Appendix 8: Utility of the model(s) for predictive purposes

A8.1 Introduction

This Appendix describes how the statistical models that have been developed may be used to predict the toxicity of untested petroleum streams and how these predictions compare to similar prediction methods, e.g. BMD. The different applications of the models included in this document are for demonstration only and are meant to show the wide applicability of the models. The following sections show, with examples,

A8.2 Application of the model to directly predict a response
A8.3 Application of the model in a BMD type application to predict a dose associated with a response
A8.4 Comparison of a BMD and PAC model results
A8.5 Comparison of a series of BMD results with a PAC model result

A8.2 Direct Prediction of Dose-Response Curves

The most direct method of predicting the endpoint specific toxicity of an untested substance is to develop a dose response curve based on one of the eleven models that have been developed. The predicted dose response curves may be generated with any of these models by following these steps:

1. Identify the new, untested substance.
2. Identify the specific endpoint model and data set used to develop that specific model.
3. Determine if the untested substance is interpolated or extrapolated relative to the data set used to develop the specific model (see **Appendix 6**, **section A6.4** Interpolation and Extrapolation, for step-by-step instructions).
4. Using the PAC equation and coefficients that correspond to the endpoint that was chosen (information listed in **Appendix A6.6**) develop a set of predicted points for a range of doses to determine the predicted dose-response curve.

As an example consider the use of a model to generate dose-response curves for the live fetus/litter counts for two different samples. The model form is:

$$Live\,Fetus\,Count = \alpha + \beta_1 \cdot control\,live\,fetus\,count + \beta_2 \cdot number\,implants + \eta \cdot PAC_4 \cdot PAC_5 + \sum_{i=1}^{7} \gamma_i \cdot dose \cdot PAC_i + \sum_{j=1}^{7} \xi_j \cdot dose \cdot PAC_4 \cdot PAC_5 \cdot PAC_j$$

The PAC profiles for the two untested substances are:

	PAC rings (wt. %)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Samples							
NEW SAMPLE X	0.0	1.25	2.0	1.4	1.1	0.5	0.0
NEW SAMPLE Y	0.0	1.1	1.9	0.2	0.2	0.0	0.0

Checking with the data in Appendix 10 we determine that Sample X is an interpolated sample because the ring concentrations for Sample X lie within those of the data set used to develop the live fetus/litter model (samples listed in the Live Fetus/Litter section of **Appendix 10)**. Specifically, the Sample X ring concentrations are between those for Sample 50431 and 88614 (it is interpolated for 50431 and extrapolated for 88614). Table A8-1 shows the ring concentrations for the test sample and the two samples chosen from **Appendix 10** as bracketing samples.

Table A8-1 PAC Ring Concentrations for New Sample X and Bracketing Samples

Sample	Maximum dose (mg/kg/day)	1-Ring Weight %	2-Ring Weight %	3-Ring Weight %	4-Ring Weight %	5-Ring Weight %	6-Ring Weight %	7-Ring Weight %
83366	250	0.1	2.5	5.1	3.6	2.5	0.9	0.1
Sample X	-	0.0	1.25	2.0	1.4	1.1	0.5	0.0
88614	2000	0.0	0.0	0.5	0.5	0.3	0.03	0.0

Sample X is interpolated relative to 83366 since each of the ring concentrations of Sample X is less than or equal to the corresponding ring concentration of 83366. Similarly, Sample X is extrapolated relative to 88614 since each of the ring concentrations of Sample X is greater than or equal to the corresponding ring concentration of 88614. Therefore, Sample X is an interpolated sample relative to the set of samples used for the live fetus/litter model up to a dose of 250 mg/kg/day.

A similar inspection of the sample data for live fetus/litter shows that Sample Y is not interpolated relative to any of the samples.

