Office of Environmental Health Hazard Assessment

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MEMORANDUM

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FROM: Sara Hoover, M.S.
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DATE: August 27, 2009

SUBJECT: REVIEW OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR N-METHYLPYRROLIDONE AND IMPLICATIONS FOR BENCHMARK ANALYSIS

Introduction

At the request of Hazard Evaluation System and Information Service (HESIS), the Office of Environmental Health Hazard Assessment (OEHHA) staff reviewed the N-methylpyrrolidone (NMP) physiologically based pharmacokinetic (PBPK) models for rat and human gestation reported in Poet *et al.* "Quantitative Risk Analysis for N-Methyl Pyrrolidone using Physiologically Based Pharmacokinetic and Benchmark Dose Modeling" (2009). We received model code in ACSLX2.5 from Dr. Poet to allow us essentially to repeat the work reported. OEHHA has also consulted extensively with Dr. Poet about both the rat and human PBPK models to obtain key details that were not in Poet *et al.* (2009).

OEHHA's major conclusions are:

- The rat PBPK model and its internal dose AUC (area-under-the curve) metrics seem reasonable and results have been repeated by OEHHA.
- Using the rat PBPK model adds little to the dose-response analysis compared to using the applied concentrations.
- The human PBPK model is, in our view, less adequately validated than the rat model and has remaining uncertainties and limitations.
- Use of the full PBPK model for derivation of human equivalent concentrations is premature.
- The choice of the benchmark response (BMR) is critical in the analyses. A 5% relative deviation of the control mean should be used as the BMR for the fetal/pup body weight endpoint.

Below we provide highlights of our review of the PBPK modeling and illustrate the implications for the benchmark analyses. We also reiterate certain key elements in the dose-response analysis for NMP. Finally, for illustration purposes, we derive possible health-based exposure limits.

OEHHA review of rat PBPK model

After reviewing the rat model and its internal dose AUC metrics, OEHHA has concluded that the model is reasonable. OEHHA was able to repeat the results of Poet *et al*. One uncertainty in the model is the omission of pre-mating exposure. The Poet *et al*. model addresses only exposure during gestation.

In terms of use of the internal dose metrics from the rat model, this approach does not provide a benefit over using the applied concentrations. The fit is not improved and the dose-response curve remains the same, so using the rat PBPK model results does not provide a modeling advantage. This is illustrated further below in the section that discusses implications for benchmark analysis.

OEHHA review of human PBPK model

OEHHA was able to run and evaluate the human PBPK model. To examine certain aspects of the human PBPK model in detail, we constructed an alternative model based on parameters in Poet *et al*. which was simplified to track only the parent NMP and to run in Berkeley Madonna software. Based on our review and evaluation, we found that the human model is less adequately validated than the rat model. Some of the limitations and uncertainties are listed below:

- The model appears to underestimate the blood concentration of NMP in exposed workers (Xiaofei *et al*., 2000; see Figure 7 in Poet *et al.*) even though the assumption of dermal exposure (7600 cm^2) exceeds the OEHHA high end default value for adults of 5800 cm^2 (OEHHA, 2000).

- There are uncertainties in the relationship between maternal blood concentrations and concentrations in fetal tissue, which is the target for developmental toxicity.
- There should be some uncertainty analysis for the parameters chosen and how they affect the internal dose metrics. Our work with the alternative model shows that the model could be sensitive to changes in cardiac output and alveolar ventilation. Different ventilation/perfusion ratios other than 1.0 should be evaluated.
- Other comments on the report have been forwarded directly to Dr. Poet to aid in the revision of the report for possible publication in a peer-reviewed journal.

OEHHA recommends against using the human PBPK model in the assessment of health-based exposure limits for NMP.

Benchmark response (BMR)

As discussed in OEHHA (2009a; 2009b), the appropriate, biologically meaningful choice for the benchmark response (BMR) for the analysis of pup/fetal weight endpoints is a 5% relative deviation of the control mean. The U.S. Environmental Protection Agency (U.S. EPA) has applied the 5% relative deviation of the control mean as the BMR for analyzing fetal body weight data (U.S. EPA, 2003 a, 2003b; U.S. EPA, 2004). This choice of BMR should be maintained for the benchmark analysis, which is separate from any PBPK considerations.

