Technical Manual for Basic Version of the

Markov Chain Nest Productivity Model (MCnest)

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A. Brief overview of the conceptual approach used in MCnest

In the United States Environmental Protection Agency's (USEPA) current pesticide risk assessment process, a pair of laboratory avian reproduction tests with mallards (*Anas platyrhynchos*) and northern bobwhites (*Colinus virginianus*) is conducted to evaluate how dietary pesticide exposure affects a standard suite of reproduction endpoints (USEPA 1996). The results of these tests are used in calculating risk quotients (RQ) by comparing the reported no-observed-adverse-effect concentration (NOAEC) for the most sensitive measured endpoint(s) with estimates of the maximum dietary exposure expected for a given application rate. As a screening tool, RQs are compared to an established regulatory level-of-concern to categorize the potential for unacceptable risk. Because of the high degree of uncertainty in these simple tools for characterizing risk, RQs typically incorporate conservative or worst-case assumptions about exposure and toxicity to reduce the chances of concluding a chemical has an acceptable level of risk when in fact it does not (i.e., false negative conclusion). Consequently, risk quotients can be used to identify the environmental concentration above which adverse effects to avian reproduction may occur, but they cannot determine the probability or magnitude of potential reproductive effects.

An alternative conceptual framework for interpreting the results of avian reproduction tests was proposed by Bennett et al. (2005). Briefly, it involves linking the types of effects that may occur during each phase of a bird's reproductive cycle (e.g., pair formation, egg laying, incubation, nestling rearing) to selected surrogate endpoints from all three standard avian toxicity tests and relates those effects to the estimated exposure during each phase under a given pesticide-use scenario (Bennett et al. 2005). Because the great majority of avian reproduction tests do not provide quantitative dose-response information for surrogate endpoints, by necessity the alternative approach is based on a series of phase-specific deterministic decision points– essentially RQs for specific surrogate endpoints at each breeding phase–for determining whether the nest attempt fails or continues (Figure 1). Also, estimated exposure and effects endpoints are expressed as ingested doses (e.g., mg/kg/day) rather than as concentrations on food (e.g., ppm in diet). In the framework proposed by Bennett et al. (2005), if the estimated exposure during the critical exposure period is less than the established toxicity threshold (e.g., the no-observed-adverse-effect level or NOAEL) for surrogate endpoints at each phase, the nest continues without disruption. However, if exposure exceeds the toxicity threshold for a surrogate endpoint, the nest attempt is assumed to have failed and the female may be able to renest if conditions permit and sufficient time remains in the breeding season. Also, for those species that can produce multiple broods in a single breeding season, females may renest after successful nesting attempts if conditions permit. The simulated performance of a population of females in relation to the timing of pesticide applications is modeled over the course of a full breeding season (Bennett et al. 2005). Consequently, using this framework, the effects of a pesticide on annual reproductive success are not only a function of the results of avian toxicity tests, but also are quite sensitive to the timing of pesticide applications relative to a species' breeding season and to differences in life history characteristics among species.
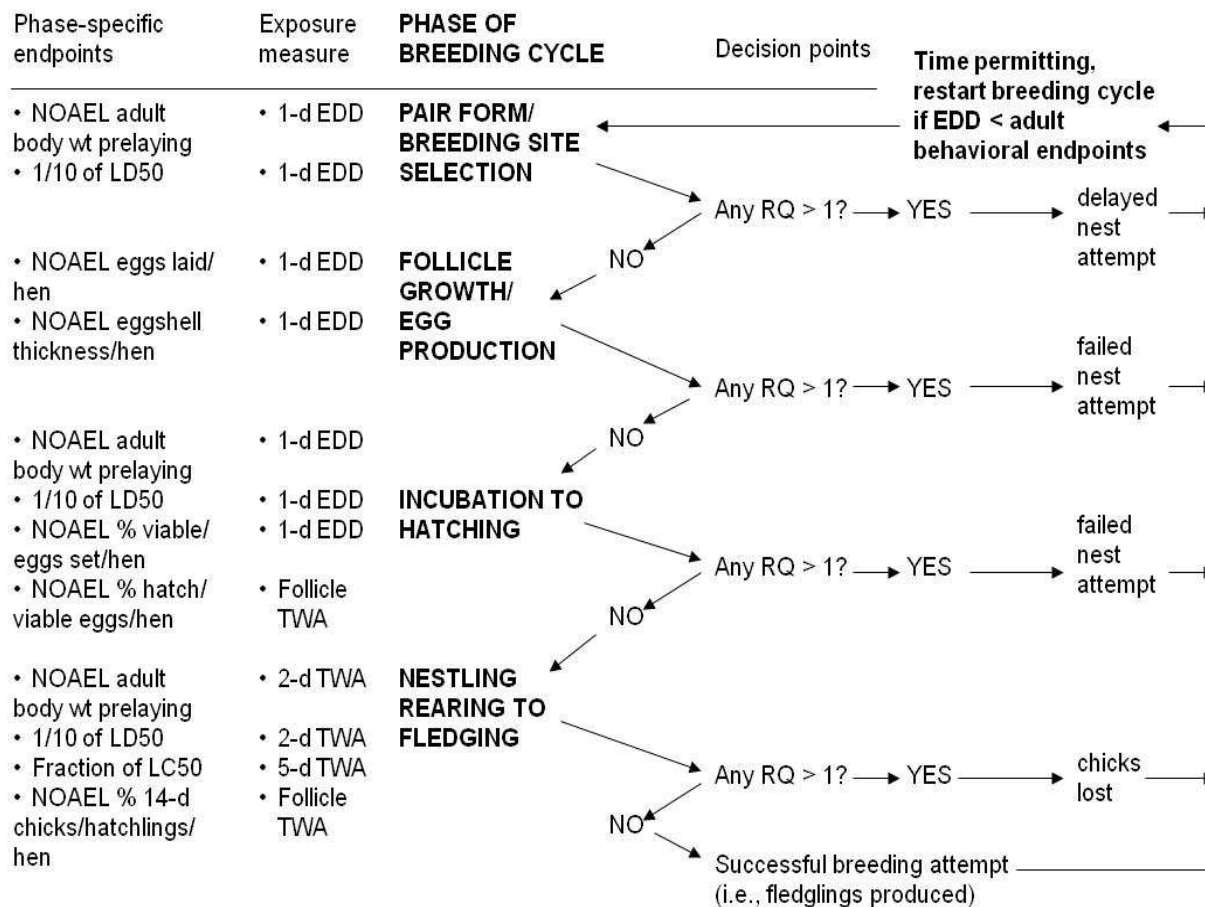
Figure 1. Four phases of avian breeding cycle with phase-specific toxicity endpoints and associated exposure estimates (i.e., estimated daily dose [EDD] or time-weighted average dose [TWA]) used in risk quotients (RQs) at each decision point.

The framework described above identifies three categories of effects resulting from direct exposures that may occur: 1) effects on adult behavior and reproductive performance from external exposure (e.g., dietary), 2) effects on nestling growth and survival from external exposure, and 3) effects on nestling growth and survival from *in ovo* exposure. Some potential effects have direct corollary measurements from the reproduction test (e.g., percent hatchability related to *in ovo* exposure), while other effects have more indirect surrogate measures (e.g., using change in adult body weight during the pre-laying period as an indicator of overall parental well-being and behavioral effects). Some effects, such as nestling toxicity from external exposures, have no surrogate directly from the reproduction test because chicks are not exposed to treated diets. However, data from other toxicity tests are used as surrogates. While indirect effects of chemicals also may reduce reproductive success, laboratory toxicity tests cannot provide adequate surrogate endpoints for these effects, and they are not addressed currently in the framework. However, on a case-by-case basis they could be included if sufficient information is available for defining appropriate decision points in the framework.