Using the coefficients for the live fetus/litter count model from **Table A6-7** of **Appendix 6**, and the mean values of the Control Live Fetus Count and Control Number Implants from **Appendix 10**, we have for Sample X at a dose value of 500 mg/kg/day:

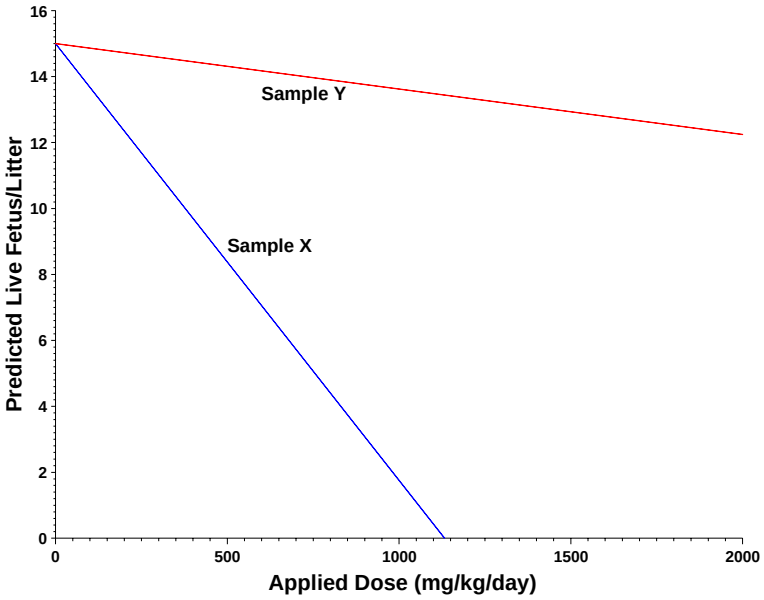
Table A8-2. Data and Model Coefficients Used Calculating Predicted Live Fetus/Litter Count for Sample X at a Dose of 500 mg/kg/day.

	Coefficient	Data Value	Coeff * Data
Intercept	1.721611494	1.0	1.72161
Control Live Fetus Count	0.122260599	14.93	1.82535
Number Implants	0.717062149	15.97	11.4515
ARC_4*ARC_5	0.000110287	1.4 * 1.1	.000169842
dose*ARC_1	0.004644289	500 * 0.0	0
dose*ARC_2	-0.000196448	500 * 1.25	-0.12278
dose*ARC_3	0.000711925	500* 2.0	0.71193
dose*ARC_4	-0.003954569	500 * 1.4	-2.76820
dose*ARC_5	0.018878324	500 * 1.1	10.3831
dose*ARC_6	-0.054180024	500 * 0.5	-13.5450
dose*ARC_7	-0.052092080	500 * 0.0	0
ARC_4*ARC_5*dose*ARC_1	-0.009190232	1.4 * 1.1 * 500 * 0.0	0
ARC_4*ARC_5*dose*ARC_2	-0.001682758	1.4 * 1.1 * 500 * 1.25	-1.61965
ARC_4*ARC_5*dose*ARC_3	-0.000133492	1.4 * 1.1 * 500* 2.0	-0.20558
ARC_4*ARC_5*dose*ARC_4	0.000700435	1.4 * 1.1 * 500 * 1.4	0.75507
ARC_4*ARC_5*dose*ARC_5	-0.000710801	1.4 * 1.1 * 500 * 1.1	-0.60205
ARC_4*ARC_5*dose*ARC_6	0.001022511	1.4 * 1.1 * 500 * 0.5	0.39367
ARC_4*ARC_5*dose*ARC_7	-0.009734692	1.4 * 1.1 * 500 * 0.0	0
sum			8.38

Using the values in Table A8-2, the model predicted live fetus/litter count is 8.38 at a dose of 500 mg/kg/day. This can be reported as 100*8.38/14.93 = 56.1% of the control value.

A plot of the predicted dose-response curve for live fetus/litter count for Sample X can be drawn by repeating the above calculations for several dose values. Figure A7-2 shows the predicted dose-response curve for Samples X. For Sample X, doses greater than 250 mg/kg/day are extrapolated because that is the maximum dose of the sample with the largest ring concentration structure relative to Sample X.

FIG A8-1 Predicted Live Fetus/Litter Count for New Samples X and Y



Any of the predicted response values can be changed to the percent of control by dividing the predicted live fetus/litter count by the control value and multiplying by 100.