Study choice

OEHHA has determined that the Staples (1990) study is the most appropriate for the dose response analysis of NMP. The study choice has been thoroughly discussed previously (OEHHA, 2009a; 2009b). OEHHA (2009b) also addressed which groups should be combined in Staples. While OEHHA concurred with The Sapphire Group (2009) and Poet *et al*. (2009) on combining certain control groups in Staples, combining any of the high dose groups in Staples is inappropriate. As previously discussed (OEHHA, 2009b), these groups are distinct experiments and should not be combined.

Because the Health Expert Advisory Committee (HEAC) is still considering using Saillenfait *et al*. (2003) as well as Staples as the basis for deriving a health-protective exposure limit for NMP, we provide results based on both studies below for the convenience of the HEAC.

Implications of PBPK modeling for benchmark analysis

Based on rat only PBPK model results

OEHHA conducted a benchmark analysis based on the internal rat tissue concentrations derived by Poet *et al*. using the rat PBPK model. In this analysis we applied a 5% relative deviation BMR and use a homogenous variance model (see OEHHA [2009b] for a discussion of problems with using this variance model for the Saillenfait study). The resulting BMCL05s expressed in terms of internal rat tissue concentrations were extrapolated to external air concentrations based on the simple linear relationship from Table 7 in Poet *et al*. A simplified human pharmacokinetic conversion was then applied to obtain the human equivalent concentrations (based on a U.S. EPA approach; see OEHHA 2008). A description of the approach and results of this analysis follows.

- The internal doses, or the AUCs, were used in the dose-response analysis (Tables 3 and 4 in Poet *et al*.).
- Benchmark concentrations were derived using the rat internal doses and the 5% relative deviation BMR (internal BMCL05). The BMDS output is attached in the Appendix. The results are summarized below:
    - Staples: internal BMCL05 = 143 mg*hr/L-day
    - Saillenfait, historical controls: internal BMCL05 = 282 mg*hr/L-day
    - Saillenfait, concurrent controls: internal BMCL05 = 255 mg*hr/L-day
- The relationship between the internal dose in rats and the external air concentration was reported by Poet *et al*. (Table 7 footnote) and was used to convert the internal rat BMCL05s to external air concentrations in ppm. The relationship between the internal doses and the external air concentrations reported by Poet *et al.* was assumed to be linear and applicable across this range of concentrations.
    - The internal rat BMCL05s calculated above were multiplied by ratio of 105 ppm in the humans to 350 mg*hr/L-day in rats and adjusted for the worker exposure scenario. An example calculation is shown below for Staples:

$$143\left(\frac{mg*hr}{L*day}\right)*\frac{105\,ppm}{350\left(\frac{mg*hr}{L*day}\right)}*\frac{6\,hours}{8\,hours}*\frac{7\,days}{5\,days}=45.1\,ppm$$

- A simplified pharmacokinetic conversion from rats to humans was used to obtain human equivalent concentrations (HECs). Because NMP is a systemically acting vapor, a default regional gas dose ratio (RGDR) of 1 was assumed for the HEC calculation (see OEHHA, 2008). The BMCL05s expressed as external air concentrations are therefore equal to the human equivalent concentrations. The resulting HECs are shown below. The results from the applied concentrations (OEHHA, 2009b) are shown in parentheses for comparison purposes.
    - Staples: 45 ppm (compared to 43 ppm)
    - Saillenfait, historical controls: 89 ppm (compared to 85 ppm)
    - Saillenfait, concurrent controls: 80 ppm (compared to 77 ppm)

The benchmark analysis using the internal rat doses did not improve the fit of the model, change the shape of the dose-response curve, or otherwise improve the ability to model the data. The results using the rat internal doses based on the Poet *et al.* PBPK model are virtually identical to the results previously obtained by OEHHA (2009b) using the applied concentrations. Therefore, the rat PBPK model, while reasonable, does not improve upon the dose-response analysis of the rat data.

