homogeneous variance model
appears to be appropriate

The p-value for Test 3 is greater than .05.  The modeled variance appears to
be appropriate here

The p-value for Test 4 is greater than .05.  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 =        2.03943


          BMDL =       1.20998
```

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## Polynomial Model with 0.95 Confidence Level
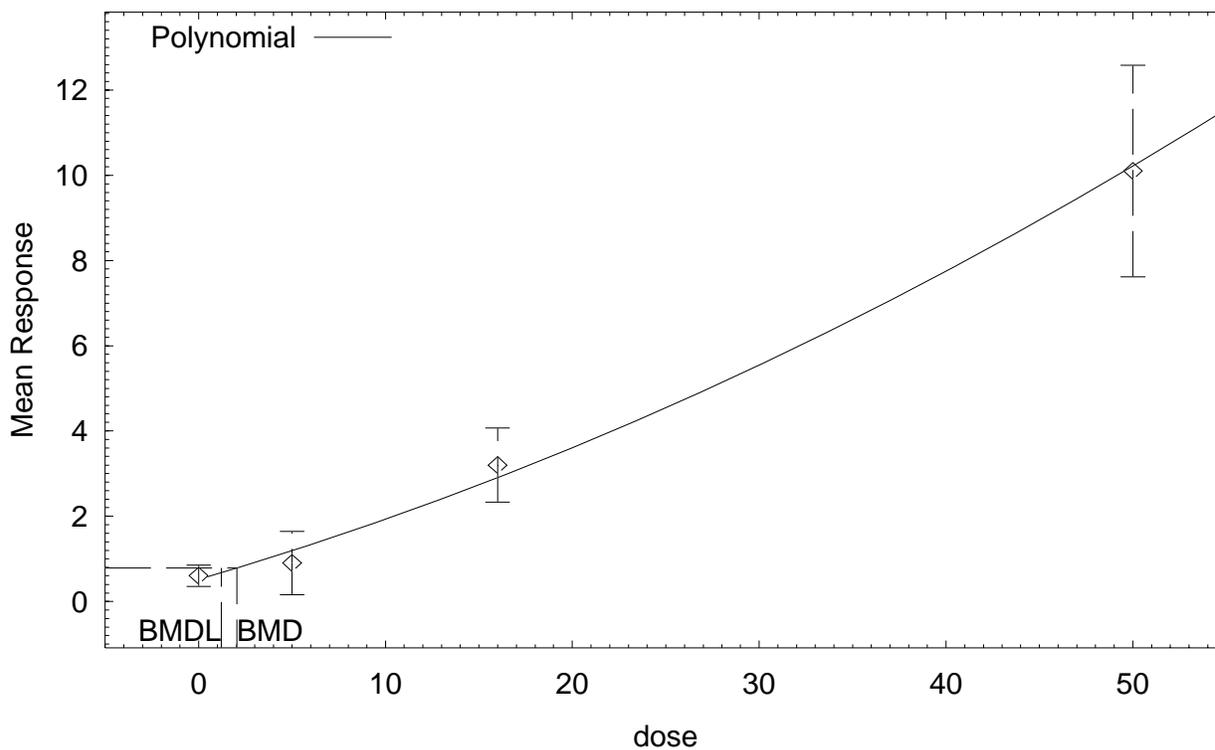


11:47 03/05 2005

```
=====================================================================
      Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
      Input Data File: C:\BMDS\UNSAVED1.(d)
      Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                    Sat Mar 05 11:48:10 2005
=====================================================================

BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = dose
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   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
```

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```
                    Default Initial Parameter Values
                          alpha =        1.2225
                            rho =             0
                        control =           0.6
                          slope =     0.0266776
                          power =       1.50184


              Asymptotic Correlation Matrix of Parameter Estimates

                     alpha           rho       control         slope         power

       alpha            1          -0.6         -0.35         0.025        -0.019

         rho         -0.6             1           0.3         -0.13           0.1

     control        -0.35           0.3             1          -0.4          0.35

       slope        0.025         -0.13          -0.4             1         -0.98

       power       -0.019           0.1          0.35         -0.98             1



                            Parameter Estimates

              Variable             Estimate              Std. Err.
                 alpha             0.160622              0.0668863
                   rho              1.30165               0.323561
               control             0.531155               0.106052
                 slope            0.0812585              0.0347427
                 power              1.22246               0.116766



         Table of Data and Estimated Values of Interest

  Dose        N     Obs Mean     Obs Std Dev    Est Mean     Est Std Dev    Chi^2
 Res.
 ------      ---    --------     -----------    --------     -----------    ---------
  -

     0        5        0.6           0.2          0.531          0.266         0.259
     5        5        0.9           0.6           1.11           0.43        -0.494
    16        5        3.2           0.7           2.94          0.809         0.321
    50        5       10.1             2           10.2           1.82       -0.0723



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)} = alpha*(Mu(i))^rho

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


                    Likelihoods of Interest

          Model       Log(likelihood)    DF        AIC
           A1            -9.777544         5      29.555088
           A2             1.150392         8      13.699216
           A3            -0.653099         6      13.306198
          fitted         -1.077589         5      12.155178
            R           -38.013168         2      80.026337


                    Explanation of Tests

 Test 1:  Does response 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)

                    Tests of Interest

   Test     -2*log(Likelihood Ratio)      d.f        p-value
   Test 1             78.3271              6          <.00001
   Test 2             21.8559              3        6.989e-005
   Test 3             3.60698              2          0.1647
   Test 4             0.84898              1          0.3568
```

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 less than .05.  A non-homogeneous variance model
appears to be appropriate

The p-value for Test 3 is greater than .05.  The modeled variance appears to
be appropriate here

The p-value for Test 4 is greater than .05.  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 =       2.63408