A flexible mathematical model, known as the Markov chain nest productivity model or MCnest, has been developed for implementing the conceptual framework of Bennett et al. (2005). It projects estimates of pesticide effects on reproductive success for a broad range of species and can be modified to incorporate either sparse or abundant life-history data. MCnest builds on over 40 years of avian nest-survival modeling in the ornithological literature. Etterson and Bennett (2005) showed that a simple Markov chain model is equivalent to the well-known Mayfield (1961, 1975) nest-survival model when similar assumptions are imposed and unifies many current formulations of nest survival estimation models (e.g., Johnson 1979, Hensler and Nichols 1981, Bart and Robson 1982, Dinsmore et al. 2002, Shaffer 2004). Etterson et al. (2009) demonstrated how alternative approaches for defining the length of the breeding season affected productivity estimates. Bennett and Etterson (2007) presented a demonstration of an earlier version of the model applied to a pesticide-use scenario. MCnest also can be applied to contaminant effects questions in other USEPA Program Offices, though at present the model is not designed to adequately estimate the effects of bioaccumulative chemicals where effects on hatchability and hatchling survival may result from chemical residues accumulated prior to the egg formation period.

The decision to develop MCnest as a Markov process was based in part on the iterative nature of avian breeding (propensity to renest after either failure or success of a nest attempt), which is naturally captured in the cyclic nature of Markov processes and easily represented in the associated transition matrices. Another important consideration is the way in which important nest survival parameters (m_1 and m_2) are estimated equivalent to the Mayfield estimator. Thus, the choice to simulate nest survival and productivity as a Markov process is consistent with way in which important model parameters are generated. We have considered other mathematical methods (specifically individual-based models and differential equations) for simulating avian reproduction (see Etterson et al. 2011) and found that the Markov approach was the most suitable for development of MCnest.

Most of the data used in MCnest are in the form of input parameters provided by the model user and represent three categories of input parameters: toxicity threshold values for surrogate endpoints, pesticide application scenarios, and species life-history parameters (Figure 2). MCnest uses information for parameterizing toxicity threshold values and application scenarios that is currently available in the risk assessment process. The model user may use default life-history parameters from a library of avian species available to MCnest or create new or modified species parameter profiles.

In the Basic Version of MCnest, the estimated exposure to a species immediately after a pesticide application (i.e., initial dietary dose) is based largely on the approach used in the Terrestrial Residue EXposure model (a.k.a. T-REX) developed by the USEPA Office of Pesticide Programs. In addition to estimating the mean and maximum dose as in T-REX, MCnest also gives the model user the option of using a distribution of initial doses based on the mean and standard deviation of residues expected on various food types in the diets of each species (discussed further in Section C below).

Once all input parameters are set, a model simulation in MCnest follows the breeding activities of a population of females each day throughout a breeding season. The temporal pattern of breeding activity of each female (i.e., transitions among breeding phases) 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 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 failures 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 (Figure 1). 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).

The primary output of each MCnest simulation is an estimate of the number of successful broods per female per year, which can be multiplied by the number of fledglings per successful nest to estimate the number of fledglings per female per year (i.e., annual reproductive success). A "successful brood" is defined as a nest attempt producing one or more fledglings. However, to put the output from a single MCnest simulation involving a pesticide exposure into perspective, it is compared to the output of a simulation(s) without pesticides. Calculating the relative difference between scenarios with and without pesticide exposure provides an estimate of the potential magnitude of pesticide effects to annual reproductive success under a specific pesticide-use scenario. MCnest also can provide information on which species are at greatest risk under a specific pesticide-use scenario or which application dates have the greatest impact throughout a breeding season. This quantitative estimate of pesticide effects on annual reproductive success is needed for use in population modeling or probabilistic risk assessments.

While MCnest provides an approach for quantifying the effects of pesticide exposure on avian reproductive success, caution is needed in interpreting the results, especially when conservative assumptions are made concerning input parameters. Because of data gaps and uncertainty in information on species life-history parameters and for quantifying toxicity and exposure, it is inevitable that assumptions will be required when using available data to determine input parameters. The ecological risk assessment process traditionally has used conservative assumptions when addressing uncertainty to reduce the probability of concluding that there is not an unacceptable risk of adverse effects when, in fact, there is an unacceptable risk. Due to limitations of the toxicity data, the model structure of MCnest has incorporated a conservative assumption by assuming that exposures that exceed a specified toxicity threshold of a surrogate endpoint will lead to a nest failure. We recognize that not every exposure exceedance of the toxicity threshold value in the field will result in a nest failure, but given the lack of information from the reproduction test for quantitatively describing the dose-response relationship, failure is assumed the worst outcome for the nest. Consequently, estimates of the reduction in reproductive success from MCnest represent a conservative estimate of potential pesticide effects that may occur in the field. Future versions of MCnest could incorporate dose-response information into the decision points when it is available. Model users also may incorporate conservative assumptions in MCnest simulations through selection of parameter inputs, such as using the maximum nomogram values instead of the estimated distribution of residue values or by selecting the 35-d default value for the residue half-life value instead of chemical-specific information of the rate of residue degradation. However, when using

conservative assumptions, model users need to be aware that not only will the model results be more conservative (i.e., estimate a greater reduction in annual reproductive success than may occur in the field), but the relative responses among species and across applications dates will change in ways that may make these comparisons less informative. This is discussed in greater detail in Section H (Model Assumptions and Uncertainties) below.

While the primary goal of MCnest is translating available toxicity data into a currency useful for population-level assessments by estimating the magnitude of change in annual reproductive success for a species exposed to a specific pesticide-use scenario, the ultimate challenge in ecological risk assessment is to estimate the effects of pesticide use on population growth rates in complex landscapes where pesticide exposure often is patchily distributed. MCnest can be an important tool in that challenge, but isn't the only tool needed. The model user must recognize that MCnest intentionally simplifies aspects of the exposure scenario, but these simplifications can be addressed by the model user within a population-modeling framework. First, in each MCnest simulation a pesticide is applied on a single day for the entire population, when in reality pesticide applications are asynchronous across a landscape in response to pest levels and weather conditions. The MCnest user can run simulations for a series of possible application dates to estimate the overall effect as a cumulative function of the relative effect on each date and the probability of an application made on that date. This gives the model user both an overall estimate of the effect of the pesticide for any distribution of possible application dates and information on which specific application dates pose the least or greatest risk. Second, all females in a simulation are exposed to the pesticide. MCnest currently does not include a spatial component that allows the user to expose only a portion of the population to the pesticide as would occur across a heterogeneous landscape, but the MCnest results can be used in spatially-implicit or spatially-explicit population models to simulate patchy exposures. Third, all females in a simulation are active breeders even though in field populations it is possible for a certain portion of the population to remain in non-breeding status (i.e., floaters). Again, this can be addressed best in a population model, especially since the proportion of active breeders each year may be a density-dependent function. The need to address any of these issues depends largely on the specific risk management question being posed.
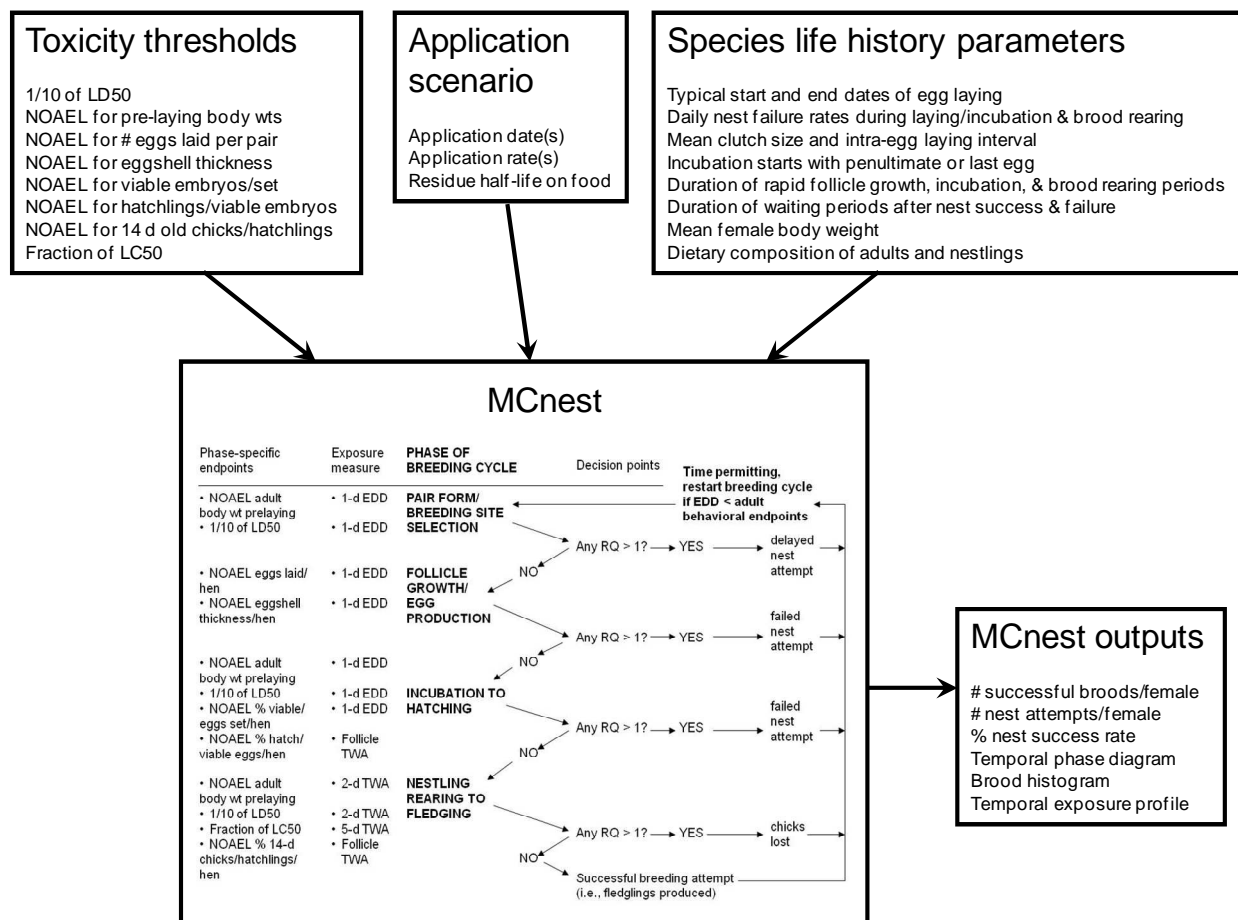
Figure 2. MCnest model inputs and outputs.