A8.3 Comparison of Values Predicted by Current Models to BMD Predicted Values

The benchmark dose (BMD) (Crump, 1984) is a dose that is associated with a pre-defined change in a response based on a set of dose response data points and a statistical model fit to the data. The models developed in this report can be applied in a similar manner..

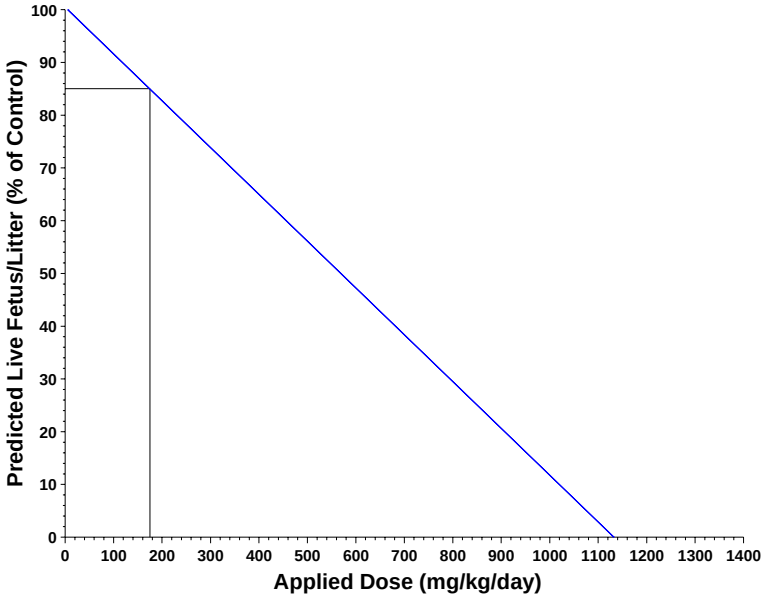
For a specific model and a defined change from the control value control, a dose can be calculated that would be associated with the defined change of that magnitude. To distinguish this value from the BMD call the value determined from the PAC model the PAC_{BMD}. Let the dose associated with a 10% change from control be noted as PAC_{BMD10}.

The PAC_{BMD} is similar to the BMD, but the PAC_{BMD} relies on only one validated model, whereas the BMD can be developed from several competing models and the BMD result is strongly dependent on the model selected (Gephart, et al, 2001). As compared to the BMD, the PAC_{BMD} has several advantages:

- the PAC_{BMD} is usually based on an interpolated dose value from the models because of the large number of data points that have been used to develop the PAC model, while the BMD value is often an extrapolated dose value.
- an additional disadvantage of the BMD is that the prediction error associated with the BMD is related to how near the observed data are to the critical response.
- the PAC_{BMD} can be used with a material that has compositional data (PAC content) and no biological response data, while the BMD cannot be used for untested materials.
- the PAC_{BMD} is that it is based on multiple studies while the BMD is based on a single study, usually with 3 to 5 data points.

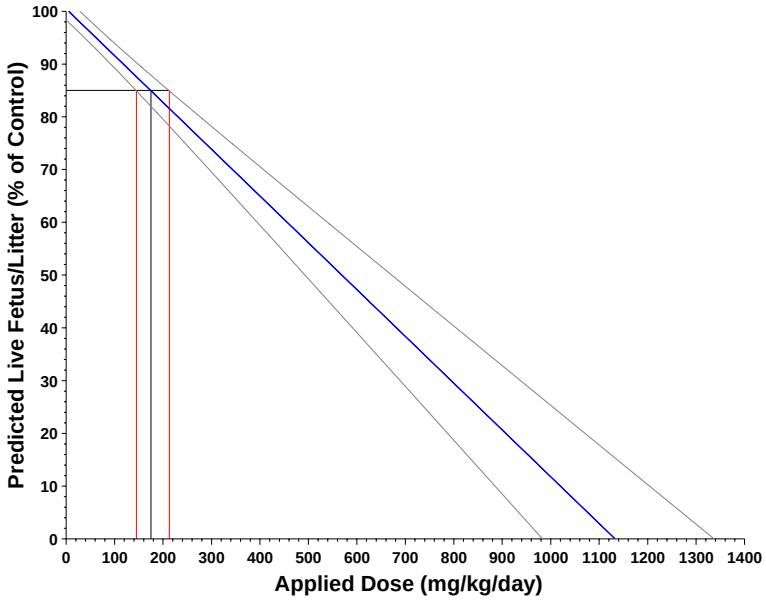
As an example, consider Sample X (an untested sample with no biological endpoint data, but with PAC content data) that was modelled in **Section A8.2.** We can determine the dose associated with a 15% decrease in live fetus/litter count, the PAC_{BMD15}, by determining the dose associated with a 15% decrease in the live fetus/litter count. Using a plot of the predicted values (**Figure A8.2**) we see that the PAC_{BMD15} is 175 mg/kg/day, based on the point where the vertical line intersects the x-axis.