          BMDL =       1.44223
```
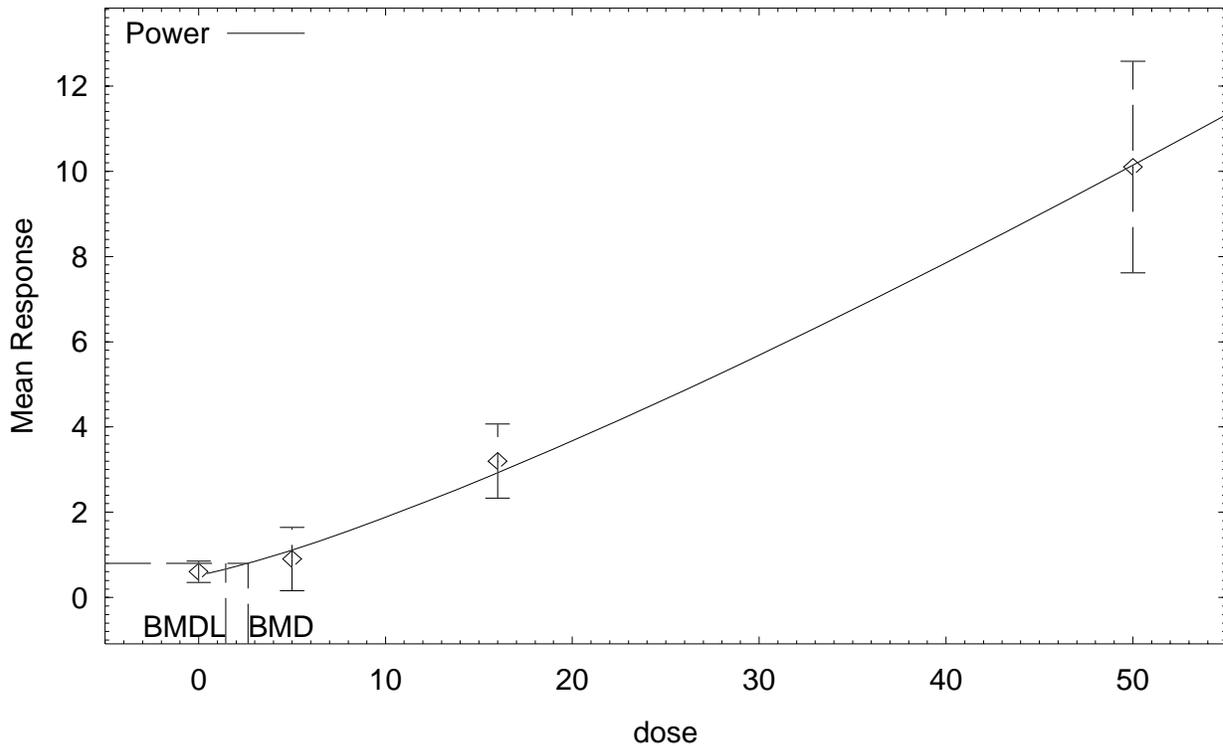
Power Model with 0.95 Confidence Level



11:48 03/05 2005

### B-2.3. Evaluation of Human Equivalent Concentrations - Methemoglobinemia

Because the RfC is a metric that addresses continuous human exposure for a lifetime, adjustments need to be made to animal data obtained from intermittent and/or less-than-lifetime exposure scenarios, as supported in the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b).  The first step required for the final RfC determination is adjustment of the intermittent inhalation exposure to continuous exposure, based on the assumption that the product of exposure concentration and exposure time is constant (U.S. EPA, 2002).  In the subchronic study (CIIT, 1984), animals were exposed for 6 hours/day and 5 days/week.  Therefore, the POD (adjusted LEC) for inhalation of nitrobenzene is as follows:

$$LEC_{(adj)} = LEC \times \text{daily exposure}/24 \text{ hours} \times \text{exposure time}/\text{lifetime}$$
$$LEC_{1SD(adj)} = 1.44 \times 6/24 \times 5/7 = 0.257 \text{ ppm}$$

Furthermore, because RfCs are expressed in $mg/m^3$, the above ppm value needs to be converted to $mg/m^3$ using the conversion factor for nitrobenzene of 1 ppm = 5.04 $mg/m^3$.  Thus, the POD value is:

$$LEC_{1SD(adj)} = 0.257 \times 5.04 = 1.295 \text{ mg/m}^3$$

### B-2.4. Human Equivalent Concentration - Methemoglobinemia

The HEC for methemoglobinemia, which is not a respiratory, but a systemic effect, is calculated based on the following. For systemic effects, nitrobenzene is considered a category 3 gas. EPA guidelines (U.S. EPA, 1994b) mandate to adjust the concentration effective in the animal to humans by a multiplicative factor based on the ratio of air:blood partition coefficients in animals (here: male CD rat) to humans:

$$HEC = LEC \times (c_{BA,a} \div c_{BA,h})$$

where:
$c_{BA,a}$ = air:blood partition coefficient for nitrobenzene in animals
$c_{BA,h}$ = air:blood partition coefficient for nitrobenzene in humans

In the absence of measured air:blood partition coefficients in both male CD rats and humans, the ratio ($c_{BA,a} \div c_{BA,h}$) defaults to unity, and the HEC for methemoglobinemia becomes:

$$HEC_{RH} = LEC_{1SD} = 1.295 \text{ mg/m}^3$$

### B-2.5. Calculation of the RfC based on Methemoglobinemia, a minimally adverse effect—Application of Uncertainty Factors

The RfC for methemoglobinemia as a minimally adverse effect is calculated from the HEC by application of UFs as follows:

$$RfC = HEC \div UF$$

$$RfC = 1.295 \div 30 = 0.0432 \text{ mg/m}^3 = \mathbf{4 \times 10^{-2} \text{ mg/m}^3}$$

The UF of 30 is composed of four parts as follows:

- An intraspecies uncertainty factor of 10 was applied to account for human variability and to protect potentially sensitive humans (e.g., G6PD deficiency) and lifestages (e.g., children).

- A UF of 3 to adjust for interspecies extrapolation from rat to human. The reduced UF is applicable because the RfC is based on an HEC, which accounts for toxicokinetic differences between rats and humans.

- A UF of 1 was applied to account for the extrapolation from a subchronic to a chronic study. The HEC was derived from a 90-day subchronic study (CIIT, 1984) because methemoglobinemia, a minimally adverse effect, appeared to exhibit a compensatory response in the chronic inhalation study. Since metHb is a sensitive measure, albeit minimally adverse at the levels observed, a value of 1 for subchronic to chronic is deemed appropriate.

- An uncertainty factor of 1 was applied to account for database deficiencies. The reduced value is based on the existence of the following studies: a 2-year (lifetime) chronic study with an interim (15-month sacrifice), two-generation reproductive and developmental studies, a subchronic (10-week) inhalation neurotoxicity study, and a 90-day inhalation study.

- A UF for use of a LOAEL instead of a NOAEL was not applied because BMD modeling was used.

## B-2.6. Study Selection – Olfactory Degeneration

For olfactory degeneration, an exposure-related response was observed in both males and females in the two-year inhalation study, with the females being more sensitive than the males (CIIT, 1993). At the highest concentration tested (50 ppm), the incidence was 62% in males 69% in females (Table B-2.6.1). In females, all three treatment groups displayed loss or degeneration of olfactory epithelium to variable degrees. Olfactory degeneration was nearly absent in male mice in the low-concentration group. In the high concentration males, frequently one side of the septum was affected more than the other.

**Table B-2.6.1.  Incidence of olfactory degeneration in mice following chronic nitrobenzene inhalation**

| | Sex | Exposure level (ppm) | | | |
|---|---|---|---|---|---|
| | | **0** | **5** | **25** | **50** |
| **Incidence** | M [a] | 1/67 | 1/66 | 32/65 [b] | 41/66 [b] |
| | F [a] | 0/52 | 19/60 [b] | 47/63 [b] | 42/61 [b] |

[a] Significant positive trend by Armitage-Cochran test, p<0.05.
[b] Significantly different from controls, Fisher Exact test, p<0.05.
Source:  Cattley et al., 1994; CIIT, 1993.

**B-2.7.  Method of Analysis — Benchmark Dose Modeling (Olfactory Degeneration)**

*Olfactory Degeneration — Mouse, Chronic*

Most of the critical effects observed in the CIIT (1984) subchronic study and the CIIT (1993; Cattley et al., 1994) 2-year study, presented in dichotomous or continuous form, were suitable for BMD modeling.  Therefore, a NOAEL/LOAEL approach was not necessary.  Since the data for olfactory degeneration were presented in dichotomous form, a BMR of 10% ER was applied.  In order to obtain an adequate fit of the olfactory degeneration data, the highest dose (50 ppm) had to be excluded.  Using the 0, 5, and 25 ppm doses only, adequate fits were obtained with several models (Table B-2.7.1).

**Table B-2.7.1.  Benchmark concentrations for olfactory degeneration in female mice following chronic nitrobenzene inhalation**

| Model used [a] | *p*-Value | Akaike Information Criterion | $EC_{10}$ (ppm) | $LEC_{10}$ (ppm) |
|---|---|---|---|---|
| Gamma | 0.50 | 149.64 | | |
| Multistage | 0.50 | 149.64 | | |
| Quantal linear | 0.50 | 149.64 | 1.75 | 1.42 |
| Weibull | 0.50 | 149.64 | | |
| Log logistic | 1.00 | 150.32 | 1.44 | 0.79 |

[a] High dose group excluded; only results for models with *p*≥0.5 shown.

The log logistic model afforded a higher *p*-value (1.00) but also a higher Akaike Information Criterion (AIC) value (150.32, difference > 0.5) and was therefore excluded from the RfC derivation.  The following values were used as equivalent concentration at 10% ER ($EC_{10}$) and its 95% lower bound ($LEC_{10}$) for female B6C3F1 mice:

$$EC_{10} = 1.75 \text{ ppm}$$
$$LEC_{10} = 1.42 \text{ ppm} \qquad \qquad \text{(olfactory degeneration)}$$

The BMD model outputs for metHb in female B6C3F1 mice are provided below.

Input Data File: C:\BMDS\UNSAVED1.(d)

Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt

==================================================================

BMDS MODEL RUN

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The form of the probability function is:

P[response]= background+(1-background)*CumGamma[slope*dose,power],
where CumGamma(.) is the cummulative Gamma distribution function

Dependent variable = Incidence

Independent variable = Concentration

Power parameter is restricted as power >=1

Total number of observations = 3

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 (and Specified) Parameter Values
        Background =    0.00943396
            Slope =    0.091108
            Power =        1.3

Asymptotic Correlation Matrix of Parameter Estimates

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

        Slope

    Slope        1

            Parameter Estimates

| Variable | Estimate | Std. Err. |
|---|---|---|
| Background | 0 | NA |
| Slope | 0.0602508 | 0.00791851 |
| Power | 1 | NA |

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

### Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -73.159 | | | |
| Fitted model | -73.8205 | 1.3229 | 2 | 0.5161 |
| Reduced model | -115.963 | 85.6088 | 2 | <.0001 |

AIC:     149.641

### Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|---|---|---|---|---|---|
| 0.0000 | 0.0000 | 0.000 | 0 | 52 | 0 |
| 5.0000 | 0.2601 | 15.607 | 19 | 60 | 0.9986 |
| 25.0000 | 0.7783 | 49.031 | 47 | 63 | -0.6159 |

Chi-square =     1.38    DF = 2     P-value = 0.5025

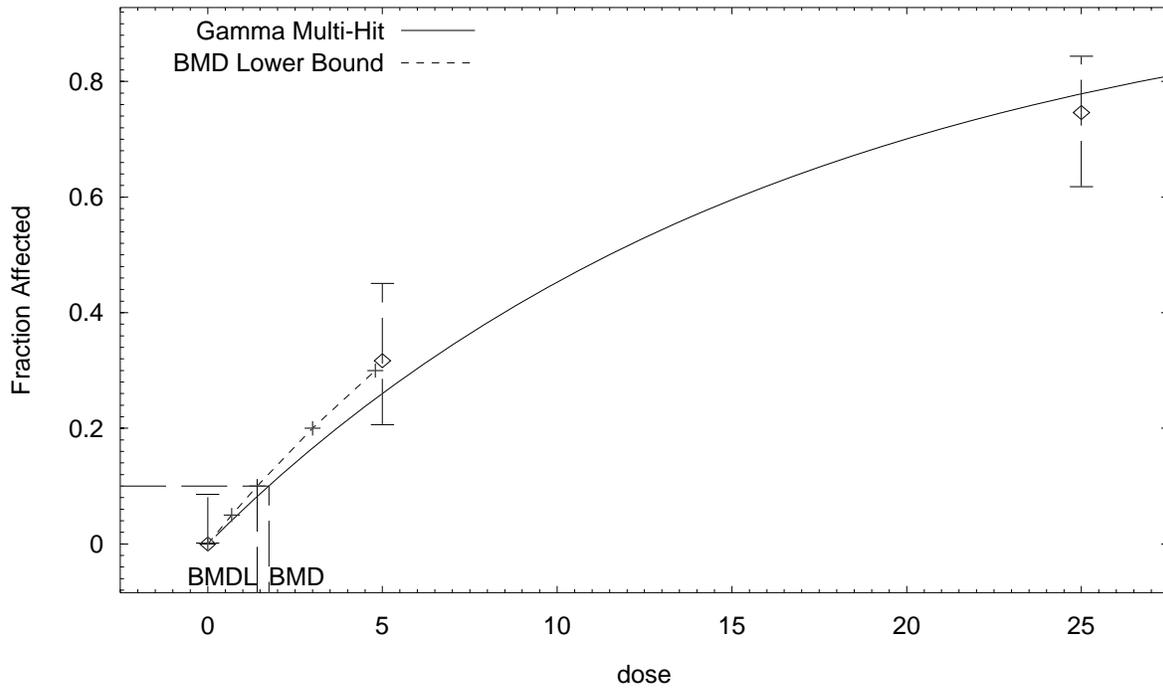### Benchmark Dose Computation

Specified effect =     0.1

Risk Type     =     Extra risk

Confidence level =     0.95

BMD =     1.7487

BMDL =     1.41665

Gamma Multi-Hit Model with 0.95 Confidence Level



12:31 06/16 2006

====================================================================

    Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $

    Input Data File: C:\BMDS\UNSAVED1.(d)

    Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt

                                      Fri Jun 16 12:34:52 2006

====================================================================

 BMDS MODEL RUN

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Observation # < parameter # for Multistage model.

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(

-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

  The parameter betas are restricted to be positive

  Dependent variable = Incidence

Independent variable = Concentration

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3

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
Background =        0
Beta(1) =    0.0814877
Beta(2) =        0
Beta(3) =        0

Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s)  -Background    -Beta(2)    -Beta(3)
    have been estimated at a boundary point, or have been specified by the user,
    and do not appear in the correlation matrix )

        Beta(1)

Beta(1)        1

Parameter Estimates

| Variable | Estimate | Std. Err. |
|---|---|---|
| Background | 0 | NA |
| Beta(1) | 0.0602508 | 0.0101428 |
| Beta(2) | 0 | NA |
| Beta(3) | 0 | NA |

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

Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -73.159 | | | |
| Fitted model | -73.8205 | 1.3229 | 2 | 0.5161 |
| Reduced model | -115.963 | 85.6088 | 2 | <.0001 |

AIC:      149.641

Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Chi^2 Res. |
|---|---|---|---|---|---|
| i: 1 | | | | | |
| 0.0000 | 0.0000 | 0.000 | 0 | 52 | 0.000 |
| i: 2 | | | | | |
| 5.0000 | 0.2601 | 15.607 | 19 | 60 | 0.294 |
| i: 3 | | | | | |
| 25.0000 | 0.7783 | 49.031 | 47 | 63 | -0.187 |

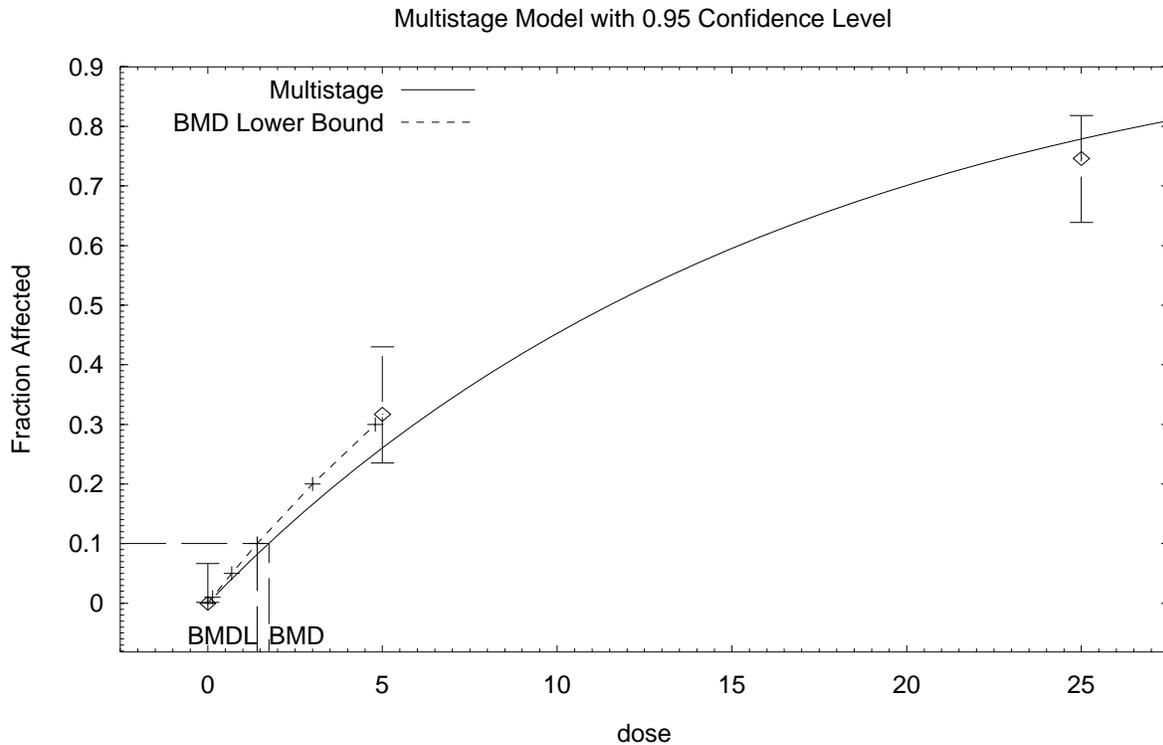Chi-square =     1.38    DF = 2      P-value = 0.5025

Benchmark Dose Computation

Specified effect =        0.1

Risk Type      =     Extra risk

Confidence level =        0.95

BMD =      1.7487

BMDL =      1.41665

## Multistage Model with 0.95 Confidence Level



12:34 06/16 2006

=====================================================================

 Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
 Input Data File: C:\BMDS\UNSAVED1.(d)
 Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt

                                        Fri Jun 16 12:36:40 2006

=====================================================================

 BMDS MODEL RUN

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 The form of the probability function is:

 P[response] = background + (1-background)*[1-EXP(-slope*dose)]


 Dependent variable = Incidence
 Independent variable = Concentration

 Total number of observations = 3
 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 (and Specified) Parameter Values
Background = 0.00943396
Slope = 0.0538418
Power = 1 Specified

Asymptotic Correlation Matrix of Parameter Estimates

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

        Slope

Slope        1

Parameter Estimates

| Variable | Estimate | Std. Err. |
|---|---|---|
| Background | 0 | NA |
| Slope | 0.0602508 | 0.00791851 |

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

Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -73.159 | | | |
| Fitted model | -73.8205 | 1.3229 | 2 | 0.5161 |
| Reduced model | -115.963 | 85.6088 | 2 | <.0001 |

    AIC:     149.641

Goodness of Fit

| Dose | Est._Prob. | Expected | Scaled Observed | Size | Residual |
|------|-----------|----------|-----------------|------|----------|
| 0.0000 | 0.0000 | 0.000 | 0 | 52 | 0 |
| 5.0000 | 0.2601 | 15.607 | 19 | 60 | 0.9986 |
| 25.0000 | 0.7783 | 49.031 | 47 | 63 | -0.6159 |

Chi-square = 1.38   DF = 2   P-value = 0.5025

Benchmark Dose Computation
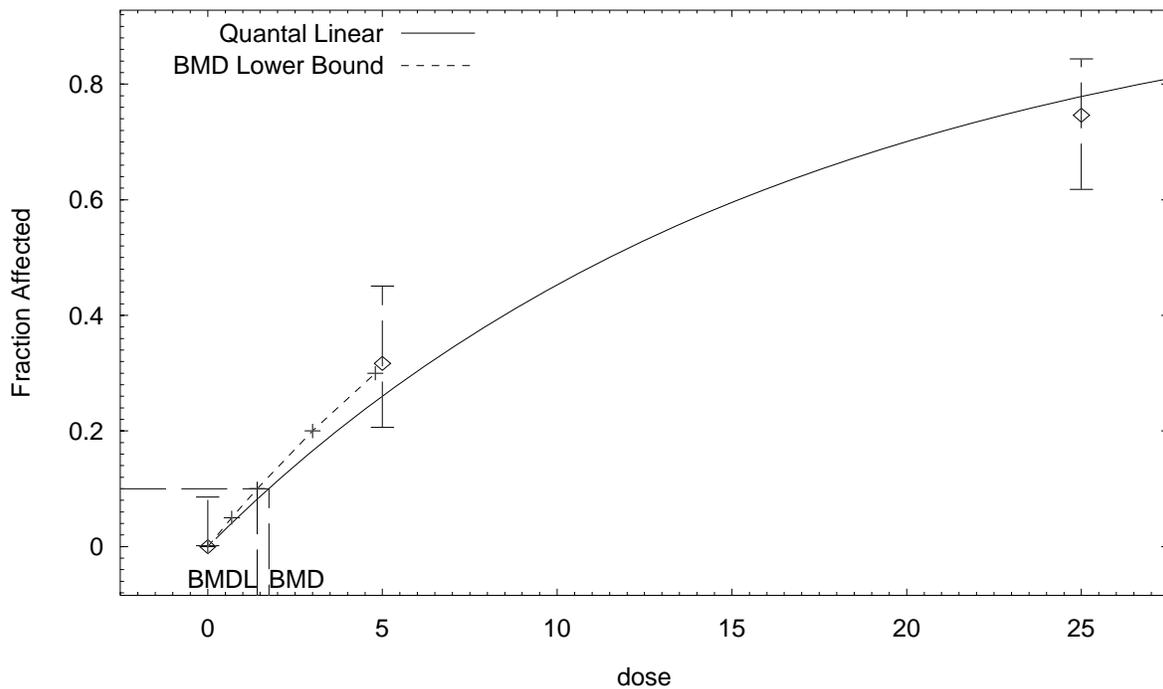
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.7487

BMDL = 1.41665

## Quantal Linear Model with 0.95 Confidence Level



12:36 06/16 2006

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```
==================================================================
       Weibull Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
       Input Data File: C:\BMDS\UNSAVED1.(d)
       Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                        Fri Jun 16 12:37:59 2006
==================================================================
```

