PETROTOX User's Guide
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FIGURES

SECTION 1

INTRODUCTION

This spreadsheet-based model, PETROTOX, is designed to calculate the toxicity of petroleum products to aquatic organisms. Petroleum hydrocarbons are complex mixtures that exert a narcotic mode of toxic action, which is assumed to be additive. This model calculates the solubility of a petroleum product and then uses the Target Lipid Model (TLM) (Di Toro et al. 2000) and toxic unit theory of additivity to calculate the toxicity or environmental risk limits of these mixtures.

The spreadsheet uses a three-phase (air, water, free product) oil solubility calculation (McGrath et al. 2004) that is coupled with a database of physical and chemical properties of typical petroleum hydrocarbons to calculate the distribution of petroleum hydrocarbons among the exposure water, headspace and free product phases. The properties database was developed by CONCAWE and contains physical/chemical properties such as boiling point, solubility and octanol-water partition coefficients (K_{ow}) for 1457 hydrocarbon structures that are possibly found in petroleum products. This version of the Petrotox model performs the solubility and toxicity calculations with physiochemical properties derived from the database for each hydrocarbon block.

The computed dissolved hydrocarbon concentrations are then used by the TLM to calculate aquatic toxicity. A modification to the TLM is the use of membrane-water partition coefficients (K_{MW}) rather than K_{OW} to describe the partitioning between the water phase and the organism (Parkerton et al 2011). This modification is critical for petroleum products that have very hydrophobic compounds ($\log(K_{OW}) > 6.0$).

SECTION 2

FRAMEWORK AND CONCEPTUAL MODEL

2.1   COMPOSITION AND MAPPING

The model can accommodate two types of inputs, low resolution or high resolution, which depend on the information known about the mass distribution of the petroleum product. In the low-resolution approach, the mass distribution of two general chemical classes (aliphatic and aromatic) is entered over user-defined boiling point intervals. This format is patterned after the information given from simpler hydrocarbon block analyses. In the high-resolution approach, the mass distribution for up to 16 chemical classes can be entered: *n*-paraffins, *iso*-paraffins, *n*-substituted cylcohexanes, *n*-substituted cylcopentanes, other mono-naphthenics, di-naphthenics, poly-naphthenics, *n*-olefins, *iso*-olefins, sulfur-bearing aliphatics, mono-aromatics, naphthenic mono-aromatics, di-aromatics, naphthenic di-aromatics, poly-aromatics and sulfur-bearing aromatics. This format is patterned after the information derived from highly detailed GCxGC that provides mass distribution information for the 16 chemical classes over discrete carbon number (C#) intervals. These hydrocarbon blocks (C# and chemical class) are used for environmental fate and effects estimates for complex petroleum substance. The CONCAWE database contains structures for each of these 16 chemical classes over a range of physic-chemical properties

The library structures are assigned to a hydrocarbon block and the mass fraction given to that block is evenly distributed among all of the assigned structures. For example, a hydrocarbon block in the *iso*-paraffin class is assigned a mass fraction of 5.0% with an initial carbon number (CNo) interval of C9-C10. The CONCAWE library is sorted for structures within the *iso*-paraffin class that fall within the defined CNo interval. In this case there are eight *iso*-paraffins in the database within this CNo interval (ID: 676, 677, 678, 679, 681, 682, 683, 684). The mass fraction of this hydrocarbon block (5.0%) is evenly distributed among the candidate structures so that each has 0.625% of the total mass. The mass fraction that is assigned to each structure is later used to determine the mole fraction of a given hydrocarbon, which impacts the aqueous solubility of that structure.

If a hydrocarbon block has a chemical class and/or CNo interval configuration that does not identify any representative structures in the database the mass fraction of this so-called 'orphan' block is reassigned to a neighboring block. The unassigned mass in these 'orphan' blocks is added to a neighboring block generally within the same chemical class where there are structures available for assignment depending on the boiling point configuration of the block. If the CNo interval for the 'orphan' block is above C14 in high resolution mode, or the boiling point interval above 275.2 ºC in low resolution mode, the unassigned mass is added to the first available hydrocarbon block at higher boiling point intervals. This is done to account completely for all of the mass at any given

loading. See Figure 1 for a simple depiction of the mass reassignment procedure. 'Orphan' blocks below this BP value are added to the first available hydrocarbon block at a lower boiling point interval. This is based on the distribution of hydrocarbon structures in the database (See Appendix 1). In a few cases where there are no hydrocarbon structures within the same chemical class available for the mass reassignment, the unassigned mass is added to the first available chemical class to the left (unassigned PolyAr mass added to structures in NDiAr block, etc. See Figure 1) within the same boiling point interval. The mass in orphan blocks from the *n*-Paraffin class is reassigned to the available block to the right (unassigned *n*-Paraffin mass added to *iso*-Paraffin mass).

2.2 THREE-PHASE DISTRIBUTION (SOLUBILITY) MODEL

The following equations describe a multi-phase (petroleum product, water, air) dissolution model that considers the changes in product volume and composition as a result of dissolution. Several models for computing equilibrium concentrations in multiphase systems have been presented elsewhere (McGrath et al. 2005 and references therein) and only a brief outline of the equations are presented here. The mass balance for component *i* in the system is

$$m_{T,i} = m_{G,i} + m_{W,i} + m_{A,i} \tag{1}$$

where $m_{T,i}$ is total mass of component *i* in the system and $m_{G,i}$, $m_{W,i}$, and $m_{A,i}$ refer to the mass of component *i* in the product, water, and air phases, respectively. The mass balance can be expressed in terms of volume and concentration of component *i*

$$m_{T,i} = V_G \, C_{G,i} + V_W \, C_{W,i} + V_A C_{A,i} \tag{2}$$

where

$C_{G,i}$ = petroleum product phase concentration of *i*, mol/L$_G$
$C_{W,i}$ = water phase concentration of *i*, mol/L$_W$
$C_{A,i}$ = air phase concentration of *i*, mol/L$_A$
V_G = volume of petroleum product, L$_G$
V_W = volume of water, L$_W$
V_A = volume of air, L$_A$

Assuming petroleum products are an ideal mixtures (Di Toro et al., 2007), the equilibrium between the product-phase concentration and the total water-phase concentration ($C_{W,i}$) is described by Raoult's Law

$$C_{W,i} = x_i \, S_i \tag{3}$$

where x_i is the mole fraction of component i in the product phase and S_i is the aqueous solubility of component i. The concentration of the monomeric species is calculated in a similar manner

$$C_{D,i} = x_i \, A_i \tag{4}$$

where x_i is the mole fraction of component i in the product phase and A_i is the aqueous activity (e.g., monomer solubility) of component i. For many compounds with $\log(K_{OW})$ less than approximately 5.5 the activity is equal to the sub-cooled solubility indicating that these compounds do not form polymeric species. And in for some compounds with $\log(K_{OW})$ greater than 5.5 the activity can be a very small fraction of the sub-cooled solubility (< 0.001) indicating that the monomer species is a small fraction of the total dissolved chemical.

The Henry's Law describes the equilibrium between the air phase concentration and the water phase concentration of the monomer species

$$H_i = C_{A,i} / C_{D,i} \tag{5}$$

where H_i is the unitless Henry's Law constant for component i.

The relationship between the product phase concentration on a mole fraction basis and a mol/L basis is via the petroleum product density, ρ_G (mol/L)

$$C_{G,i} = x_i \, \rho_G \tag{6}$$

Substituting equations 3, 4, 5, and 6 into equation 2 and solving for the mole fraction yields

$$x_i = \frac{m_{T,i}}{(V_G \, \rho_G) + V_W \, S_i + V_A \, H_i A_i} \tag{7}$$

There is one three additional equation needed to describe the system. The mole fractions of the components in the petroleum product sum to 1

$$\sum_i x_i = 1 \tag{8}$$

There are a total of $n + 1$ equations and $n + 1$ unknowns (n x_i, and the product of $V_G\rho_G$). This system of equations is solved numerically as described below. The inputs are the total mass

($m_{T,i}$) the solubility (S_i), Henry's Law constant (H_i) and density (ρ_i) of each component i, as well as the volumes of air and water in the system (V_A and V_W).

The strategy is to minimize the error in Equation 9. The procedure is coded into a Microsoft Excel™ 2010 spreadsheet using the Visual Basic for Applications (VBA) programming language. The starting value for $V_G \rho_G$ (the mass of petroleum product) is obtained by assuming all the mass is in the product phase.

$$(V_G \rho_G) = \sum_i m_{T,i} \tag{9}$$

An iterative method is used to vary the V_G until the overall sum of the working mole fractions is 1.0.

2.3 TARGET LIPID MODEL

For the risk assessment of petroleum-derived products, it is accepted that the mode of action of petroleum related hydrocarbon components is via narcosis (CONCAWE, 1996). Chemicals that act via narcosis are referred to as narcotics. It has been demonstrated experimentally that the effects of narcotics are strictly additive. This is important for petroleum products since they consist of mixtures of hydrocarbons. The toxic unit (TU) approach is used to express the toxicity of mixtures with components that exert a similar mode of action. The TLM (Di Toro et al. 2000) and the TU concept have been adopted for use in computing environmental risk limits for mineral oil (Verbruggen, 2004).

The TLM predicts the aquatic toxicity of narcotic chemicals to a variety of aquatic species, including fish, algae and invertebrates. It is based on the inverse relationship observed between the log(LC50) (lethal concentration to 50% of test organisms) and log(K_{ow}) and the observation by McCarty et al. 1991 that this relationship can be understood as the result of a constant body burden of narcotic chemical that causes adverse effects. In the TLM a single universal slope for the log (LC50)-log (K_{ow}) relationship has been demonstrated, independent of species. The TLM equation for computing the LC50 (mmol/L) for a particular chemical for a specific species is

$$\log LC50 = -0.936 \log Kow + \Delta c + \log C_l^* - \log ACR \tag{10}$$

where Δc_ℓ is the chemical class correction factor for chemicals that were found to be slightly more potent than baseline chemicals (e.g, PAHs), -0.936 is the universal slope, and C_L^* is the species-specific critical target lipid body burdens (CTLBB) for narcosis effects (µmol/goctanol) and ACR is the acute-to-chronic ratio. The ACR is used to convert acute effects (e.g., LC50, ACR of 1) to

chronic effects levels (e.g., NOEC, LC10, ACR of 3.83). The TLM has been recalibrated with current log(K_{ow}) values extracted from SPARC v4.2 in May 2008 (Hillal et al., 2004). The revised TLM parameters are provided in the worksheet labeled 'CTLBB' for 42 organisms including daphnids, fish, algae and other species. The parameters include the CTLBB, universal narcosis slope, chemical class corrections and their associated standard errors. Chemical classes that require a correction include the mono-, di- and polyaromatic hydrocarbon classes. The acute-to-chronic ratio can be applied here to calculate chronic effect concentrations.