FIG A8-2. Determination of PAC_{BMD15} for an UntestedSample



Additionally, if we develop 95% confidence limits on the PAC model, it is possible to determine an approximate 95% confidence interval (CI) on the PAC_{BMD15}. From **Figure A8.3,** the CI is (145, 213 mg/kg/day) based on the points where the vertical red lines intersect the x-axis. Note that the PAC_{BMD15} and associated CI are within the dose range for interpolation (0 to 250 mg/kg/day).

FIG A8-3. Determination of PAC_{BMD15} and 95% CI for an Untested Sample



This process can be used with any of the 11 models that have been developed and any degree of change. The example has shown that the PAC_{BMD} can be used with a material that has compositional data (PAC content) and no biological response data; this is one of the main advantages it has over the BMD.

A8.4 Comparison of a BMD and PAC Model Results

We have seen in **Section A8.3** how the PAC models can be used to develop a PAC_{BMD} that is similar in purpose to a BMD. Both methods result in a dose that is associated with a response that is proportional to the control or base value. It is possible to make a general comparison of the results of the two methods, but because the two measures are not exactly alike the comparisons are not meant to be exact.

The methods differ because the BMD analysis is based only on the biological results of (usually) one study of the selected material and a selected model from a set of relatively simple dose-response models; the coefficients in the model are optimised to fit the biological data for that calculation. The complexity of the BMD model is limited by the number of available biological data points for the substance under study. The PAC_{BMD} is based on the compositional data of the selected material and a fixed, complex model whose coefficients were developed from many samples. There is no model fitting (estimation of model coefficients) involved with the determination of the PAC_{BMD}.

For the comparisons, the BMD was calculated using the US EPA National Center for Environmental Assessment (NCEA) BMD Software Program V 1.4.1.c. The latest version of the program is available from the EPA BMDS website http://ww.epa.gov/ncea/bmds.htm. The PAC_{BMD} was calculated as shown in Section A8.3. Representative endpoints were chosen, and samples were selected with the goal of choosing those that had 4 or more dose levels in a sample. The 10% level was selected as the relative change. The BMD_L and PAC_{BMD} _L are the lower 95% confidence limits for the corresponding estimates.

In all cases, the continuous linear BMD model fit the data best (or was not inferior to other models). **Table A8-3** shows the results of the models

Table A8-3 Comparison of BMD and PAC_{BMD} For Selected Samples and Endpoints

Study Type	Dependent Variable	Sample	BMD10 (mg/kg/day)	BMD10_L (mg/kg/day)	PAC_{BMD10} (mg/kg/day)	PAC_{BMD10}_L (mg/kg/day)
Repeat –dose	Thymus Weight	F179-Male	141.6	96.8	131	-
Developmental Toxicity Studies (Prenatal)	Live Fetuses/Litter	86270	64.5	50.7	107	69
		8281	329.9	175.4	318	189
	Fetal Body Weight	83366	127.3	104.6	110	89
		89645	1870.1	822.2	1810	755
Developmental Toxicity Studies (Postnatal)	Pup Body Weight	89645	1482.3	805.7	598	234

With the exception of Pup Body Weight, the results are similar for the two methods. The lower 95% limit of the PAC_{BMD} for the thymus weight was not estimated because the curve decreased so rapidly that the lower limit of the curve never crossed the 85% line. The largest difference between the BMD10 and the PAC_{BMD10} is for the estimated pup body weight for sample 89645. The PAC based regression curve declined more rapidly than the data, so the PAC_{BMD10} is an underestimate for this sample.

