

**User's Manual MCnest –
Markov Chain Nest Productivity Model
Version 2.0**

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This model and user's manual have been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

I. Introduction

A. What is MCnest?

The Markov chain nest productivity model, or MCnest, is a set of algorithms for integrating the results of avian toxicity tests with reproductive life-history data to project the relative magnitude of chemical effects on avian reproduction (Bennett et al. 2005, Bennett and Etterson 2007, Etterson & Bennett 2013). MCnest was designed and optimized in MATLAB (www.mathworks.com), but most users will run a compiled version as a standalone program that does not require the user to have MATLAB software. The mathematical foundation of MCnest is in the analysis of Markov chains, which provides a flexible template for modeling the variation in avian breeding cycles among species.

B. What has changed from previous MCnest releases?

There are two versions of MCnest available, MCnest v1.0 (Basic MCnest) and MCnest v2.0 (TIM/MCnest). Both versions are available for download at: <https://www.epa.gov/chemical-research/markov-chain-nest-productivity-model>. Version 2.0 extends the basic version to enable the use of exposure profiles generated using the Terrestrial Investigation Model (TIM, USEPA 2015). MCnest now accounts for background mortality to breeding females as well as the potential for adult mortality due to pesticide exposure. However, the MCnest breeding season algorithms themselves have not changed between Basic MCnest (Bennett & Etterson 2013b-d) and this version, and simulations run in Basic MCnest can be replicated in TIM/MCnest.

C. How to use this Manual

This manual is organized into four major sections. Section I gives some basic background on MCnest and the justification for its development. Section II is a quick start guide to running simulations in MCnest that walks users through several examples. Section III describes the MCnest outputs and their interpretation. Section IV is a complete list of user inputs that can be modified in MCnest. Parameterization of the TIM model, which can now be invoked by MCnest, is described in the [user's guide to TIM](#).

D. What does MCnest do?

The purpose of MCnest is to quantitatively estimate the relative change in the number of successfully fledged broods per female per year of avian species exposed to a specific pesticide application scenario. The relative change in the number of successful broods is estimated by comparing model results based on a defined pesticide application scenario with a no-pesticide scenario. To express the results in terms of annual reproductive success, the model estimate of the number of successfully fledged broods per female is multiplied by the expected number of fledglings per successful nest, which is taken from the literature.

Each model simulation follows the breeding activities of a population of females each day throughout a breeding season. The breeding activity is described by a series of phases through which the female transitions (i.e., pair formation, egg laying, incubation, nestling rearing, and waiting periods prior to starting a new nest attempt). The temporal pattern of breeding activity of each female varies due to differences in the initiation date of the first nest attempt and due to a

specific probability each day that the nest attempt could fail from ecological causes such as predation or adverse weather. When a nest attempt fails, each female can make a new attempt if there is time remaining in the breeding season, and for many species, females make a new attempt after completing a successful brood. If the simulation incorporates one or more pesticide applications, the pesticide exposure may represent an additional cause of nest failure depending on the types of pesticide effects observed in tests and the timing of the application relative to the phase of the nesting attempt for each female. When a nest attempt fails due to pesticide exposure, each female may make a new attempt if there is time remaining in the breeding season and pesticide residues decline to levels that would not affect parental well-being. As MCnest follows each female of the population through the breeding season, it tabulates the number of nest attempts and successful broods (i.e., broods surviving to fledging) per female in the population.

As in the previous version of MCnest (Bennett & Ettoreson, 2013), our goal has been to limit the number of model inputs so that the model is applicable to as many species as possible. We have also focused on using model inputs that are readily available from published literature and from toxicity data submitted as part of the current pesticide registration process under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). MCnest is designed to accommodate the structure of quantitative dose-response data from reproduction tests if it becomes available.

E. Why is MCnest needed?

Prior to the adoption of Basic MCnest (Bennett & Ettoreson 2013) the pesticide registration process used deterministic risk quotients (RQ) as the primary metric for assessing the potential risks of pesticide exposure to avian reproduction. While RQs can be used to classify the potential risk, they do not quantify the magnitude of risk nor provide information on differences in risk among species or pesticide application scenarios. MCnest offers an approach for more quantitatively estimating the magnitude of avian reproductive effects when the ultimate goal is to probabilistically describe the risk of reproductive effects or to estimate the magnitude of effects to a population.

In population modeling, we need to know how a pesticide application scenario affects the annual reproductive success of exposed birds compared to a scenario without pesticides. Annual reproductive success, also known as the fecundity rate, is here defined as the number of fledglings produced per female in the population over an entire breeding season. It represents the cumulative production of fledglings during a breeding season, including re-nesting attempts after nest failures and multiple successful nesting attempts. In MCnest, fecundity can be estimated as the average number of successfully fledged broods per female per season times the average number of fledglings per successful brood.

F. What information is needed to use MCnest?

The basic version of MCnest requires data from avian toxicity tests, avian species life-history profiles, and a pesticide-use scenario that defines the timing and temporal pattern of exposures. The most common sources of toxicity data are the results from three standard avian toxicity tests: 1) the acute oral LD50 test (USEPA 2012a), 2) the dietary LC50 test (USEPA 2012b), and 3) the avian reproduction test (USEPA 2012c). When available, additional toxicity data from

alternative sources or from alternative interpretations of standard toxicity tests may be useful. The selection and use of toxicity data in the model is discussed in the MCnest Technical Manual (Etterson & Bennett 2017).

In the basic version of MCnest, model simulations are run on avian species of interest (i.e., life-history profiles developed for specific species). A series of life-history parameters is required for each species of interest. The user can select one of the species for which a suite of default life-history parameters has been developed, create a new species, or modify the profiles of existing candidate species by directly inputting the list of life-history parameters. The use of default species profiles and the creation of new or modified species profiles are discussed later in the manual.

Finally, MCnest requires that the user define a pesticide-use scenario by parameterizing an exposure model. With this release, MCnest offers two options for parameterizing exposure, the Terrestrial Residue Exposure Model (T-Rex, USEPA 2012d) and the Terrestrial Investigation Model (TIM, USEPA 2015). The first option is identical to that of Basic MCnest and requires, at a minimum, specifying the date(s) of application, the application rate(s), and the half-life of residue degradation on foods relevant to the species of interest. The user also has four options for how the application rate is converted to the ingested daily dose on the day of each application, which are explained later in Section IV.C. The second option, TIM, requires more information on physical chemical properties and test results beyond the three standard avian tests mentioned above. Parameterization of TIM is described in the TIM user's manual and that information is not repeated here. User's wishing to employ the TIM option will need to cross reference with the [User's Guide for TIM](#) (USEPA 2015).

II. Quick start guide

A. Installation of MCnest

Probably the most efficient way to understand MCnest is to begin using the model with default life-history data. To begin using the program, extract the MCnest files from "MCnest2.0.zip" to an appropriate directory on your computer. MCnest2.0.zip should contain six files, all of which must be in the same directory (Table 2.1).

Table 2.1. Files included in the MCnest v 2.0 release ("MCnest2.0.zip")

| File name | Function |
|---------------------------------|---|
| MCnest.exe | Main MCnest executable. Run this file to start MCnest |
| TIM.MCnest.Species.Library.xlsx | Draft species profiles for use in MCnest |
| CallTim.bat | batch file for implementing TIM from Matlab |
| StopTim.bat | batch file for interrupting TIM from Matlab |
| TIM3.0_Beta_3-18-15b.exe | Main TIM executable |
| readme.txt | Matlab compiler information |

If this is your first time using MCnest, you will need to install the MATLAB compiler runtime library (MCR). It can be downloaded from Mathworks at:

<http://www.mathworks.com/products/compiler/mcr/> by selecting the Windows 64-bit R2016b

version. Installation of the MCR requires administrator privileges. To install, double-click “MCR_R2016b_win64_installer.exe” or select “Run...” and browse to the MCR file. Installation may take several minutes due to the very large file size. Once the MCR is installed, you may run “MCnest.exe” file without administrator privileges. Be patient; it can take up to 60 seconds to open!

B. Sample Data Files

With this release of MCnest we are including 14 sample data files on seven insecticides (Table 2.2). Each insecticide has two files, one for the toxicity test results and one for TIM parameters. These files should be extracted from the zip file, but can be placed anywhere on your computer. For ease of access it is recommended to put them in a subfolder of the directory containing the MCnest executable.

Table 2.2. Sample data files included with MCnest 2.0.

| Insecticide | Class | Mode of Action |
|--------------------|-----------------|--|
| carbaryl | carbamate | AChE inhibition |
| chlorpyrifos | organophosphate | AChE inhibition |
| indoxacarb | oxadiazine | Voltage-dependent sodium channel blocker |
| λ-cyhalothrin | pyrethroid | Sodium channel modulator |
| malathion | organophosphate | AChE inhibition |
| methomyl | carbamate | AChE inhibition |
| permethrin | pyrethroid | Sodium channel modulator |

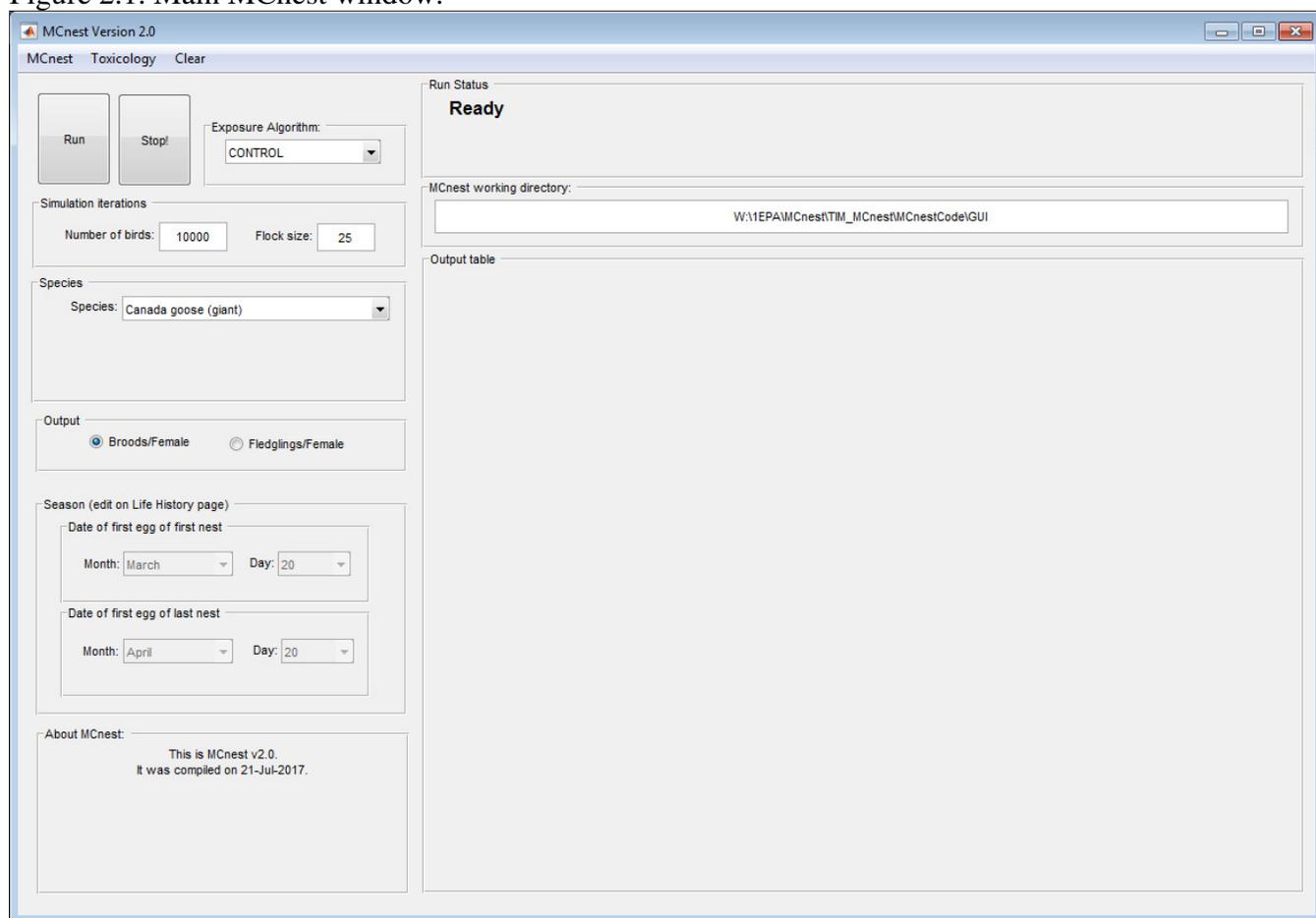
C. MCnest startup window

Once the MATLAB compiler runtime library is installed, MCnest is called by double-clicking the executable file (MCnest2.0.exe). Doing so brings up the main MCnest screen (Figure 2.1). MCnest opens ready to run a control simulation (i.e., no pesticide exposure or effects).

Number of birds: This parameter defines the simulation sample size (number of breeding females). The associated **Flock size** parameter defines the level of replication associated with the output metrics (number of successful broods or number of offspring fledged). For example, the default values of 10,000 birds with a flock size of 25 results in 400 replicate estimates of the output metrics.

Species: The Species block contains a dropdown menu of species names for which life-history profiles have been pre-coded for use in MCnest. The default is the first species in the list, Canada goose. Specific details about the life-history of species in the library, and functions to edit life-history parameters to create new species are found on the Life History page, which can be accessed under the main MCnest menu. More details about editing and creating life-histories are given in Section III.