BMDS MODEL RUN
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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

Dependent variable = Incidence
Independent variable = Concentration
Power parameter is restricted as power >=1

Total number of observations = 3
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 (and Specified) Parameter Values
            Background =   0.00943396
                Slope =    0.0538418
                Power =          1

       Asymptotic Correlation Matrix of Parameter Estimates

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

            Slope

     Slope        1

                                33

Parameter Estimates

| Variable | Estimate | Std. Err. |
|---|---|---|
| Background | 0 | NA |
| Slope | 0.0602508 | 0.00791851 |
| Power | 1 | NA |

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

Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -73.159 | | | |
| Fitted model | -73.8205 | 1.3229 | 2 | 0.5161 |
| Reduced model | -115.963 | 85.6088 | 2 | <.0001 |

AIC:     149.641

Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|---|---|---|---|---|---|
| 0.0000 | 0.0000 | 0.000 | 0 | 52 | 0 |
| 5.0000 | 0.2601 | 15.607 | 19 | 60 | 0.9986 |
| 25.0000 | 0.7783 | 49.031 | 47 | 63 | -0.6159 |

Chi-square =     1.38    DF = 2      P-value = 0.5025
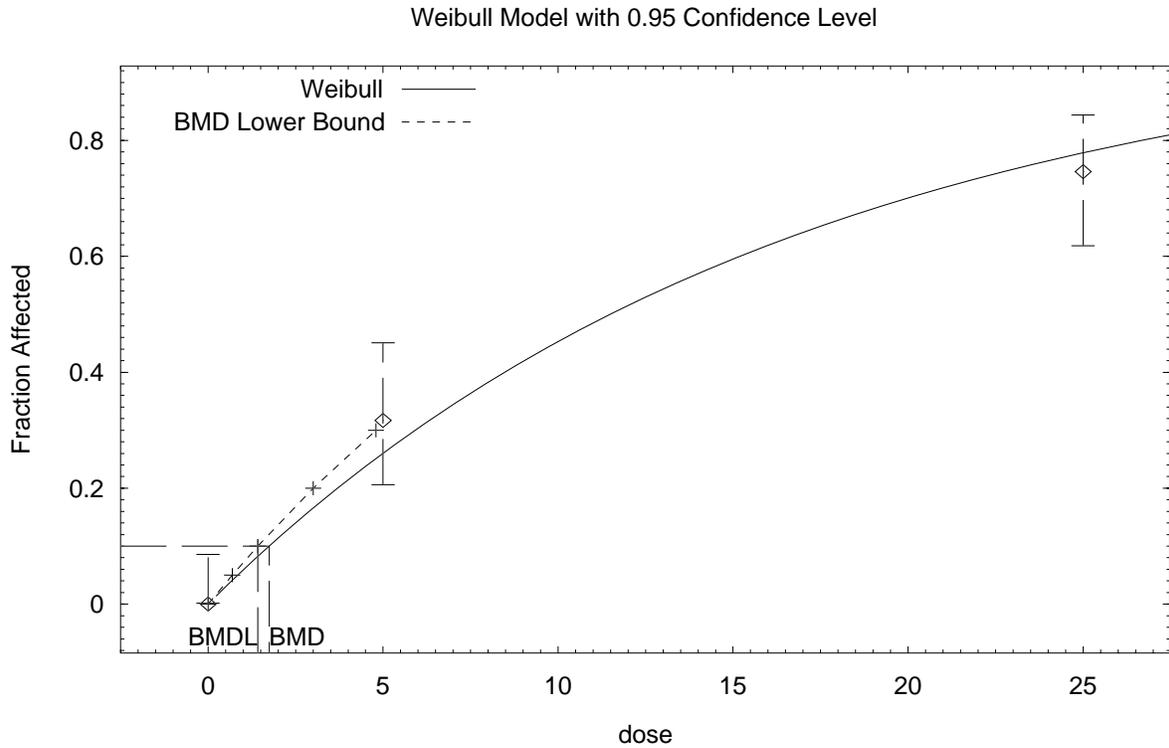
Benchmark Dose Computation

Specified effect =      0.1

Risk Type      =    Extra risk

Confidence level =      0.95

BMD =        1.7487

BMDL =       1.41665

### Weibull Model with 0.95 Confidence Level



12:37 06/16 2006

====================================================================

Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $

Input Data File: C:\BMDS\UNSAVED1.(d)

Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt

                                        Fri Jun 16 12:33:53 2006

====================================================================

BMDS MODEL RUN

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The form of the probability function is:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = Incidence

Independent variable = Concentration

Slope parameter is restricted as slope >= 1

Total number of observations = 3

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

User has chosen the log transformed model

Default Initial Parameter Values
background =        0
intercept =    -2.61583
slope =    1.14741

Asymptotic Correlation Matrix of Parameter Estimates

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

|  | intercept | slope |
|---|---|---|
| intercept | 1 | -0.95 |
| slope | -0.95 | 1 |

Parameter Estimates

| Variable | Estimate | Std. Err. |
|---|---|---|
| background | 0 | NA |
| intercept | -2.61583 | 0.625987 |
| slope | 1.14741 | 0.249152 |

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

Warning: Likelihood for the fitted model larger than the Likelihood for the full model.

Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|-------|-----------------|----------|---------|---------|
| Full model | -73.159 | | | |
| Fitted model | -73.159 | -2.84217e-014 | 1 | -1 |
| Reduced model | -115.963 | 85.6088 | 2 | <.0001 |

AIC:      150.318

Goodness  of  Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|------|-----------|----------|----------|------|-----------------|
| 0.0000 | 0.0000 | 0.000 | 0 | 52 | 0 |
| 5.0000 | 0.3167 | 19.000 | 19 | 60 | 1.443e-008 |
| 25.0000 | 0.7460 | 47.000 | 47 | 63 | 1.383e-008 |

Chi-square =      0.00    DF = 1      P-value = 1.0000

Benchmark Dose Computation

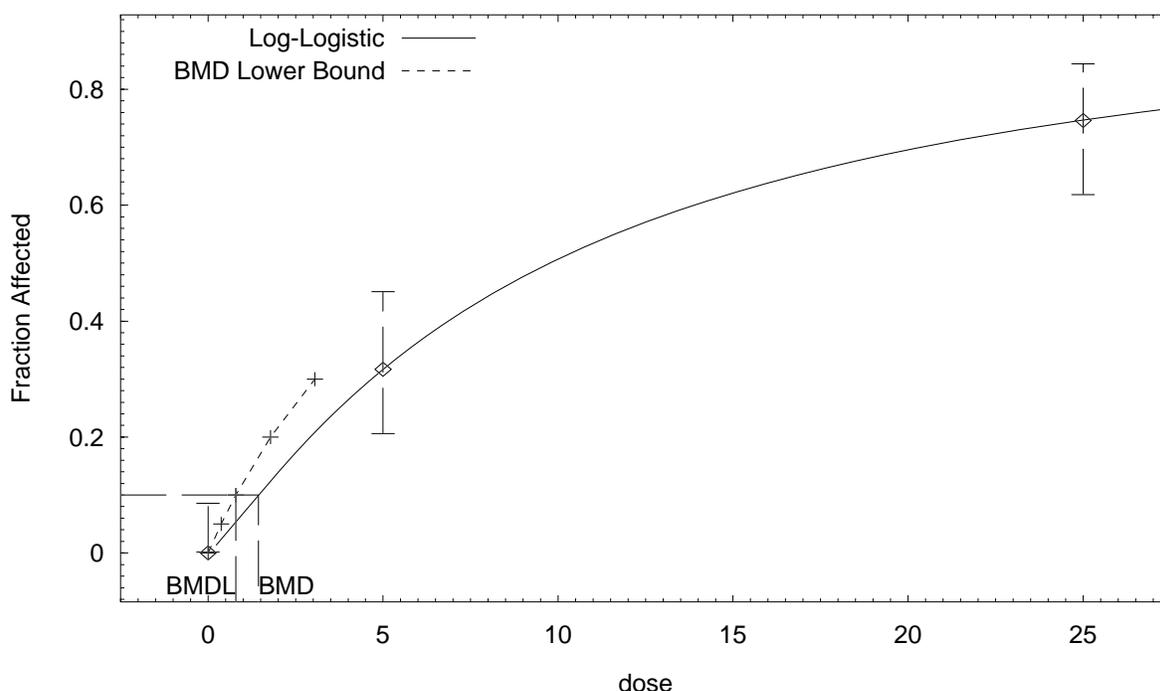Specified effect =       0.1

Risk Type      =    Extra risk

Confidence level =       0.95

BMD =      1.44026

BMDL =     0.791577

Log-Logistic Model with 0.95 Confidence Level



12:33 06/16 2006

## B-2.8. Evaluation of Human Equivalent Concentrations - Olfactory Degeneration

Because the RfC is a metric that addresses continuous human exposure for a lifetime, adjustments need to be made to animal data obtained from intermittent and/or less-than-lifetime exposure scenarios, as mandated in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). The first step required for the final RfC determination is adjustment of the intermittent inhalation exposure to continuous exposure, based on the assumption that the product of exposure concentration and exposure time is constant (U.S. EPA, 2002). In both the subchronic (CIIT, 1984) and the chronic studies (Cattley et al., 1994; CIIT, 1993), animals were exposed for 6 hours/day. In the subchronic study, animals were exposed 5 days per week, and in the chronic study 505 days of an assumed 2-year, or 730 day, lifetime (to adjust for weekends as holidays, as stated by the authors). Therefore, the PODs (adjusted LEC) for chronic inhalation of nitrobenzene are as follows:

$$LEC_{(adj)} = LEC \times \text{daily exposure/24 hours} \times \text{exposure time/lifetime}$$

$$LEC_{10(adj)} = 1.42 \times 6/24 \times 5/7 = 0.253 \text{ ppm} \qquad \text{(olfactory degeneration)}$$

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Furthermore, since RfCs are expressed in mg/m$^3$, the above ppm values need to be converted to mg/m$^3$ using the conversion factor for nitrobenzene of 1 ppm = 5.04 mg/m$^3$. Thus, the POD values are:

$$\text{LEC}_{10(\text{adj})} \;=\; 0.253 \times 5.04 \;=\; 1.275 \text{ mg/m}^3 \qquad\qquad \text{(olfactory degeneration)}$$

### B-2.9. *Human Equivalent Concentration — Olfactory Degeneration*

EPA guidance for RfC evaluation provides procedures for determining a human equivalent concentration (HEC) from the duration-adjusted POD [here: LEC$_{10(\text{adj})}$] obtained from animal data (U.S. EPA, 1994b). The approach considers the physicochemical characteristics of the gas or vapor in question as well as the toxicological specifics of the target tissue (viz., respiratory vs. systemic and, in the former case, extrathoracic, thoracic, tracheobronchial, or pulmonary). The effect considered, degeneration of the olfactory epithelium, is an extrathoracic effect. Nitrobenzene qualifies as a category 2 gas: moderately water soluble, reactive in respiratory tissue, and toxicologically active at remote sites (U.S. EPA, 1994b).

*Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b) suggest that HECs be estimated by applying to the duration-adjusted POD [here: the LEC$_{10(\text{adj})}$], a factor that is specific for the affected region of the respiratory tract and the breathing characteristic of the species to be compared. This factor, the regional gas dose ratio (RGDR), as detailed in the RfC guidance (U.S. EPA, 1994b) is determined for the extrathoracic (ET) region as follows:

$$\text{RGDR}_{\text{ET}} \;=\; (\text{MV}_a/\text{S}_{a,\text{ET}}) \div (\text{MV}_h/\text{S}_{h,\text{ET}})$$

where:
MV$_a$ = minute volume for animals, here: mice $=$ 0.06 m$^3$/day
MV$_h$ = minute volume for humans $=$ 20 m$^3$/day
S$_{a,\text{ET}}$ = default surface area for respiratory effects in the murine extrathoracic area $=$ 3 cm$^2$
S$_{h,\text{ET}}$ = default surface area for respiratory effects in the human extrathoracic area $=$ 200 cm$^2$

The minute volume, MV$_a$, for female B6C3F1 mice in chronic studies was calculated as:

$$\ln V_E \;=\; b_0 + b_1 \times \ln \text{BW}$$

where:

$V_E$ = minute volume

$b_0$ = intercept from algorithm to calculate the default minute volume in mice = 0.326

$b_1$ = coefficient from algorithm to calculate the default minute volume in mice = 1.050

BW = default body weight for female B6C3F1 mice in chronic studies = 0.0353 kg

Hence:

$$\ln V_E = 0.326 + 1.05 \times \ln 0.0353 = 0.326 + 1.05 \times \text{-}3.34$$
$$\ln V_E = \text{-}3.19$$
$$V_E = 0.0414 \text{ L/min} = 0.06 \text{ m}^3/\text{day}.$$

Substituting these values into the $RGDR_{ET}$ equation, the RGDR is calculated as:

$$RGDR = 0.06/3 \div 20/200 = 0.20$$

Finally, the HEC is derived as follows:

$$HEC = LEC_{10} \times RGDR$$
$$HEC_{OD} = 1.275 \times 0.20 = 0.255 \text{ mg/m}^3 \qquad \text{(olfactory degeneration)}$$

**B-2.10.  Calculation of the RfC based on Olfactory Degeneration, a portal of entry effect—Application of Uncertainty Factors**

*Olfactory Degeneration*

The RfC for olfactory degeneration as the critical effect is calculated from the HEC by application of UFs as:

$$RfC = HEC \div UF$$
$$RfC = 0.255 \div 30 = 0.0085 \text{ mg/m}^3 = \mathbf{9 \times 10^{\text{-}3} \text{ mg/m}^3}$$

The UF of 30 is composed of four parts:

- An intraspecies uncertainty factor of 10 was applied to account for human variability and to protect potentially sensitive humans and lifestages (e.g., children).

- An interspecies uncertainty factor of 3 was applied to adjust for interspecies extrapolation from mouse to human. The reduced UF is applicable because the RfC is based on a HEC (U.S. EPA, 1994b).

- A subchronic-to-chronic uncertainty factor for extrapolation to lifetime exposure was not applied since the data used originated from a 2-year (lifetime) chronic study.

- An uncertainty factor of 1 was applied to account for database deficiencies. The reduced value is based on the existence of the following studies: a 2-year (lifetime) chronic study with an interim (15-month sacrifice), two-generation reproductive and developmental studies, a subchronic (10-week) inhalation neurotoxicity study, and a 90-day inhalation study.

**APPENDIX B–3:**

**Dose-Response Modeling of Carcinogenicity Data for Nitrobenzene**

## B–3.1  METHODS

All data sets in rats and mice exposed to nitrobenzene vapor by inhalation for up to 2 years (CIIT, 1993; Cattley et al., 1994)showing at least a statistical trend for increased tumor incidence with increasing exposure were fit using models for quantal data in U.S. EPA's Benchmark Dose (BMD) software (version 1.3.2).  The data modeled are shown in Table B-3.1.1.  The exposure levels used were those reported in the study.  They were not adjusted for duration of exposure or converted to human equivalent concentrations (HECs) prior to modeling. In accordance with the U.S. EPA (2000) BMD methodology, a benchmark response (BMR) of 10% increase in extra risk was used kidney adenomas and carcinomas, and a 5% increase in extra risk was used for liver and thyroid adenomas and carcinomas.  Models were run using the default restrictions on parameters built into the BMDS.

**Table B–3.1.1  Tumorigenic Responses in Experimental Animals Exposed to Nitrobenzene via Inhalation for up to 2 Years**

| Animal/Strain/Site | Incidence of Neoplasms | | | |
|---|---|---|---|---|
| | Concentration of Nitrobenzene (ppm) | | | |
| Rats | 0 | 1 | 5 | 25 |
| **F-344 Rats (male)** Liver: hepatocellular adenoma or carcinoma | 1/43[t] | 4/50 | 5/47 | 16/46 |
| Thyroid: follicular cell adenoma or adenocarcinoma | 1/43[t] | 1/50 | 5/47 | 8/46 |
| Kidney: tubular adenoma and carcinoma | 0/43 | 0/50 | 0/47 | 6/46 |
| | Concentration of Nitrobenzene (ppm) | | | |
| Mice | 0 | 5 | 25 | 50 |
| **B6C3F1 Mice (male)** Lung: A/B adenoma or carcinoma | 8/42 | 16/44 | 20/45 | 21/48 |
| Thyroid: follicular cell adenoma | 0/41 | 4/44 | 1/45 | 6/46 |

[t] Significant positive exposure-related trend in incidence, by Cochran-Armitage trend test (p<0.05).

Source: CIIT, 1993; Cattley et al., 1994.

## B–3.2  RESULTS

The BMD modeling results are summarized in Table B-3.2.1.  This table shows the BMDs and BMDLs derived from each endpoint modeled.  The most suitable endpoint for use as point of departure for derivation of the inhalation unit risk appears to be liver tumors in male rats.  The BMDL for this endpoint was the lowest for any endpoint, and was calculated from a model with good fit to the data (p=0.63).  The remainder of this section shows detailed summaries of the modeling results for each endpoint, presented sequentially.  The BMDS outputs for all model runs are presented below.  The multistage model results were adequate for each endpoint, and provided the basis for the final cancer risk estimates.

**Table B–3.2.1.  Summary of BMD Modeling Results for Nitrobenzene Cancer Data**

| Tumor | Species | Sex | BMD (ppm) | BMDL (ppm) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Liver: hepatocellular adenoma and carcinoma | rat | male | 3.3-9.3[b] | 1.7-7.4 |
| Thyroid: follicular cell adenoma and adenocarcinoma | rat | male | 6.6-14.4[b] | 3.3-10.3 |
| Kidney: tubular cell adenoma and carcinoma | rat | male | 22.2-24.3[c] | 13.0-16.8 |
| Lung: A/B adenoma and carcinoma | mouse | male | 6.0-22.3[a] | 2.9-15.4[a] |
| Thyroid: follicular cell adenoma | mouse | male | 29.5-45.7[a] | 12.5-26.1[a] |
| [a] based on models with only marginally adequate fit (0.10>p>0.05) [b] based on a BMR of 5% [c] based on a BMR of 10% | | | | |
| | | | | |

## PART I. Male F344 rat liver tumors (hepatocellular adenoma and carcinoma)

| model fit to means | p-value for model fit | AIC for fitted model | BMD (ppm) | BMDL (ppm) |
|---|---|---|---|---|
| gamma (power >=1) | 0.63 | 133.591 | 3.29 | 2.17 |
| logistic | 0.63 | 133.557 | 2.79 | 1.68 |
| **multistage** | **0.63** | **133.591** | **3.29** | **2.17** |
| probit | 0.39 | 134.815 | 7.80 | 5.49 |
| quantal linear | 0.63 | 133.591 | 3.29 | 2.17 |
| quantal quadratic | 0.38 | 134.902 | 9.32 | 7.43 |
| weibull (power >=1) | 0.63 | 133.591 | 3.29 | 2.17 |

## PART II. Male F344 rat thyroid tumors (follicular cell adenomas and adenocarcinomas)

| model fit to means | p-value for model fit | AIC for fitted model | BMD (ppm) | BMDL (ppm) |
|---|---|---|---|---|
| gamma | 0.37 | 99.429 | 6.64 | 3.78 |
| logistic | 0.41 | 99.2686 | 6.05 | 3.26 |
| multistage | 0.37 | 99.429 | 6.64 | 3.78 |
| probit | 0.10 | 101.733 | 14.05 | 8.98 |
| quantal linear | 0.37 | 99.429 | 6.64 | 3.78 |
| quantal quadratic | 0.12 | 101.493 | 14.41 | 10.32 |
| weibull (power >=1) | 0.37 | 99.429 | 6.64 | 3.78 |

**PART III.  Male F344 rat kidney tumors (tubular adenoma and carcinomas)**

| model fit to means | p-value for model fit | AIC for fitted model | BMD (ppm) | BMDL (ppm) |
|---|---|---|---|---|
| gamma | 1.00 | 39.6235 | 23.84 | 16.04 |
| logistic | 1.00 | 39.6235 | 24.31 | 16.03 |
| multistage | 0.99 | 37.7291 | 22.81 | 16.78 |
| probit | 1.00 | 39.6235 | 23.69 | 15.54 |
| quantal linear | 0.65 | 40.4462 | 23.85 | 13.03 |
| quantal quadratic | 0.96 | 38.1592 | 22.19 | 16.40 |
| weibull (power >=1) | 1.00 | 39.6235 | 24.34 | 16.30 |

**PART IV.  Male B6C3F1 mouse lung tumors (A/B adenomas and carcinomas)**

| model fit to means | p-value for model fit | AIC for fitted model | BMD (ppm) | BMDL (ppm) |
|---|---|---|---|---|
| gamma (power >=1) | 0.18 | 233.658 | 7.51 | 4.14 |
| logistic | 0.21 | 233.337 | 6.02 | 2.94 |
| multistage | 0.18 | 233.658 | 7.51 | 4.14 |
| probit | 0.08 | 235.228 | 18.43 | 11.15 |
| quantal linear | 0.18 | 233.658 | 7.51 | 4.14 |
| quantal quadratic | 0.07 | 233.566 | 22.26 | 15.38 |
| weibull (power >=1) | 0.18 | 233.658 | 7.51 | 4.14 |

**PART V.  Male B6C3F1 mouse thyroid tumors (follicular cell adenoma)**

| model fit to means | p-value for model fit | AIC for fitted model | BMD (ppm) | BMDL (ppm) |
|---|---|---|---|---|
| gamma (power >=1) | 0.07 | 82.0142 | 45.67 | 16.06 |
| logistic | 0.03 | 83.3018 | 29.51 | 12.44 |
| multistage | 0.05 | 82.528 | 41.08 | 14.77 |
| probit | 0.02 | 84.009 | 29.94 | 13.37 |
| quantal linear | 0.03 | 83.2877 | 29.94 | 13.37 |
| quantal quadratic | 0.04 | 83.0044 | 38.96 | 26.07 |
| weibull (power >=1) | 0.03 | 83.2877 | 29.94 | 13.37 |