B. Selection of surrogate endpoints

The conceptual approach described in Bennett et al. (2005) recognizes that the current avian reproduction test is not sufficient on its own to directly estimate effects on annual reproductive success, but it provides data for measured endpoints that may represent several of the specific types of effects that occur in the field. They define these test endpoints as surrogate endpoints and briefly describe the rationale for their use as a surrogate for effects in the field. However, there are several types of potential effects in the field that are not represented in the reproduction test (Mineau et al. 1994a, Mineau 2005, Bennett and Etterson 2006), though there may be endpoints in the avian reproduction test that can act indirectly as surrogate endpoints in these cases. For other types of field effects there are no suitable surrogate endpoints available from the reproduction test. In such cases, suitable surrogate endpoints may be available from other laboratory tests or pen and field studies. For example, Bennett et al. (2005) proposed using data from the 5-day LC50 test as a surrogate for mortality of chicks from direct pesticide exposure, since chicks are not fed pesticide-treated diets in the reproduction test.

Pesticides may cause a variety of proximate effects (e.g., effects on parental behavior, eggshell thickness, or hatchability of eggs) through all the breeding phases that can result ultimately in a change in annual reproductive success. There are several major categories of effects resulting from different pathways of exposure (Table 1). Bennett et al. (2005) described three of these categories for effects resulting from direct exposures: 1) effects on adult behavior and reproductive performance, including egg production and eggshell quality, from external exposure (adult extrinsic); 2) effects on juvenile growth and survival from external exposure (juvenile extrinsic); and 3) effects on juvenile growth and survival from *in ovo* exposure (juvenile intrinsic). There are two additional categories involving indirect effects, namely reduced food resources to adult and juveniles, which were not previously discussed, but could be important to the assessment of overall effects on avian reproductive success.

During each of the breeding phases, effects may result via one or more of these exposure pathways (Table 1). To fully assess the potential risks of pesticide exposure on overall reproductive success, all potential effects of a pesticide should be identified and, where data exist, surrogate endpoints should be defined for use in a model of reproductive effects. For many types of effects, surrogate endpoints would need to be derived from sources other than the laboratory avian reproduction test because, as Mineau et al. (1994) describe, the test measures "a very unnatural and truncated reproductive performance." While the test simulates an extended period of egg laying, there are many types of effects related to changes in adult behaviors that cannot be observed, including behaviors affecting nest construction, clutch completion, incubation, or rearing of nestlings that are important in determining overall reproductive success.

Table 1. Types of effects possible during each avian breeding phase by major categories of exposure.

Breeding phase	Adult direct	Juvenile *in ovo*	Juvenile direct	Adult indirect	Juvenile indirect
Pair formation/ Breeding site selection	• Territory loss or nest abandonment due to sublethal effects or death	• Not applicable		• Territory loss or abandonment due to reduced food availability/ habitat	• Not applicable
Follicle growth/ Egg production	• Reduced clutch size • Nest abandonment due to sublethal effects or death of adults or eggshell failures			• Reduced clutch size • Nest abandonment due to reduced food availability/ habitat	
Incubation to hatching	• Nest abandonment due to sublethal effects or death of adults • Reduced hatch due to infertility	• Embryotoxicity due to *in ovo* exposure	• Embryotoxicity due to external eggshell exposure	• Nest abandonment due to reduced food availability/ habitat	
Nestling rearing to fledging	• Brood abandonment due to sublethal effects or death • Reduced juvenile growth and survival due to reduced parental care and defense	• Reduced juvenile growth and survival due to *in ovo* exposure	• Reduced juvenile growth and survival due to direct post-hatch exposure	• Brood abandonment due to reduced food availability/ habitat • Reduced juvenile growth/survival due to reduced parental foraging success	• Reduced juvenile growth and survival due to reduced food availability/ habitat

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The Basic Version of MCnest focuses on surrogate endpoints for direct effects though it is capable of including decision points for all types of potential effects, assuming that a meaningful surrogate endpoint and exposure period can be defined. There are many issues still to resolve on how best to integrate information on indirect effects into this model. Also, because the Basic Version of MCnest focuses on the female as the subject of the model, rather than individual eggs or chicks, it does not follow the survival of juveniles after fledging or their sexual maturation as adults. Post-fledging performance is better addressed using a separate modeling approach.

1. <u>Attributes of surrogate endpoints</u>

Surrogate endpoints must have certain attributes to be useful in MCnest or in other models of reproductive effects such as those presented in Roelofs et al. (2005) and Topping et al. (2005). Not all measured endpoints of effects possess the attributes to be useful as surrogate endpoints in estimating pesticide effects on overall avian reproductive success.

First, surrogate endpoints must be measurements of effect that can be linked to an exposure concentration or dose. While this is relatively straightforward in controlled-dose laboratory studies, linking effects to an estimated exposure can be more difficult in pen and field studies, not only because exposure may be more difficult to measure, but also because exposure can be very dynamic over time. To be most effective for pesticide risk assessments, the estimated exposure concentration or dose should also be relatable to an application rate (i.e., application rate x $\rightarrow$ exposure dose y $\rightarrow$ effect z).

Second, surrogate endpoints must be measurements of effect that can be related directly or indirectly to field effects that ultimately may lead to changes in reproductive success (See Table 2). For example, an observed reduction in hatching rate from *in ovo* exposure in a laboratory test may relate quite closely to an observed reduction in hatching rate measured in the field from comparable pesticide concentrations in eggs. Reduced hatching success can result directly in changes in reproductive success. However, some measurement endpoints (e.g., change in a biochemical concentration or in a behavioral measurement) may require additional information to demonstrate their relationship to changes in reproductive success. Measurement endpoints that are proposed as indirect surrogates of effects on reproductive success need to be examined on a chemical-by-chemical basis to ensure the plausibility of these relationships. A measured endpoint should not be used as a surrogate endpoint when there is no plausible linkage between the endpoint and effects on overall reproductive success. Establishing plausibility may require reliance on completely separate–and unrelated–models; e.g., an efficacy model relating a given application rate to the proportion of invertebrate kill in the case of indirect effects.

Third, for use in deterministic phase-specific decisions, surrogate endpoints of direct effects must be expressed as a daily dose (i.e., μg active ingredient [AI]/g body weight/day) below which the risk of adverse effects is considered acceptable. This dose is referred to as the toxicity threshold value. In Bennett et al. (2005) these toxicity threshold values were primarily represented by the NOAEL for the surrogate endpoints from the avian reproduction test.