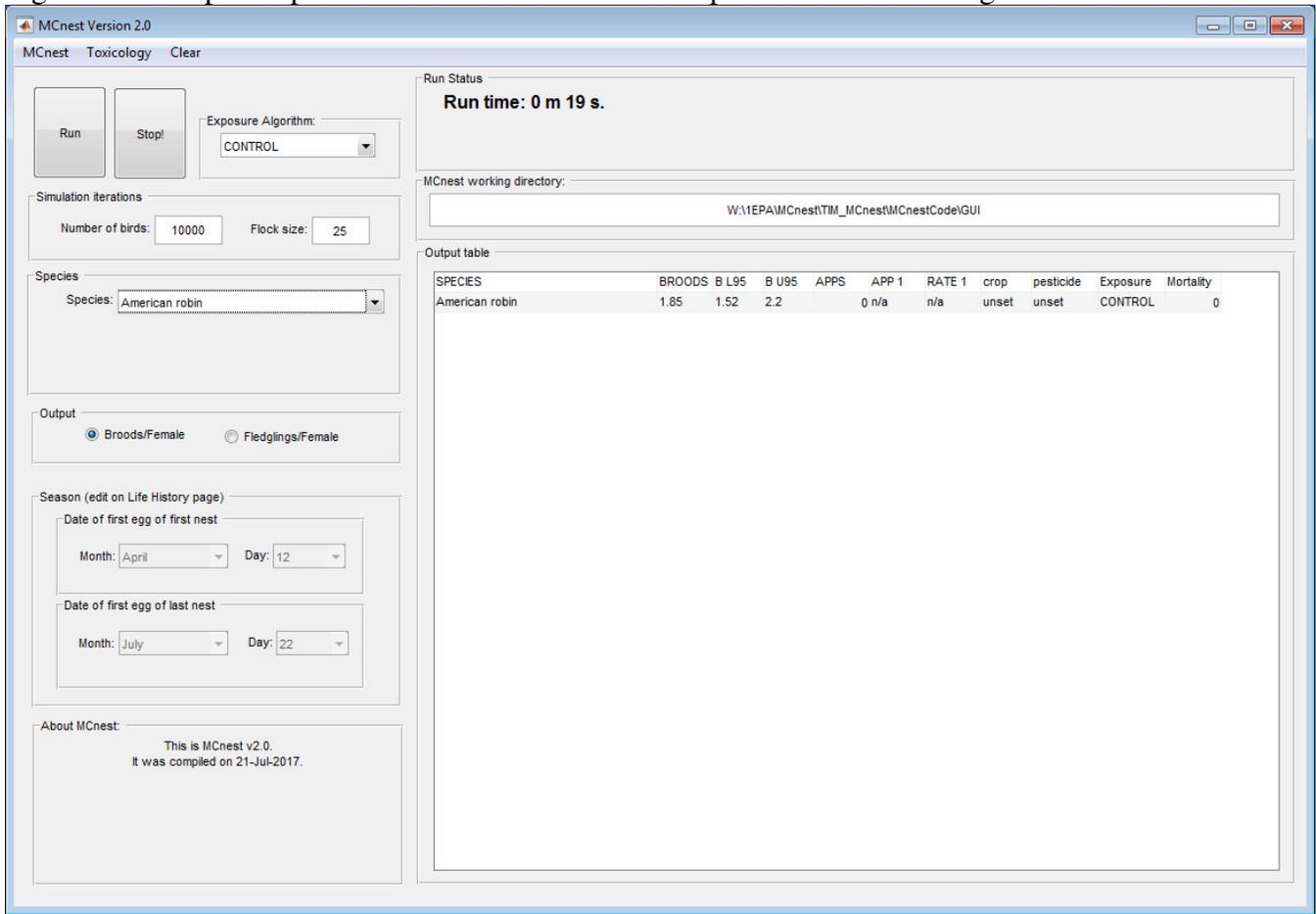
Figure 2.1. Main MCnest window.



D. Running a simulation with no pesticide exposure for a selected species

Once the program is loaded, verify that **Number of birds** edit box and the **Flock size** are set to 10,000 and 25, respectively and that **Exposure Algorithm** is set to CONTROL. Under **Species**, click American robin. Then click **Run** in the upper left corner. While the simulation is running, the **Run Status** box in the upper right of the main MCnest window will initially display the message “Simulation running, please wait...” while the Markov chain transition matrix is constructed. Once the simulation begins to run, the **Run Status** box will display the updating message “Iteration X of Y,” where X is the recently completed iteration and Y is the total number of iterations (breeding females). When the simulation finishes, the window will revert to a run time message (Figure 2.2).

Figure 2.2. Sample output from a simulation with 400 replicates of 25 breeding American robins.



The default tabular output to a MCnest simulation gives the species name, the expected number of successful broods per female (BROODS), the upper and lower 95th percentile confidence intervals for BROODS (based on the 400 replicates), and the number of pesticide applications simulated (APPS), which is 0 in this case. As more simulations are run, new output is appended to this table.

Note that the numerical results on your screen may differ slightly from Figure 2.2 because of variation in the actual sequences of random numbers utilized by the program. MCnest does give you the ability to set the random seed so that simulations may be replicated exactly. These functions are covered in detail below in Section IV.

E. Running a simulation with a pesticide exposure

MCnest is designed to simulate the effects of pesticide applications on the reproductive output of breeding birds. There are many options in MCnest for how this is done, but all involve essentially a two-step procedure. Test results must be entered into the model for computing the surrogate endpoints (Bennett and Etersson 2013) and an exposure model must be parameterized.

MCnest offers two choices for exposure models, T-REX (USEPA 2012d) and TIM (USEPA 2015).

1. TEST RESULTS

With the MCnest main screen still displaying the American robin simulation, choose **Test Results** under the main **Toxicology** menu. After a short delay, the **Test Results** window should appear (Figure 2.3)

Figure 2.3. The **Test Results** window in MCnest.

The screenshot shows the 'MCnest Version 2.0: Test Results' window. At the top, there are buttons for 'Done', 'Clear form', 'Load data', and 'Save data'. A 'Half-life on dietary items' field is set to '35 days'. The 'Pesticide' field is 'unset'.

Avian Reproduction Test: Includes fields for 'Avian reproduction test source (MRID):', 'Test species:', and 'Treatment group data:'. The treatment group data is a table with 5 columns (1-5) and 6 rows of parameters, all currently showing 'NaN'.

| Treatment levels: | 1 | 2 | 3 | 4 | 5 |
|--|-----|-----|-----|-----|-----|
| Measured concentration (mg/kg diet): | NaN | NaN | NaN | NaN | NaN |
| Average food consumption (g/bird/day): | NaN | NaN | NaN | NaN | NaN |
| Average initial female body weight (g): | NaN | NaN | NaN | NaN | NaN |
| Average initial male body weight (g): | NaN | NaN | NaN | NaN | NaN |
| Average final female body weight (g): | NaN | NaN | NaN | NaN | NaN |
| Average final male body weight (g): | NaN | NaN | NaN | NaN | NaN |
| Estimated average daily dose (mg/kg bw/d/day): | NaN | NaN | NaN | NaN | NaN |

Avian LD50 Test (adults): Includes fields for 'Mineau scaling factor: 1.15', 'Body weight: NaN', 'LD50 (mg/kg bw/d): NaN', 'Scaled: NaN', 'LD50 test source (MRID):', and 'Test species:'.

Avian LC50 Test (juveniles): Includes fields for 'LC50: NaN', 'Fraction of LC50 (i.e., 0.1 or): NaN', 'Mean body weight (g): NaN', 'Mean food ingestion rate (g/d): NaN', 'LC50 source (MRID):', and 'Test species:'.

Test NOAELs: A table with two columns for 'Endpoint NOAEL' and 'Endpoint NOAEL', and six rows of parameters, all currently showing '>'.

| Endpoint NOAEL | Endpoint NOAEL |
|---------------------------------------|------------------------------|
| Number of eggs laid: > | % 14-d chicks of eggs set: > |
| % viable eggs of eggs set: > | shell thickness: > |
| % live 3-wk embryos of viable eggs: > | hatch weight: > |
| % hatchlings of live 3-wk embryos: > | 14-d chick wt: > |
| % hatchlings of eggs set: > | prelaying female weight: > |
| % 14-d chicks of hatchlings: > | prelaying male weight: > |

MCnest Toxicity Thresholds (mg/kg/d): A list of parameters with values: 'Alternative behavioral threshold: 9999', 'Adult prelaying body weight: 9999', 'Eggs laid per hen: 9999', 'Mean eggshell thickness per pen: 9999', 'Viable eggs set per pen: 9999', 'Hatchlings per viable egg per pen: 9999', '14-d chicks per hatchling per pen: 9999', and 'Fraction of juvenile 5-d LC50: 9999'.

A full description of the **Test Results** controls is given in Section IV. For now, choose the Load Data option at the top left and navigate to the folder in which you stored the sample data files. Select the data file for Lambda Cyhalothrin (“LambdaCyhalothrinTestResults.mat”) and click **Open**. The controls on the TestResults page should now look like Figure 2.4, below. Some

additional context for understanding the controls on the **TestResults** page is presented immediately below Fig. 2.4. To continue with the examples, skip to **2. EXPOSURE MODEL**.

Figure 2.4. **TestResults** window with data parameterized for λ -Cyhalothrin

The screenshot shows the 'Test Results' window for MCnest Version 2.0. The window is divided into several sections for data entry:

- Buttons:** Done, Clear form, Load data (highlighted), Save data.
- Half-life on dietary items:** 35 days.
- Pesticide:** Lambda Cyhalothrin.
- Avian Reproduction Test:**
 - Avian reproduction test source (MRID): R2048984
 - Test species: Mallard
 - Treatment group data:**

| Treatment levels: | 1 | 2 | 3 | 4 | 5 |
|--|---------|---------|-----|-----|-----|
| Measured concentration (mg/kg diet): | 4.63 | 50.8 | NaN | NaN | NaN |
| Average food consumption (g/bird/day): | 225.8 | 218.3 | NaN | NaN | NaN |
| Average initial female body weight (g): | 962 | 962 | NaN | NaN | NaN |
| Average initial male body weight (g): | 1087 | 1075 | NaN | NaN | NaN |
| Average final female body weight (g): | 1135 | 1113 | NaN | NaN | NaN |
| Average final male body weight (g): | 1249 | 1251 | NaN | NaN | NaN |
| Estimated average daily dose (mg/kg bw/day): | 0.94334 | 10.0792 | NaN | NaN | NaN |
 - Test NOAELs:**

| Endpoint NOAEL | Endpoint NOAEL |
|---------------------------------------|------------------------------|
| Number of eggs laid: 1 | % 14-d chicks of eggs set: > |
| % viable eggs of eggs set: 2 | shell thickness: 2 |
| % live 3-wk embryos of viable eggs: 2 | hatch weight: > |
| % hatchlings of live 3-wk embryos: 2 | 14-d chick wt: > |
| % hatchlings of eggs set: > | prelaying female weight: 2 |
| % 14-d chicks of hatchlings: 2 | prelaying male weight: 2 |
- Avian LD50 Test (adults):**
 - Mineau scaling factor: 1.15
 - Body weight: 1049
 - LD50 (mg/kg bw): 50000
 - Scaled LD50: 33812.7587
 - LD50 test source (MRID): R2084922.pdf
 - Test species: Mallard
- Avian LC50 Test (juveniles):**
 - LC50: 2354
 - Fraction of LC50 as dose (mg/kg bw): 0.5
 - Mean body weight (g): 17.4
 - Mean food ingestion rate (g/d): 14.96
 - LC50 source (MRID): R2048970.pdf
 - Test species: Northern Bobwhite
- MCnest Toxicity Thresholds (mg/kg/d):**
 - Alternative behavioral threshold: 9999
 - 1/10 LD50: 3381.2759
 - Adult prelaying body weight: 10.0792
 - Eggs laid per hen: 0.94334
 - Mean eggshell thickness per pen: 10.0792
 - Viable eggs set per pen: 10.0792
 - Hatchlings per viable egg per pen: 10.0792
 - 14-d chicks per hatching per pen: 10.0792
 - Fraction of juvenile 5-d LC50: 1011.95

On the **TestResults** window, results pertaining to the Acute Oral Toxicity Test (USEPA 2012a) and to the Dietary Toxicity Test (USEPA 2012b) are entered on the right-hand side and results pertaining to the Avian Reproduction Test (USEPA 2012c) are entered on the left-hand side (Fig. 2.4). For the Avian Reproduction Test and the Dietary Toxicity Test, results must be converted from dietary concentrations (mg/kg – diet) to doses (mg/kg - body weight), which requires knowledge of the body weights and food consumption rates of tested animals. Details on these conversions are provided in the technical manual.

For the Avian Reproduction Test, the No Observed Adverse Effects Levels (NOAELs) are entered in the bottom left panel, which has drop-down menus for 12 NOAELs, though only 8 are currently used by the model. These drop-down menus contain only integers, which correspond to the potential test levels of the Avian Reproduction Test. For each measured test endpoint, the user should choose the integer corresponding to the NOAEL for that endpoint. For Lambda-Cyhalothrin, only 2 dietary concentrations (and control) were tested, thus the highest possible integer would be 2. If the NOAEL is unbounded then the symbol '>' can be chosen to indicate this and the program will use 9999 for the value of surrogate endpoints to which this test endpoint is connected.

For the Acute Oral Toxicity Test, scaling factors suggested by Mineau et al. (1996) can be used to rescale the LD50 from the tested species (typically Northern Bobwhite or Mallard) to the body

weight of the modeled species. This is done using an allometric equation with an empirically determined exponential parameter. Mineau et al. (1996) published values for many insecticides, primarily organophosphates and carbamates, and suggested an overall mean exponential parameter among insecticides of 1.15, which is the default value used by MCnest. A value of 1 will result in no rescaling.

For the Dietary Toxicity Test, a choice for the fraction of the LC50 must be made to determine the surrogate endpoint corresponding to this measured endpoint. The default is 0.5. The choice of fraction to use is up to the user and discussion of the choice of appropriate value can be found in Bennett et al. (2005) and Bennett & Etterson (2013b,d).

The toxicity data entered into the **TestResults** panels are used to calculate the surrogate endpoints (Fig. 2.4, bottom right panel), which are compared to time-weighted averages of dose equivalents on a daily basis to determine whether a given nest attempt fails or succeeds. Much more detail on calculation of surrogate endpoints and the time-weighted averages to which they are compared are provided by Bennett & Etterson 2013b,c,d.

2. EXPOSURE MODEL

The examples below parameterize the two exposure models (T-REX and TIM) under the same scenario for comparison.