For application to petroleum products, the term LL50 (lethal loading concentration) is used instead of the LC50 due to the insoluble nature of most petroleum products. Additionally, for application to petroleum products, the K_{TLW} (target lipid-water partition ratio) is used in place of K_{OW} as follows

$$\log K_{TLW} = \begin{bmatrix} 0.947 \log K_{OW} + \Delta C & ; & for \ \log K_{OW} < 6 \\ 6 + \Delta C & ; & for \ \log K_{OW} \geq 6 \end{bmatrix} \tag{11}$$

The overall result is that the maximum log(K_{TLW}) is 6.0 even for structures with very high log(K_{OW}) values (>15). This adjustment dramatically limits the impact that super-hydrophobic hydrocarbons have on the calculated toxicity. Without the adjustment, the predicted LL50s were orders of magnitude lower than observed LL50s indicating that the model over estimated the toxicity. The reader is referred to Verbruggen 2004 and Parkerton et al 2011 for additional details on the derivation of the K_{TLW} cutoff. There is an option available to the user where the cutoff and slope of the cutoff can be customized to meet specific the needs of a specific application.

The toxicity of the petroleum product (hydrocarbon mixture) can be expressed using the concept of TUs (Sprague and Ramsay, 1965). A TU is defined as the ratio of the exposure concentration of the freely dissolved chemical (equation 4) to the effect concentration for a specific medium (e.g. water). The monomer species is assumed to be the bioavailable form of the total dissolved hydrocarbons. A toxic unit posed by each specific hydrocarbon block i in a mixture is computed as

$$TU_i = \frac{C_{W,i}}{LC50_i} \tag{12}$$

where $C_{W,i}$ is the aqueous freely dissolved concentration of the hydrocarbon block (mmol/L) and $LC50_i$ is the aqueous effect concentration (mmol/L) for that block for a specific species as derived from equation 12 or 13. To compute the toxicity of the mixture, the TUs for all hydrocarbon

components in the mixture are summed. When the sum of the TUs for the mixture equals one, the mixture is expected to be toxic to 50% of the organisms.

Loading data are not required for the model to calculate median acute or chronic effect endpoint (e.g., LC50). The model starts with an initial guess at the loading and calculates solubility and the subsequent toxic units, iteratively changing the load with each pass until the final toxic units equal 1.0 according to the following definition:

$$\text{Acute Toxic Units} = 1 = \text{Load}(i)/\text{model} - \text{calculated LL50} \tag{13}$$

Or alternatively for chronic effects (e.g., ACR = 3.83):

$$\text{Chronic Toxic Units} = 1 = \text{Load}(i)/\text{model} - \text{calculated chronic effects level} \tag{14}$$

2.3.1 Bioavailability correction

Under certain exposure conditions the partitioning of hydrocarbons to the test organisms can reduce the bioavailability of dissolved hydrocarbons. For example, in algal exposures there can be substantial particulate organic carbon (POC) concentrations (1-2 mg/L) from the algal cells. The particulate algal carbon acts as a sorbent for dissolved hydrocarbons. The organic carbon partition coefficient (K_{oc}) and the fraction of dissolved hydrocarbon are calculated following equilibrium partitioning theory (EqP) (Di Toro et al. 1991):

$$\log(K_{OC}) = 0.00028 + 0.983 * \log(K_{OW}) \tag{15}$$

$$\text{Fraction dissolved} = 1/(1 + \text{POC} * K_{OC}) \tag{16}$$

If lipid concentrations are entered with the corresponding biodilution option selected then the $\log(K_{MW})$ value is used to calculate the fraction of dissolved hydrocarbons in a similar manner

$$\text{Fraction dissolved} = 1/(1 + \text{Lipid Concentration} * K_{MW}) \tag{17}$$

There are four bioavailability correction options in the model. The first option, Option 1, is to proceed with the calculations and assume that no correction occurs. Option 2 allows the user to enter POC concentrations in units of mg POC / L water. Under typical test conditions algal cell density can reach 10^5 cells/ml (i.e., CONCAWE-kerosene data), which corresponds to a POC concentration near 2 mg/L assuming a cellular C-content of 20 pg/cell (Mayer et al. 1998). The model will calculate the fraction of dissolved hydrocarbons that is bioavailable according to the POC partitioning model described by equations 17 and 18. Option 3 will perform the same calculation with a default POC concentration of 2 mg/L, representing a typical upper bound. Options 2 and 3

are usually enabled during calculations for algal endpoints but the calculation can be performed for any species in the TLM database, including the user-defined option. If Option 2 is selected but no POC concentrations are entered, the model will proceed with POC concentrations of zero. Option 4 allows the user to enter lipid concentrations in place of POC concentrations. A convenient estimate of the lipid concentration by calculating an organism loading (g wet weight / L water) from the exposure condition and assuming at lipid content of 5% or with other estimation techniques or measurements. The fraction of bioavailable hydrocarbons is calculated with membrane-water partition coefficients as described in equation 19.

2.3.2 Predicted No Effect Concentration (PNEC)

Aquatic PNECs were calculated for each of hydrocarbon structure according to the HC_5 equation as described in (McGrath et al., 2004). The equation was modified to calculate the HC_5 with target lipid-water partition coefficients (K_{TLW}) instead of K_{OW} as described in equation 11.

$$\log(HC_5) = E\{m \log(K_{TLW})\} + E\{\log(C_L^*)\} - E\{\log(ACR)\} - k_Z \sqrt{V\{m \log(K_{TLW})^2\} + V\{\log C_L^*\} + V\{\log(ACR)\}} \quad (18)$$

m   = universal target lipid slope (-0.936)
HC_5 = hazardous concentration to 5% of test species, mmol/L
C_L^* = mean critical target lipid body burden, µmoles/g octanol
ACR = mean Acute to Chronic Ratio
$\Delta c_{\ell,i}$ = chemical class correction
k_Z = 95% confidence extrapolation factor

where the $E\{\log(C_L^*)\}$ is the logarithmic value of the geometric mean of all critical target lipid body burdens (µmoles / g octanol) in the TLM database (Di Toro et al. 2000a with Sept. 2005 recalibration) and has a value of 2.13. The mean ACR for all species in the toxicity database is 3.83 for hydrocarbons (McGrath and Di Toro. 2009). The term 'k_Z' is the 95% confidence sample size-dependent extrapolation factor and has a value of 2.21. The variance in the universal target lipid slope ($V(m)$) is 2.25E-4 and the variance in the C_L^* ($V(\log(C_L^*))$) is 0.015. The variance in the ACR ($V(\log(ACR))$) in the ACR is 0.126. In addition, a chemical class-correction of -0.352 for di- and polyaromatic hydrocarbons and a correction of –0.109 for monoaromatic hydrocarbons are included in these calculations since these compounds exhibit slightly higher toxicity than other hydrocarbon classes that conform to "baseline" ecotoxicity. Additional validation of PETROTOX are provided in Redman et al., (2011).

SECTION 3

SPREADSHEET MODEL OVERVIEW/ORGANIZATION

This version of the spreadsheet model is password protected so that critical portions of the model are not inadvertently lost or modified. The spreadsheet interface and visual basic code are all protected so that error messages will appear if text or calculations are attempted in the interface sheets in locations that are not specifically intended for data entry. Colored cells indicate the data entry fields. The password for the spreadsheet interface, in all caps, is: CONCAWE. The password protection will be reinstated after every calculation. This is done to prevent inadvertent deletion of key data required to run the model. The nine worksheets in this spreadsheet are briefly described in this section.

3.1 INPUT

This worksheet is used to enter product-specific or application-specific data that are required to use the model (Figure 2). These data includes mass fraction distribution by chemical class and boiling point range, product loading information, and exposure conditions. Also, in this sheet the user can select the target species endpoint, input resolution, and bioavailability correction options. The colored boxes in the 'Input' worksheet are where the user inputs information necessary to run the model. There are two modes for inputting compositional data that correspond to GCxGC (High resolution) and TPH (Low resolution) analytical approaches. Compositional data for High resolution is organized by discrete chemical class (n=16) and carbon number. Compositional data for Low resolution is organized by total aromatic and total aliphatic general chemical classes and boiling point. The orange-shaded section indicates an input range for carbon number or boiling point intervals. The light blue (cyan) color indicates a section for entering mass fraction information. The purple shaded section is for entering dose response information: loadings, effect data, or particulate organic carbon concentrations. The pink cells are for entering the dimensions of the exposure chamber: water and headspace volumes. The user may also input date and title information specific to the petroleum product being analyzed in the top two cells in column C. These features will be discussed in more detail later on in this guide.

3.2 OUTPUT LL50

This output sheet (Figure 3) has information related to the calculated acute or chronic endpoint. The product loading is reported at the LL50 as well as the distribution of toxicity via toxic units for different chemical classes and boiling point intervals (hydrocarbon blocks). Average physical/chemical properties of the hydrocarbon blocks are also reported in this sheet. Average PNEC values calculated with the HC5 equation (McGrath et al. 2004) for each hydrocarbon block are reported. PNEC values that are greater than the computed water solubility of a given

hydrocarbon block are indicated in bold red numbers. The concentrations of hydrocarbons in the three different phases (water, headspace, free product) are provided here. If the headspace volume was set to zero in the model run, then the headspace results will return concentrations of zero. Likewise, if the product completely dissolves (no product phase) the model will report concentrations of zero for this phase of hydrocarbon blocks.

There is a 'Chart' button that will create stacked bar and pie charts that portray the distribution of toxic units among different chemical classes and hydrocarbon blocks. These charts are printed in the 'Charts' worksheet. There is a 'Copy Input/Output' button that will copy the input and output pages from the output sheets to a new workbook so that different model scenarios can be recorded. This worksheet is hidden when dose-response calculations are performed.