Finally, although not strictly an attribute of the surrogate endpoint itself, the toxicity threshold value for each surrogate endpoint needs to be compared to an estimate of

environmental exposure during an appropriate exposure period. A key issue is determining what constitutes an "appropriate" exposure period. For example, if effects can occur very rapidly, such as changes in adult behavior leading to nest abandonment, the appropriate exposure period may be as short as a single day immediately preceding the effect. On the other hand, for effects such as reduced hatchability or chick survival due to *in ovo* exposure, the appropriate exposure period for rapidly-metabolized pesticides is during the time of egg formation, which occurs days or weeks before the effect can be observed, and may be defined as a time-weighted average (TWA) dose based on the length of the rapid follicle growth period for each egg. The selection of appropriate exposure periods is discussed in greater detail below.

2. Default surrogate endpoints from existing laboratory studies

The Basic Version of MCnest is programmed with a series of eight default surrogate endpoints from standard laboratory toxicity tests done primarily on northern bobwhite and mallards (Table 2), each of which can be used or not used at the discretion of the model user. Many of the surrogate endpoints were proposed in Bennett et al. (2005). A few additional surrogate endpoints were included in MCnest based on the reasoning presented in a recent revision of the European Union (EU) Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2008). This section provides additional guidance for the use of default surrogate endpoints from existing laboratory tests in MCnest. Depending on the nature of a particular chemical, additional surrogate endpoints may be appropriate for representing the same or other potential direct effects. The Basic Version of MCnest has the capability to modify surrogate endpoints during each breeding phase when they can be justified; however, it is very important to assure that new surrogate endpoints can be properly linked to an appropriate exposure period. This is particularly critical where there is a lag time between exposure and expression of the effect (e.g., *in ovo* exposure-related effects).

In MCnest the surrogate endpoints are expressed as toxicity threshold doses (i.e., mg/kg/day), which represent the dose below which unacceptable adverse effects are not expected. For surrogate endpoints from the avian reproduction test, the toxicity thresholds may be based on the NOAEC for that endpoint. However, test endpoints from the avian reproduction test and the LC50 test are usually reported as the dietary concentration (mg/kg in diet), rather than as a daily dose. There are multiple possible approaches for making this conversion, but no standardized approach, so at this point MCnest does not attempt to standardize the method for the conversion. In the Basic Version of MCnest, it is the responsibility of the model user to convert dietary concentration for test endpoints into an estimate of the comparable dose using the information on body weights and food ingestion rates provided in the test reports.

Table 2. Types of effects and corresponding surrogate endpoints used in the Basic Version of MCnest for each avian breeding phase.

Breeding phase	Phase-specific effect of concern	Type of effect	Test endpoint used as surrogate	Comparable exposure period for phase-specific RQ
Pair formation/ breeding site selection	Adult behavioral effects leading to territory abandonment or delayed breeding	Extrinsic adult	1/10 of LD_{50}	1-day maximum estimated daily dose (EDD)
		Extrinsic adult	NOAEL for adult body wt pre-laying	1-day EDD
Follicle development and egg laying	Adult behavioral effects leading to abandonment of nest attempt	Extrinsic adult	NOAEL for the number of eggs laid per hen	1-day EDD
	Reduced eggshell quality leading to abandonment of nest attempt	Extrinsic adult	NOAEL for mean eggshell thickness	1-day EDD
	Reduced clutch size	Extrinsic adult	NONE	NONE
Incubation and hatching	Adult behavioral effects leading to abandonment of nesting attempt or reduced nest attentiveness	Extrinsic adult	1/10 of LD_{50}	1-day EDD
			NOAEL for adult body wt pre-laying	1-day EDD
	Reduced fertility	Extrinsic adult	NOAEL for proportion of viable eggs per eggs set per hen	1-day EDD during follicle development and egg laying
	Embryotoxicity from *in ovo* exposure leading to reduced hatchability	Intrinsic juvenile	NOAEL for proportion of hatchlings per viable eggs per hen [1]	Follicle development time-weighted average (TWA)
	Embryotoxicity from external eggshell exposure leading to reduced hatchability	Extrinsic juvenile	NONE	NONE
Nestling rearing until fledging	Adult behavioural effects leading to brood abandonment or abnormal parental care	Extrinsic adult	1/10 of LD_{50}	2-day TWA
			NOAEL for adult body wt pre-laying	2-day TWA
	Reduced nestling survival from direct exposure	Extrinsic juvenile	1/10 of LD_{50}	1-day EDD (juvenile diet)
			Fraction of 5-d LC_{50}	5-day TWA (juvenile diet)
	Reduced nestling survival and growth from *in ovo* exposure	Intrinsic juvenile	NOAEL for proportion of 14-day-old juveniles per number of hatchlings per hen	Follicle development TWA

[1] Alternatively, if the NOAEL for proportion of hatchlings per number of viable eggs is not available, use the lower of the NOAEL for proportion of 3-week live embryos per number of viable eggs or the NOAEL for proportion of hatchlings per number of 3-week live embryos.

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The surrogate endpoints used in the Basic Version of MCnest are as follows:

a. Adult pre-laying body weight

Bennett et al. (2005) proposed using a change in adult body weight during the pre-laying period of the avian reproduction test as a surrogate endpoint for parental well-being during all breeding phases from territory establishment to fledging, except the egg-laying phase. This proposal now has been refined to focus on changes in body weight observed in the first two weeks after the onset of treatment because it is a surrogate for physiological or behavioral responses resulting in nest and territory abandonment or reduced nest attentiveness that may occur rapidly after an initial pesticide exposure. Adult body weight is the surrogate endpoint with the most indirect connection between the measured effect and the potential responses of birds in the field. The avian reproduction test is designed so that many of the pesticide-related responses potentially expressed by breeding adults in the field cannot be observed in the laboratory. While we cannot observe many of these adult behavioral changes that threaten the success of a nest, we do measure pesticide-related changes in parental food consumption and body weight. Food consumption measurements can be highly variable and biased by unmeasured spillage, whereas changes in body weight can be more accurately measured. Consequently, a rapid change in body weight during the first two weeks of the pre-laying period in the avian reproduction test is considered to be an appropriate surrogate of possible effects on the overall well-being of the adult females in the field that could ultimately lead to nest failure. Because parental responses may occur rapidly following an initial pesticide exposure, Bennett et al. (2005) proposed that the NOAEL of the body weight surrogate endpoint be compared to the expected dietary dose on each day during a breeding phase (i.e., 1-day estimated daily dose or EDD). For some pesticides, this may lead to a very conservative decision point if the NOAEL for change in body weight is considerably lower than a dose causing behavioral effects leading to a nest failure, though in most cases information on this field effect would be unknown. However, other pesticides may cause effects on adult behavior without impacting body weight in the laboratory test, leading to decisions that underestimate risks. The adequacy of using a change in adult body weight as a surrogate endpoint should be evaluated on a pesticide-by-pesticide basis.

During the nestling-rearing phase, Bennett et al. (2005) argued that the change in pre-laying body weight should be compared to the 2-day TWA for exposures throughout the phase because nestlings can withstand reduced parental care for approximately one day, but may not be able to withstand longer periods of reduced attentiveness.

The comparison of changes in adult body weight during the pre-laying period to single day (or 2-day TWA) exposure doses has been criticized because the measured effect (i.e., change in body weight) takes more than a 1-day exposure to be observed. In determining the appropriate period of exposure to compare with a surrogate endpoint, it is important to focus on how rapidly the field effect of concern is expressed after an initial exposure, rather than the time course of the effect measured in the laboratory. In this case, the concern is over sublethal behavioral and/or physiological effects that can cause nest failures soon after initial exposures, such as the nest or brood abandonments observed shortly after application by Busby et al. (1990) and Brewer et al. (1988). Consequently, despite using a change in adult body weight as the surrogate endpoint, the concern is not over how quickly birds lose weight following a pesticide

application, because weight loss may not be relevant to the response of wild birds if they abandon the area or change feeding sites. However, if the laboratory birds show a significant change in body weight in the first two weeks of exposure, then it is likely that sublethal effects, such as reduced food consumption or reduced metabolic efficiency, began shortly after the initial exposure, and that sublethal effects such as these may be indicative of other effects that threaten the success of the nest attempt.