First, under the **Toxicology** menu choose **Exposure** and then **TREX**. Once the T-REX page opens, set **Pesticide applications** to 4 and the first application to occur on 20 May, with subsequent applications separated by 7 days. Finally, set all application rates to 0.03 lbs AI/acre, so that the T-REX page looks like Fig. 2.5, below. MCnest uses algorithms from T-REX (USEPA 2012d) to translate the application rate (0.03 pounds AI/acre) into doses (expressed as mg/kg body wt./day) for both adult and juveniles, taking into account the species' typical diet. This produces the values for "Adult Initial Dose" (1.53 mg AI/kg body weight) and "Juvenile Initial Dose" (2.28 mg AI/kg bodyweight) that appear in the two associated boxes. These initial doses will be realized on the application date (May 20) and will decay geometrically with a residue half-life of 35 days. Click "Done" to close this window and return to the main MCnest window and set the **Exposure Algorithm** to T-REX, then click **Run**. When finished, the main window should resemble Figure 2.6 (though again, results may differ slightly due to stochastic variation).

Figure 2.5. T-REX window parameterized for λ-Cyhalothrin

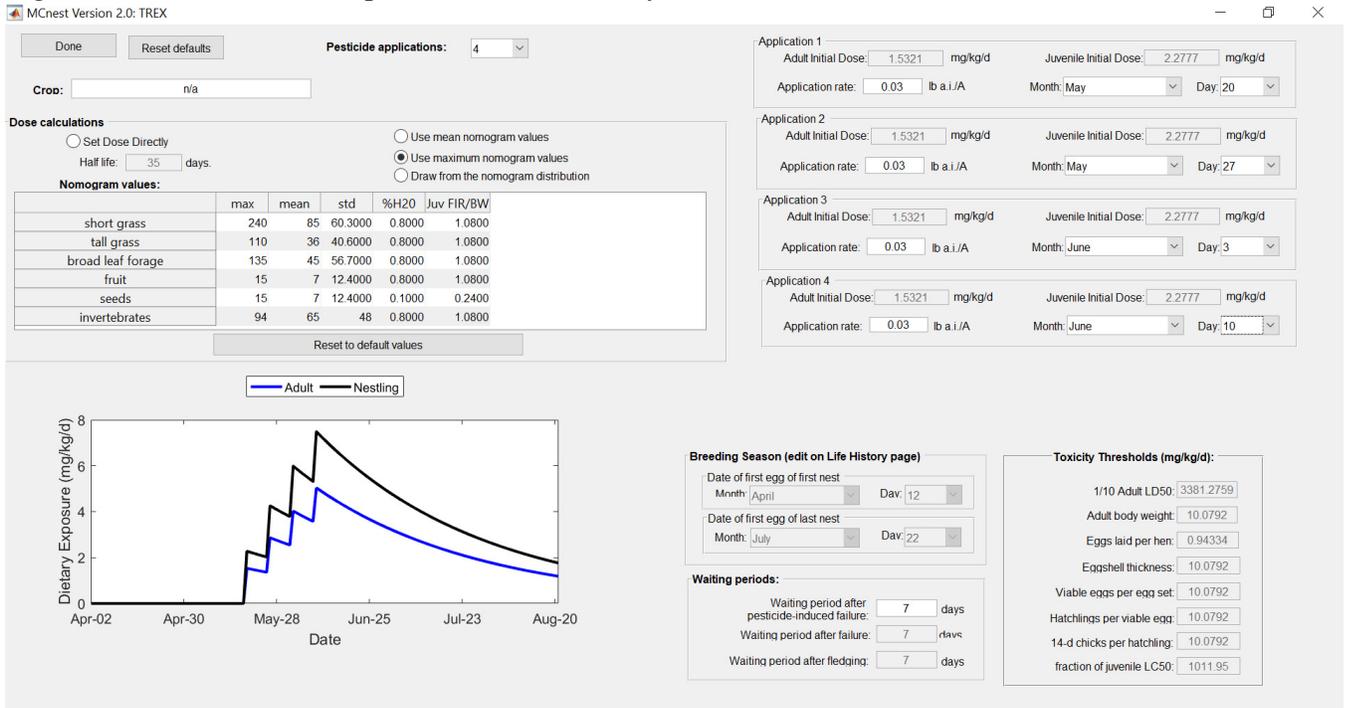
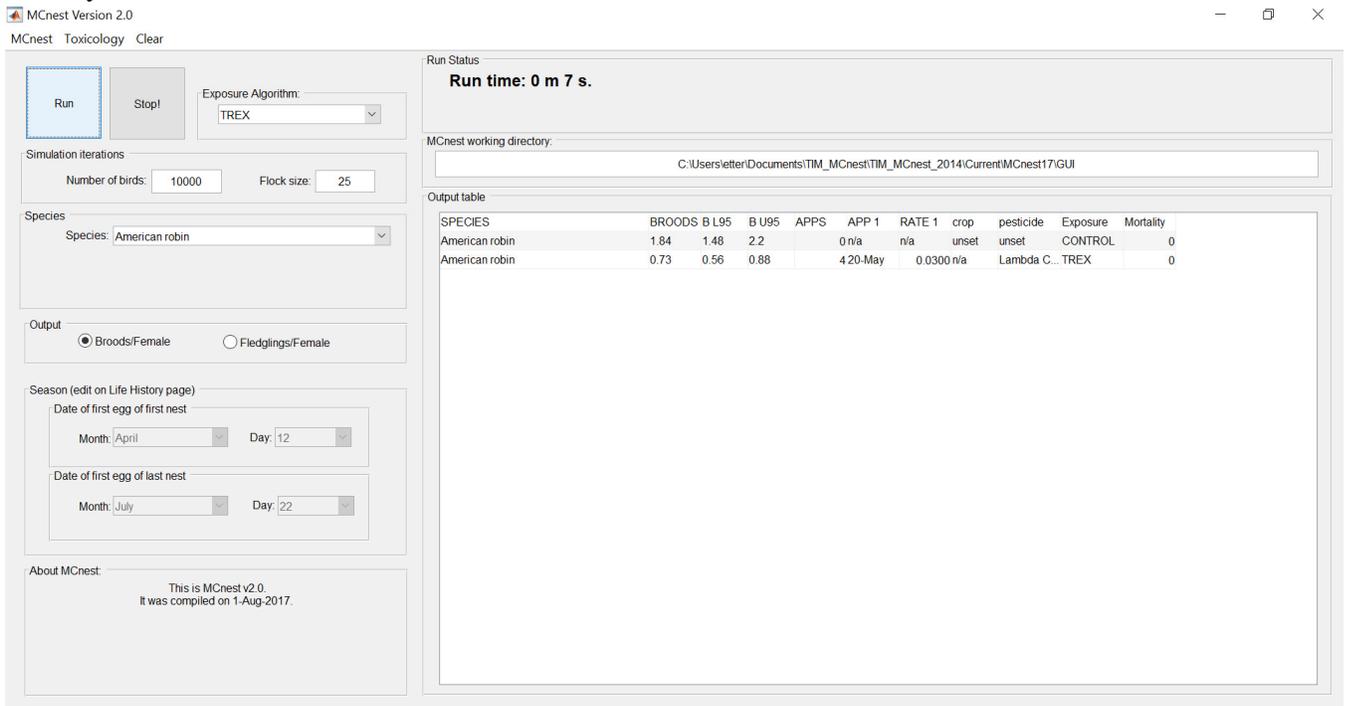


Figure 2.6. The main MCnest window after running an American robin simulation with exposure to λ-Cyhalothrin.



Next, choose **Toxicology > Exposure > TIM** to bring up the **TIM** window. On the TIM window, choose **File > Load TIM Parameters** and navigate to the folder where the sample data are located. Choose the file **LambdaCyhalothrinTIMParameters.mat** and the TIM window should look like Figure 2.7. Click **Close**. This will cause an error message to display (Fig. 2.8). The error message is displayed to warn you that you have loaded a mismatched data set. The parameter set that you have just loaded was created with Canada Goose as the species. To run the American Robin simulation, you will need to first set the species to Canada Goose (giant) and then load the TIM parameters. Once loaded, you can close the TIM window and switch species back to American Robin. *This bug will be fixed in a future version. 15 Sept. 2017.*

Figure 2.7. The **TIM** window parameterized for Lambda-Cyhalothrin.

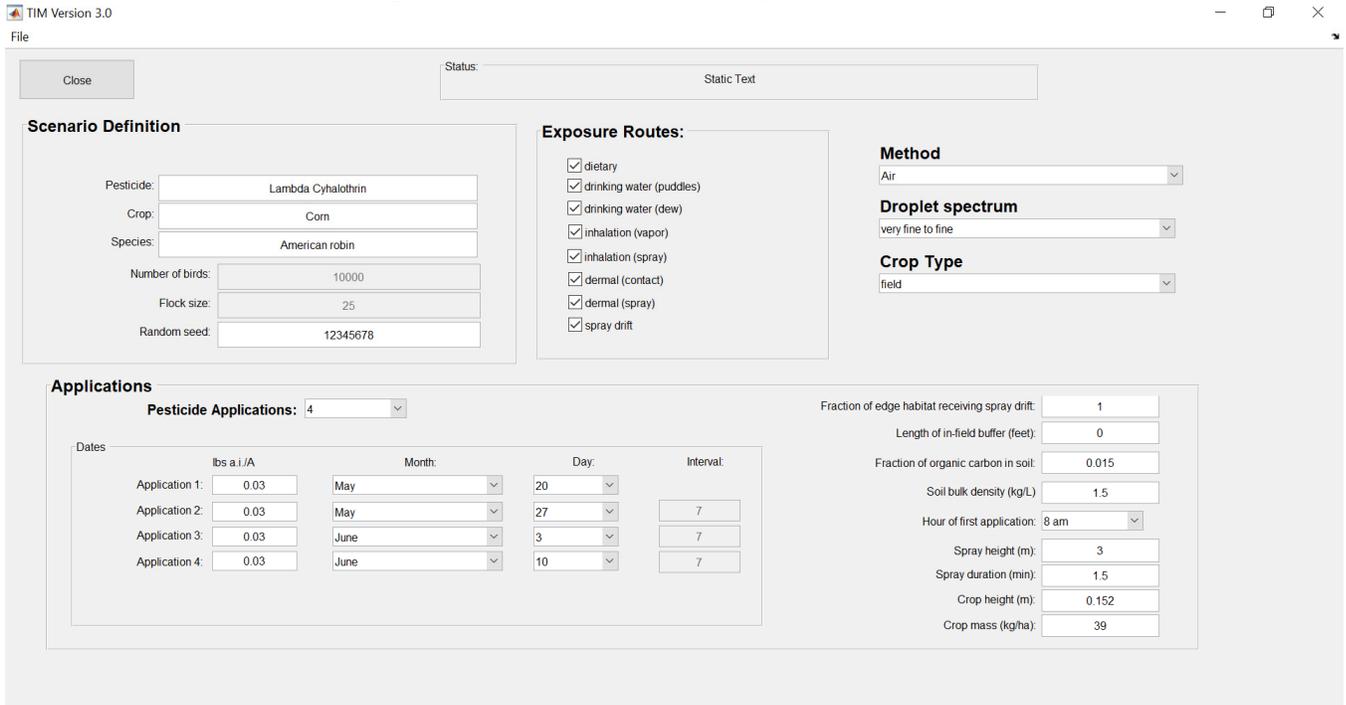
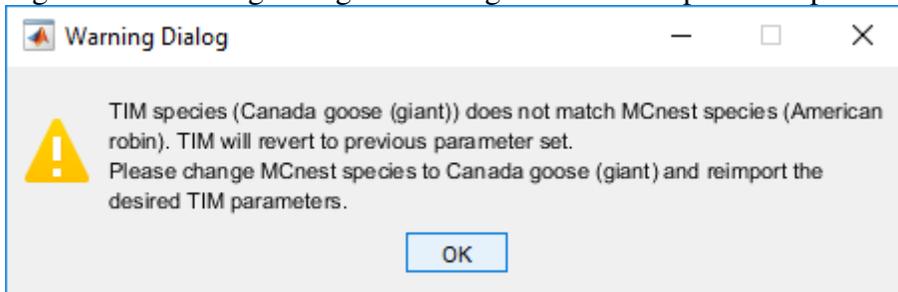


Figure 2.8. Warning dialog concerning mismatched species on parameter import for TIM



Once you have gone through this process and closed the TIM window without an error, change Exposure Algorithm on the main MCnest window to TIM and click Run. This will call up a TIM window, that will immediately minimize. While TIM is running you can watch the TIM spool by

clicking on the minimized TIM icon (Fig. 2.9). While TIM is running, MCnest is paused, waiting for the TIM exposure output. When TIM finishes, the TIM window will close automatically and MCnest will start running, conditional on the TIM output. Finally, once the MCnest simulation is complete, the main MCnest window should resemble Figure 2.10.