3.3   OUTPUT LOADS

The output loads worksheet (Figure 4) is similar to the 'Output LL50' worksheet in that the average physical/chemical properties of the hydrocarbon blocks and the concentrations of the hydrocarbons in the different phases are reported. The results are provided in this sheet for each product loading specified in the 'Input' worksheet, rather than at the LL50. A 'Chart' button similar to the one found in 'Output LL50' can be used to generate similar stacked bar and pie charts for each of the product loadings and the 'Copy Output/Input' button is also available. The avgPNEC is reported here for comparison to the computed water solubilities. Note, the PNEC is independent of product loading. This worksheet is hidden when LL50 calculations are performed.

3.4   DETAILEDOUT

This worksheet contains detailed output of the assignment and mass allocation of the library structures to the user-input hydrocarbon blocks. The outputs indicate which block and chemical class an individual compound is assigned to as well as the mass fraction allocated to that compound. For reference the compound ID is also given along with the $\log(K_{OW})$ and PNEC. The results for predicted air, water and oil-phase concentrations are also given along with the TLM-predicted toxic unit. These data can be compared to the summarized outputs in the 'Output - …' worksheets. Only the results for the last loading analyzed from the dose response or LL50 calculation modes. This worksheet is intended to report the mapping of representative structures to the hydrocarbon blocks and the subsequent mass distribution among the assigned structures.

3.5   CHARTS

This sheet will include charts that are generated from the output worksheets. The charts will remain in place until the next set of calculations is performed – at that time the existing charts are cleared to make space for a new series generated with the 'Chart' button. Only a bar chart is produced for each product loading showing toxic unit distribution over the hydrocarbon blocks. The charts have a number of text boxes that reflect the details of a given model calculation. The

data plotted in the charts are found in the output sheet where the 'Chart' button was activated. See Figure 5. The charting feature is only active under the high resolution mode.

3.6   CTLBB

This worksheet lists the CTLBB for 42 species that are in the TLM database. There is also a drop-down menu where the user can select the ACR. There is the default option of 4.47 (McGrath et al. 2004) or a user-defined option for specific applications. This worksheet also displays the assignment of chemical class corrections that are used for toxicity modeling as well as the constants used in the HC5 calculation that is described in greater detail elsewhere (McGrath et al. 2004). See Figure 6. Also in this sheet are options for entering user-defined target lipid-water partitioning parameters (near cell H39).

3.7   CONCAWE LIBRARY

Physical and chemical properties data computed with SPARC for 1512 library structures are stored in this database. The TLM is calibrated with SPARC $\log(K_{ow})$ values and to maintain internal consistency within the spreadsheet model, physicochemical properties extracted from SPARC are used in all solubility and toxicity calculations. All physicochemical parameters correspond to conditions of standard temperature (25 $^{\circ}$C) and pressure. There are several columns of property data: Molecular Weight, Boiling Point, Sub-Cooled Solubility, Activity, $\log(K_{OW})$, Henry's Law Constant and a few other data fields necessary to run the model calculations. Physical/chemical properties in this database were calculated with EPA's properties calculator SPARC at standard temperature and pressure. PNEC values are computed with the HC5 equation and those PNEC values that are greater than the sub-cooled solubility are highlighted in a light blue color with bold numbers. See Figure 7.

3.8   DEFINITIONS AND SUPPORTING INFORMATION

This worksheet contains a list of the parameters and their definitions used in the spreadsheet. Acronyms are defined and the units of the various input/output parameter concentrations are described here as well. A key to the chemical class assignments and toxicity corrections is provided so that the user will be able to track the mapping of library structures to the input description. See Figure 8.

SECTION 4

STEPS FOR MODEL EXECUTION

This section contains generalized step-by-step instructions for entering data and running the model. There are detailed examples provided in the Demonstration files of the SETAC short course materials (SETAC 2011). The command buttons and input data fields on the 'Input' sheet are all labeled on Figure 9.

1.   Select input resolution. See Figure 10.

Near cell B17 there is a button labeled 'Set Input Resolution' that, when activated, gives the user two options related to the input format of mass fraction: high-resolution and low-resolution. These input formats determine how the model assigns structures from the library database to the hydrocarbon blocks as discussed earlier. Whenever this button is activated the previous inputs will be cleared to prepare the spreadsheet for a new set of inputs in the selected format. The user must save their input data prior to switching resolution formats since the input/output data will be lost with each new calculation. Once a calculation is performed there are command buttons in the output sheets that enable the user to copy the input and output sheets to a new worksheet so that the user can track their progress through different analyses.

The high-resolution format displays 16 chemical classes where mass fraction data can be entered. This data field appears between cells E19 and T70 and is highlighted in light blue. There are 100 possible entry lines for hydrocarbon blocks. The model expects data to be entered in the first available row of the data field (Row 21). Additional hydrocarbon blocks must be entered in the next available row. If there are gaps in the blocks of entered data the model will not read the inputs correctly.

The low-resolution option will clear the previous format and data and display two columns with light blue shading in cells E19 to F70. These two columns allow the user to enter mass fraction information for the general aliphatic and aromatic chemical classes.

2.   Enter mass fractions and boiling point

The data that the user enters into the light blue cells are the mass fractions of a given hydrocarbon block in units of weight percent so that the sum of all of the mass fractions equal 100%. See the Tutorials for detailed examples. The number of entries in the first orange-shaded boiling point data field (Cells C21 to C70) sets the number of hydrocarbon blocks that the model works with internally. All mass fraction and boiling point interval information for a given block must be entered on the same line. Each block can have a value between 0 and 100% but the sum of mass fractions in all of chemical classes in each hydrocarbon block must add up to 100%. If the

sum of the mass fractions does not add to 100% then the model will interrupt the model calculations and prompt the user to correct the inputs. See Step 8.

The orange-shaded cells in columns C and D are used to enter CNo or boiling point information that defines the interval used to extract library structures from the Concawe library database for mapping and assignment purposes. The initial CNo or BP is entered in column C and the final CNo or BP in column D. Interval entries that are above or below the maximum or minimum CNo or BP of the database are highlighted with bold blue or bold red coloring, respectively. Structures with a CNo or BP greater than or equal to the initial interval mark and less-than the final interval mark is mapped to this hydrocarbon block. Then, these structures are assigned mass fractions according to the user-defined distribution of mass fractions among the different chemical classes. The model expects the intervals to be entered with the lowest range in the first hydrocarbon block row (row 21) and the next highest range in the second row and so forth. Otherwise the reassignment of unassigned mass in the 'orphan' blocks will not be performed properly.

3.   Enter volume of exposure conditions

Once the mass fractions and boiling point intervals are entered, the user must set the volume dimensions of the exposure scenario. This information is entered in the pink-shaded cells C6 and C7. If no values are entered the model assumes default values of 0.9 L of exposure water with a 0.1 L headspace for a total system volume of 1.0L, which is equivalent to a system with a 10% headspace. The minimum headspace volume can be 0.0 L. The headspace can be an important phase for particularly volatile petroleum products like gasoline products where much of the product load volatilizes to the headspace.

4.   Select species/ACR

The next step is to select the species-specific CTLBB from the drop-down menu located near cell F5. If the CTLBB for a desired species is not available, a user-defined CTLBB can be entered. The units of the CTLBB values are μmoles/g octanol. Similarly, on the 'CTLBB' sheet there is a drop down menu where the user can select either the default ACR or enter a custom ACR in cell K5.

Also in this spreadsheet, the user may enter application-specific parameters to describe the membrane-water partition cutoff. There is a drop down menu and a series of notes explaining the parameters and their typical applications near cells H39 in the 'CTLBB' sheet.

5.   Select bioavailability correction options. See Figure 11.

Once the species is selected, a dialog box will appear and prompt the user to select from one of four bioavailability correction options: (1) No correction, (2) User-defined particulate organic carbon (POC) concentrations, (3) default POC concentration of 2.0 mg/L and (4) lipid

concentration in mg/L. The bioavailability options can be reset at any time by activating the button labeled "Bioavailability Mode" near cell G9. Activating this button will cause the dialog box to reappear so the options can be set. The current options are displayed in cells F11 and G11. These cells are critical to the model's performance and their contents should not be changed independently of the command buttons or drop-down menus. If option 1 is selected the model will proceed under the assumption that all of the model-calculated dissolved hydrocarbons are bioavailable. The model will ignore any particulate organic carbon (POC) concentration values entered in the data field in column P rows 6 to 15, shaded in purple in the 'Input' sheet.

During dose response calculations POC or lipid concentrations need to be entered in every line where loading information is entered when options 2 or 4 are selected. During acute or chronic endpoint calculations with these options, the user will enter the POC or lipid concentration in cell P6 as indicated by the message that appears in cell Q6.

6.   Enter loading information

This step depends on the calculation option that is selected in Step 7. If the user desires to run a dose response for a series of product loadings then these values are entered in the purple-shaded cells in column N, rows 6 to 15 in units of mg product/L exposure water. The model can accommodate up to 10 loads. The user may also input effect data (i.e. mortality) in column O. The effect data are not used in the model calculations and are only used to generate charts in the 'Output – Loads' sheet of effect data versus model-calculated toxic units. The units of the effect data column are percent response (%) but the actual interpretation of these values is left to the user. These data can be used for analyses of the model results.

7.   Select calculation button

There are two calculation buttons: 'Calculate End Point' and 'Calculate Dose Response'. Activating either button will direct the model to read in the data entered in steps 1 through 6 and will immediately display several dialog boxes in series while setting up the calculations (See Figure 12). The first box will prompt the user to select either the acute or chronic calculation mode. The acute mode is the default toxicity calculation mode.

8.   Mass scaling options. See Figure 13.

After the calculation type is selected, a second dialog box will appear if there are imbalances in the mass fraction entries. If the sum of the mass fractions as entered in Step 2 is greater than 100.005%, the model will stop the calculations so that the user can correct the inputs and retry the calculation. If the mass fractions are substantially less than 100% (< 99.995 %) then the model displays a warning message stating that there is a mass imbalance and reports the summed mass fractions (Figure 13,A). Immediately following this message another dialog box appears with 3 options: (1) Scale up to 100%, (2) Apply to non-toxic n-paraffin, (3) Stop Calculations.