## B–3.3 BMDS OUTPUTS FOR SELECTED MODEL RUNS

**PART I.  Male F344 rat liver tumors (hepatocellular adenoma or carcinoma)**

```
==================================================================
        Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                        Mon Jul 10 11:35:35 2006
==================================================================
```

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

  The parameter betas are restricted to be positive


  Dependent variable = Incidence
  Independent variable = Concentration

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3


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
              Background =    0.0425205
                Beta(1) =    0.0148284
                Beta(2) =          0
                Beta(3) = 8.47487e-007


      Asymptotic Correlation Matrix of Parameter Estimates

    ( *** The model parameter(s)  -Beta(2)   -Beta(3)
          have been estimated at a boundary point, or have been specified by the user,
          and do not appear in the correlation matrix )

        Background      Beta(1)

Background        1      -0.57

Beta(1)      -0.57          1


### Parameter Estimates

| Variable | Estimate | Std. Err. |
|---|---|---|
| Background | 0.0410129 | 0.0921379 |
| Beta(1) | 0.0155752 | 0.00868085 |
| Beta(2) | 0 | NA |
| Beta(3) | 0 | NA |

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


### Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -64.3357 | | | |
| Fitted model | -64.7955 | 0.919545 | 2 | 0.6314 |
| Reduced model | -75.2506 | 21.8296 | 3 | <.0001 |

AIC:      133.591


### Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Chi^2 Res. |
|---|---|---|---|---|---|
| i: 1 | | | | | |
| 0.0000 | 0.0410 | 1.764 | 1 | 43 | -0.451 |
| i: 2 | | | | | |
| 1.0000 | 0.0558 | 2.792 | 4 | 50 | 0.458 |
| i: 3 | | | | | |
| 5.0000 | 0.1129 | 5.304 | 5 | 47 | -0.065 |
| i: 4 | | | | | |
| 25.0000 | 0.3503 | 16.114 | 16 | 46 | -0.011 |

Chi-square =      0.92    DF = 2      P-value = 0.6314


### Benchmark Dose Computation

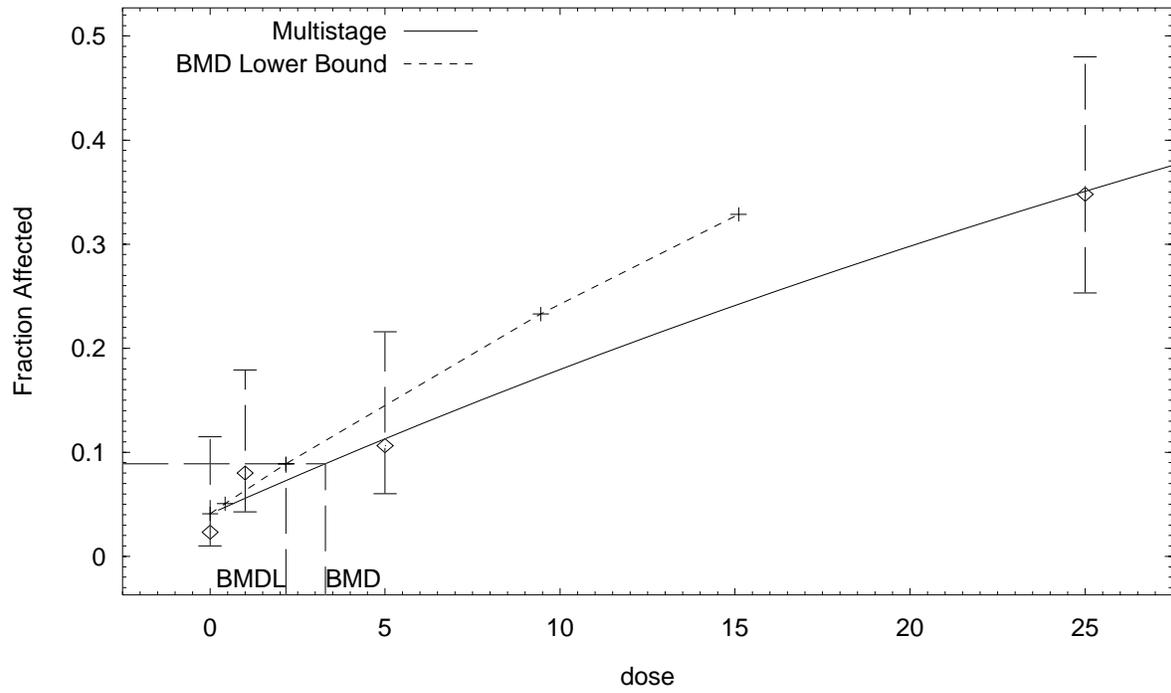Specified effect =      0.05

Risk Type    =    Extra risk

Confidence level =      0.95

BMD =      3.29326

BMDL =      2.17164

Multistage Model with 0.95 Confidence Level

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**PART II.  Male F344 rat thyroid tumors (follicular cell adenoma and adenocarcinoma)**

```
=====================================================================
        Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                        Mon Jul 10 13:17:19 2006
=====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Incidence
   Independent variable = Concentration

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 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
                    Background =    0.0358259
                      Beta(1) =    0.0064946
                      Beta(2) =           0
                      Beta(3) =           0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)    -Beta(3)
                 have been estimated at a boundary point, or have been
specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)
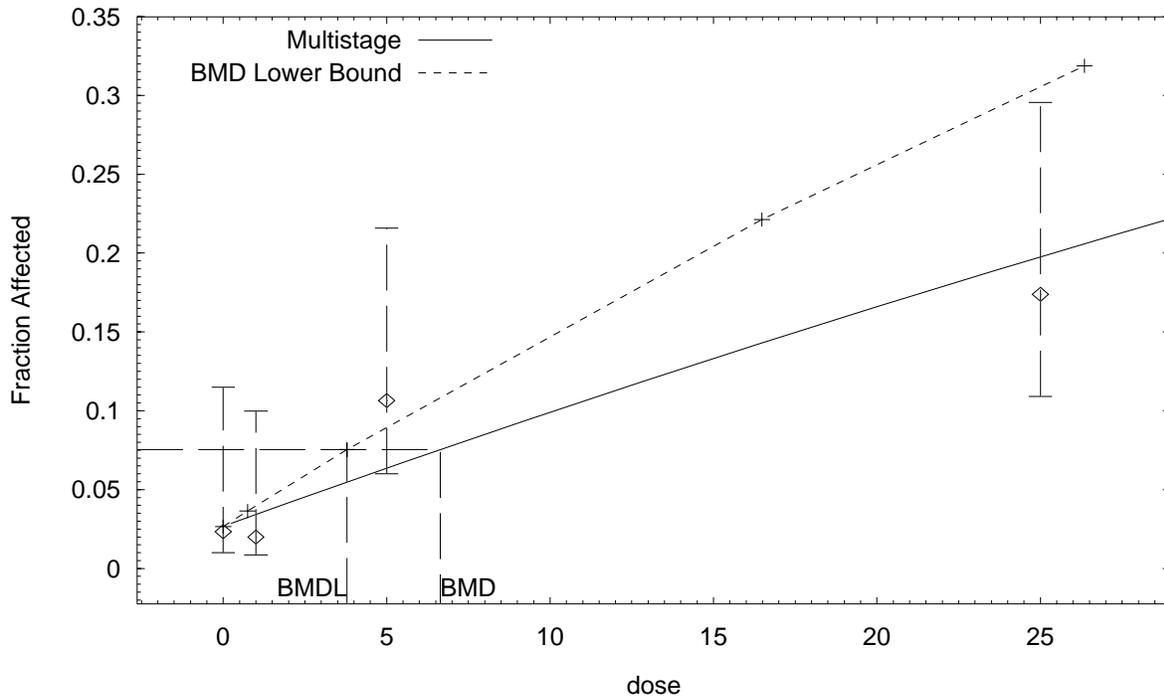
Background            1       -0.62
```

```
   Beta(1)          -0.62              1
```

                         Parameter Estimates

        Variable          Estimate            Std. Err.
       Background          0.0266522          0.0949823
         Beta(1)          0.00772486         0.00856726
         Beta(2)              0                  NA
         Beta(3)              0                  NA

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

                      Analysis of Deviance Table

        Model       Log(likelihood)  Deviance  Test DF     P-value
      Full model        -46.8328
     Fitted model       -47.7145      1.76351      2         0.4141
    Reduced model       -52.1437     10.6218       3         0.01396

           AIC:          99.429

                        Goodness   of   Fit

     Dose     Est._Prob.    Expected    Observed    Size     Chi^2 Res.
    --------------------------------------------------------------------
  i: 1
    0.0000     0.0267        1.146         1          43        -0.131
  i: 2
    1.0000     0.0341        1.707         1          50        -0.429
  i: 3
    5.0000     0.0635        2.986         5          47         0.720
  i: 4
   25.0000     0.1976        9.089         8          46        -0.149

   Chi-square =      1.94     DF = 2       P-value = 0.3799

     Benchmark Dose Computation

Specified effect =          0.05

Risk Type        =       Extra risk

Confidence level =          0.95

          BMD =        6.64003

         BMDL =        3.78919

## Multistage Model with 0.95 Confidence Level



13:17 07/10 2006

**PART III.  Male F344 rat kidney tumors (tubular adenoma and carcinomas)**

```
====================================================================
        Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                        Mon Jul 10 13:25:30 2006
====================================================================
```

BMDS MODEL RUN

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

The parameter betas are restricted to be positive

Dependent variable = Incidence
Independent variable = Concentration

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3

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
Background =          0
Beta(1) =        0
Beta(2) =        0
Beta(3) = 8.96836e-006


Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s)  -Background   -Beta(1)   -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix )

Beta(3)

Beta(3)          1


Parameter Estimates

| Variable | Estimate | Std. Err. |
|---|---|---|
| Background | 0 | NA |
| Beta(1) | 0 | NA |
| Beta(2) | 0 | NA |
| Beta(3) | 8.86633e-006 | 1.00717e-005 |