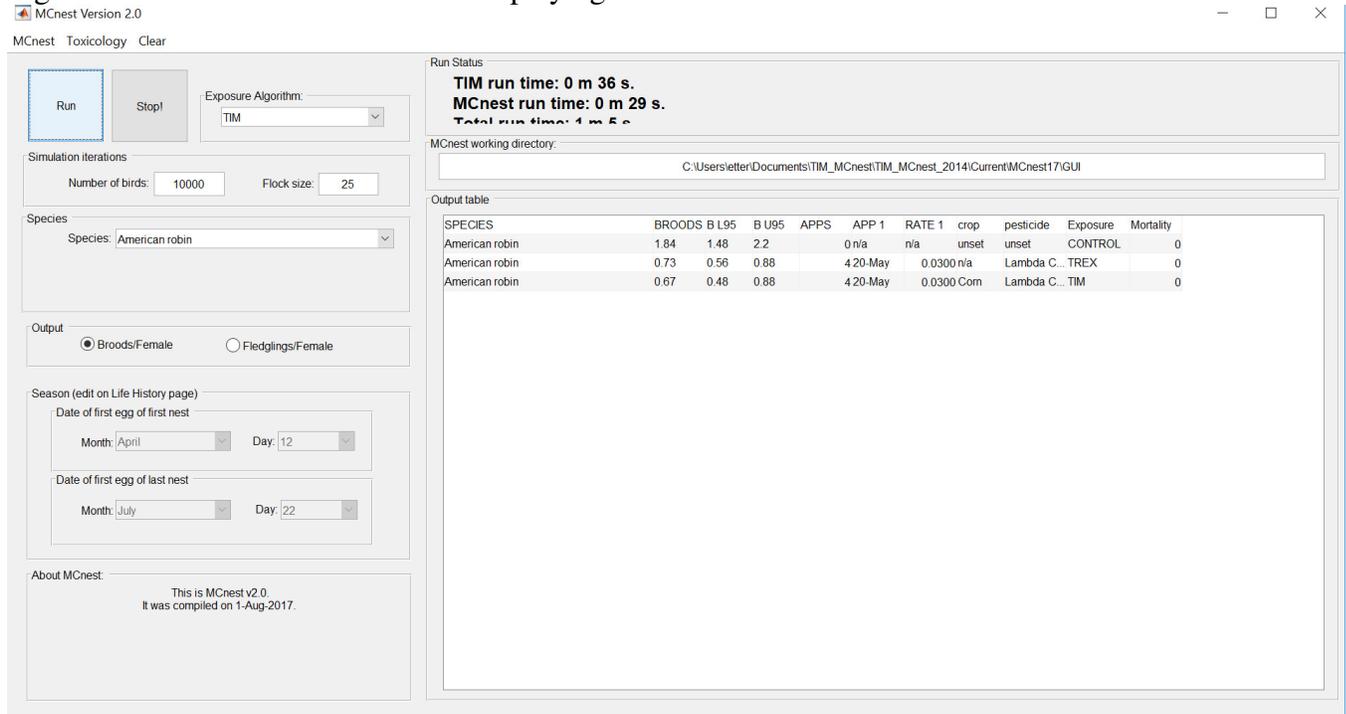
Figure 2.9. The TIM spool for an American Robin simulation with λ -Cyhalothrin

```

-----
                Food Ingestion  1  On
                  Puddle      1  On
                   Dew         1  On
    Inhalation: Vapor  1  On
    Inhalation: Spray  1  On
      Dermal Contact  1  On
      Dermal Spray   1  On
        Spray Drift  1  On
-----

-----
    Bird  Dead  Hour  Tolerance  Total Retained Dose  Population Risk
-----
    250    0    2256  71228.869  67.082  0.00000
    500    0    2256  44022.556  32.163  0.00000
    750    0    2256  47250.294  33.703  0.00000
   1000    0    2256  71072.769  52.482  0.00000
   1250    0    2256  76210.846  90.675  0.00000
   1500    0    2256  43902.472  67.589  0.00000
   1750    0    2256  47969.791  91.854  0.00000
   2000    0    2256  73004.489  79.494  0.00000
   2250    0    2256  81643.381  117.676  0.00000
   2500    0    2256  74889.817  60.251  0.00000
   2750    0    2256  71648.051  78.531  0.00000
   3000    0    2256  69112.458  32.027  0.00000
   3250    0    2256  79376.322  86.125  0.00000
   3500    0    2256  83881.613  104.344  0.00000
   3750    0    2256  40071.580  70.048  0.00000
-----
    
```

Figure 2.10. Main MCnest window displaying the results of three simulations



This is the end of the Quick Start Guide. Congratulations! You now know how to run elementary simulations in MCnest. However, we recommend you continue through the next section to see how to extract more information from MCnest simulations. The above three simulations will be used as examples throughout Section III to demonstrate the diagnostic and output features of MCnest.

III. MCnest outputs

A. Using the table

The Output table on the main MCnest screen provides a snapshot of the overall results for each simulation as well as functional access to more detailed information about MCnest results. Because each MCnest simulation is separate, MCnest does not automatically calculate the percent reduction in seasonal productivity due to a pesticide exposure relative to a no-pesticide

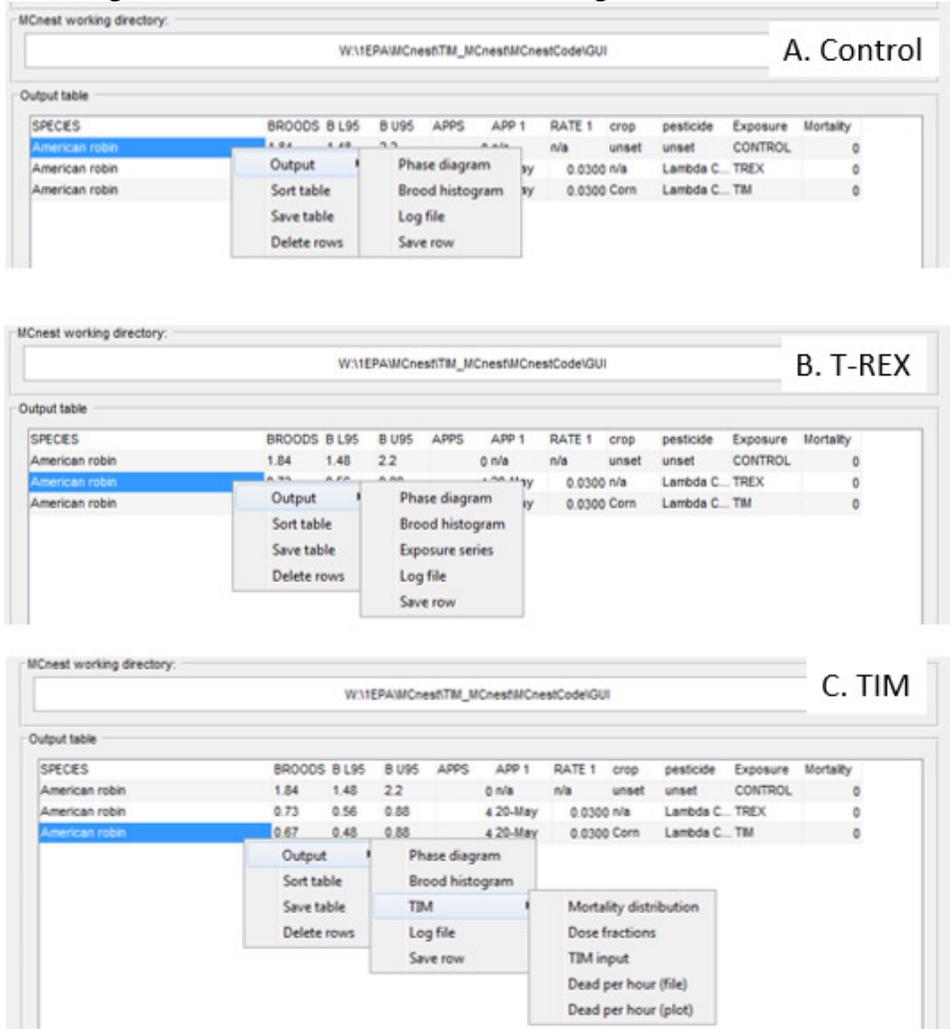
scenario. However, the contents of the Output table can be copied into a spreadsheet, such as Excel, to calculate comparisons among simulations using the standard windows commands (Ctrl-C and Ctrl-V).

Additional output functions can be accessed by selecting any cell in the desired output row and then right-clicking the mouse within the outlined results block. Most of the output is accessed under the **Output** submenu; the remaining menu items are for sorting, saving or deleting the output table. The examples below make use of the three simulations run above in Section II (Quick Start Guide). Throughout this section on model outputs it is assumed that you have three similar results available.

B. The Output menu

After selecting the first row, right-click and place the cursor on **Output** in the results menu to get access to a sub-menu with 4 choices (Fig. 3.1). When T-REX is run, this menu has 5 choices, with the new third menu option being **Exposure series**, whereas when TIM is run, the new third menu option is **TIM** (which has, in turn five sub-choices). Below, each submenu choice is described in turn, with the exception of the TIM output options, which are described in the [TIM User's Guide](#).

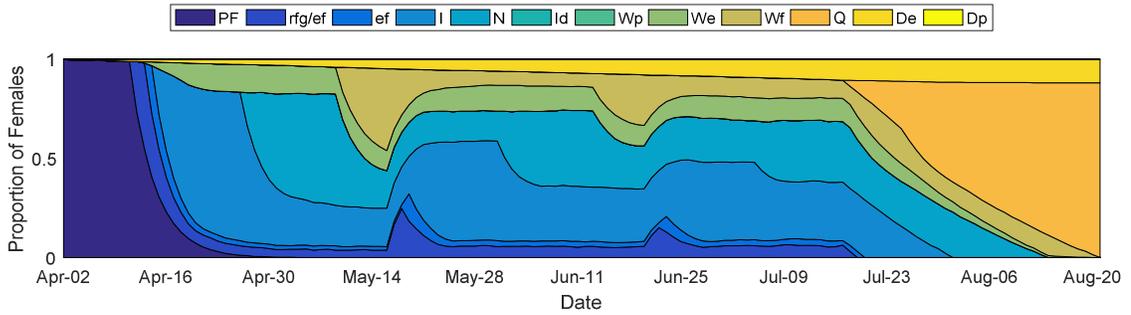
Figure 3.1. The **Output** submenu of the context menu for a. Control simulation, b. a simulation run using T-REX, and c. a simulation run using TIM.



1. PHASE DIAGRAM

Clicking **Phase diagram** under the Output menu brings up an area plot of the proportion of females (vertical axis) in different developmental stages (colors) as the breeding season progresses (horizontal axis) (Figure 3.2).

Figure 3.2. Phase diagram for American robin with no pesticide exposure.



The phase diagram is the primary diagnostic tool for understanding how the simulated chemical exposure was translated by the model into projected reproductive effects. Because there is no chemical exposure in the example simulation, there are no corresponding diagnostic effects in the phase diagram. Nevertheless the diagram is useful for tracing the general progress of females unexposed to pesticides through various breeding phases over the course of the simulated season. Table 3 provides a more detailed explanation of the phases in the phase diagram. Each of the phases is defined in greater detail in the technical support document.

Notice that three phases in Table 3.1 do not occur in the phase diagram pertaining to the first simulation above (Fig. 3.2). These are the doomed incubation period (Id: greenish blue), the waiting period after pesticide-induced failure (Wp: green), and the phase corresponding to killed by pesticide (Dp: yellow). These three states can only be entered into after exposure to a pesticide exceeding the corresponding threshold. However, highlighting the second row in the Output table and opening the phase diagram will produce a figure (Fig. 3.3) that shows a portion of females in the waiting period after pesticide induced failure (Wp).

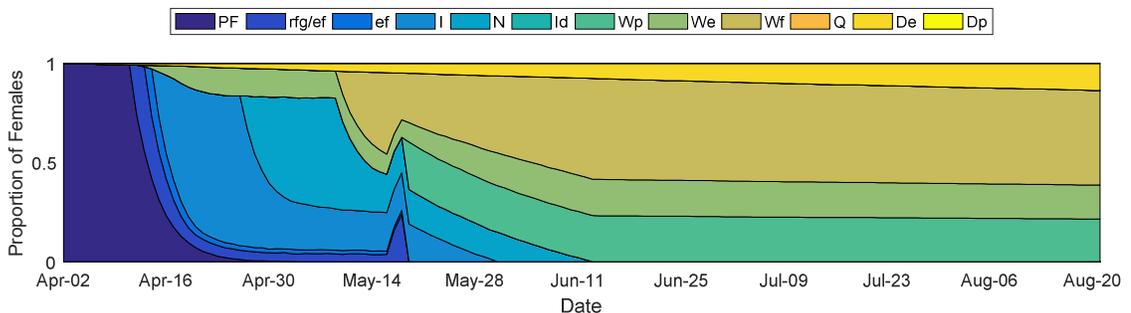
Table 3.1. Nest development phases in MCnest

| Phase | Legend abbreviation | Legend color (approx.) |
|--|---------------------|------------------------|
| Pair Formation | PF | Dark Purple |
| Rapid Follicle Growth | rfg | Blue |
| Overlap of Rapid Follicle Growth and Egg Formation | rfg/ef | Light Blue |
| Egg Formation | ef | Medium Blue |
| Incubation | I | Light Cyan |
| Nestling | N | Teal |
| Doomed Incubation | Id | Light Green |
| Waiting after pesticide-induced failure | Wp | Light Green |
| Waiting after ecological failure | We | Light Green |
| Waiting after successful fledging | Wf | Light Green |
| Quit | Q | Orange |
| Adult female dead due to background causes | De | Yellow |
| Adult female dead due to pesticide exposure | Dp | Yellow |

Hint: you do not need to close the phase diagram for a previous simulation before generating one for the next. Thus for example, the two phase diagrams could be open concurrently for direct comparison.

Further guidance on the interpretation of phase diagrams can be found in Appendix A of Bennett & Etterson 2013c.

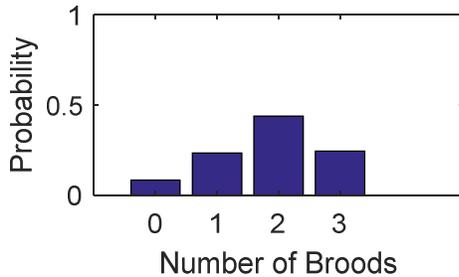
Figure 3.3. Phase diagram for an American Robin simulation with λ -Cyhalothrin using T-REX



2. BROOD HISTOGRAM

This sub-menu item generates a histogram of the number of successful broods produced per female in the simulation (Fig. 3.4). It is useful for gaining an intuitive sense for the expected variance in female reproductive success associated with a simulation scenario.

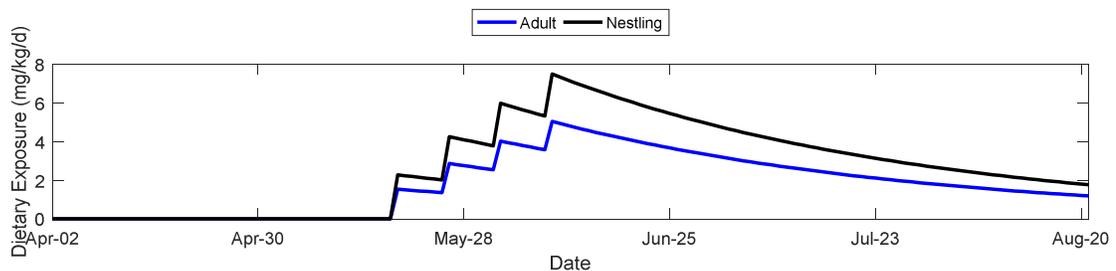
Figure 3.4. Sample brood histogram for default American robins with no pesticides.