One issue to consider is that while original avian reproduction study reports present analyses on treatment-related differences in body weights measured bi-weekly prior to the onset of egg-laying, the data and analyses on pre-laying body weights are not currently included as part of the OPP Data Evaluation Records (DER). While the body weight data at the end of the test is included in the DERs, this endpoint IS NOT an adequate substitute for pre-laying weight as a surrogate endpoint. Also, there are at least two methods for analyzing the data on changes in pre-laying body weights. Many avian reproduction study reports simply perform an analysis of variance on the actual body weights at each pre-laying measurement period. A more statistically sensitive analysis for treatment effects on body weights would be to use a repeated measures analysis of variance or to conduct the analysis of variance on the change in weights since test initiation (i.e., difference in weight between weeks 0 and 2 for each individual) among treatments.

b. 1/10 of the LD_{50}

One concern raised about the original proposal for using pre-laying body weight as a surrogate endpoint for adult well-being is that if changes in body weight are not observed until several weeks after the onset of treatment, this period may be much longer than a realistic exposure period with most current pesticides. Largely for that reason, the recent revisions to the EU Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2008) proposed that an alternate surrogate endpoint of adult well-being be derived from results of the avian acute toxicity (LD_{50}) test. Ideally, the test could provide information on the single oral dose below which mortality and/or overt signs of poisoning are not observed. However, many LD_{50} tests produce mortality and other signs of toxicity at each dose tested and do not adequately document presence and severity of sublethal signs of poisoning. A review of LD_{50} studies showed that severe signs of toxicity likely to interfere with a bird's normal activities tend to be recorded at dosing levels greater than 1/10 of the LD_{50} (Callaghan and Mineau 2000). On the basis of this work, it is proposed that, as a default, 1/10 of the LD_{50} be used as a surrogate endpoint for effects on adult behavior leading to disruption on nesting success and that it be compared to the expected dietary dose on each day during a breeding phase (i.e., 1-day EDD), except during the nestling rearing phase where it is compared to the 2-day TWA exposure dose. However, if a model user has chemical-specific data on which to derive a refined estimate of the dose below which mortality and/or overt signs of poisoning are not observed, this value can be used as an alternative to the 1/10 of the LD_{50} surrogate endpoint. The model user is responsible for providing the rationale for alternative values.

c. Eggshell thickness and number of eggs laid

Mean eggshell thickness per hen and the number of eggs laid per hen are surrogate endpoints reflecting effects to adults from direct pesticide exposure during the egg-laying phase.

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A reduction in eggshell thickness is a surrogate for nest failures related to cracked and broken eggs with reduced eggshell quality. Although the effects on eggshell thickness observed in the avian reproduction test occur after an extended period of pre-laying exposure of the parents, other studies have shown that pesticides may affect eggshell quality rapidly after the initial exposure (Bennett et al. 1990, Bennett and Bennett 1990). Adverse effects of reduced eggshell thickness, such as egg breaking or punctures, may be expressed in the field during either the egg-laying or incubation phases, but it is used in MCnest as a surrogate endpoint during the earlier breeding phase where effects may occur and affect the outcome of the nest. A reduction in the number of eggs laid is a surrogate for effects on adult well-being that can lead to nest abandonment or reduced nest attentiveness. The cluster analysis conducted by Mineau et al. (1994a) showed that these two endpoints segregated into different categories of responses observed in avian reproduction tests, and both are needed as surrogate endpoints to represent the range of parental effects possible during egg laying. However, reduced egg production in the laboratory test is not an appropriate indicator of reduced clutch size in the field, because it is unclear if reduced production in the laboratory translates into a proportional reduction in clutch size, complete abandonment of the nest, or a longer period of time to complete a normal-size clutch (Mineau 2005). The determinants of clutch size in the field involve hormonal and sensory cues that are not present in a laboratory test where eggs are removed daily for artificial incubation. For this reason, reduced egg production should be seen as a broader indicator of adult well-being during the egg-laying phase that could ultimately affect reproductive success and that may be expressed in several ways in the field. Because some pesticides can affect egg production and eggshell thickness rapidly after initial exposures, Bennett et al. (2005) proposed that both endpoints be compared to the estimated dietary dose (i.e., 1-day EDD) on each day during the egg-laying phase. If evidence exists for a pesticide indicating that a longer period of exposure is necessary to produce effects on these endpoints, an exposure estimate based on a longer TWA may be appropriate, but the Basic Version of MCnest does not include this option. The existing avian reproduction test itself does not provide information on the rapidity of onset of effects for the reproductive endpoints because of the extended period of pre-laying exposure.

d. Proportion of viable eggs per eggs set

Bennett et al. (2005) also proposed that the percentage of viable eggs per number of eggs set per hen be used in decisions during the egg-laying phase as a surrogate for direct parental effects leading to reduced egg viability. While this endpoint is intended to be a surrogate measure for pesticide effects on fertility (i.e., production of infertile eggs), egg viability is determined by candling eggs at approximately 11 days of incubation, and it is very difficult using this approach to separate infertility due to parental exposure from early embryo mortality due to *in ovo* exposure. Consequently, without detailed analysis of failed eggs, this endpoint potentially represents a combination of infertility and early embryo death. An additional factor is that infertility due to parental exposure may be due to the direct chemosterilant activity of the chemical to males or to sublethal intoxication that reduces or stops mounting behavior by the male. Without additional testing or knowledge of the chemical, it is difficult to determine from current practices in the avian reproduction test why eggs are not viable. Also, adverse effects on egg viability usually would not be detected by the parent until late in incubation, making it more appropriately a surrogate endpoint for effects observed at the end of the incubation phase (Table 2), rather than during egg laying, as proposed in Bennett et al. (2005).

Bennett et al. (2005) proposed that this surrogate endpoint be compared to the expected dietary dose on each day (i.e., 1-day EDD) during the follicle growth and egg production phase. Jones and Jackson (1972) and Jones et al. (1972) demonstrated that male fertility in Japanese quail can be decreased within days after single-dose exposures to certain chemicals. Consequently, using single-day exposure doses during the egg-laying period (from start of the rapid follicle growth period through egg laying) as the default exposure period is intended to represent chemicals that can rapidly affect fertility. However, some chemicals can depress fertility rates for many weeks or even permanently (Jones and Jackson 1972, Jones et al. 1972, Schafer et al. 1976), in which case, chemical exposures occurring well before the start of egg laying could lead to higher rates of male infertility. The default exposure period in the Basic Version of MCnest does not address this situation, and because the effects on the pattern of fertility vary among chemicals, it would be difficult to establish a single default exposure period that was suitable for all chemicals. When information exists on the specific effects of a chemical on the proportion of viable eggs, it is possible that MCnest could be modified to incorporate that information by modifying the exposure period for comparison with the toxicity threshold value. The use of the 1-day EDD in the Basic Version of MCnest may represent a conservative estimate of exposure for comparing with toxicity threshold of this surrogate endpoint.

e. Proportion of hatchlings per viable egg

During the incubation phase, the proportion of hatchlings per number of viable eggs per hen is proposed as a surrogate for effects on hatchability in the field. This endpoint has been modified from the proposal in Bennett et al. (2005). In currently accepted protocols, viability is assessed by candling at 11 days (bobwhite) or 14 days (mallard) of incubation (USEPA 1996). Originally, this endpoint was proposed as the percentage of hatchlings per number of eggs set per hen. However, the proportion is more appropriately based on the number of viable eggs to separate the effects of *in ovo* exposure on late embryotoxicity from the combined effects of reduced fertility and early embryo mortality. This surrogate endpoint should be compared to the time-weighted average of exposures occurring during the period of rapid follicle growth of each egg prior to laying.