Based on full PBPK model results

The HEC proposed by Poet *et al*. is 480 ppm, based on an internal BMCL 1SD of 350 mg*hr/L-day in rats and conversion to humans using the full PBPK model. OEHHA's internal BMCL05 of 143 mg*hr/L-day for Staples is less than half that derived by Poet *et al.* using the 1 SD BMR. For illustration purposes, OEHHA estimated HECs from Staples and Saillenfait by using a conversion based on the full model results from Table 7 in Poet *et al*. and assuming linearity over this range of concentrations:

- An example calculation based on the Staples result is shown below (the model accounts for the worker exposure scenario of 8 hours per day, 5 days per week):

$$143\left(\frac{mg*hr}{L*day}\right)*\frac{480\ ppm}{350\left(\frac{mg*hr}{L*day}\right)}=196.3\ ppm$$

- Using this simple conversion from Poet *et al.*, the approximate human equivalent concentrations that correspond to the internal dose BMCL05s derived by OEHHA are:
    - Staples: 143 mg*hr/L-day for Staples corresponds approximately to an HEC of 196 ppm
    - Saillenfait, historical controls: 282 mg*hr/L-day corresponds approximately to an HEC of 387 ppm
    - Saillenfait, concurrent controls: 255 mg*hr/L-day corresponds approximately to an HEC of 350 ppm

Based on the uncertainties and limitations discussed above for the human component of the full PBPK model, OEHHA recommends against using results from the full PBPK model of Poet *et al*.

Possible health-based exposure limits

Choice of uncertainty factors (UF)

A general discussion of interspecies and intraspecies uncertainty factors is provided below. The cumulative uncertainty factor is discussed later for each assessment approach (applied concentrations and rat PBPK model).

Interspecies uncertainty factor

By using the U.S. EPA human equivalent concentration (HEC) approach, the toxicokinetic portion of the interspecies factor can be reduced to 2 (OEHHA, 2008). The toxicodynamic portion remains at √10, for a total interspecies factor of 6 (OEHHA, 2008). Using results from the rat PBPK model would not affect the interspecies UF.

Intraspecies uncertainty factor

The default value of the intraspecies uncertainty factor is 10. For workers, intraspecies UFs ranging from 1 to 10 have been applied, depending on various factors (OEHHA, 2007; Hoover, 2008). In the case of developmental toxicants, concerns for the developing fetus have been used to justify retaining an intraspecies UF of 10 (OSHA, 1993). The HEAC document on NMP adopted this approach (see http://www.dir.ca.gov/dosh/DoshReg/5155Meetings_2009.htm), and an intraspecies UF of 10 has therefore been retained for the example calculations below.

Possible exposure limits based on benchmark concentration analysis of applied concentrations

OEHHA (2009b) derived a BMCL05 of 43 ppm from the pup body weight data of Staples (1990) using applied concentrations and a 5% relative deviation for the BMR. For comparison purposes, the BMCL05 derived from the Saillenfait data were 85 ppm using historical controls and 77 ppm with concurrent controls. The BMCL05s are equal to the human equivalent concentrations, based on the fact that NMP is a systemically acting vapor and assuming a default RGDR of 1. The uncertainty factors applied to the HECs are 6 for the interspecies UF (toxicokinetic portion reduced to 2, toxicodynamic portion of $\sqrt{10}$ retained) and 10 for the intraspecies UF, giving a cumulative UF of 60.

Application of the cumulative UF of 60 and the BMCL05s calculated by OEHHA using a 5% relative deviation BMR, gives the following possible health-based exposure limits:

- Staples: 0.72 ppm
- Saillenfait, historical controls: 1.4 ppm
- Saillenfait, concurrent controls: 1.3 ppm

Possible exposure limits based on rat only PBPK model results

Using results from the rat PBPK model does not alter the uncertainty factors discussed above for applied concentrations. Possible health-based exposure limits for NMP based on results from the rat model as discussed above, and a cumulative UF of 60 are:

- Staples: 0.75 ppm
- Saillenfait, historical controls: 1.5 ppm
- Saillenfait, concurrent controls: 1.3 ppm

These are virtually identical to the exposure limits derived based on applied concentrations.

Notes on use of full PBPK model for derivation of exposure limits

Because of the uncertainties and limitations in the full PBPK model proposed by Poet *et al.* OEHHA does not recommend using it for deriving health-based exposure limits. If the HEAC chooses to use results from the full PBPK model, OEHHA would recommend retaining the same uncertainty factors as above (*i.e.*, a total UF of 60). For Staples, applying a total UF of 60 to the estimated BMCL05 of 196 ppm would give an exposure limit of approximately 3 ppm.

Conclusions

- OEHHA recommends using a BMR of 5% relative deviation and the Staples dataset for the benchmark analysis.