Option 1 will evenly scale up all of the entered mass fractions until the sum of the mass fractions is 100%. Option 2 will assign the difference between the sum of the mass fractions and 100% to a non-toxic *n*-paraffin. Under this option, the input sheet will be modified to include an additional hydrocarbon block that immediately follows that last user-input block. The narrow boiling point range that is displayed brackets the boiling point of *n*-Hentriacontane, a C31 *n*-paraffin (ID: 1021). Although this structure has a particularly high log(K_{ow}) of 17.78 and very low solubility of ~10^{-15} mg/L ensure that this hydrocarbon block will not appreciably affect the toxicity calculations and that it is essentially inert.

9.   Output sheets

There are two output sheets that correspond to the calculation command buttons on the 'Input' sheet. The 'Calculate End Point' button prints results to the 'Output – LL50' sheet. The model calculated critical product loading (in mg product / L water) for acute or chronic endpoints is reported in a purple-shaded cell C7. The toxic units from the calculation are in cell C8 and should be 1.0 for this calculation mode. In a few cases when petroleum products are not acutely (or chronically) toxic and the toxic units do not reach 1.0, a message is displayed indicating that the desired endpoint was not reached and the maximum toxic units are reported. The acute or chronic endpoint is reported as >1000 mg/L in cell C7 and the maximum toxic units are displayed in cell C8. The maximum toxic units can be reached at loadings substantially lower than 1000 mg/L and a targeted dose-response calculation can be performed to identify the range of loadings where the maximum TUs are reached.

The concentration of free product that remains is reported in cell C13 near the other exposure chamber dimensions. The model is constrained so that ratio of the free product phase volume is never less than 10^{-10} L oil/L total system. The constraint ensures model stability during calculations with highly soluble petroleum products and has a negligible impact on the mass balance at low loadings or for materials that are highly soluble. The weighted-average physical/chemical properties of the reference structures that have been mapped to each hydrocarbon block are reported in the orange-shaded cells in columns C to F. The outputs are the geometric means of the octanol-water partition coefficient, sub-cooled solubility, molecular weight, and air-water partition

coefficients. Columns G through I report the computed concentrations of hydrocarbons in the different phases: product, air, water. The units of the concentration outputs are mass/L phase (e.g., mg/L product, mg/L headspace, mg/L water). If there is no headspace or free product phase then the model reports concentrations of zero for these phases.

A unique result in this sheet is the PNEC output in column J with units of mg/L water. The PNEC values for the hydrocarbon blocks reported in column J are the calculated as the geometric mean of the PNEC values for the representative structures from the database that were mapped to a given hydrocarbon block. The PNEC for the individual structures are found in column Y of the library database sheet: 'CONCAWE Library' and are calculated with the HC5 equation described in the 'CTLBB' sheet and in McGrath and Di Toro (2009). These values change as a function of the hydrocarbon block definition but are independent of product loading. These values are provided for quick reference only. Block-averaged values can introduce error into risk assessments, which is why PETROTOX uses individual compounds to perform fate and effects estimated. These values will be printed in red bold font in cases where the PNEC (column J) is greater than the sub-cooled solubility reported in column D.

The light blue-shaded data fields report the toxic units that are contributed from each hydrocarbon block for 6 grouped chemical classes. The output format will change as a function of the input resolution. Under high resolution the TUs and PNECs are reported for five output classes as follows:

- Paraffinic and Olefinic – sum of toxic units from sub classes 1, 2 (*n-* and *iso-* paraffins)

- Naphthenics – sum of toxic units from sub classes 3, 4, 5, 6, 9 (all cycloalkanes)

- Mono-Aromatics – sum of toxic units from sub classes 11, and 12 (Mono- and Naphthenic Mono-Aromatics)

- Di-Aromatics – sum of toxic units from sub classes 13, and 14 (Di- and Naphthenic Di-Aromatics)

- Poly-Aromatics – sum of toxic units from sub class15 (Poly-Aromatics)

Under low resolution calculations TU and PNEC results are printed for only total aliphatic (sub classes 1, 2, 3, 4, 6 and 9) and total aromatic (sub classes 11, 12, 13, 14 and 15) chemical classes by block.

The 'Output – Loads' sheet has a very similar format, except that it does not have the PNEC output since this output does not vary with product loading. The output fields repeat across the sheet, beginning in columns C to N for the first load and ending in columns GA to GL for the 10th load. A summary table of the loads and total toxic units is given in cells I5 to K14, shaded in purple. There are two charts that are found on this page in columns O to T. The first plots the effect data

versus the model-calculated toxic units and the second displays total toxic units versus product loading. They are given as a convenient representation of the modeling results.

10.   Charts and copy buttons

For more detailed analysis of the model results there are two buttons available in each of the output sheets. The first is labeled 'Chart LL50' (or 'Chart Loads') that creates two charts for each product loading (LL50 calculations have one set of charts and Dose Response calculations have one set for each load). The charts read data from the output sheets and are displayed in the 'Charts' sheet. This feature is only active in high resolution mode.

One chart is a stacked bar chart of the toxic units in each of the 6 grouped chemical classes versus the hydrocarbon block position. Also, a pie chart is generated to show the contribution of the 6 grouped chemical classes to the overall toxic units. These charts contain a few text boxes that indicate the product name, target organism and product loading and toxic units associated with each chart. The charts are erased with each new calculation.

For more detailed data analysis the input and output sheets can be copied to a new spreadsheet so that the user can save the model results in a separate workbook. A command button labeled 'Copy Input/Output' is found on each output sheet. The copied spreadsheet will have three tabs: Output, Input, and a general comments page. The command buttons are removed from these spreadsheets and the sheets are no longer part of the functional model.

SECTION 5

APPLICATIONS

These spreadsheet models can be used to analyze dose response data, calculate LL50s, or toxic units for user-input dose response data as a means of analyzing and designing toxicity experiments. The models can be used to analyze the results of fate models to calculate environmental toxicity and potential risk of hydrocarbon emissions in aquatic compartments. The environmental toxicity of individual hydrocarbon blocks can also be evaluated using this version of PETROTOX.

SECTION 6

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SECTION 7

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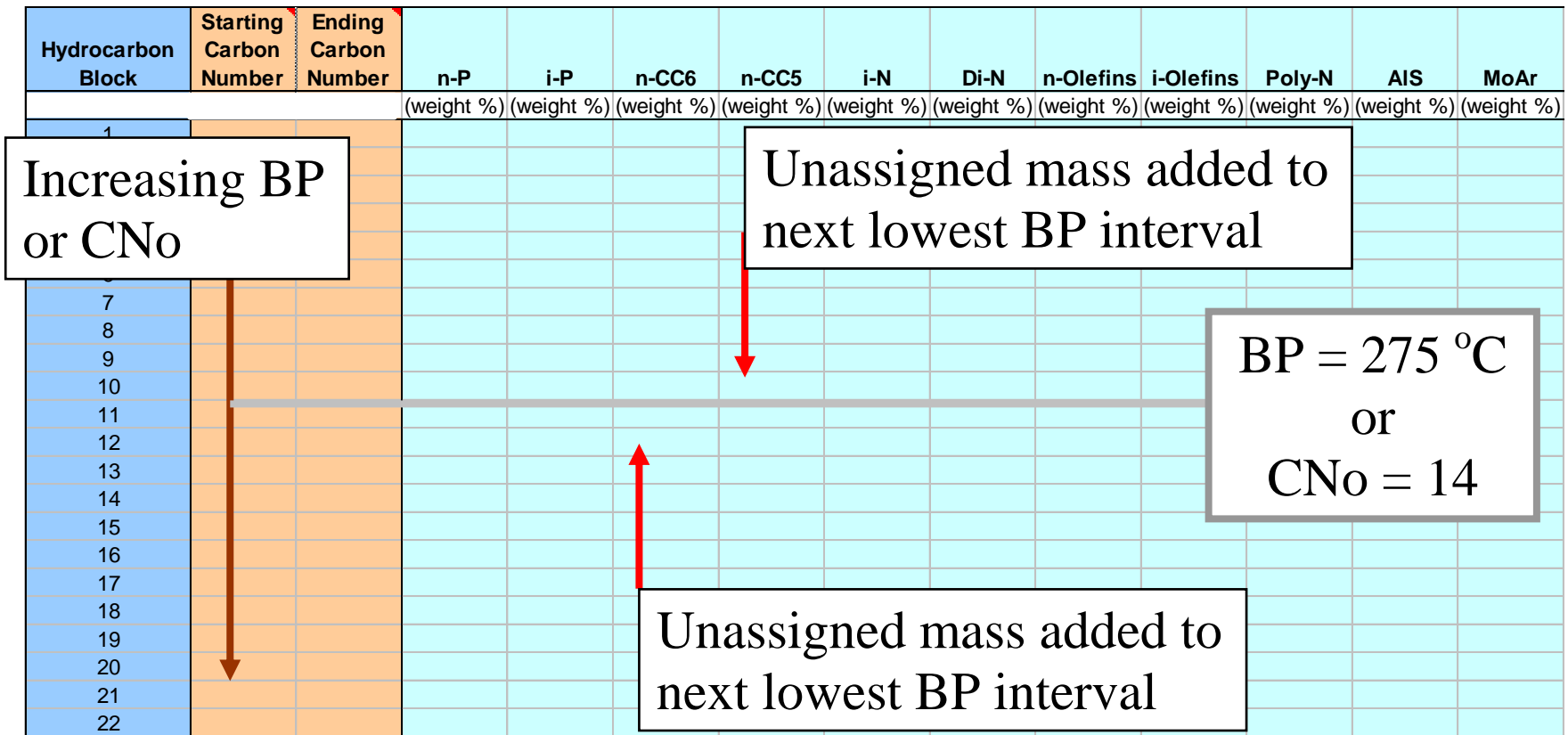Phone: +32-2-566.91.65
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FIGURES