Many studies do not specifically report the proportion of hatchlings per number of viable eggs per hen, but rather report the proportion of 3-week live embryos per number of viable eggs and the proportion of hatchlings per number of 3-week live embryos. In such cases, the more sensitive of the two endpoints should be used as the surrogate endpoint to represent the effects of *in ovo* exposure on hatchability. Regardless of which of these endpoints is used as the surrogate endpoint, it should be compared to the TWA of exposures occurring during the period of rapid follicle growth prior to laying. Consequently, the duration for the TWA will vary among species based on the length of their rapid follicle growth period (See additional background in Appendix A). Because the Basic Version of MCnest estimates the exposure only during the period of rapid follicle growth, it may not be suitable for bioaccumulative pesticides where the deposition of the pesticide into yolk may be a function of a longer period of dietary exposure occurring prior to egg formation.

f. Proportion of 14-day-old chicks per hatchling

During the juvenile rearing phase, the proportion of 14-day-old chicks per number of hatchlings per hen is an indicator of effects to chick growth and survival from *in ovo* exposure. This endpoint also should be compared to the TWA of exposures occurring during the period of rapid follicle growth of each egg prior to laying.

g. Fraction of juvenile dietary LC_{50}

Since chicks are raised on untreated diets in the avian reproduction test, the test does not provide information on their sensitivity to direct pesticide exposures after hatching. As a surrogate endpoint for direct pesticide exposure to hatchlings and fledglings, Bennett et al. (2005) proposed using a dietary exposure dose derived from the 5-day dietary toxicity (LC_{50}) test with juveniles that does not result in adverse effects—essentially an effects threshold.

There are important issues to be addressed when using an endpoint derived from the 5-day toxicity test in a reproductive success model. First, the 5-day toxicity test is not designed specifically to determine a toxicity threshold value below which adverse effects on juvenile survival are not expected, because the emphasis is on selecting treatment concentrations that would produce some level of mortality between 0 and 100%. In the Basic Version of MCnest we are proposing that a fraction of the LC_{50} be used to represent a toxicity threshold based on the levels of concern (LOCs) as defined by USEPA's Office of Pesticide Programs for classifying risk to birds from short-term dietary exposure. The three LOCs related to the 5-day toxicity test are 0.5 of the LC_{50} for acute risk, 0.2 for acute restricted-use risk, and 0.1 for acute endangered species risk (See additional discussion on LOCs at http://www.epa.gov/oppefed1/ecorisk_ders/toera_risk.htm Last accessed 11 January 2013). The model user is responsible for using the fraction of the LC_{50} that is appropriate to the specific pesticide-use scenario. Second, many concerns have been raised about the adequacy of the avian 5-day toxicity test as a quantitative measure of toxicity for use in risk assessment (Hill 1995; Mineau et al. 1994b). It is considered to be a test of vulnerability instead of toxicity, where vulnerability is the product of the willingness to consume treated feed, feeding rate, sensitivity to the pesticide, and temporal pattern of pesticide availability (Hill 1995). Two studies designed to directly compare the results of the laboratory 5-day toxicity test with same age birds in the field observed that not only was the mortality rate higher in the field than in the laboratory at comparable exposure levels, but the timing and nature of mortality was very different (Matz et al. 1998, Vyas et al. 2006). Consequently, the adequacy and use of a surrogate endpoint derived from the 5-day toxicity test should be evaluated and decided on a pesticide-by-pesticide basis.

Bennett et al. (2005) argued that the surrogate endpoint from the LC_{50} should be compared to a 5-day TWA of dietary exposure to juveniles. Consequently, comparisons between the toxicity threshold and the 5-day TWA begin once nestlings have had 5 days of dietary exposure (i.e., at beginning of 6th day since hatching) and continue each day until fledging. It could be argued that some pesticides act very quickly (i.e., most mortalities occur in the first day or two) so the toxicity threshold does not require a full 5 days of exposure before effects are observed. However, LC_{50} test reports may not adequately document the time course of mortalities, and the analysis of time to death is not a primary purpose of the test. Given the other

limitations of the test discussed above, we have not tried to tailor the duration of the TWA to the temporal pattern of observed mortalities.

>   *h. 1/10 of the LD$_{50}$ (as it pertains to juveniles–not a separate input)*

The EU Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2008) alternatively proposed to use 1/10 of the adult LD$_{50}$ to assess the ability of juveniles to grow and develop. This is based on the assumption that for precocial young, at least, there is no systematic difference between the relative sensitivity of juveniles and adults (Hudson et al. 1972). There may be differences on a substance-by-substance basis, but no systematic correction factor is available. It should be noted that this may not be the case for altricial young (i.e., species where the young hatch blind and are tended by their parents, such as passerines). For example, altricial juveniles have been shown to be more sensitive to cholinesterase-inhibiting chemicals than adults (Wolfe and Kendall, 1998). However, it is not known whether this difference applies to pesticides with other modes of action. In the absence of any further information, it is proposed that 1/10 of the LD$_{50}$ be used as the surrogate endpoint for direct toxicity to juveniles, and it should be compared to the expected dietary dose to juveniles on each day during the nestling rearing phase (i.e., 1-day EDD).

>   *i. Other integrative endpoints*

There are other endpoints measured in the avian reproduction test that have not been mentioned as possible surrogate endpoints; however, there are situations where they may be adequate substitutes for the default surrogate endpoints. These endpoints are primarily expressed as counts (i.e., the number of eggs set, viable eggs, 3-week live embryos, hatchlings, or 14-day-old chicks per hen) and integrate information from a combination of factors including different types of possible effects and husbandry issues. For example, the number of eggs set per hen represents the number of eggs laid minus the number of eggs removed for eggshell quality measurement and the number of eggs cracked for any number of reasons. The number of 14-day-old chicks per hen integrates many of the types of effects on parents via direct exposure and on embryos/chicks via *in ovo* exposure into a single metric. Surrogate endpoints are most effective in models of reproductive success when they represent a specific type of field effect. Usually a response observed in integrative endpoints should also be observed in the more effect-specific endpoints that are proposed as surrogate endpoints. However, in some avian reproduction tests the data analysis may indicate that an integrative endpoint is more sensitive (i.e., lower NOAEL) than each of the proposed surrogate endpoints that form the basis for the integrative endpoint. An example of this would be if the NOAEL for the number of hatchlings per hen was lower than the NOAELs determined for the number of eggs laid per hen, the proportion of viable embryos per egg set, and the proportion of hatchlings per viable egg. In some cases, this may result from differences in statistical power among dissimilar endpoints. In other cases, it may be the cumulative impact of integrating several types of specific effects. When this occurs, it is critical to determine if the lower NOAEL in the integrative endpoint simply reflects significant parental effects, such as a treatment-related decrease in the number of eggs laid, or a true measure of *in ovo* effects independent of parental effects.   Unless there is a clear indication of *in ovo* effects independent of possible parental effects, an integrative endpoint could be a very misleading substitute for surrogate endpoints already proposed.

3. Selecting toxicity threshold values for each surrogate endpoint from toxicity tests

For each of the default surrogate endpoints, a toxicity threshold value is determined that represents the daily dose below which the risk of adverse effects is considered acceptable. In most avian reproduction tests with experimental designs based on hypothesis testing, the toxicity threshold value may be defined as the no-observed-adverse-effect level (NOAEL). Where quantitative dose-response relationships have been defined, the toxicity threshold level may be expressed as an EC_{xx}. In the conceptual approach presented in Bennett et al. (2005), the toxicity threshold values for each surrogate endpoint derived from the avian reproduction test were defined as the NOAEL determined in the test. This reflects the common practice of using the NOAEL of avian reproduction endpoints for characterization of risks via risk quotients. When avian reproduction tests were conducted for two species (e.g., northern bobwhite and mallard), Shore et al. (2005) used the lower of the two NOAELs for each surrogate endpoint. However, the selection of the toxicity threshold value to use in decisions is a combination of science (e.g., what is a biologically-meaningful description of level of effect?) and policy (e.g., what is the intended level of protection?). A European Union opinion paper on pesticide risks to mammals presents an argument for why a higher value than the NOAEL may be appropriate for some surrogate endpoints (EFSA 2006). Specifically, they argue that for sensitive endpoints a statistically-significant difference may not equate to a biologically-significant effect and that the acceptable toxicity threshold may be at the LOAEL or higher. In other cases, it may be argued that due to low statistical power in a test, the NOAEL for a surrogate endpoint may not be considered suitably protective, so a lower value may be more appropriate. Ultimately, risk assessors need to evaluate the toxicity threshold value associated with each surrogate endpoint to insure it is suitable for the intended level of protection in the assessment.