- The rat PBPK model for exposures during gestation is reasonable, but does not improve the dose-response modeling.

- The full PBPK model is not ready for use in risk assessment.

- OEHHA recommends use of the applied concentrations in deriving health-based exposure limits for NMP.

References

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Appendix: BMDS output for Staples and Saillenfait using the internal doses (AUC) from the rat PBPK model

Staples 1990 data

```
 BMDS MODEL RUN
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   The form of the response function is:

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

   Dependent variable = RESPONSE
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   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 =     0.464224
                            rho =            0   Specified
                         beta_0 =      7.31399
                         beta_1 =  -0.00163899

           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    -3.1e-010    -1.9e-010
    beta_0    -3.1e-010            1        -0.62
    beta_1    -1.9e-010        -0.62            1

                                 Parameter Estimates
                                     95.0% Wald Confidence Interval
Variable    Estimate   Std. Err.  Lower Conf. Limit Upper Conf. Limit
alpha     0.464164    0.0684373          0.33003          0.598299
beta_0     7.38187    0.0905621          7.20437           7.55937
beta_1 -0.00186263  0.000451295      -0.00274715        -0.00097811

     Table of Data and Estimated Values of Interest
```

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	39	7.48	7.38	0.701	0.681	0.918
31.8	16	7.03	7.32	0.705	0.681	-1.75
162	15	7.13	7.08	0.695	0.681	0.302
387	22	6.66	6.66	0.616	0.681	0.0179

```
 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	-8.655329	5	27.310658
A2	-8.375428	8	32.750855
A3	-8.655329	5	27.310658
fitted	-10.694246	3	27.388493
R	-18.508588	2	41.017176

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	20.2663	6	0.002483
Test 2	0.559803	3	0.9056
Test 3	0.559803	3	0.9056

```
   Test 4              4.07783          2          0.1302

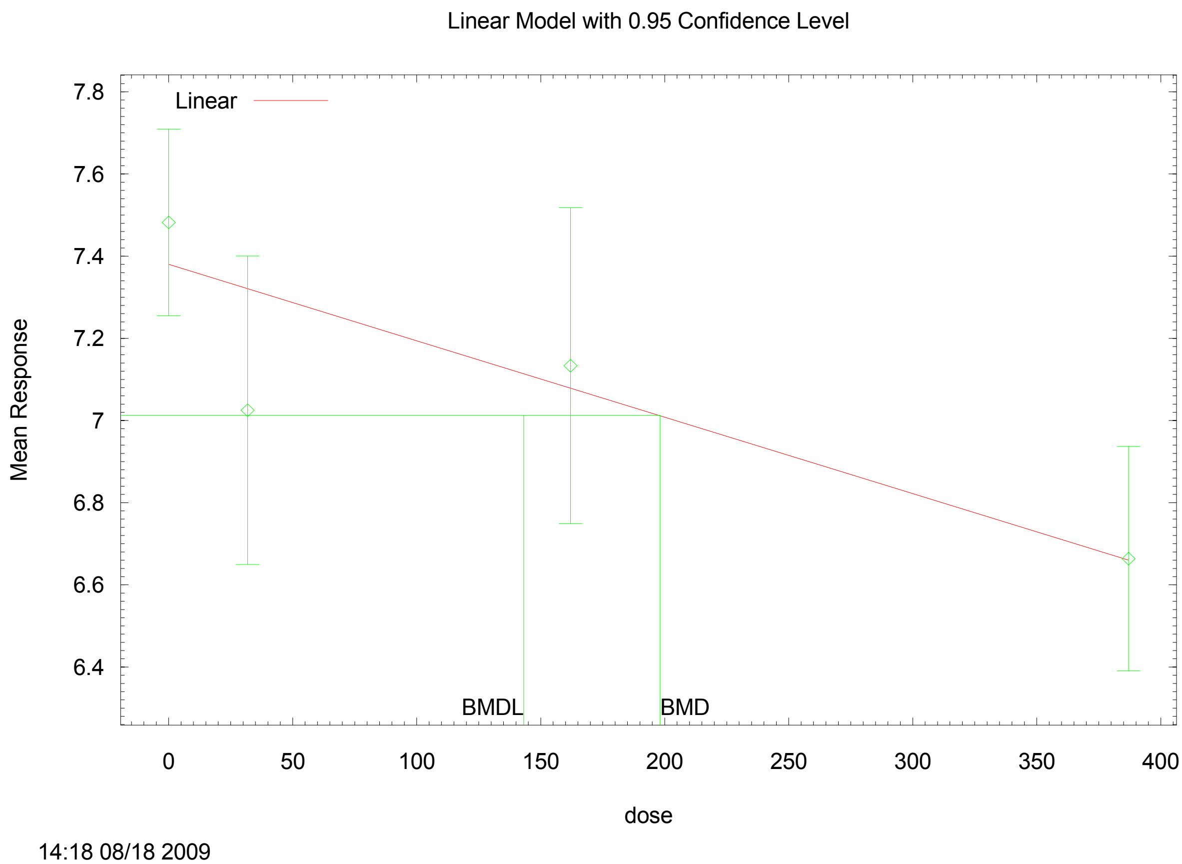
The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