Figure 1. Schematic of redistribution of unassigned mass

Figure 2. Input Sheet Sheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		Product Name:	Gas Oil A											
2		Date:	16-Apr-09				LL50/NOEL and PNEC Report							
3														
4											Copy Input/Output			
5														
6		Species:	Daphnia magna		Acute TUs									
7		Model LL50 (mg/L water)	3.17											
8		Total Toxic Units:	1.00											
9														
10		Test Conditions:												
11		Volume of water (L):	0.9											
12		Volume of headspace (L):	0.1											
13		Volume of free product (µl):	2.0											
14								Chart LL50						
15														
16														
17														
18														
19		Hydrocarbon Block	Average log Kow	Average Sub-Cooled Solubility	Average Molecular Weight	Average Henry's Law Constant	Product Phase	Air Phase	Water Phase	Avg PNEC n,i-Paraffins	Avg PNEC Naphthenics	Avg PNEC Mono-Aromatic	Avg PNEC Di-Aromatics	Avg PNEC Poly-Aromatics
20				(mg/L)	(g/mol)	Log(unitless)	(mg/L product)	(mg/L headspace)	(µg/L water)	(µg/L water)	(µg/L water)	(µg/L water)	(µg/L water)	(µg/L water)
21		1	2.77	1.92E+02	70.47	0.94	3.5E-01	1.9E-03	8.3E-02	1.2E+02	1.0E+03	0.0E+00	0.0E+00	0.0E+00
22		2	3.15	7.67E+01	83.66	0.90	1.2E+01	1.7E-02	1.8E+00	4.2E+01	3.3E+02	2.1E+03	0.0E+00	0.0E+00
23		3	3.79	1.54E+01	98.19	1.35	2.3E+02	1.2E-01	1.0E+01	2.0E+01	1.1E+02	7.7E+02	0.0E+00	0.0E+00
24		4	4.00	1.01E+01	114.14	0.76	3.5E+03	6.2E-01	5.8E+01	3.9E+00	3.6E+01	3.3E+02	0.0E+00	0.0E+00
25		5	4.47	3.22E+00	126.68	0.88	2.8E+04	1.6E+00	1.4E+02	2.3E+00	1.3E+01	1.9E+02	0.0E+00	0.0E+00
26		6	5.00	8.92E-01	140.33	1.00	1.1E+05	2.0E+00	1.5E+02	7.1E-01	5.8E+00	6.9E+01	1.1E+02	0.0E+00
27		7	5.48	2.79E-01	154.50	1.02	1.8E+05	1.0E+00	1.1E+02	5.3E-01	2.3E+00	3.0E+01	4.5E+01	0.0E+00
28		8	5.53	2.68E-01	166.87	0.30	1.9E+05	3.7E-01	6.1E+01	5.6E-01	2.2E+00	1.4E+01	2.3E+01	6.2E+01
29		9	6.06	7.11E-02	180.68	0.41	1.8E+05	1.1E-01	2.0E+01	6.6E-01	1.1E+00	6.6E+00	1.0E+01	2.9E+01
30		10	6.56	1.88E-02	195.35	0.45	1.2E+05	2.7E-02	3.9E+00	7.1E-01	1.1E+00	2.6E+00	5.4E+00	1.1E+01
31		11	6.99	6.93E-03	207.28	0.49	5.7E+04	4.5E-03	4.4E-01	6.8E-01	1.1E+00	1.2E+00	2.3E+00	4.9E+00
32		12	7.02	6.55E-03	220.37	-0.35	2.0E+04	8.7E-04	4.0E-02	1.0E+00	1.2E+00	9.9E-01	1.0E+00	2.2E+00
33		13	6.99	8.04E-03	231.24	-1.12	6.0E+03	5.3E-05	5.1E-03	1.1E+00	1.4E+00	9.9E-01	7.1E-01	9.9E-01
34		14	7.09	6.12E-03	243.24	-1.72	2.4E+03	6.0E-06	6.7E-04	1.1E+00	1.6E+00	1.0E+00	6.5E-01	8.2E-01
35		15	7.77	1.13E-03	254.23	-1.06	1.3E+03	9.9E-07	8.1E-05	1.1E+00	2.1E+00	1.1E+00	6.6E-01	6.4E-01
36		16	8.07	4.80E-04	268.51	-1.61	4.5E+02	8.9E-08	5.9E-06	1.1E+00	1.8E+00	1.1E+00	6.9E-01	6.4E-01
37		17	8.25	3.05E-04	280.85	-1.98	2.3E+02	1.1E-08	6.6E-07	1.1E+00	2.1E+00	1.1E+00	7.3E-01	6.7E-01
38		18	8.62	1.15E-04	294.78	-2.20	1.7E+02	1.7E-09	1.5E-07	1.1E+00	2.0E+00	1.1E+00	6.9E-01	6.8E-01
39		19	9.14	3.34E-05	306.42	-1.74	1.5E+02	5.3E-10	6.4E-09	1.1E+00	1.9E+00	1.2E+00	7.3E-01	7.0E-01

Input / DetailedOut / **Output - LL50** / Charts / CTLBB / Concawe Library / Definitions&SuppInfo

Draw ▾ AutoShapes ▾

Figure 3. Output – LLL50 Sheet

Figure 4. Output – Loads Sheet

Figure 5. Charts Sheet

		Intercept	Log(Standard Error)	CTLBB	Standard Error						
				(µmol / g octanol)							
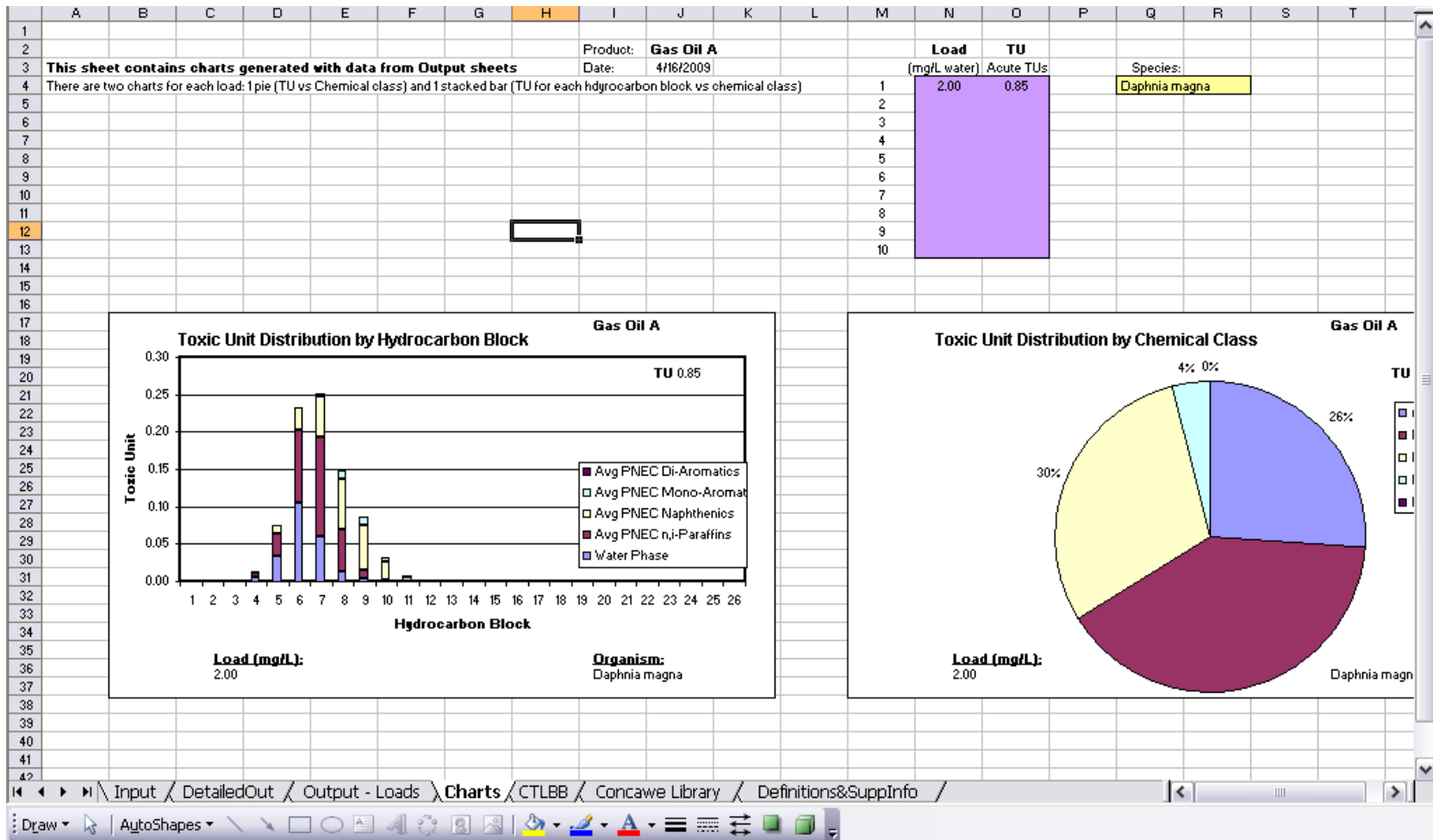
Most recent Narcosis Analysis (Sept 2005)

ID	Species	Intercept	Log(Standard Error)	CTLBB (µmol / g octanol)	Standard Error
1	USER-DEFINED				
2	Aedes aegypti	2.415	0.174	260.0	117.7
3	Alburnus alburnus	2.234	0.147	171.4	63.3
4	Ambystoma mexicanum	2.388	0.174	244.3	110.6
5	Ankistrodesmus falcatus	2.699	0.139	500.0	173.1
6	Artemia salina nauplii	2.288	0.079	194.1	36.2
7	Carassius auratus	2.18	0.067	151.4	23.8
8	Chlamydomonas angulosa	2.524	0.083	334.2	65.7
9	Chlamydomonas reinhardtii	1.536	0.141	34.4	12.1
10	Chlorella vulgaris	2.65	0.078	446.7	82.3
11	Culex pipiens	2.333	0.174	215.3	97.5
12	Cyprinodon variegatus	2.055	0.094	113.5	25.5
13	Danio rerio	2.099	0.101	125.6	30.5
14	Daphnia cucullata	2.394	0.174	247.7	112.2
15	Daphnia magna	2.062	0.050	115.3	13.4
16	Daphnia pulex	1.957	0.089	90.6	19.2
17	Gambusia affinis	2.31	0.147	204.2	75.4
18	Hydra oligactis	2.329	0.174	213.3	96.6
19	Ictalurus punctatus	1.864	0.149	73.1	27.4
20	Jordanella floridae	1.827	0.101	67.1	16.3
21	Lepomis macrochirus	2.117	0.059	130.9	18.1
22	Leptochirus plumulosus	1.634	0.203	43.1	23.8
23	Leucisus idus melanotus	2.183	0.078	152.4	28.1
24	Lymnaea stagnalis	2.288	0.174	194.1	87.9
25	Menidia beryllina	2.465	0.143	291.7	104.4
26	Mysidopsis bahia	1.535	0.087	34.3	7.1
27	Neanthes arenaceodentata	2.26	0.202	182.0	99.8
28	Nitocra spinipes	2.256	0.158	180.3	72.6
29	Oithona davisae	2.151	0.142	141.6	50.2
30	Oncorhynchus mykiss	1.82	0.067	66.1	10.4
31	Orconectes immunis	2.137	0.160	137.1	56.0
32	Oryzias latipes	2.104	0.174	127.1	57.5
33	Palaemonetes pugio	1.758	0.127	57.3	17.9
34	Pimephales promelas	2.087	0.044	122.2	12.5
35	Poecilia reticulata	2.402	0.108	252.3	65.8
36	Portunus pelagicus	1.727	0.203	53.3	29.4