3. EXPOSURE SERIES

Figure 3.5 shows the simulated Seasonal Exposure Profile for the second American robin simulation, in which the threshold (i.e., 0.94 mg/kg/d, per the Test Results input screen; Fig. 2.4) for number of eggs laid was exceeded immediately on application date and for the remainder of the breeding season. Note the separate exposure series for adults versus juveniles. This menu choice is not available with control simulations (no pesticide). When TIM is used, this menu choice is replaced by the standard TIM outputs (see below and USEPA 2015).

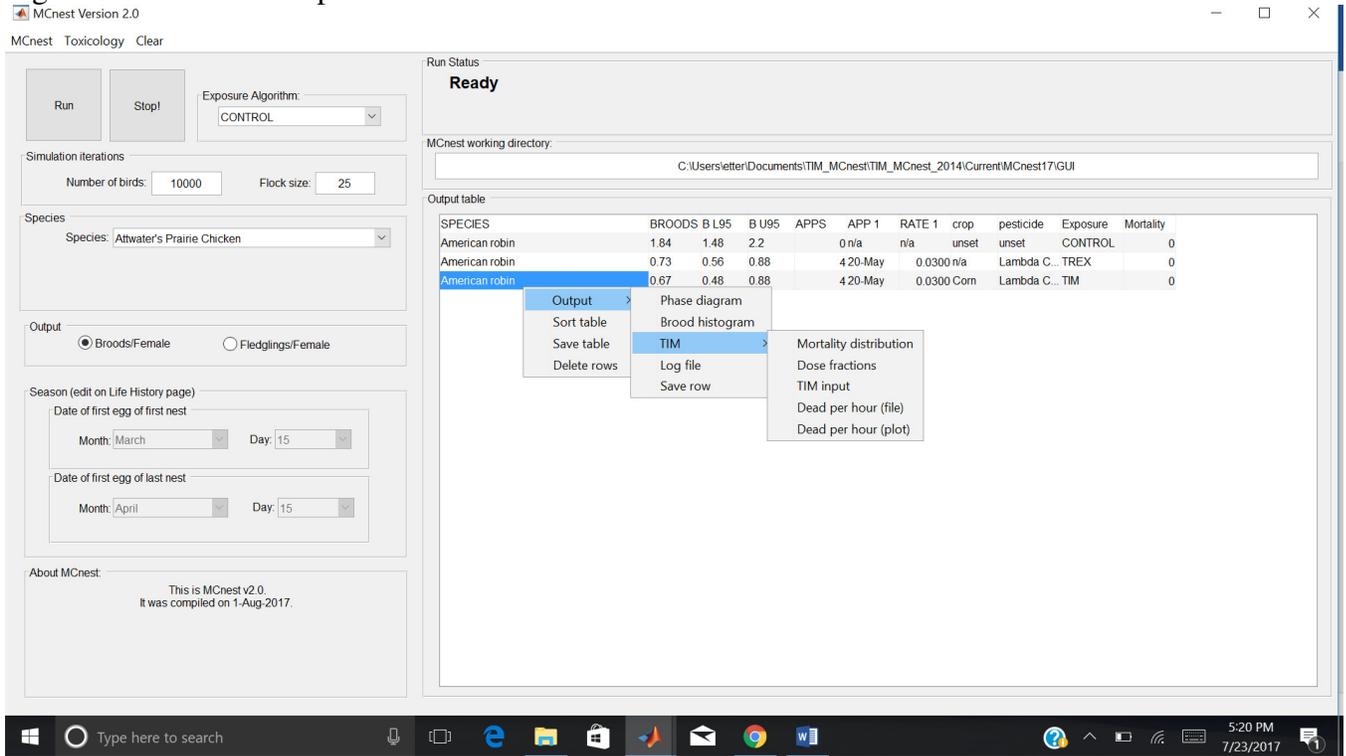
Figure 3.5. Example Seasonal Exposure Profile for American robins with the application dates and rates set as described in the pesticide examples of Section II (Quick Start Guide).



4. TIM

When TIM is used as the exposure algorithm, the **Exposure series** choice is replaced by TIM, which has 5 submenu choices (Fig. 3.6). These outputs are described in the TIM technical document, Appendix A, (<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#terrestrial>).

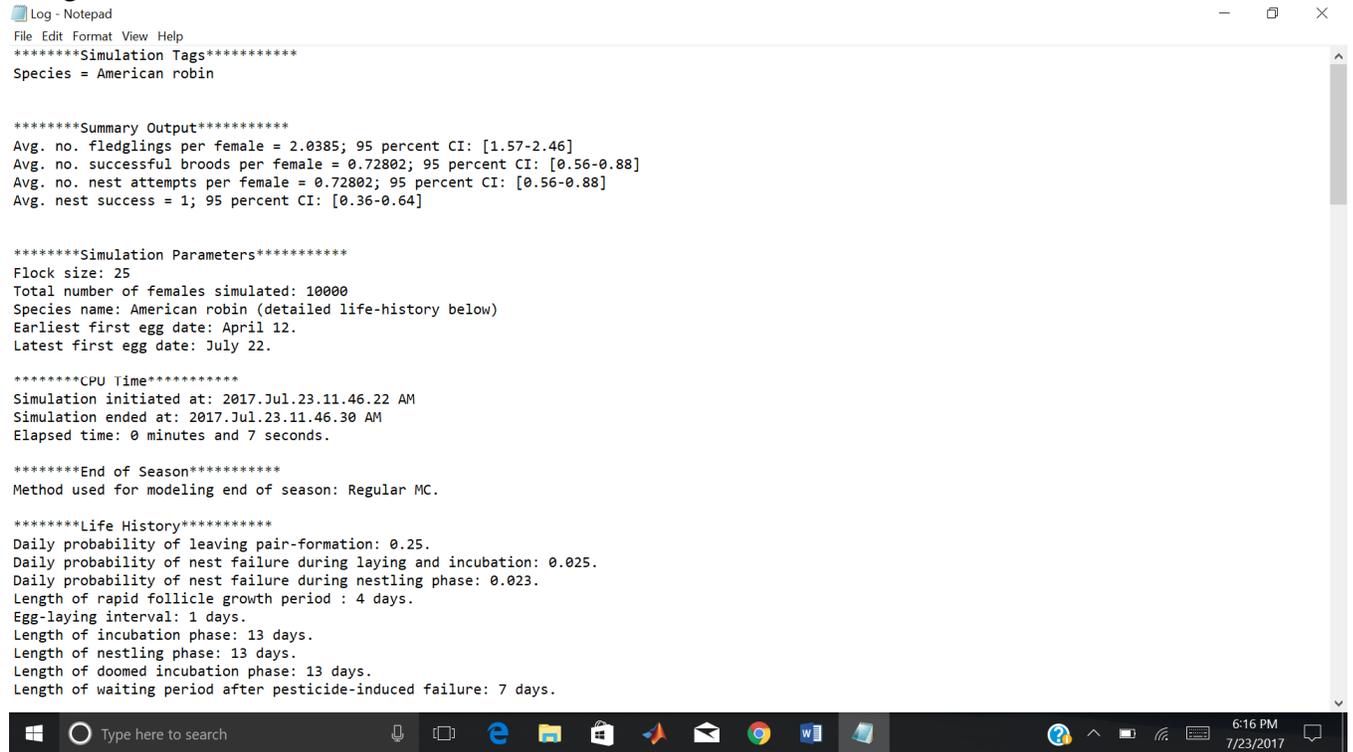
Figure 3.6. The TIM output menu



5. LOG FILE

This sub-menu choice opens a text file (using the computer's default text editor) and prints the main results and all of the input parameters used in the simulation (Fig. 3.7). This file is virtual (it can be regenerated at any time, but it does not exist until generated). Therefore, if it is needed for documentation, it should be explicitly generated and saved or printed.

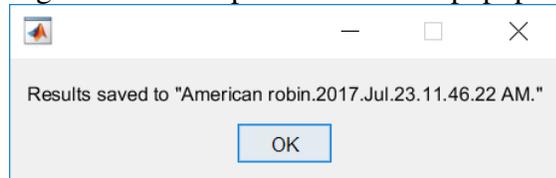
Figure 3.7. Sample log file for American robin simulation with λ -Cyhalothrin exposure and using T-REX



6. SAVE ROW

This sub-menu choice saves the simulation from the selected row to an output file that can later be opened in MCnest. The file is assigned a name by the program corresponding to the species name and the date and time that the simulation was run. A confirmation pop-up window is displayed (Fig. 3.8). Files are saved into the directory specified in the “MCnest working directory” listed above the “Output table.”

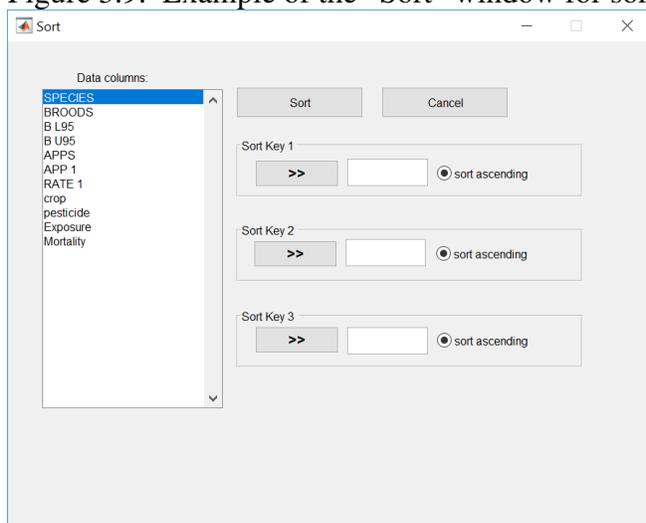
Figure 3.8. Example filename and popup window when a single row is saved to an output file.



C. Sort table

This menu choice provides functions to sort the displayed Output table using any of the table headers as sort keys (Fig. 3.9).

Figure 3.9. Example of the “Sort” window for sorting simulations in the Output table.



D. Save table

This sub-menu choice allows the entire output table to be saved to disk. For this option you are prompted to provide a name for the table. You are responsible for changing the default name of “*.mat” to a unique name with a “.mat” extension. It can later be reopened in MCnest, at which point it would be appended to the bottom of any existing simulations in the Output table.

E. Delete rows

This sub-menu choice deletes the selected row(s) from the table. Multiple rows can be selected and all selected rows will be deleted. They cannot be recovered.

IV. Systematic description of MCnest controls

The descriptions below contain minimal background and justification for the design of MCnest. For more details about the algorithms underlying MCnest and the technical justification for programming choices, please see the MCnest Technical Manual and scientific publications. MCnest has three basic categories of user controls, drop-down menus, window controls, and the table context menus. Not all windows contain all types of controls. Most MCnest windows include various controls that will be familiar to users of windows driven programs, including push buttons, radio buttons, check boxes, list boxes, text boxes, menus, and context menus. The presentation below is organized by window, beginning with the main MCnest window.

A. MCnest window

MCnest opens to its main window upon launching and most of the controls are accessed through this window. The main menu bar contains three choices (**MCnest**, **Toxicology**, and **Clear**). The MCnest window also contains several user-input controls.

1. USER-INPUT CONTROLS

Run: The Run button initiates a simulation.

Stop! The Stop button terminates a simulation currently in progress.

Simulation Iterations: This form contains two user-input boxes labeled “Number of birds” and “Flock size,” respectively. These two inputs determine the scale of the simulation and degree of replication for output statistics. The default values are 10,000 birds with a flock size of 25, resulting in 400 replicates of 25 birds. This replication is what determines the width of the confidence limits around model outputs (broods per female, offspring fledged per female). Differences among outcomes for replicate females arises only from stochasticity induced by the probabilities of nest survival and female survival.

Species: The species block contains a listbox menu of species names for which life-history profiles have been pre-coded for use in MCnest. For additional information about the species and their profiles, see the companion document on Avian Life History Profiles for Use in the Markov Chain Nest Productivity Model (Bennett & Etterson 2013a). The default is the first species in the list, i.e., Canada Goose. Specific details about the life history of species in the library, and functions to edit life-history parameters to create new species are found on the Life History window, which can be accessed under the main MCnest menu and is described in Section IV.B (Life History window) below.

Note: all life-history parameters are fixed. Experimental work is under way to explore systematic and stochastic variation in life history parameters, which may be included in future versions of the model.

Output: Once simulations have been run, this box allows the user to toggle between the number of successful broods/female/year and the number of fledglings/female/year in the Output table. Results for both metrics are captured in the Log file.

Season (edit on Life History page): this is simply a display of the breeding phenology of the species chosen in the **Species** listbox. It can be edited on the Life History window, which can be accessed under the MCnest menu.

About MCnest: status box giving the version and date of compilation of the model. This control is for display only.

Run Status: status box showing the state of the model and progression through the simulation when a simulation is running.

MCnest working directory: status box showing the current working directory for MCnest. This is the directory into which MCnest will save results or initially look for files when you attempt to load data (you can always navigate to another directory). Clicking in the text box and hitting return will bring up a browser to change the working directory.

Output table: This frame contains the outputs of individual runs of MCnest. When no previous runs are available it is empty.

In Figure 4.1, the Output table shows the results for the three simulations run in Section II with the first row highlighted. The first column gives the species name (American robin). The second column gives the global average (across all 400 flocks) of the number of successful broods produced per female (1.84). The third and fourth columns give the lower and upper confidence limit for a 95% confidence interval (1.48 – 2.2) constructed around the global average number of successful broods per female using the 400 replicates of 25 birds.

Figure 4.1. Main MCnest window showing output of two simulations from Section II (Quick Start Guide).