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


Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -17.8118 | | | |
| Fitted model | -17.8645 | 0.105529 | 3 | 0.9912 |
| Reduced model | -26.5061 | 17.3886 | 3 | 0.0005879 |

AIC:      37.7291


Goodness of Fit
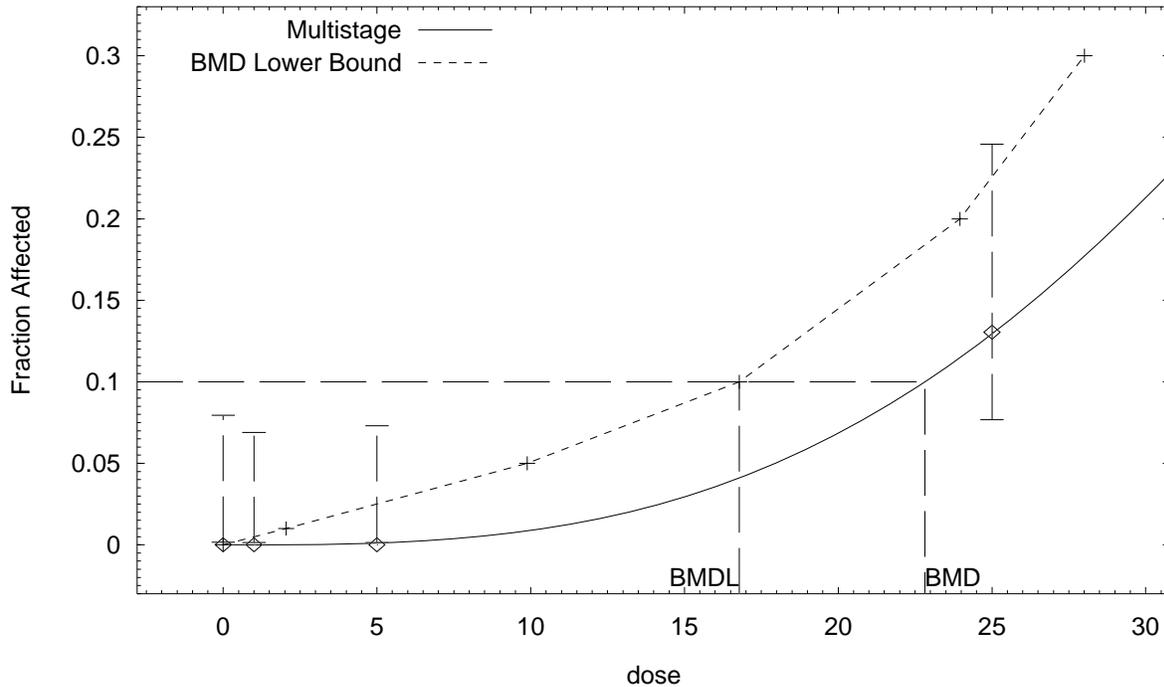
| Dose | Est._Prob. | Expected | Observed | Size | Chi^2 Res. |
|---|---|---|---|---|---|
| i: 1 | | | | | |
| 0.0000 | 0.0000 | 0.000 | 0 | 43 | 0.000 |
| i: 2 | | | | | |
| 1.0000 | 0.0000 | 0.000 | 0 | 50 | -1.000 |
| i: 3 | | | | | |