Right-side panel:

Selected Acute-to-Chronic Ratio:	Default	User-Defined	Working Value
	4.47	0	4.47

NOTE:
If values are left blank the model will assume default conditions

PNEC calculation based with HC5 formulation

LogHC5 = UNS * LogKmw + (geomean of CTLBB + Chemical Class Correction) - Log(geomeanACR) - kz * (Varia

Universal Narcosis Slope	-0.936	
Geomean of CTLBB	119.0	(µmol / g octanol)
ACR	4.47	
kz	2.30	
Variance of UNS	2.25E-04	
Variance of logCTLBB	0.112	
Variance of logACR	0.080	

**These parameters cannot be modified at this time

Chemical Class corrections (additive)

ketone correction	0.000	None in database
halogenated chemical correction	-0.339	None in database
PAHs correction	-0.352	Applied to PolyAr, DiAr, NDiAr structures
Monoaromatic hydrocarbon correcti	-0.109	Applied to MoAr, NMoAr and ArS structures
Aliphatic correction	0.000	Applied to n-P, I-P, n-CC6, n-CC5, I-N, Di-N, PolyN,

Sheet tabs: Input / DetailedOut / Output - Loads / Charts / CTLBB / Concawe Library / Definitions&SuppInfo

Figure 6. CTLBB Sheet

	A	B	C	D	E	F	G	H	I	J	K	L
1	WORKSHEET PROTECTED: CONCAWE											
2	1516	CAS	Name	Smiles	Carbon #	Class	SubClass	General Clas	Mol Wt	Boil Pt (C)	Sat Sol (mg/l	Log Kaw
3	ID		Name	SMILES			See key 'CTL	r = 2, S- and	g/mol	C	mg/L	air/water
1482	1479		2-methyl-3-heptene	CC(C)C=CCCC	8	Olefin, branche	8	3	112.214	114.843	2.70E+00	1.803
1483	1480		2,4-dimethyl-1-hexene	C=C(C)CC(C)CC	8	Olefin, branche	8	3	112.214	111.927	3.40E+00	1.768
1484	1481		2-methyl-1-heptene	C=C(C)CCCCC	8	Olefin, branche	8	3	112.214	126.687	3.24E+00	1.546
1485	1482		2-methyl-1-octene	C=C(C)CCCCCC	9	Olefin, branche	8	3	126.241	151.534	7.52E-01	1.732
1486	1483		Tetratriacontane	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	34	Alkane, linear	1	1	478.927	482.628	6.25E-17	5.320
1487	1484		Hexatriacontane	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	36	Alkane, linear	1	1	506.981	500.846	5.06E-18	5.134
1488	1485		2-methyl Octacosane	CC(C)CCCCCCCCCCCCCCCCCCCCCCCCCCC	30	Alkane, branch	2	1	422.820	423.504	2.29E-14	4.972
1489	1486		2-methyl Tetratriacontane	CC(C)CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	31	Alkane, branch	2	1	436.847	432.057	5.31E-15	5.101
1490	1487		n-Pentacosylcyclohexane	C(CCCC1)(C1)CCCCCCCCCCCCCCCCCCCCCCCCC	31	Alkane, mono	3	1	434.831	431.157	1.70E-14	4.242
1491	1488		n-Hexacosylcyclohexane	C(CCCC1)(C1)CCCCCCCCCCCCCCCCCCCCCCCCCC	32	Alkane, mono	3	1	448.858	437.737	3.91E-15	4.371
1492	1489		1-cyclohexyl 4,8,12,16 pentamethyl t	C1CCCCC1CCCC(C)CCCC(C)CCCC(C)CCCC(C)CCCC(	34	Alkane, mono	3	1	476.911	394.739	5.77E-16	5.009
1493	1490		1-cyclohexyl 4,8,12,16 pentamethyl p	C1CCCCC1CCCC(C)CCCC(C)CCCC(C)CCCC(C)CCCC(	36	Alkane, mono	3	1	504.965	407.861	3.16E-17	5.241
1494	1491		1-3methyltetraacosyl,2-methlcyclo	C1CCCCC1CCCC(C)CCCC(C)CCCC(C)CCCC(C)CCCC(	34	Alkane, mono	3	1	476.911	394.739	5.77E-16	5.009
1495	1492		1-3methylhexacosyl,2-methlcycloh	C1CCCCC1CCCC(C)CCCC(C)CCCC(C)CCCC(C)CCCC(	36	Alkane, mono	3	1	504.965	407.861	3.16E-17	5.241
1496	1493		2-(2,6,10,14 tetramethylhexadecyl)-9	C1CCC2CCC(CC(C)CCCC(C)CCCC(C)CCCC(C)CC)CC2	37	Alkane, di-cycl	6	1	516.976	402.904	1.84E-17	5.237
1497	1494		2-(2,6,10,14 tetramethyhexadecyl)-9	C1CCC2CCC(CC(C)CCCC(C)CCCC(C)CCCC(C)CC)CC2	39	Alkane, di-cycl	6	1	545.029	417.522	9.72E-19	5.493
1498	1495		2-ethyl,6-methylundecyl-hydro-chry	C1CCC2CCC3C4CCC(CC(CC)CCCC(C)CCCCC)CC4CC	32	TetraN	9	1	442.810	393.889	1.41E-13	3.055
1499	1496		2-propyl,6-methylundecyl-hydro-ch	C1CCC2CCC3C4CCC(CC(CCC)CCCC(C)CCCCC)CC4C	33	TetraN	9	1	456.837	397.044	3.64E-14	3.147
1500	1497		2-isopentyl,6-ethylundecyl-hydro-c	C1CCC2CCC3C4CCC(CC(CC)CC)CCCC(CC)CCCCC)	36	TetraN	9	1	498.917	390.248	8.35E-16	3.727
1501	1498		2-(3,6-dimethylheptyl)-6-methyl hyc	C4C(CCC(C)CCC(C)CCCCC)CC3CC(C)C2C1CCCCC1C(	32	TetraN	9	1	442.810	391.713	1.13E-13	3.319
1502	1499		2-(3,6-dimethylheptyl)-6-butyl-10-(2	C4C(CCC(C)CCC(C)CCCCC)CC3CC(CCCC)C2C1CC(C(	41	TetraN	9	1	569.051	422.313	3.22E-19	4.925
1503	1500		Dimethylheptyl-hydro benzo(a)pyre	C(C(C(CC1)CCC2)C2CC3)(C3CC(C4CCC5CC(C)CCCC((	29	PentaN	9	1	398.714	378.900	2.20E-11	2.328
1504	1501		Isohexyl hydro-benzo-perylene	CCC(C)CCC6CC2C(CC6)C1CCCC4C1C(C3CCCC5C3C4(	30	HexaN	9	1	410.725	400.279	8.00E-12	1.629
1505	1502		BENZO(P)NAPHTHO(1,8,7-GHI)(	c1cc6ccccc7c6c2c(c4c7c3c(c5c4cccc5)cccc3)cccc12	28	HexaAr	15	2	352.434	706.425	3.48E-05	-7.007
1506	1503		Hydro-BENZO(P)NAPHTHO(1,8,7	C1CC6CCCC7C6C2C(C4C7C3C(C5C4CCCC5)CCCC3)C	28	HexaN	9	1	380.656	419.590	1.61E-10	0.299
1507	1504		DIBENZO(B,K)CHRYSENE	c5c6ccccc6cc2c5c1c(c3cc4c(cc3cc1)cccc4)cc2	26	HexaAr	15	2	328.412	669.407	2.32E-04	-7.000
1508	1505		Hydro-DIBENZO(B,K)CHRYSENE	C5C6CCCCC6CC2C5C1C(C3CC4C(CC3CC1)CCCC4)CC	26	HexaN	9	1	354.618	378.228	2.68E-09	1.087
1509	1506		n-Butyl dimethyl hydroperylene	CCCCC2CC1CC(C)CC4C1C(C3CC(C)CC5C3C4CCC5)C2	26	PentaN	9	1	356.634	362.066	1.43E-09	2.141
1510	1507		2,2,4,6,6-pentamethylheptane	C(C)(C)(C)CC(C)CC(C)(C)C	12	Alkane, branch	2	1	170.337	208.362	4.86E-03	3.247
1511	1508		2,2,3-trimethyl decane	CCCCCCCC(C)C(C)(C)C	13	Alkane, branch	2	1	184.364	234.644	1.45E-03	2.773
1512	1509		3,3,6,6-Tetramethyloctane	CCC(C)(C)CCC(C)(C)CC	12	Alkane, branch	2	1	170.337	228.451	5.42E-03	2.921
1513	1510		2,2,4,4,6,8,8-heptamethylnonane	CC(C)(C)CC(C)CC(C)(C)CC(C)(C)C	16	Alkane, branch	2	1	226.445	287.525	2.11E-05	4.054
1514	1511		6-n-Butyl-2,3-dimethylnaphthalene	Cc2cc1ccc(CCCC)cc1cc2C	16	Naphthalene	13	2	212.334	349.611	1.08E-01	-1.729
1515	1512		2,3 Dimethy-5(4methylpentyl) naph	Cc2cc1c(CCCC(C)C)cccc1cc2C	18	Naphthalene	13	2	240.388	354.416	1.37E-02	-1.574
1516	1513		2-(4,8,14,18-Tetramethylnonadecyl)	C(C)CCCC(C)CCCC(C)CCCC(C)CCCCCCc1ccc2ccccc2	33	Naphthalene	13	2	450.790	456.459	1.41E-10	-0.726
1517	1514		n-phenylcyclohexane	c(cccc1)(c1)C(CCCC2)C2	12	PentaN	9	1	160.258	232.810	5.85E+00	-1.126
1518	1515		cyclopropylbenzene	c1ccccc1C2CC2	9	Naphthenic M	12	2	118.178	166.112	2.26E+02	-1.227
1519	1516		2-methyloctyl-hexadecahydropicer	c12ccc3c(c2CCC5C1CCC(CC(C)CCCCCC)C5)CCC4C3C	31	Naphthenic M	12	2	420.720	433.324	6.62E-08	-3.145
1520												