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 =          0.05
Risk Type        =     Relative risk
Confidence level =          0.95
             BMD =        198.157
            BMDL =         143.11
```



Saillenfait *et al*. (2003) data: historical controls

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 BMDS MODEL RUN
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   The form of the response function is:

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

   Dependent variable = MEAN
   Independent variable = COLUMN1
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   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 =     0.119026
                            rho =            0   Specified
                         beta_0 =      5.66242
                         beta_1 =  -0.00072353


           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     5.5e-011     1.4e-011
    beta_0     5.5e-011            1        -0.36
    beta_1     1.4e-011        -0.36            1

                                 Parameter Estimates

                                     95.0% Wald Confidence Interval
Variable   Estimate  Std. Err.   Lower Conf. Limit   Upper Conf. Limit
alpha    0.117992      0.00850427       0.101324            0.13466
beta_0     5.6692       0.0187509        5.63245            5.70595
beta_1  -0.000735105  0.000165418    -0.00105932        -0.000410891
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	321	5.67	5.67	0.34	0.343	0.0416
94.6	20	5.62	5.6	0.359	0.343	0.265
193	19	5.47	5.53	0.251	0.343	-0.727
403	25	5.39	5.37	0.446	0.343	0.248

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	219.230727	5	-428.461454
A2	222.773839	8	-429.547677
A3	219.230727	5	-428.461454
fitted	218.899139	3	-431.798278
R	209.269837	2	-414.539673

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	27.008	6	0.0001443
Test 2	7.08622	3	0.0692
Test 3	7.08622	3	0.0692
Test 4	0.663176	2	0.7178

```
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 .1.  Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

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

             Benchmark Dose Computation
Specified effect =          0.05
Risk Type        =     Relative risk
Confidence level =          0.95
             BMD =        385.605
            BMDL =        281.838
```

Saillenfait *et al*. (2003) data: concurrent controls

```
 BMDS MODEL RUN
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   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = COLUMN1
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   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 =      0.13697
                            rho =            0   Specified
                         beta_0 =      5.66242
                         beta_1 =  -0.00072353

           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     1.7e-010    -6.5e-011
    beta_0     1.7e-010            1        -0.75
    beta_1    -6.5e-011        -0.75            1

                                 Parameter Estimates
                                        95.0% Wald Confidence Interval
Variable Estimate    Std. Err.  Lower Conf. Limit   Upper Conf. Limit
alpha     0.131613      0.0198415     0.0927248            0.170502
beta_0      5.66306      0.058399        5.5486             5.77752
beta_1 -0.000715687   0.000246308   -0.00119844        -0.000232932
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	24	5.67	5.66	0.37	0.363	0.0938
94.6	20	5.62	5.6	0.359	0.363	0.304
193	19	5.47	5.52	0.251	0.363	-0.66
403	25	5.39	5.37	0.446	0.363	0.212

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	45.518737	5	-81.037475
A2	48.847927	8	-81.695854
A3	45.518737	5	-81.037475
fitted	45.227012	3	-84.454024
R	41.196007	2	-78.392015

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	15.3038	6	0.01802
Test 2	6.65838	3	0.08362
Test 3	6.65838	3	0.08362
Test 4	0.583451	2	0.747

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 .1.  Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

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

             Benchmark Dose Computation
Specified effect =          0.05
Risk Type        =     Relative risk
Confidence level =          0.95
             BMD =        395.638
            BMDL =        255.105
```