Run Status
Ready

MCnest working directory:
C:\Users\letter\Documents\TIM_MCnest\TIM_MCnest_2014\Current\MCnest17\GUI

Output table

| SPECIES | BROODS | B L95 | B U95 | APPS | APP 1 | RATE 1 | crop | pesticide | Exposure | Mortality |
|----------------|--------|-------|-------|------|----------|--------|-------|-------------|----------|-----------|
| American robin | 1.84 | 1.48 | 2.2 | 0 | n/a | n/a | unset | unset | CONTROL | 0 |
| American robin | 0.73 | 0.56 | 0.88 | | 4 20-May | 0.0300 | n/a | Lambda C... | TREX | 0 |
| American robin | 0.67 | 0.48 | 0.88 | | 4 20-May | 0.0300 | Corn | Lambda C... | TIM | 0 |

About MCnest:
This is MCnest v2.0.
It was compiled on 1-Aug-2017.

2. THE MCNEST MENU

Of the three drop-down menus on the main MCnest window, the first two (**MCnest** and **Toxicology**) are the most important, giving access to virtually all of the functionality of MCnest. The MCnest menu contains 7 choices (Table 4.1).

Table 4.1. Menu choices under the main MCnest menu.

| Menu Item | Function |
|--------------------------------|---|
| Load Species Library | Loads a different species library into memory for use in simulations |
| Life History | Loads the life history window for editing species life history values |
| Batch Mode | Allows you to set up multiple simulations to run sequentially along systematic changes in species identity, application date, and/or application rate |
| Random numbers | Allows you to set the random seed and choose among random number generators |
| Load MCnest File | Loads previously saved MCnest results into memory for examination |
| Import time varying parameters | Allows you to explore the importance of systematic changes in the value of a parameter over the course of a breeding season |
| Exit | Closes the model |

Load Species Library: Although MCnest opens up to the default species profile library, the menu option allows you to select other species profile libraries, if they exist. Custom species libraries may be useful for 1) tailoring species profiles to a geographic region of interest; 2) exploring known variation in model parameters associated with different habitat types, climates, or background stressors; or 3) sensitivity analysis. Custom species libraries must use the same structure as the default library provided with MCnest. Further details on the species library and structure can be found in (Bennett & Etersson 2013a).

Life History: Choosing this menu option loads the Life History window. Controls for the Life History page are described in Sub-section B (Life History window), below.

Batch Mode: Choosing this menu option loads the Batch mode page. Controls for the Batch mode page are described in Sub-section D (Batch window), below.

Random Numbers: Choosing this menu option loads the Random Numbers window. Controls for the Random Numbers page are described in Sub-section E (Random Numbers window), below.

Load MCnest file: Choosing this menu option loads the results of previous simulations into the output table on the main MCnest window. Previous results will be appended to the end of any currently displayed results.

Import time-varying parameters: Choosing this option loads the time-varying parameters page. NOTE – this feature is still under construction. It currently has limited features available. See section E.3. below.

Exit: Choosing this option exits MCnest. MCnest can also be closed by clicking the upper right hand corner of the main MCnest window.

3. THE TOXICOLOGY MENU

The toxicology menu has two choices, **Test Results** and **Exposure**, which in turn has two choices, **TREX** and **TIM**. Choosing **Test Results** brings up the **Test Results** window, which is covered in detail below in section IV.C. Choosing **Exposure > TREX** brings up the **TREX** page, which is covered in detail below in section IV.D. Finally, choosing **Exposure > TIM** brings up the **TIM** window, which is covered in detail below in the [TIM technical documentation](#).

4. THE CLEAR MENU

The Clear menu is used to remove existing results. It has only three choices.

Summary Table: Choosing this menu option clears the summary table from memory and from display. It cannot be recovered unless the data were first saved to a file.

Figures: Choosing this menu option closes all open figures. They can be regenerated by repeating the appropriate commands.

All: Choosing this menu option clears the summary table and closes all open figures. Neither the table nor figures can be recovered unless the data were first saved to file.

B. The Life History window

The life history window is invoked by selecting the **Life History** option under the **MCnest** menu.

The Life History window has five control frames and a button for returning to the main MCnest window (Fig. 4.2). The species library contains default parameters for all of the controls on the Life History page. Thus, if you are using default species you need not use this window. If you invoke this window with a default species selected, it will display the default parameters for that species. This can be useful at runtime to see what those values are. However, changing any parameter on this page will automatically change the default species to “-new-.” It will also make visible a new box on the main MCnest window for naming the new species. The default name for the new species is “Bird.” Mandatory renaming will be removed in a future version of the model.

Figure 4.2. The Life History window with default data for American robin.

MCnest Version 2.0: LifeHistory

Season

Date of first egg of first nest
Month: April Day: 12

Date of first egg of last nest
Month: July Day: 22

Return to Main Screen

Transition Probabilities

Initiation prob: 0.25
Nest failure (pre-hatch): 0.025
Nest failure (post-hatch): 0.023
Adult survival: 0.999

Phase Durations (days)

Rapid Follicle Growth: 4
Egg-laying Interval: 1
Incubation Period: 13
Nesting Period: 13
Waiting period after failure: 7
Waiting period after fledging: 7

Other Life History

Clutch Size: 3

Incubation Onset
 Penultimate Egg
 Last Egg

Fledglings per successful nest: 2.8

Diet & body weight (exposure)

Body Weight (g): 77.3

| | Adults | Nestlings |
|--------------------------|--------|-----------|
| Short grass: | 0 | 0 |
| Tall grass: | 0 | 0 |
| Broadleaf forage plants: | 0 | 0 |
| Fruit: | 0.28 | 0.3 |
| Seeds: | 0 | 0 |
| Invertebrates: | 0.72 | 0.7 |
| Sums (must be 1): | 1 | 1 |

Season: This frame allows you to change the length of the breeding season, which is defined in MCnest by two dates: the date of the first egg in the first and last nests for the season of each species. Implicit in this definition is the ability of females to complete any nesting attempts initiated by this later date, assuming the nest is not lost due to ecological or pesticide-induced failure.

The end date (date of first egg in last nest) must be at least two weeks later than the beginning date (date of first egg in first nest). If you try to set a breeding season shorter than two weeks, you will get an error and the end date will be automatically set to two weeks after the beginning date.

Phase Durations (days): This frame contains eight text boxes for entering the phase durations. These durations determine the time required to complete various processes in the nesting cycle and are described in greater detail in the MCnest Technical Manual. With the exception of the rapid follicle growth period and the egg-laying interval, these parameters must be specified as integers.

Transition Probabilities: This frame contains three text boxes for model parameters. The first, p , is the daily probability that a female in the pair formation stage initiates rapid follicle growth for her first egg of her first nest of the season. This parameter applies only to a female's first nest attempt of the year. The second two parameters are the daily probabilities of background nest failure in egg-laying and incubation (pre-hatch) and nestling rearing (post-hatch) periods of the nest cycle. These failure rates should include all causes of failure except for failures arising from the pesticide algorithms in MCnest. Pesticide failures are handled separately, as described below in Sub-section C and in the MCnest Technical Manual (Etterson & Bennett 2017). The final

variable in this frame is the daily background rate of adult female survival (again excluding pesticide mortality). All parameters in this frame are restricted to the interval (0,1).

Other Life History: This frame contains two text boxes and two radio buttons. The first text box is for average clutch size, the average number of eggs laid in a nest. It must be an integer. The two radio buttons indicate whether the species generally begins incubation with the penultimate versus the final egg. Whichever button is selected; all females are assumed to follow the same pattern. The second text box is for an estimate of the mean number of fledglings per successful brood. It does not need to be an integer. Clutch size should serve as an upper bound to this parameter.

Diet & body weight (exposure): You do not need to use this frame unless you are simulating pesticide effects. Dietary information is only used to estimate dose, based on residue analyses of different food types. Body weight must be provided in grams. Dietary proportions must be between 0 and 1 for all categories and they must sum to 1 (down columns). Specifying proportions outside the range (0,1) will generate an immediate warning and that category will be set to $1/6 \approx 0.17$. However, specifying sums of dietary categories that do not sum to 1 will not generate errors until you attempt to click **Return to Main Screen**.

C. The Test Results window

The **Test Results** window (Fig. 4.3) is invoked by selecting the **Test Results** option under the **Toxicology** menu. The **Test Results** window has several large frames pertaining to three different toxicity tests, the Acute Oral Toxicity test (USEPA 2012a), the Dietary Toxicity Test (USEPA 2012b), and the Avian Reproduction Test (USEPA 2012c) as well as a frame for surrogate endpoints calculated from these tests results. There are also input boxes at the top of the window for typing the name of the pesticide and its degradation half-life on dietary items. The USEPA/OPP default half-life of 35 days will automatically be displayed when opening the window, though an empirical value may be substituted, if known. Finally, the row of four buttons at the top of the window provide additional functionality. In particular, the **Load data** and **Save data** functions allow you to load test result data from a previously saved file or to save the data (once the window is completely filled out) for easy use in the future.

Figure 4.3. The Test Results window

The screenshot shows the 'MCnest Version 2.0: Test Results' window. At the top, there are buttons for 'Done', 'Clear form', 'Load data', and 'Save data'. A 'Half-life on dietary items' field is set to 35 days, and a 'Pesticide' field is set to 'unset'. The window is divided into several sections:

- Avian Reproduction Test:** Includes fields for 'Avian reproduction test source (MRID)' and 'Test species'. Below is a 'Treatment group data' table with 5 columns (1-5) and rows for 'Measured concentration (mg/kg diet)', 'Average food consumption (g/bird/day)', 'Average initial female body weight (g)', 'Average initial male body weight (g)', 'Average final female body weight (g)', 'Average final male body weight (g)', and 'Estimated average daily dose (mg/kg bwt/day)'. All cells in this table contain 'NaN'.
- Avian Acute Oral Toxicity Test:** Includes fields for 'LD50 test source (MRID)', 'Test species', 'Mineau scaling factor' (1.15), 'Body weight' (NaN), 'LD50 (mg/kg bwt)' (NaN), and 'Scaled LD50' (NaN).
- Avian Dietary Toxicity Test:** Includes fields for 'LC50 source (MRID)', 'Test species', 'LC50' (NaN), 'Fraction of LC50 (i.e., 0.1 or ...)' (NaN), 'Mean body weight (g)' (NaN), 'Mean food ingestion rate (g/d)' (NaN), and 'Fraction of LC50 as dose (mg/kg bwt)' (NaN).
- Test NOAELs:** A table with two columns for 'Endpoint NOAEL' and rows for various endpoints: 'Number of eggs laid', '% viable eggs of eggs set', '% live 3-wk embryos of viable eggs', '% hatchlings of live 3-wk embryos', '% hatchlings of eggs set', '% 14-d chicks of hatchlings', '% 14-d chicks of eggs set', 'shell thickness', 'hatch weight', '14-d chick wt.', 'prelaying female weight', and 'prelaying male weight'. All dropdown menus are set to '>'.
- MCnest Toxicity Thresholds (mg/kg/d):** A table with columns for 'Alternative behavioral threshold', '1/10', and '9999'. Rows include 'Adult prelaying body weight', 'Eggs laid per hen', 'Mean eggshell thickness per pen', 'Viable eggs set per pen', 'Hatchlings per viable egg per pen', '14-d chicks per hatchling per pen', and 'Fraction of juvenile 5-d LC50'. All values are 9999.

1. AVIAN REPRODUCTION TEST

The Avian Reproduction Test Frame occupies the full left-hand side of the **Test Results** window. Within the frame, there are two smaller frames, the first for entering treatment-group data, and the second for entering the NOAELs from the analyzed test results reported in the USEPA/OPP Data Evaluation Record (DER). There are also two text boxes at the top of the frame for entering USEPA/OPP Master Record Identification number (MRID) as well as the species tested.

In the treatment-group data panel, there are seven rows of input boxes for entering treatment-level data (except control). They should be self-explanatory and are typically reported in USEPA/OPP DERs. The first row is for measured concentrations in the feed. The second row is for entering average food consumption of tested birds, by treatment. The third and fourth rows are for entering the average initial and weights of males and females, and the fifth and sixth rows are for entering the average final weights of males and females respectively. The food consumption and body weight data are used to convert the measured concentrations (mg/kg diet, row 1) to doses (mg AI/kg body weight/day) displayed in row 7, which cannot be directly edited. The equation used for calculating daily dose is:

$$\text{Daily dose} = \frac{\text{Dietary concentration (mg AI/kg diet)} \times \text{food ingestion rate (g food/day)}}{\text{Body weight (g)}}$$

The conversion of dietary concentrations to daily doses is an approximation because body weight and food ingestion rates are changing during the course of both the reproduction test and the LC50 test. Also, studies vary in the degree to which they quantify food spillage during the tests. For converting dietary concentrations to daily doses, the model user can calculate the average daily food ingestion rate per bird and the average body weight at the beginning and end of the test period for each bird. The model user should consult the MCnest Technical Manual for more background information about the selection of toxicity threshold values prior to use.

In the Test NOAELs panel, there are 12 drop-down boxes for test endpoints from the Avian Reproduction Test. However, only eight of these are currently used by the model and the remaining four (%hatchlings of eggs set, %14-d chicks of eggs set, hatch wt, and chick wt) can be ignored. They may be used by a future version of the model. Each of the remaining eight NOAELs should be set to the highest level (dietary concentration, row 1 in treatment-group panel) at which that endpoint did not differ from the control. If no statistical difference from control was observed in any of the tested dietary concentrations, for a given endpoint, the drop-down box can be set to '>' to indicate that it is an unbounded NOAEL.

2. AVIAN ACUTE ORAL TOXICITY TEST

The acute oral toxicity test frame occupies the upper right of the **Test Results** window. It has six input boxes, only five of which are editable. The first two are for the tested species and referencing the source of the test (MRID). The next two boxes are for rescaling the LD50 to account for differences in body weight between the tested species and the simulated species (Mineau et al. 1996). The default value of 1.15 will automatically be displayed when opening the window. If no scaling is desired, then change this value to 1. Finally, the last editable input box is for entering the estimated LD50 from the test, which is then converted internally to the scaled LD50.