Input / DetailedOut / Output - LL50 / Charts / CTLBB \ **Concawe Library** / Definitions&SuppInfo /

Figure 7. CONCAWE 1457 Sheet

	A	B
2	**Description of Parameters in Model**	
4	Cw	Sum of library structure's aqueous concentration in mg per liter of water
5	Ca	Sum of library structure's concentration in mg per liter of headspace
6	Cn	Sum of library structure's concentration in mg per liter of free product
7	ACR	Acute-to-Chronic Ratio: Default value is 4.47 but there is a user-defined options in the 'CTLBB' tab
8	CTLBB	Critical Target Lipid Body Burden in units of micromoles / kilogram octanol - representative of toxicity endpoint of the challenge organisms
9	Product Loading	Mass of product per liter of water in units of mg/L water
10	Effect Data	Mortality, survival or other types of effect data in % of control
11	Cell Density	Particulate organic carbon concentration from microbial cell growth in units of mg carbon per L exposure water
12	Lipid concentration	Lipid concentration in exposure chamber calculated from organism loading (g/L water) and lipid fraction (flipid) in units of mg lipid per L exposure water
13	TU	Toxic Units, by definition a TU = 1 is the aqueous concentration of a challenge hydrocarbon to the LL50: TU = Cw / LL50
14	Chemical Class Correction (CC)	Adjustment factor applied to certain chemical classes that exhibit greater than baseline narcosis toxicity
15	Degree of Resolution	Number of chemical classes for assignment purposes: High resolutions has all 16 classes, Low resolution has to general groups: Aliphatic and Aromatic without olefinic and S-bearing structures
16	LL50	Median Lethal Loading (mg/L water)
17	TLM	Target Lipid Model - calibrated with physical/chemical properties from SPARC
18	PNEC	Predicted No Effect Concentrations calculated with HC5 equation described in 'CTLBB' tab
19	BP	Boiling Point in degrees Celcius
20	n-P	Linear alkanes: n-Paraffins
21	i-P	Branched alkanes: iso-Paraffins
22	n-CC5	Parent and Substituted cyclo-pentanes
23	n-CC6	Parent and Substituted cyclo-hexanes
24	i-N	Other naphthenic structures
25	DiN	Di-naphthenic structures
26	n-Olefin	Linear olefinic structures
27	i-Olefin	Branched olefinic structures
28	PolyN	Poly Naphthenics (cyclic alkanes) including Tri-, Tetra-, Penta- and Hexa-naphthenics
29	AIS	Sulfur-bearing aliphatic compounds
30	MoAr	Parent and Substituted Mono-Aromatics
31	NMAr	Naphthenic Mono-Aromatic structures
32	DiAr	Di-Aromatic structures including parent and substituted biphenyl and naphthalene structures
33	NDiAr	Naphthenic Di-Aromatic structures
34	PolyAr	Poly-Aromatic compounds includes: Tri-, Ntri-, Tetra-, Ntetra-, Penta- and Hexa-aromatic structures
35	ArS	Sulfur-bearing aromatic compounds
36	Vair	Volume of headspace in exposure chamber
37	Vwater	Volume of water in exposure chamber
38	Vsystem	Total system volume calculated as sum of Vair and Vwater
39	BP interval	Boiling Point range in degrees Celsius for any given hydrocarbon block: Defined by Starting and Ending Boiling Point entries
40	Hydrocarbon block	There can be up to 50 individual hydrocarbon blocks defined by boiling point interval and mass distribution by chemical classes
41	Target species selection menu	Drop-down menu to select CTLBB for any species/endpoint in the Target Lipid Model database
42	Biodilution	Partitioning of dissolved petroleum hydrocarbons to particulate organic carbon or lipid tissue from organism in exposure water

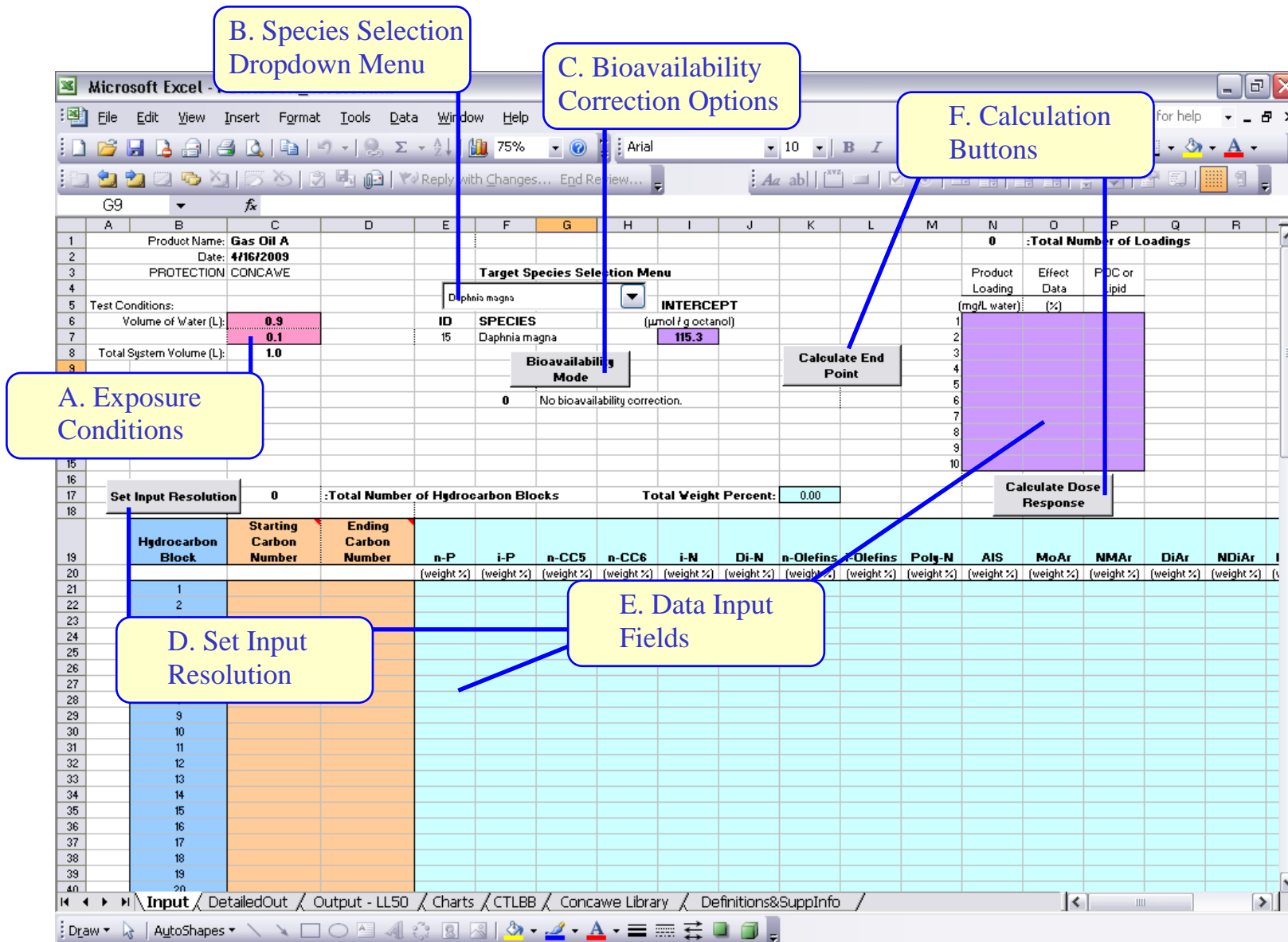Tabs: Input / DetailedOut / Output - LL50 / Charts / CTLBB / Concawe Library / **Definitions&SuppInfo**

Figure 8. Parameters Sheet

Figure 9. Command Button Labels

Figure 10. Resolution Selection Form

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1		Product Name:	Gas Oil A											0	:Total Number of Loadings			
2		Date:	4/16/2009															
3		PROTECTION	CONCAWE				Target Species Selection Menu							Product	Effect	POC or		
4														Loading	Data	Lipid		
5	Test Conditions:					Daphnia magna			INTERCEPT					(mg/L water)	(%)			
6		Volume of Water (L):	0.9		ID	SPECIES			(µmol / g octanol)				1	2				
7			0.1		15	Daphnia magna			115.3				2					
8		Total System Volume (L):	1.0					Bioavailability		Calculate End		3					
9								Mode		Point		4					
10												5					
11							0					6					
12												7					
13												8					
14												9					
15												10					
16		Set Input Resolution	26									Calculate Dose					
17					:Total Number of Hydrocarbon							Response					
18																	
19		Hydrocarbon Block	Starting Carbon Number	Ending Carbon Number	n-P	i-P						i-Olefins	Poly-N	AIS	MoAr	NMAr	DiAr	NDiAr
20					(weight %)	(weight %)						(weight %)	(weight %)	(weight %)	(weight %)	(weight %)	(weight %)	(weight %)
21		1	5.00	6.00	0.008	0.001						0.000	0.008	0.000	0.000	0.000	0.000	0.000
22		2	6.00	7.00	0.029	0.021						0.000	0.008	0.000	0.008	0.000	0.000	0.000
23		3	7.00	8.00	0.170	0.128						0.000	0.008	0.000	0.159	0.000	0.000	0.000
24		4	8.00	9.00	0.752	0.593						0.000	0.008	0.000	1.325	0.000	0.000	0.000
25		5	9.00	10.00	2.627	2.182						0.000	0.008	0.000	3.467	0.078	0.000	0.000
26		6	10.00	11.00	4.094	4.865	0.324	0.455	3.917	1.789	0.000	0.000	0.008	0.000	3.367	0.457	0.162	0.000
27		7	11.00	12.00	3.966	5.082	0.228	0.313	4.022	2.286	0.000	0.000	0.008	0.000	2.257	0.946	0.515	0.000
28		8	12.00	13.00	3.129	4.112	0.167	0.250	3.524	1.953	0.000	0.000	0.008	0.000	1.404	0.975	0.556	0.002
29		9	13.00	14.00	2.465	3.785	0.126	0.167	2.937	1.906	0.000	0.000	0.008	0.000	0.882	0.691	0.218	0.012
30		10	14.00	15.00	1.475	2.870	0.055	0.096	1.845	0.987	0.000	0.000	0.008	0.000	0.441	0.283	0.073	0.007
31		11	15.00	16.00	0.618	1.702	0.022	0.031	0.928	0.312	0.000	0.000	0.008	0.000	0.176	0.090	0.016	0.004
32		12	16.00	17.00	0.182	0.686	0.006	0.010	0.338	0.084	0.000	0.000	0.008	0.000	0.052	0.023	0.004	0.001
33		13	17.00	18.00	0.056	0.187	0.002	0.002	0.114	0.024	0.000	0.000	0.008	0.000	0.014	0.006	0.001	0.000
34		14	18.00	19.00	0.016	0.092	0.001	0.001	0.034	0.007	0.000	0.000	0.008	0.000	0.004	0.002	0.000	0.000
35		15	19.00	20.00	0.005	0.058	0.000	0.000	0.010	0.002	0.000	0.000	0.008	0.000	0.002	0.000	0.000	0.000
36		16	20.00	21.00	0.002	0.016	0.000	0.000	0.003	0.000	0.000	0.000	0.008	0.000	0.001	0.000	0.000	0.000
37		17	21.00	22.00	0.002	0.004	0.000	0.000	0.001	0.000	0.000	0.000	0.008	0.000	0.001	0.000	0.000	0.000
38		18	22.00	23.00	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
39		19	23.00	24.00	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
40		20	24.00	25.00	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000