The EFSA (2006) document states that the use of "acceptable levels" in place of NOAELs would require:

1) quantification of the dose-response relationship and its uncertainty;

2) knowledge of the functional relationship between the measured parameter and individual reproductive success and survival;

3) knowledge of the relationship between individual reproductive success and impacts on population dynamics;

4) knowledge of how these functional relationships in 2) and 3) vary between mammal species with different life-history and developmental traits, and vary between captive (often inbred) and wild mammals; and

5) a risk management judgment about what types and magnitude of effects are acceptable.

Although these requirements were written regarding mammals, they are equally relevant for birds. Adoption of such an approach for selecting "acceptable levels" is currently hampered by poor definition of the dose-response relationship for most pesticides and by lack of knowledge in areas 2), 3) and 4) above.

Although toxicity threshold values are expressed as daily dose (µg AI/g body wt/day), the surrogate endpoints from the avian reproduction test and the dietary LC50 test are typically reported as dietary concentrations (µg AI/g food). Dietary concentrations need to be converted to daily doses by the model user for use in MCnest. The conversion from concentration to dose

can be accomplished using information on body weights and daily food ingestion rates (FIR) from the toxicity test:

$$\text{Daily dose (mg AI/kg /day)} = \frac{\text{Dietary concentration (mg AI/kg food) x FIR (g food/day)}}{\text{Body weight (g)}}.$$

This conversion is an approximation because body weight and food ingestion rates are changing during the course of both the reproduction test and the LC50 test. In the reproduction test, both body weights and food ingestion rates increase as birds move into egg production. The LC50 uses juvenile birds that grow rapidly during the test. Also, laboratory studies vary in the degree to which they quantify food spillage during the tests. One option for converting dietary concentrations to daily doses is to 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. Because of differences among tests in exactly how body weights and food ingestion rates are reported, it may be difficult to standardize a specific algorithm for making this conversion. Consequently, the Basic Version of MCnest does not convert dietary concentrations into daily doses. This is the responsibility of the model user.

Another issue that complicates the selection of toxicity threshold values for some surrogate endpoints from the avian reproduction test is not having "bounded NOAELs," defined as the next lower tested dietary treatment group below the lowest-observed-adverse-effect level, or LOAEL, determined by analysis of variance. Even in well-designed avian reproduction studies, some endpoints may not be affected by the pesticide within the range of dietary treatments tested (i.e., the mean responses for the endpoints at all treatment levels are not statistically different from controls). In such cases, the NOAEL is reported as being the highest tested treatment level (i.e., an "unbounded NOAEL"), but it is not known if a statistically-significant effect would occur at levels just above those tested or not at all because the endpoint is insensitive to the chemical. This highest treatment level could be selected as a conservative estimate of the toxicity threshold value for a surrogate endpoint, but this becomes problematic if a proposed application rate results in an estimated exposure dose that is higher than the highest treatment level used in the test. When this occurs at a MCnest decision point, the nests would be considered to have failed even though there may be no evidence from the test to indicate that such failures might occur or be possible. Consequently, the model user should evaluate each surrogate endpoint on a case-by-case basis. An alternative approach when a surrogate endpoint has an unbounded NOAEL is to not designate a toxicity threshold value (e.g., leave it at the default value of 9999) that would lead to a nest failure, unless there is additional evidence on which to base an appropriate toxicity threshold value.

4. Addressing interspecies variability in selecting toxicity thresholds

There is little guidance for addressing interspecies variability for effects on reproductive success. Development of species sensitivity distributions has focused primarily on acute toxicity data since avian reproduction tests are conducted on so few species–typically northern bobwhite and mallards. Mineau et al. (2001) considered that variation among species in reproductive tests would be at least as great as that observed in acute tests and recommended that extrapolation factors derived from acute toxicity data could be applied to reproductive endpoints. Luttik et al. (2005) summarize possible approaches for addressing interspecies variability in effects on avian

reproductive endpoints and recommend a method for estimating extrapolation factors proposed by Luttik and Aldenberg (1995, 1997).

The Basic Version of MCnest does not explicitly address modifications of toxicity thresholds based on differences in toxicological sensitivity among species. It is the responsibility of the model user to determine if interspecies extrapolation factors (or other means of addressing interspecies variability) are warranted. If they are, the model user may modify the input values for the toxicity thresholds accordingly. Shore et al. (2005) present an example of this approach.

5. __Modifying or adding surrogate endpoints__

The list of default surrogate endpoints in the Basic Version of MCnest was developed to address most types of direct effects to reproduction. However, for some pesticides it may be appropriate to modify existing surrogate endpoints or add new surrogate endpoints based on knowledge of the chemical. Users should clearly articulate the rationale for changes to the default list of surrogate endpoints and realize that changes appropriate for a specific pesticide may not be appropriate for others.

Some modifications to existing surrogate endpoints have already been discussed above, including:

1. Using a toxicity threshold value different from a NOAEL from the avian reproduction test for surrogate endpoints where there is information to justify using a higher or lower value.

2. Using related endpoints that make up one of the existing surrogate endpoints (e.g., using the proportion of 3-week live embryos per number of viable eggs and/or the proportion of hatchlings per number of 3-week live embryos in place of the proportion of hatchlings per number of viable eggs).

3. Using related integrative endpoints (i.e., counts rather than proportions) to replace an existing surrogate endpoint when an argument can be made that the integrative endpoint is a better reflection of the response to the pesticide.

For many pesticides, there may be additional information available from non-standard laboratory studies and field studies for forming the basis of a new surrogate endpoint, especially if it can be argued that an endpoint based on this information is a better, more direct indicator of effects in the field. For example, if a field study provided data on the rate of nest abandonment during incubation caused by a pesticide application of known application rate, this information may provide a more direct surrogate endpoint than using changes in body weight or a fraction of the LD50. Similarly, pen or field studies may provide more useful information on juvenile mortality from direct exposure (i.e., not *in ovo* exposure) than can be provided from the LC50 or LD50 test.

A critical element in creating a new surrogate endpoint is making sure the endpoint is matched up with the appropriate exposure period. This is relatively straightforward if the endpoint represents an effect that can occur very rapidly after exposure. In these cases the surrogate endpoint might be matched with single-day exposures occurring during each day of

that particular breeding phase. However, if a surrogate endpoint reflects a delayed response after a period of exposure (i.e., effects on hatchability occurring from *in ovo* exposure during the time of egg formation), the user must make sure that the endpoint is matched with the appropriate time-weighted average during egg formation.

The Basic Version of MCnest is not designed to insert completely new surrogate endpoints in addition to the current default list. If a user had data sufficient to create a new surrogate endpoint that is essentially a replacement for an existing one and using the same exposure period, then the user can simply insert the toxicity threshold value for the new surrogate endpoint in place of the existing endpoint. For example, if data from a field study indicated that the pesticide had a greater impact on hatchability via *in ovo* effects compared to using the NOAEL for hatchability from the reproduction test, the toxicity threshold value derived from the field could be used to parameterize the hatchability endpoint in the model. However, if a new surrogate endpoint requires a different exposure period than exists for the default surrogate endpoints, the Basic Version of MCnest does not have this capability, though future versions of MCnest could be modified to include additional surrogate endpoints as needed.

C. Parameterizing the pesticide-use scenario

In addition to selecting pesticide toxicity information for use in phase-specific decisions in MCnest, the pesticide-use scenario needs to be specified for each simulation run, including the timing of application(s), the application rate(s) expressed in pounds active ingredient per acre, and the half-life of residue degradation on foods relevant to the species of interest. The Basic Version of MCnest can simulate single or multiple applications of a pesticide by specifying one to five dates of applications and the application rate for each date. In each model simulation, all individuals in a population of breeding birds are exposed to the pesticide application(s) on the specified date(s).

It is recognized that application dates are not synchronized within a geographical unit, and that the individual birds within a free-ranging population may experience applications occurring throughout a range of dates. MCnest does not try to simulate a distribution of application dates, which can be quite variable over time and space due to factors such as weather and pest levels. Also, running simulations based on a distribution of application dates can obscure information about the potential effects on specific dates when an avian species may be at greatest risk to a particular chemical. Instead it is recommended that simulations be run for a series of specific dates (using the batch function) covering the range of possible application dates. By examining the model results for a series of possible application dates, the user has more information from which to determine which specific dates can lead to the greatest impacts on each species. The user also gains sufficient information to estimate the overall effect to a population exposed to any assumed distribution of application dates.