3. AVIAN DIETARY TOXICITY TEST

The dietary toxicity test frame occupies the middle right of the **Test Results** window. It has seven input boxes, only six of which are editable. The first two are for the tested species and referencing the source of the test (MRID). The next box is for entering the estimated LC50 from the test, followed by the fraction of the LC50 desired for use as a toxicity threshold (default is 0.5, Bennett and Etterson 2013b). The last two are mean body weight and food consumption rates of tested animals for converting from dietary concentration (mg/kg diet/day) to dose (mg/kg body weight/day).

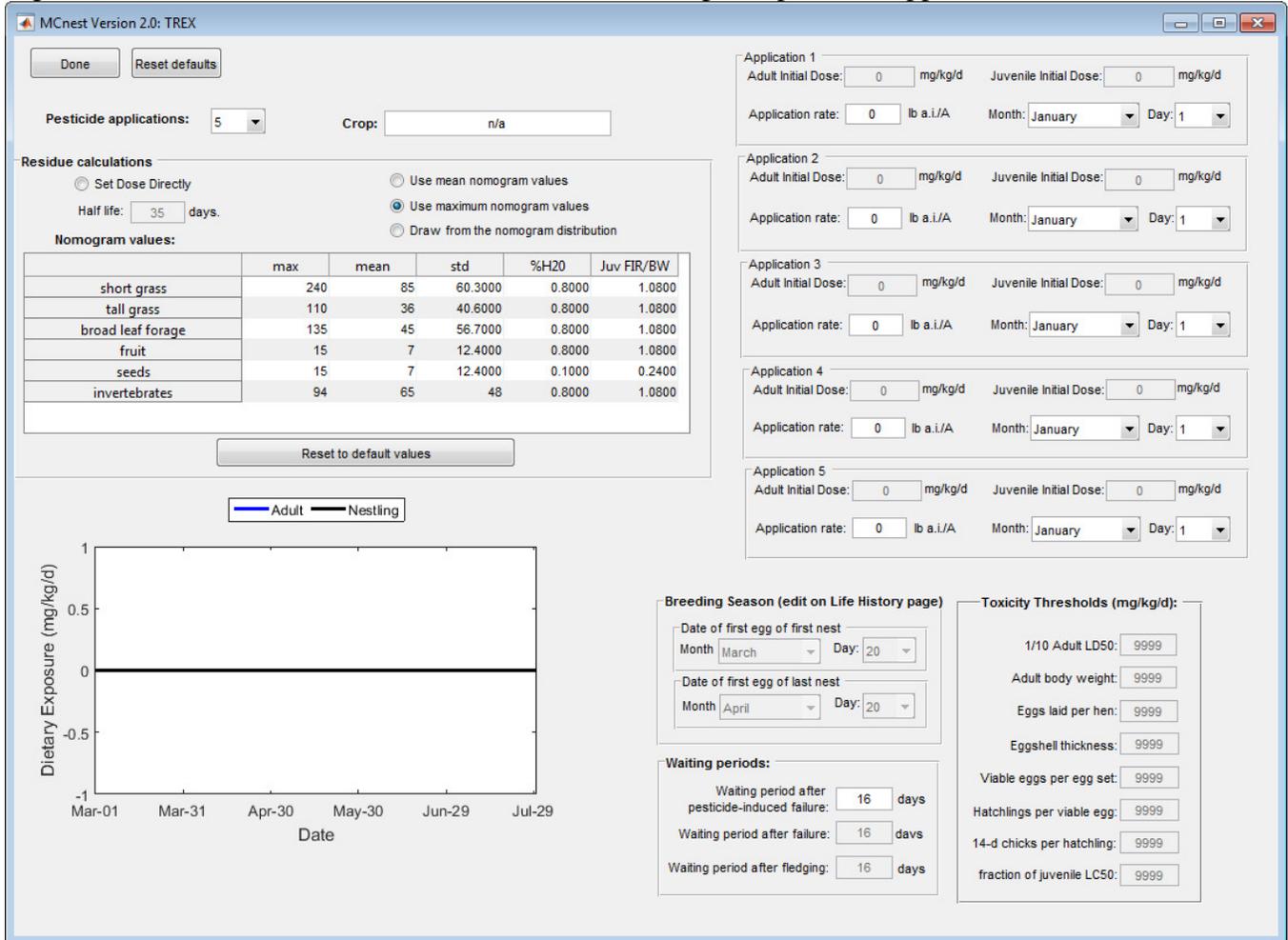
4. TOXICITY THRESHOLDS

This control frame contains nine text boxes for the toxicity thresholds (values which, if exceeded by exposure, will trigger nest failure or prevent a bird from initiating a new nest). These values are calculated from the data entered in the three test results panes and are expressed as daily dose (mg AI/kg body wt/day). The calculated surrogate endpoints are not directly editable, except for an auxiliary input box for an **alternative behavioral threshold**, which can be used if there were behavioral effects noted at one or more tested dietary concentrations or doses of one of the three

tests. If used, this alternative threshold applies only to those phases for which the default surrogate endpoint for behavior (1/10 LD50) is used (Bennett & Ettoreson 2006, Bennett & Ettoreson 2013b,d). In practice, the model uses the lower of the two values as the surrogate endpoint for behavior.

D. The TREX window

Figure 4.4. The default Pesticides window when selecting five pesticide applications.



Pesticide applications: This popup menu determines the number of pesticide applications that will be used. By default, the window opens with 0 applications.

Crop: This input box is for inputting the name of the modeled crop scenario, for future reference. It's value is reported in the log file.

Residue calculations: The controls in this frame include four radio buttons and a text box. The first text box is for setting the half-life of pesticide residue degradation of food items (expressed in days). The default is 35 days, but the value can be set to any positive value. The radio buttons

are mutually exclusive (only one can be checked) and indicate how pesticide application rates are translated into exposure doses. The first choice “Set Dose Directly” allows the user to manually set the adult and juvenile initial dietary dose of the pesticide on application day. The final three choices translate the application rate into estimates of adult and juvenile initial dietary doses at application using the approach in OPP’s Terrestrial Residue EXposure model or T-REX (USEPA 2012). The T-REX approach is based on the estimated mean or maximum residue concentration on each of the six food type categories immediately following an application of 1 pound active ingredient (AI) per acre. The user can choose **Use maximum nomogram values**, **Use mean nomogram values**, or **Draw from the nomogram distribution**, using a log-normal distribution based on the mean and standard deviation. Residue concentrations on food types are translated into dose using body weight and dietary information on the Life History window. Additional detail is provided about these calculations in the MCnest Technical Manual. Whichever method is used to determine the initial dietary dose on the day of application, subsequent exposure will decay according to the specified half-life. When two or more applications are simulated, it should be noted that the initial dietary dose presented for each application represents only the contribution from that application, and does not add the contribution from earlier applications. The additivity of overlapping decay curves from multiple applications can be observed in the Seasonal Exposure Profile figure, even though this is not reflected in the adult and juvenile initial doses shown for each application.

The **nomogram values** table is also editable. This table lists the default maximum, mean, and standard deviation nomogram concentrations on each of the six food type categories used in T-REX Version 1.5. It also includes the default percent moisture value from T-REX and the juvenile food ingestion rate per body weight (i.e., FIR/BW) used in the calculation of juvenile doses, as described in the MCnest Technical Manual. All of these values are editable by the user. If changes are made to any values within the **nomogram values** table, there is a button to restore all default values.

NOTE. There is a minor bug in the program that prevents the exposure series from being calculated and displayed when application rates are set to 0 and the radio button for **Draw from the nomogram distribution is chosen**. This will be fixed in a future release. Current workaround is to set desired application rates to non-zero values before choosing the radio button for **Draw from the nomogram distribution is chosen**. 28 July 2017.

Seasonal Exposure Profile (graph): As information on application rate, application date, and residue half-life are modified in the Pesticides window, a Seasonal Exposure Profile will be generated automatically. The model user can use this to visualize the exposure profile during the breeding season of a species and/or to examine the degree of residue carry-over from one application into the next. After a simulation, the Seasonal Exposure Profile also is available by right-clicking on a highlighted simulation in the Output table and under “Row,” click “Exposure series.”

Application 1: All of the pesticide application frames operate identically to Application 1. These frames each contain three text boxes and two list boxes. The top two text boxes are for the adult and juvenile initial dose (i.e., the dose on application day), respectively. These boxes are not editable when any of the final three radio buttons in the Residue calculations frame are selected. In this case, initial doses are calculated using the application rate and the dietary

information and body weight information for the species selected on the main MCnest page, as described above and in more detail in the MCnest Technical Manual. The Application rate text box can be set to any non-negative value. It will not be visible if the “Set Dose Directly” radio button is selected in the Residue calculations frame, above. Finally, the two listboxes allow the user to specify the date on which the pesticide is applied to the environment for each application (i.e., the day on which the initial doses will be experienced).

The Application frames operate independently from each other, though they all use the same half-life for defining the decay curve. Applications can be specified in any order (e.g., Application 2 could be specified as occurring prior to Application 1), and they need not occur within the window defined by the Breeding Season (i.e., an application may occur prior to or after the breeding season of some species). Regardless of how many pesticide applications are chosen on the Pesticides window, the adult and juvenile initial doses shown for each application are calculated based only on information for that application. However, when more than one application is specified, the decay curves for each application may overlap. Consequently, the estimated initial doses at application for birds in the simulation may reflect the combination of the pesticide residues produced by each application plus any carry-over residues from previous applications. The additivity of overlapping decay curves from multiple applications can be observed in the Seasonal Exposure Profile figure, even though this is not reflected in the adult and juvenile initial doses shown for each application.

Breeding Season: The controls in this frame are redundant with the same controls on the Life History window. They are not operational on the Pesticides window, but are provided for reference when setting the dates of pesticide applications.

Waiting Periods: This frame lists the three waiting periods after nest success or failure before the first egg is laid in a subsequent nest. The two lower periods are redundant with waiting periods on the Life History profile for each species. The top period is the “Waiting period after pesticide-induced failure.” The default value for this waiting period is set equal to the “Waiting period after ecological failure” for each species. The waiting period after pesticide failure must be an integer and can be set to a longer duration than for ecological failure, but it cannot be shorter.

E. The TIM windows

TIM includes three windows for model input parameters (Figs 4.5-4.7). These are identical to those presented in the TIM User's Guide ([USEPA 2015: Appendix A](#)) and model guidance is the same. That document is the definitive technical document for TIM and can be found at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/tim-version-30-beta-technical-description-and-user-7>. The main TIM window (Fig. 4.5) contains a **File** menu with five choices (Table 4.3).

Table 4.3. Choices available on the File menu on the main TIM window

| Menu Choice | Function |
|----------------------|---|
| Pesticide Parameters | Loads the TIM Pesticide Parameters window (Fig. 4.6) |
| Species | Loads the TIM Species window (Fig. 4.7) |
| Save TIM Parameters | Loads a file browser for saving a *.mat file with the currently entered TIM parameters (parameters for all TIM windows will be saved) |
| Load TIM Parameters | Loads a file browser for loading a *.mat file with TIM parameters (parameters for all TIM windows will be loaded) |
| About | Displays a message with information about TIM and where to find further resources |

Figure 4.5. The Opening TIM window parameterized with the sample data provided for λ -cyhalothrin

The screenshot shows the TIM Version 3.0 software interface. The window title is "TIM Version 3.0". The main area is divided into several sections:

- Scenario Definition:**
 - Pesticide: Lambda Cyhalothrin
 - Crop: Corn
 - Species: Canada goose (giant)
 - Number of birds: 10000
 - Flock size: 25
 - Random seed: 12345678
- Exposure Routes:**
 - dietary
 - drinking water (puddles)
 - drinking water (dew)
 - inhalation (vapor)
 - inhalation (spray)
 - dermal (contact)
 - dermal (spray)
 - spray drift
- Method:** Air
- Droplet spectrum:** very fine to fine
- Crop Type:** field
- Applications:**
 - Pesticide Applications: 4
 - Fraction of edge habitat receiving spray drift: 1
 - Length of in-field buffer (feet): 0
 - Fraction of organic carbon in soil: 0.015
 - Soil bulk density (kg/L): 1.5
 - Hour of first application: 8 am
 - Spray height (m): 3
 - Spray duration (min): 1.5
 - Crop height (m): 0.152
 - Crop mass (kg/ha): 39

| Dates | lbs a.i./A | Month: | Day: | Interval: |
|----------------|------------|--------|------|-----------|
| Application 1: | 0.03 | May | 20 | |
| Application 2: | 0.03 | May | 27 | 7 |
| Application 3: | 0.03 | June | 3 | 7 |
| Application 4: | 0.03 | June | 10 | 7 |

Figure 4.6. The TIM Pesticide Parameters window

TIM Version 3.0: Pesticide Parameters

Close

Toxicity

Avian acute oral LD50: 50000

Mean body weight of tested animals: 1049

Mineau scaling factor: 1.15

Scaled LD50: 59940.99

Slope of avian oral LD50: 10

Avian acute inhalation LD50 (0 if not avail.): 0

Rat inhalation LD50: 229

Rat acute oral LD50 (mg a.i./kg-bw): 56

Respiratory physiology adjustment factor: 3.4

Avian dermal LD50 (0 if not avail.): 0

Food matrix adjustment factor: 1

Fraction of pesticide retained from on hour to next: 0.998

Ratio of juvenile to adult toxicity: 1

Fate Parameters

Pesticide half life (puddle): 76.2

Koc (L/kg-oc): 333200

Kow: 10000000

Henry's law constant (atm/m³-mol): 1.9e-07

Solubility in water (mg a.i./L): 0.005

Dislogable foliar residue adjustment factor: 0.62

Dermal absorpption fraction: 1

Diet:

| | Contaminated Fraction | Half life: |
|------------|-----------------------|------------|
| Insects: | 1 | 35 |
| Seeds: | 1 | 35 |
| Fruit: | 1 | 35 |
| Grass: | 1 | 35 |
| Broadleaf: | 1 | 35 |