A8.5 Comparison of a series of BMD results with a PAC model result

Another method of demonstrating the inherent relationship between PAC content and SIDS mammalian toxicity endpoints (the second goal of this project) is to show that the PAC content is related to an accepted measure of toxicity, other than the usual directly measured toxicity endpoints as used in the 11 models of this report.

In this section it will be shown that the PAC content is related to the BMD measures for the studies included in the current analyses. The example is based on the fetal body weight response from the pre-natal studies. There are 23 studies with sufficient data to estimate a BMD; 21 of them have the PAC content data. For each sample the BMD10 and the lower 95% limit of the BMD were calculated using the US EPA National Center for Environmental Assessment (NCEA) BMD Software Program V 1.4.1.c. The latest version of the program is available from the EPA BMDS website http://www.epa.gov/ncea/bmds.htm. The data are shown in **Table A8-4**.

Table A8-4 Fetal Body Weight Data from Prenatal Studies

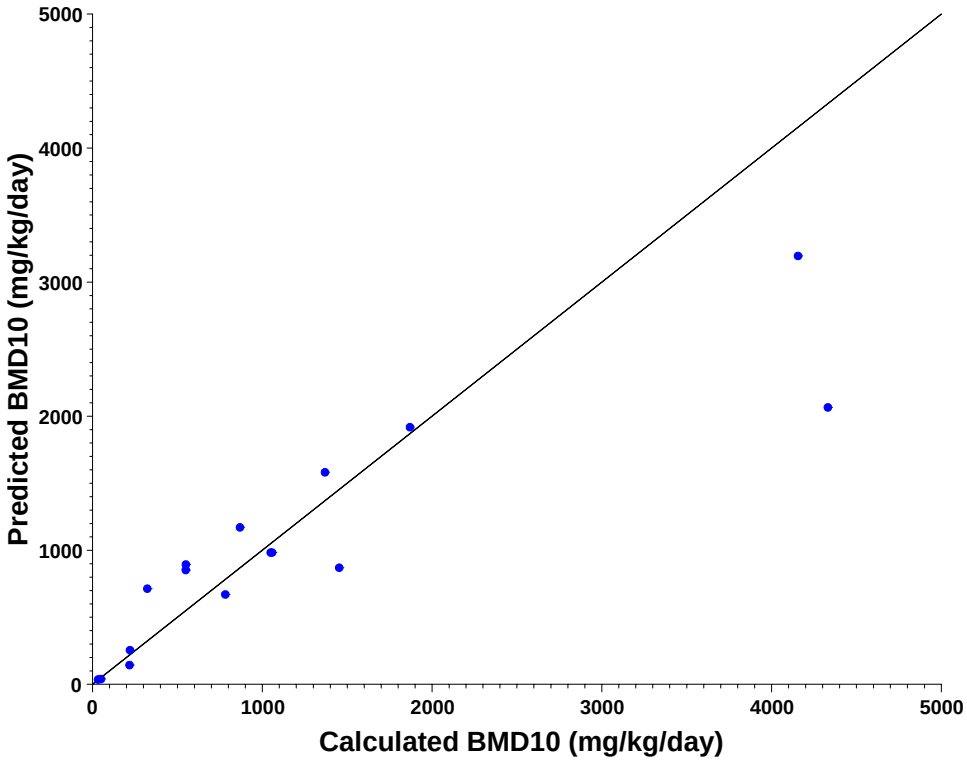
Sample No.	Study No.	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	Dose (mg/kg/day)	n	Mean	Std. Dev.
8281	50511	1869.7	1040.8	0	9	3.5	0.3
				25	10	3.4	0.3
				50	8	3.4	0.2
				125	10	3.5	0.3
				250	10	3.4	0.3
				500	9	3.2	0.4
83366	50431	322.6	261.3	0	9	3.6	0.2
				8	9	3.5	0.2
				30	10	3.5	0.3
				125	10	3.1	0.3
				250	10	2.9	0.3
85244	61801	1453.0	1172.7	0	9	3.5	0.2
				30	10	3.6	0.2
				125	8	3.6	0.2
				500	10	3.2	0.2
				1000	6	3.0	0.2
86001	50541	32.7	24.2	0	10	3.5	0.2
				8	9	3.4	0.3
				30	8	2.7	0.6
				125	1	2.3	0
				250	0	NA	NA
86181	64168	217.7	172.1	0	15	3.6	0.3
				8	13	3.5	0.2
				30	15	3.4	0.1
				125	13	2.9	0.5
				250	2	2.9	0.2
86187	62884	220.4	166.3	0	12	3.5	0.2
				8	13	3.5	0.2
				30	14	3.3	0.2
				125	8	3.0	0.4
86193	64643	NC	NC	0	13	3.7	0.3
				30	12	3.8	0.3
				125	15	3.7	0.3
				250	14	3.8	0.3
86270	62328	1049.6	802.6	0	10	3.7	0.6
				30	8	3.5	0.2