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```
  5.0000    0.0011     0.052      0      47    -1.001
i: 4
 25.0000    0.1294     5.951      6      46     0.009
```

Chi-square =     0.05    DF = 3      P-value = 0.9968


  Benchmark Dose Computation

Specified effect =        0.1

Risk Type       =    Extra risk

Confidence level =        0.95

        BMD =     22.8198

       BMDL =     16.7833



Multistage Model with 0.95 Confidence Level

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**PART IV.  Male B6C3F1 mouse lung tumors (A/B adenomas and carcinomas)**


========================================================================
       Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
       Input Data File: C:\BMDS\UNSAVED1.(d)
       Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                   Mon Jul 10 13:35:51 2006
========================================================================

BMDS MODEL RUN

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

The parameter betas are restricted to be positive

Dependent variable = Incidence
Independent variable = Concentration

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3

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
Background = 0.286791
Beta(1) = 0.00593155
Beta(2) = 0
Beta(3) = 0

Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s) -Beta(2)  -Beta(3)
     have been estimated at a boundary point, or have been specified by the user,
     and do not appear in the correlation matrix )

           Background    Beta(1)

Background      1       -0.71

  Beta(1)     -0.71        1

Parameter Estimates

   Variable        Estimate        Std. Err.
  Background       0.271318        0.0973641
   Beta(1)       0.00682817       0.00513018
   Beta(2)            0              NA
   Beta(3)            0              NA

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

Analysis of Deviance Table

| Model | Log(likelihood) | Deviance | Test DF | P-value |
|---|---|---|---|---|
| Full model | -113.1 | | | |
| Fitted model | -114.829 | 3.45823 | 2 | 0.1774 |
| Reduced model | -117.28 | 8.36076 | 3 | 0.03912 |

AIC:      233.658

Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Chi^2 Res. |
|---|---|---|---|---|---|
| i: 1 | | | | | |
| 0.0000 | 0.2713 | 11.395 | 8 | 42 | -0.409 |
| i: 2 | | | | | |
| 5.0000 | 0.2958 | 13.014 | 16 | 44 | 0.326 |
| i: 3 | | | | | |
| 25.0000 | 0.3857 | 17.355 | 20 | 45 | 0.248 |
| i: 4 | | | | | |
| 50.0000 | 0.4821 | 23.140 | 21 | 48 | -0.179 |

Chi-square =     3.40    DF = 2      P-value = 0.1828
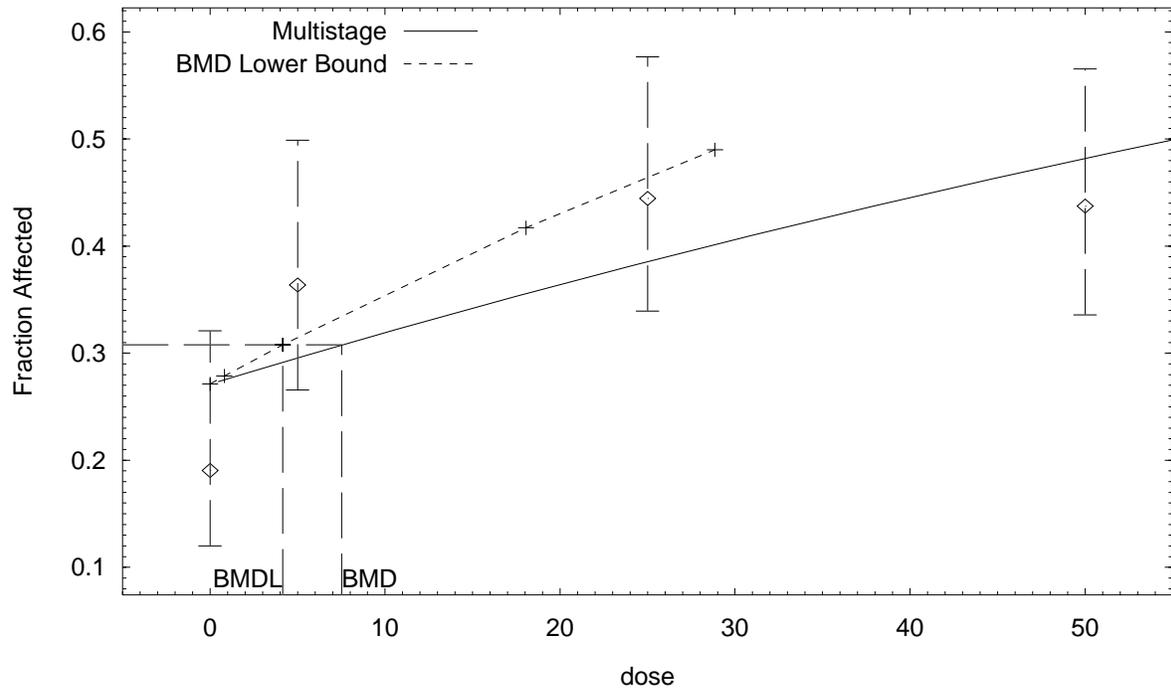
Benchmark Dose Computation

Specified effect =        0.05

Risk Type      =     Extra risk

Confidence level =        0.95

BMD =      7.51201

BMDL =      4.1493

Multistage Model with 0.95 Confidence Level

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**B-3.4**. **Deriving a Summed Inhalation Unit Risk (IUR) for Nitrobenzene by Aggregating Potency Estimates Across Multiple Tumor Sites**

In the CIIT (1993) bioassay that was selected for use in the cancer dose-response modeling of nitrobenzene, increased tumor incidences were observed at multiple sites in the rat following inhalation exposure to nitrobenzene (i.e., in the kidney, thyroid, and liver). With this multiplicity of tumors, the concern is that a potency or risk estimate based solely on one tumor site (e.g., hepatocellular adenomas or carcinomas) may underestimate the overall cancer risk associated with exposure to this chemical. The most recent U.S. EPA cancer guidelines (U.S. EPA, 2005) identify two ways to approach this issue—analyzing the incidences of tumor-bearing animals, or summing the potencies associated with significantly elevated tumors at each site.

In practice, this approach has meant summing potencies or unit risks derived from separate tumor sites. However, potencies are typically upper bound estimates. Summing such upper bound estimates across tumor sites is likely to overstate the aggregate potency or resulting risk. Therefore, in this appendix, following the recommendations of the NRC (1994) and the most recent *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), a statistically valid upper bound on aggregate potency was derived in order to gain some understanding of the overall risk resulting from tumors occurring at multiple sites. It is important to note that this estimate of overall potency describes the risk of developing tumors at any combination of the sites considered, and is not just the risk of developing tumors at all three sites simultaneously.

In general, this method consists of summing the central tendency potency estimates across sites, and then generating an upper bound on this summed value. More specifically, this method involves the following steps:

1. BMC and BMCL values based on the incidence of tumors at each tumor site in the animal study are derived using standard BMD modeling procedures (BMDS 1.3.2). BMD modeling for this specific type of analysis typically employs a small BMR (e.g., 0.000001) that ensures estimation of BMC and BMCL values in the linear part of the dose-response curve, where the slope is reasonably constant and the upper bound estimate is still numerically stable.[1]

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[1] Although this step appears to differ from the explicit recommendation in the most recent cancer guidelines (U.S. EPA, 2005) to estimate cancer risk from a point of departure "near the lower end of the observed range, without significant extrapolation to lower doses," this method is recommended in the guidelines as a method for combining multiple extrapolations. For this purpose, a quantitative combination of individual risks within the range of observation is not generally practicable. For example, use of a common point of departure across the sites requires moving away from the low end of the data range. In this particular case, the kidney tumors yield a point of departure near the highest exposure level. More significantly, numerical combination of risks in the range of observation does not lead to a numerically unique result, due to the different dose-response relationships. When risk

2. The BMCs and BMCLs (in ppm) generated in the first step from the animal data are each transformed to human equivalent concentrations (HECs) (in $\mu g/m^3$) using appropriate conversion factors to account for differences in exposure duration and/or differences in the air:blood partition coefficient between animals and humans.

3. Potencies are then calculated from each of the HECs derived in the previous step using the formula BMR/HEC, to correspond to a central tendency and upper bound risk for each site.

4. For each tumor site, an underlying normal distribution is assumed in order to derive the upper bound of the summed risk. That is, a standard deviation ("sd") of the risk distribution for each site is estimated using the following formula:

$$95\% \text{ UCL} = \text{MLE} + 1.645 \times \text{sd}$$
$$\text{sd} = [95\% \text{ UCL} - \text{MLE}]/1.645,$$

where MLE, corresponds to the maximum likelihood estimate or BMC, 95% UCL, corresponds to the lower bound on exposure, or BMCL, and 1.645 is the t-statistic corresponding to a one-sided 95% confidence interval with >120 degrees of freedom.Each of the standard deviations estimated in the previous step is then squared to yield a variance, and then these variances are summed across the different tumor sites to give an estimate of the total variance. This total variance estimate is derived under the assumption that tumors at different sites occur independently of one another, allowing calculation of the total variance as the sum of each individual variance, ignoring the covariance, which may be difficult or impossible to determine. This independence assumption cannot currently be verified, and if not correct could lead to an overestimate of risk from summing across tumor sites. The NRC (1994) has stated that a general assumption of statistical independence of tumor occurrences across sites within animals was not likely to introduce substantial error in assessing carcinogenic potency from rodent bioassay data.

5. An estimate of the total standard deviation is then derived by taking the square root of the total variance generated in the previous step.

6. Finally, the aggregate central tendency potency is calculated by simply summing each of the estimated central tendency potencies generated in step three across all tumor sites. Then, the upper bound on this sum is calculated by multiplying the total standard deviation derived in step six by 1.645, and adding this value to the summed central tendency potency.

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is expected to be linear at low doses, the approach followed here leads to the most stable estimate of the summed risk. Sensitivity analyses conducted at BMRs closer to the observed data should provide some perspective on the impact of using low BMRs.

Table B-3.4.1 presents the aggregate central tendency potency estimate for nitrobenzene generated via the procedures outlined above. This estimate is $2.59 \times 10^{-5}$ $(\mu g/m^3)^{-1}$. Table B-3.4.2 presents the total variance estimate for this aggregate central tendency potency, also derived employing the procedures outlined above. This variance estimate is $2.95 \times 10^{-11}$. Using this total variance estimate from Table B-3.4.2, the 95% UCL on the aggregate central tendency potency in Table B-3.4.1can be generated employing the following equation:

$$95\% \text{ UCL} = \text{MLE} + 1.645 \times sd$$
$$95\% \text{ UCL} = 2.59 \times 10^{-5} \, (\mu g/m^3)^{-1} + 1.645 \times (2.95 \times 10^{-11})^{0.5}$$
$$95\% \text{ UCL} = \mathbf{3.49 \times 10^{-5} \, (\mu g/m^3)^{-1}}$$

**Table B-3.4.1.  Derivation of a Aggregate Central Tendency Potency Based on Kidney, Thyroid, and Liver Adenomas or Carcinomas in F344 rats**

| Tumor Site and Type | Benchmark Concentration $(BMC_R)$ (ppm) | Human Equivalent Concentration (HEC) $(\mu g/m^3)$ | Central Tendency Potency Estimate $(\mu g/m^3)^{-1}$ [a] |
|---|---|---|---|
| Kidney, adenoma or carcinoma | $4.83 \times 10^{-1}$ | 435 | $2.30 \times 10^{-9}$ |
| Thyroid, follicular cell adenoma or carcinoma | $1.29 \times 10^{-3}$ | 1.16 | $8.58 \times 10^{-6}$ |
| Liver, hepatocellular adenoma or carcinoma | $6.42 \times 10^{-5}$ | 0.058 | $1.73 \times 10^{-5}$ |
| Aggregate  Central Tendency Potency Estimate[b] | | | **$2.59 \times 10^{-5}$** |

[a] The central tendency potency = BMR/HEC, where BMR = $1 \times 10^{-6}$ for kidney and liver tumors, $1 \times 10^{-5}$ for thyroid tumors, using the models developed and reported in section B-3.3 (additional output not shown).  The units conversion and duration adjustments are the same as those used for the IURs in Table 5.8 in the text of the report.
[b] The aggregate central tendency potency estimate is derived by summing the central tendency potency estimates across tumor sties.

**Table B-3.4.2.  Derivation of an Estimated Total Variance for the Aggregate Potency Based on Kidney, Thyroid, and Liver Adenomas or Carcinomas in F344 rats**

| Tumor Site and Type | 95 Percent Lower Bound on Benchmark Concentration $(BMCL_R)$ (ppm) | Human Equivalent Concentration (HECL) $(\mu g/m^3)$ | Inhalation Unit Risk (IUR) [a] $(\mu g/m^3)^{-1}$ | Central Tendency Unit Potency Estimate [b] $(\mu g/m^3)^{-1}$ | Estimated Variance of the Central Tendency Unit Potency Estimate |
|---|---|---|---|---|---|
| Kidney, adenoma or carcinoma | $2.04 \times 10^{-4}$ | 0.184 | $5.44 \times 10^{-6}$ | $2.30 \times 10^{-9}$ | $1.09 \times 10^{-11}$ |
| Thyroid, follicular cell adenoma or carcinoma | $7.39 \times 10^{-4}$ | 0.665 | $1.50 \times 10^{-5}$ | $8.58 \times 10^{-6}$ | $1.52 \times 10^{-11}$ |
| Liver, hepatocellular | $5.47 \times 10^{-5}$ | 0.049 | $2.03 \times 10^{-5}$ | $1.73 \times 10^{-5}$ | $3.33 \times 10^{-12}$ |

| | | | | | |
|---|---|---|---|---|---|
| adenoma or carcinoma | | | | | |
| Estimated Total Variance [c] | | | | | $2.95 \times 10^{-11}$ |

[a] The inhalation unit risk (IUR) = BMR/HECL, where R = 1 x 10$^{-6}$ for kidney and liver tumors, 1 x 10$^{-5}$ for thyroid tumors. The BMCL estimates were generated using the models developed and reported in section B-3.3 (additional output not shown). The units conversion and duration adjustments are the same as those used for the IURs in Table 5.9 in the text of the report.
[b] These central tendency potency estimates are taken from Table B-3.4.1.
[c] The estimated total variance is derived by summing the individual variance estimates across tumor sties.

Despite the relatively large difference between the BMC and BMCL for kidney tumors (BMCL/BMC ~ 1000), this endpoint contributed a little over one-third to the total variance of the aggregate risk distribution primarily because its central tendency estimate was approximately 1000-fold lower than the central tendency estimates based on the other two tumor sites. On the other hand, thyroid tumors contributed more than 50 percent to the total variance of the aggregaterisk distribution, with liver tumors contributing a relatively minor 11 percent.

As presented above, the upper bound on the aggregate potency estimate for nitrobenzene is 3 x 10$^{-5}$ ($\mu$g/m$^3$)$^{-1}$, rounding the value derived above to one significant figure. Although this estimate is based on low-dose extrapolation via the multistage model, not the POD approach more explicitly recommended by the most recent cancer guidelines (U.S. EPA, 2005) for single-site cancer risk estimates, this estimate is recommended because it reflects the exposure-response relationships across multiple tumor sites. Sensitivity analyses considering BMRs up to and including 0.1 showed no difference in the overall sum when rounded to one significant digit.

This analysis using combined tumor sites suggests a higher potency than when considering only the most sensitive tumor site alone (i.e., liver). In this case, however, there is no appreciable difference between the two approaches when the potencies are rounded to one significant figure ($3 \times 10^{-5}$ vs. $2 \times 10^{-5}$ ($\mu$g/m$^3$)$^{-1}$). Regardless, neither of these potency estimates should be used with nitrobenzene exposures greater than $2.0 \times 10^3$ $\mu$g/m$^3$, the point of departure defined for the male rat liver tumors, because the observed dose-response curve is not likely to be linear at higher doses.

As in most risk assessments, extrapolation of data from animals to estimate potential risks to human populations has generated some uncertainty in the results. This uncertainty generally falls into two major categories: 1) model uncertainty, and 2) parameter uncertainty. Model uncertainty, "refers to a lack of knowledge needed to determine which is the correct scientific theory on which to base a model", whereas parameter uncertainty, "refers to a lack of knowledge about the values of a model's parameters" (U.S. EPA, 2005). In the absence of a biologically-based model in this instance, a multistage model was selected because it has some concordance with the multistage theory of carcinogenesis, as well as serves as a benchmark for comparison with other cancer dose-response analyses. That being said, it is still unknown how well this

particular model or the linear low-dose extrapolation generated from it, predicts low-dose cancer risks from nitrobenzene exposure.  Also, while the male mice did not appear to have as strong a carcinogenic response to nitrobenzene exposure as the male rats, it is not known which species is most relevant for extrapolation of risk to humans.

The second source of uncertainty, parameter uncertainty, can be assessed through confidence intervals and probabilistic analysis.  Parameter uncertainty assumes that the underlying model and associated assumptions are valid.  Uncertainty in the animal dose-response data can be assessed through the ratio of BMCs to their BMCLs.  For the tumor sites evaluated here, these ratios were generally below a factor of 2, which is a fairly typical degree of uncertainty.