Bioavailability

- ○ No Bioavailability correction - Proceed as is
- ○ Enter POC data - Typically for algal exposures
- ○ Default: 2.0 mg/L POC - Common value in algal exposures
- ○ Enter lipid concentration -typically for fish or daphind exposures

Apply

Input / DetailedOut / Output - Loads / Charts / CTLBB / Concawe Library / Definitions&SuppInfo

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Figure 11. Bioavailability Correction Options Form

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1		Product Name:	Gas Oil A											0	:Total Number of Loadings			
2		Date:	4/16/2009															
3		PROTECTION	CONCAWE				Target Species Selection Menu							Product	Effect	POC or		
4							Daphnia magna							Loading	Data	Lipid		
5	Test Conditions:								INTERCEPT					(mg/L water)	(%)			
6	Volume of Water (L):		0.9		ID	SPECIES			(μmol / g octanol)					1	2			
7			0.1		15	Daphnia magna			115.3					2				
8	Total System Volume (L):		1.0								Calculate End			3				
9							Bioavailability				Point			4				
10							Mode							5				
11							0	No bioavailability correction.						6				
12														7				
13														8				
14														9				
15														10				
16															Calculate Dose			
17		Set Input Resolution		26	:Total Number of Hydrocarbon Blocks							99.56			Response			
18																		

EndPoint Mode

- ⦿ Acute – LL50
- ○ Chronic Effects

Apply

Hydrocarbon Block	Starting Carbon Number	Ending Carbon Number	n-P (weight %)	i-P (weight %)	n-CO (weigh				n-Olefins (weight %)	i-Olefins (weight %)	Poly-N (weight %)	AIS (weight %)	MoAr (weight %)	NMAr (weight %)	DiAr (weight %)	NDiAr (weight %)
1	5.00	6.00	0.008	0.001	0.00				0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
2	6.00	7.00	0.029	0.021	0.025	0.035	0.000	0.000	0.000	0.000	0.008	0.000	0.008	0.000	0.000	0.000
3	7.00	8.00	0.170	0.128	0.035	0.256	0.064	0.000	0.000	0.000	0.008	0.000	0.159	0.000	0.000	0.000
4	8.00	9.00	0.752	0.593	0.254	0.391	0.770	0.069	0.000	0.000	0.008	0.000	1.325	0.000	0.000	0.000
5	9.00	10.00	2.627	2.182	0.218	0.499	2.245	0.536	0.000	0.000	0.008	0.000	3.467	0.078	0.000	0.000
6	10.00	11.00	4.094	4.865	0.324	0.455	3.917	1.789	0.000	0.000	0.008	0.000	3.367	0.457	0.162	0.000
7	11.00	12.00	3.966	5.082	0.228	0.313	4.022	2.286	0.000	0.000	0.008	0.000	2.257	0.946	0.515	0.000
8	12.00	13.00	3.129	4.112	0.167	0.250	3.524	1.953	0.000	0.000	0.008	0.000	1.404	0.975	0.556	0.002
9	13.00	14.00	2.465	3.785	0.126	0.167	2.937	1.906	0.000	0.000	0.008	0.000	0.882	0.691	0.218	0.012
10	14.00	15.00	1.475	2.870	0.055	0.096	1.845	0.987	0.000	0.000	0.008	0.000	0.441	0.283	0.073	0.007
11	15.00	16.00	0.618	1.702	0.022	0.031	0.928	0.312	0.000	0.000	0.008	0.000	0.176	0.090	0.016	0.004
12	16.00	17.00	0.182	0.686	0.006	0.010	0.338	0.084	0.000	0.000	0.008	0.000	0.052	0.023	0.004	0.001
13	17.00	18.00	0.056	0.187	0.002	0.002	0.114	0.024	0.000	0.000	0.008	0.000	0.014	0.006	0.001	0.000
14	18.00	19.00	0.016	0.092	0.001	0.001	0.034	0.007	0.000	0.000	0.008	0.000	0.004	0.002	0.000	0.000
15	19.00	20.00	0.005	0.058	0.000	0.000	0.010	0.002	0.000	0.000	0.008	0.000	0.002	0.000	0.000	0.000
16	20.00	21.00	0.002	0.016	0.000	0.000	0.003	0.000	0.000	0.000	0.008	0.000	0.001	0.000	0.000	0.000
17	21.00	22.00	0.002	0.004	0.000	0.000	0.001	0.000	0.000	0.000	0.008	0.000	0.001	0.000	0.000	0.000
18	22.00	23.00	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
19	23.00	24.00	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
20	24.00	25.00	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000

Input / DetailedOut / Output - LL50 / Charts / CTLBB / Concawe Library / Definitions&SuppInfo
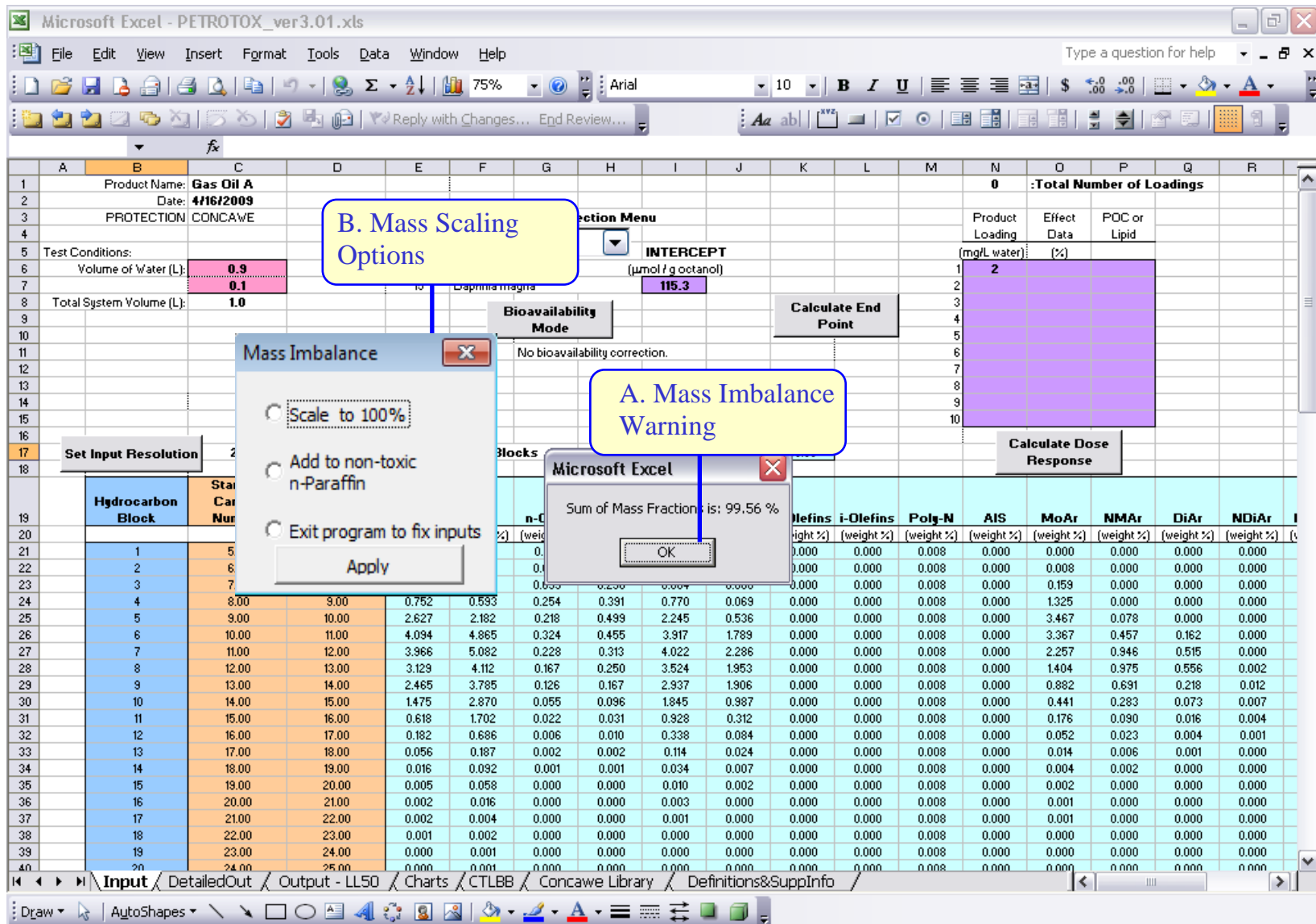
Draw ▾ AutoShapes ▾

Figure 12. Acute or Chronic Mode Selection Form

Figure 13. Mass Scaling Options