				125	10	3.5	0.2
				500	7	3.1	0.3
				1000	6	2.8	0.3
86271	64146	549.0	459.4	0	10	3.8	0.2
				8	11	3.8	0.2
				30	11	3.8	0.2
				125	12	3.6	0.2
				500	7	3.0	0.4
86484	62934	49.6	37.3	0	11	3.5	0.3
				8	14	3.2	0.3
				30	16	2.9	0.3
				125	0	NA	NA
87213	61998	NC	NC	0	10	3.4	0.2
				15	10	3.6	0.2
				60	9	3.6	0.2
89106	63264	549.6	466.9	0	14	3.6	0.2
				125	14	3.5	0.2
				500	8	2.8	0.2
89645	63836	4155.8	2829.3	0	12	3.9	0.2
				125	11	3.7	0.4
				500	11	3.8	0.3
				2000	9	3.4	0.3
89646	63848	1369.1	921.6	0	11	3.7	0.2
				30	12	3.7	0.2
				125	12	3.8	0.3
				500	11	3.4	0.3
F-179	910042	NC	NC	0	23	3.51	0.41
				0.05	24	3.54	0.34
F-193	920011	868.4	713.1	0	24	3.47	0.22
				50	24	3.48	0.23
				250	25	3.18	0.29
				500	22	2.99	0.29
F-195	920156	4331.5	1255.6	0	20	3.53	0.23
				50	21	3.57	0.37
				150	20	3.62	0.38
				300	19	3.47	0.24
F-196	920012	782.2	578.8	0	24	3.41	0.22
				75	22	3.27	0.20
				150	25	3.22	0.35
				300	23	3.05	0.26
F-197	920154	1057.7	709.7	0	20	3.60	0.16
				50	19	3.62	0.24
				100	19	3.68	0.21
			.	250	21	3.41	0.22
F-199	920013	NC	NC	0	25	3.71	0.24
				50	22	3.76	0.24
				100	25	3.80	0.34
F-215	920155	NC	NC	0	24	3.73	0.21
				50	20	3.83	0.26
				250	20	3.87	0.23
				500	22	3.75	0.27

NC Not calculated because response was not decreasing with dose.

A linear regression analysis was calculated using the 16 samples that had a calculated BMD_{10}; the log of the BMD_{10} was the dependent variable and the 7 PAC ring concentrations were the independent variables. The regression fit well with multiple correlation coefficient, r, of 0.96. Figure A8-4 shows the calculated and predicted BMD_{10} from the model.

FIG A8-4 Calculated and Predicted BMD_{10}



The fit is good, except for the two upper BMD values. The goal was not to fit the data exactly, but to demonstrate the relationship. The model fitting the $BMDL_{10}$ was also successful with an r of 0.97.

In this example the familiar BMD_{10} has been shown to be related to the PAC content of the samples. This regression differs from the other models that have been presented in that there is one data point per study, rather than several points per study each representing individual dose groups. This regression method is not meant to replace the current models, but is another demonstration of the inherent relationship between PAC content and a familiar SIDS mammalian toxicity endpoint. The PAC content is not useful for directly predicting the BMD because the model predicts the BMD, which is from a model that 'predicts' the specific endpoint (fetal pup weight). This is a model predicting a model. It is more parsimonious to predict the endpoint directly, as is done in the 11 models developed in this report.