Figure 4.7. The TIM Species window

The screenshot shows the 'TIM Version 3.0: Species' window. At the top left is a 'Done' button. To its right is a 'Choose species:' dropdown menu currently set to '0: Custom Species'. Below this are several input panels:

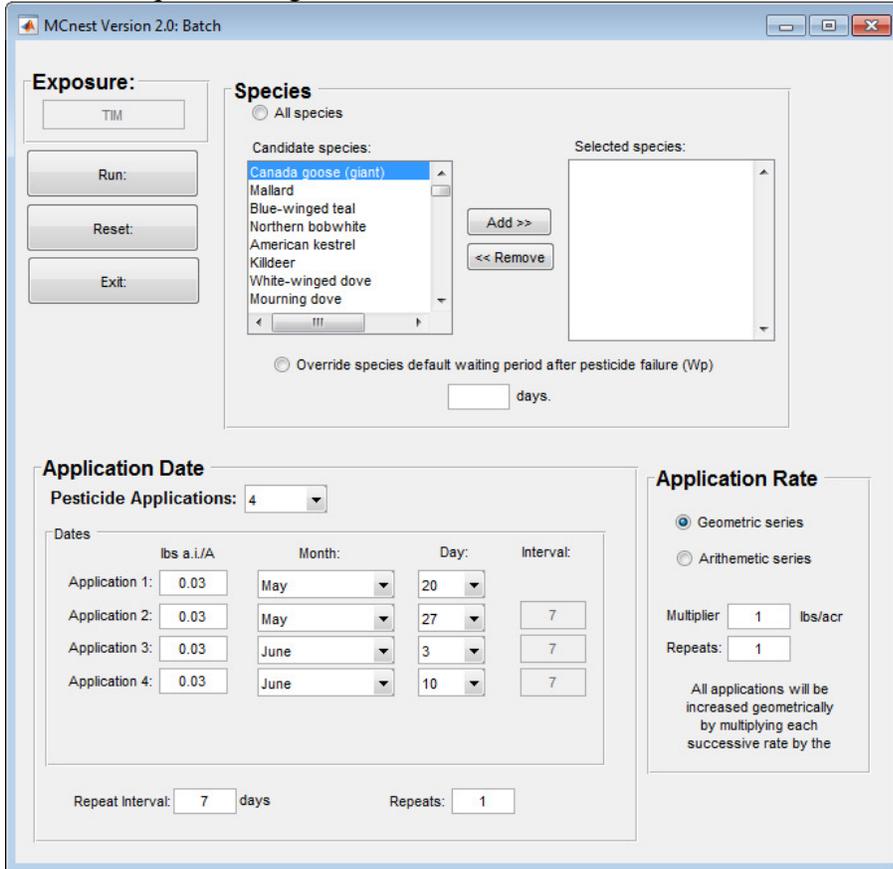
- Feeding times:** A grid of input boxes for AM and PM periods. AM: Start min: 4, Start max: 5, End min: 6, End max: 10, Prop min: 0.4, Prop max: 0.6. PM: Start min: 16, Start max: 19, End min: 20, End max: 21, Gorging factor: 1.
- Body weight:** Input boxes for mean (3514), stdev (256.522), min. (3062), and max. (3912).
- Field fidelity parameters:** Includes a 'Field resident' checkbox (unchecked), a 'Field fidelity factor' input (0.6), and a 'Frequency on' row with mean (1), min. (0), and max. (1) inputs.
- Species Type:** Includes checkboxes for 'Passerine' (unchecked) and 'Precocial' (checked), and a dropdown menu set to '2. Herbivore'.
- Dietary proportions (must sum to 1):** A table with columns for Insects, Seeds, Fruit, Grass, Broadleaf, and Sum.

| | Insects | Seeds | Fruit | Grass | Broadleaf | Sum |
|------------|---------|-------|-------|-------|-----------|-----|
| Adults: | 0 | 0 | 0 | 1 | 0 | 1 |
| Juveniles: | 0 | 0 | 0 | 1 | 0 | 1 |

F. The Batch window

The Batch window is invoked by selecting **Batch Mode** under the main MCnest menu. This window offers the ability to set up multiple simulations in advance according to three different types of progressions (Fig. 4.8). If the **Exposure Algorithm** drop-down menu is set to CONTROL, then the batch window will show only the top **Species** panel. If the Exposure Algorithm is set to either TIM or TREX, then the bottom panels for **Application Date** and **Application Rate** will be visible.

Figure 4.8. Batch window with starting conditions set to the exposure scenario described in Section II, quick start guide.



Exposure: This display panel notifies you which exposure model is chosen on the main MCnest window. It cannot be changed here. You must go back to the main MCnest window to change the exposure algorithm.

Run: This pushbutton starts the simulation through the batches you've set up.

Reset: This pushbutton removes any batches currently designed and returns the window to its opening state.

Exit: Returns to the main MCnest window without running the batches you've designed.

1. SPECIES

The **Species** frame contains two radio buttons, two listboxes, two push buttons, and an edit box. The **All species** radio button moves all species from the **Candidate species** listbox to the **Selected species** listbox. Deselecting the **All species** button will restore them to the **Candidate species** listbox. The **Add** and **Remove** buttons will move selected individual species back and forth between listboxes. When batching on species, separate simulations will be run on the species in the **Selected species** listbox and species in the **Candidate species** listbox will be

ignored. Selecting the radio button **Override species default waiting period after pesticide failure (Wp)** will allow you to set a unique value for the waiting period for pesticide failure in the associated edit box. This value will then apply to all species in the designed batch, unless the specified value is shorter than the species' value of **We** (the waiting period after ecological failure), in which case the species-specific value for **We** will be used for that species.

Note that if the “Override species default” radio button has been selected and you wish to return to using the default values for **Wp** for each of the selected species, the **Override species default...** radio button needs to be deselected and then hit **Exit**.

2. APPLICATION DATE

The **Application Date** frame contains three text boxes and additional text boxes arrayed in rows for up to 5 applications depending on the value of the **Pesticide Applications** popup menu. The input boxes within the **Dates** frame are parameterized exactly the same way they are on the **TREX** or **TIM** windows. At the bottom of the window, the two input boxes **Repeat interval** and **Repeats** determine the number of scenarios that will be run. Using these two pieces of information, MCnest simulates the application scenario described in the **Dates** frame. It then repeats the simulation, shifting all application dates by **Repeats** days each time until the total number of desired repeats is reached.

3. APPLICATION RATE

The **Application Rate** frame contains two radio buttons and two text boxes. The radio buttons determine what kind of progression will occur in changes in application rate. If arithmetic progression is chosen, then the application rates specified in the Dates frame will be changed by adding a fixed constant to each application rate with every iteration. If geometric progression is chosen, then the application rates specified in the Dates frame will be changed by multiplying by a fixed constant with every iteration. The first input box (labeled either **increment** or **multiplier**, depending on what kind of progression is chosen) indicates the fixed constant that will be either added to or multiplied by the initial application rates and all subsequent rates. The **Repeats** input box determines the number of iterations in the arithmetic or geometric progressions designed.

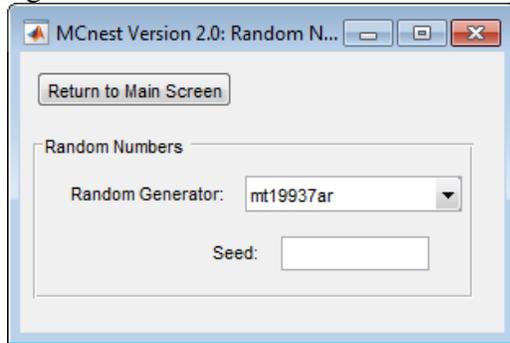
Note that the different batch panes are independent and factorial. Every combination of each iteration will be run. This can add up quickly if batching over all species in the library.

E. Other MCnest menu options

1. RANDOM NUMBERS

The Random window is invoked by selecting **Random Numbers** under the main MCnest window. This window offers the ability to choose among different types of random number generators and to set the seed for random number generation in MCnest. This may be useful for exactly repeating a particular simulation in MCnest. The Random window contains one push button, a popup menu and an input box (Figure 4.9).

Figure 4.9. The Random window in MCnest, with no seed specified.



Return to Main Screen: This pushbutton returns you to the main MCnest screen

Random Generator: The **Random Generator** popup allows you to specify which algorithm is used to generate random numbers in MCnest simulations. There are six choices (Table 4.4). We have no reason to believe that any of the algorithms below would offer significantly better performance than any other, and we recommend using the MATLAB and MCnest default option called the Mersenne twister (listed as “mt19937ar”) based on its computational efficiency (speed).

Seed: The “Seed” textbox allows you to specify a numeric seed for the random number generator. This should be a positive number. To exactly duplicate a previous simulation, find the seed value at the bottom of the Log file and enter here. If no seed is specified, MCnest will use the computer’s system clock to generate a seed. In this case, the seed is still recorded, so the simulation may be re-created even when no seed was specified.

Table 4.4. Random number generators available in MATLAB and MCnest¹.

| Option | MATLAB description | Reference ² |
|-------------|--|------------------------------|
| mt19937ar | Mersenne twister (default) | Matsumoto and Nishimura 1998 |
| mcg16807 | Multiplicative congruential generator | Park and Miller. 1998 |
| mlfg6331_64 | Multiplicative lagged Fibonacci generator | Mascagni and Srinivasan 2004 |
| mrg32k3a | Combined multiple recursive generator | L'Ecuyer et al. 2002 |
| shr3cong | Shift-register generator summed with linear congruential generator | Marsaglia 1999 |
| swb2712 | Modified subtract with borrow generator | Marsaglia and Zaman 1991 |

¹Adapted from MATLAB online documentation (Mathworks 2012).

2. LOAD MCNEST FILE

Choosing this menu option loads a standard Windows file browser. You can select and load any previously saved results file for examination using MCnest output functions, or for comparison among simulations generated in different sessions. This will work with individual simulations saved using the **Save row** option and multiple simulation saved using the **Save table** option in the **Output table** context menu. Any results loaded will be appended to any currently available results in the **Output table**.

3. IMPORT TIME-VARYING PARAMETERS

This is a very new feature with this version of MCnest and should be considered a pilot for future MCnest development. The motivation for this feature is to provide additional resolution and realism to MCnest simulations when data are available. Currently, only five parameters can be specified to vary over time. These are the quitting probabilities (q_f and q_s from an absorbing Markov Chain), the daily nest failure probabilities ($m_1 =$ pre-hatch and $m_2 =$ post-hatch) and the number of fledglings produced from a successful nest (FPSN).

To specify time-varying parameters, the desired temporal variation must first be designed in an Excel file (Fig. 4.10). In Figure 4.10, the file is displayed with the first column reserved for entering the date. This is not required, but is allowed for convenience. The next four columns are for entering date-specific values for the parameters. Note there is only one column for nest failure probability because in this case the same value will be used for both m_1 and m_2 for that day (simply by way of example, not asserting anything about American Robin nest survival).

Figure 4.10. An Excel™ worksheet with time-varying values of daily mortality rates displayed

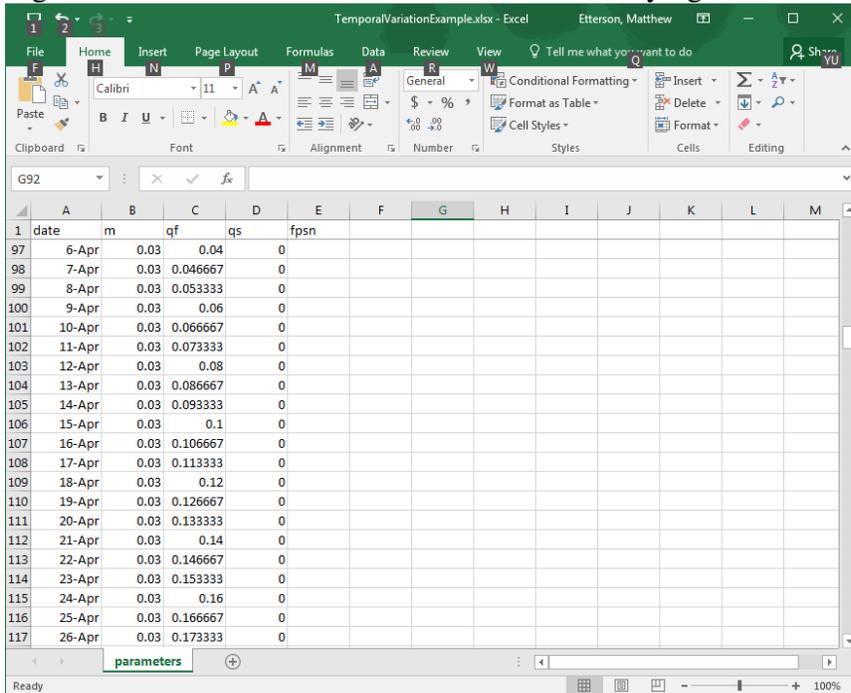
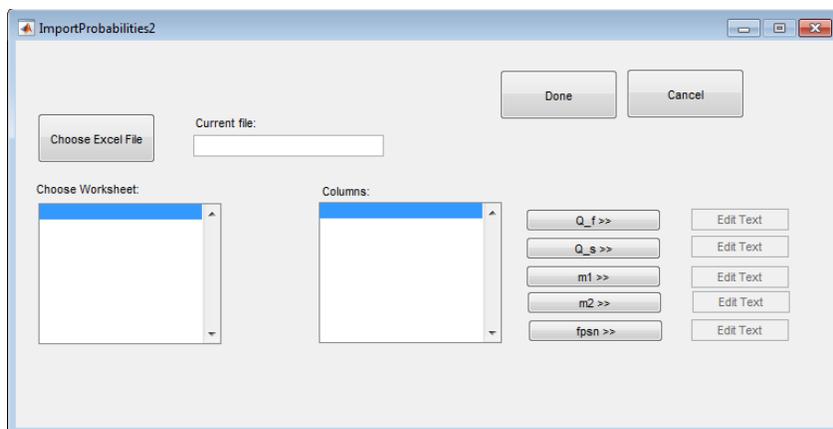


Figure 4.11. Time-varying parameter window



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