To account for the dissipation of pesticide residues over time, the model user also must specify the half-life of residues on avian food items for a particular pesticide. Residue half-life values on plant foods should be obtained from the literature or from registrant submitted studies. If no foliar residue half-life value is available for a pesticide, USEPA Office of Pesticide Programs uses a default value of 35 days based on the work by Willis and McDowell (1987) that reported foliar residue half-lives for approximately 80 pesticides, with a maximum value of 36.9

days. The average half-life (± standard deviation) across all formulations and extraction methods for organochlorine, pyrethroid, organophosphorus, and carbamate insecticides was 5.0 ± 4.6 d, 5.3 ± 3.6 d, 3.0 ± 2.7 d, and 2.4 ± 2.0 d, respectively.

In the Basic Version of MCnest, pesticide exposure to a population of birds is simulated by estimating the initial daily dose for each species on the day of application and assuming that exposure decreases over time based on the half-life of residue degradation on foods relevant to the species of interest. The model user has four options for determining the initial daily dose for each species. Three of the options are based on the approach used in OPP's Terrestrial Residue EXposure model or T-REX (USEPA 2012) for translating an application rate into the dietary concentrations on various food types and, finally, into an initial daily dietary dose (µg/g body weight/day) for each species based on its body weight, food ingestion rate, and diet composition. The dietary concentrations on plant food types used in T-REX (Table 3) are based on the reviews by Hoerger and Kenaga (1972) and Fletcher et al. (1994) that estimated the mean and maximum residue concentrations on various categories of plant food types after chemical application. OPP has developed a nomogram showing the relationship of initial residue concentrations among various plant categories as a function of application rate. In MCnest, the model user can base the initial exposure doses on: 1) the maximum residue values for each food type, 2) the mean residue values, or 3) values drawn from a log-normal distribution of residue values for each bird based on the mean and standard deviation of each food type in Fletcher et al. (1994). For the third option, once an initial value is drawn from the distribution for each female, the individually-calculated exposure dose decreases based on the residue half-life. This assumes that the distribution of initial daily dose values reflects the variation in the initial pesticide concentrations possible within and among different fields, rather than individual food items, and that the average initial pesticide concentration in some bird feeding territories is higher than in other bird's territories. Consequently, instead of having each female in the simulation receive the same mean or maximum initial dose, the third option results in each female being randomly assigned an initial dose from the distribution. It should be noted that the lognormal distribution results in values at the upper end of the distribution that exceed the maximum nomogram value. The fourth option allows the model user to directly set a specific initial dose for both adults and juveniles independent of an application rate.

In the Basic Version of MCnest, birds are assumed to get 100% of the daily food intake from treated fields. This is consistent with the approach used in T-REX. Future versions of the model could integrate species-specific information of the proportion of the diet derived from treated fields if a suitable approach is available.

Each species life-history profile includes the estimated proportion of each of six food type categories in the total diet. The six categories are: 1) short grass, 2) tall grass, 3) broadleaf forage plants, 4) fruits, 5) seeds and pods, and 6) insects and other invertebrates. Where possible, the dietary composition information is based on breeding season diets.

Table 3. Maximum and mean (plus standard deviation) residue concentrations used in T-REX for the initial dietary concentrations expected on various avian food types immediately after a pesticide application of 1 lb active ingredient/acre.

Dietary-based EECs (µg/g food)	Initial Residue Concentrations		
	Maximum	Mean	Standard deviation[1]
Short Grass	240	85	60.3
Tall Grass	110	36	40.6
Broadleaf Forage Plants	135	45	56.7
Fruits	15	7	12.4
Seeds & pods	15	7	12.4
Insects & Other Invertebrates[2]	94	65	48

[1] Standard deviations for plant food types derived from Fletcher et al 1994.
[2] Initial residue values for insects derived from Appendix B of the User's Guide for T-REX Version 1.5 (USEPA 2012)
(http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm#app_a).

Just as in T-REX, MCnest converts an application rate (expressed as pounds active ingredient/acre) into an initial daily dose for adults (expressed as µg/g body weight/day) using the information on average body weight, food ingestion rate (FIR), and diet composition for each species of interest. The FIR_{dry} (g dry weight/day) is estimated from an allometric equation for all birds ($FIR_{dry} = 0.648 *$ Body weight (g wet weight) $^\wedge 0.651$) from Nagy (1987). To convert the FIR from dry to wet weight, the diet composition must be considered because the moisture content for seeds is assumed to be lower than for other food types. In T-REX, the mass fraction of water in food types is 0.1 for seeds and 0.8 for all other food types (e.g., insects, fruits, leaves). To calculate the FIR as wet weight (FIR_{wet}):

$$FIR_{wet} = FIR_{dry} / (S * 0.9 + (1 - S) * 0.2),$$

where S = proportion of seeds in the diet. The amount of food ingested as a proportion of body weight (FIR/BW) is calculated by dividing FIR_{wet} by the average body weight. The initial daily dose (IDD) for each species is calculated as the sum across food types of the initial residue concentration for each food type (C_i) multiplied by FIR/BW and the proportion of that food type in the diet of a species (P_i):

$$IDD = \sum C_i * FIR/BW * P_i.$$

Juvenile body weights and FIRs change rapidly from hatching to fledging, and empirical information on the changes in weights and food consumption of nestlings is lacking for most avian species. Appendix R of the European Union (EU) Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2009) calculated the FIR/BW ratios for each day of the nestling period based on the work of Williams and Prints (1987) on savannah sparrows and Kendeigh et al. (1977) on house sparrows and found a peak ratio for FIR/BW of 1.08 at about day 3 after hatch. They proposed that the juvenile IDD be calculated by multiplying the residue concentrations of various food types in the diet by the 1.08 FIR/BW ratio. This may be appropriate for species that feed nestlings invertebrates and other high moisture-content foods,

but for species that feed seeds to nestlings (e.g., mourning doves, American goldfinches), this will overestimate juvenile food ingestion rates. In the EFSA (2009) analysis, the 1.08 FIR/BW rate was based on a 4.24 g nestling consuming 4.58 g of insects (wet weight) per day, but not all species feed nestlings an all invertebrate diet. Using the moisture-content assumptions in T-REX, the 4.58 g of insects (wet weight) is equivalent to 0.916 g (dry weight) per day. Assuming the moisture content of seeds is 10%, the equivalent wet weight daily consumption rate would be 1.02 g seeds, resulting in a FIR/BW ratio of 0.24 for a seed diet. Until better information is available, the Basic Version of MCnest uses a modification of the approach recommended for the EU to calculate the IDD for juveniles where the seed portion of the juvenile diet is multiplied by a FIR/BW of 0.24 and the non-seed portion is multiplied by 1.08.

MCnest also has the capability to use exposure profiles generated by EFED's Terrestrial Investigation Model (TIM) instead of the simple T-REX exposure profile described above. Details for implementing the integration with TIM exposure profiles are still being developed and are beyond the scope of the current draft of the technical manual.

1. Consequences of expressing exposure as dose instead of dietary concentration

Historically, risk quotients for avian reproductive effects have been calculated on the basis of dietary concentration of the pesticide. The lowest NOAEC from acceptable avian reproduction tests, expressed as the concentration of test substance in the diet, is compared to the estimated environmental concentration on various types of avian foods just after an application. By basing reproduction risk quotients on dietary concentrations, T-REX considers the differences in expected concentrations among food type categories listed in Table 3, but it does not address differences in moisture content among food categories or differences in the amount of food consumed as a function of body weight (i.e., FIR/BW). In MCnest, by incorporating the approach used in T-REX to calculate acute risk quotients, risks to reproductive success are based on the daily ingested dose by considering not only the residue concentrations on food types, but also moisture content and FIR/BW.

Although the allometric equation in Nagy (1987) estimates the daily ingestion rate (expressed on a dry weight basis) for each species as a function of body weight, non-seed food types have a much higher moisture content than seeds, so species that consume non-seed food types ingest a greater amount of food on a wet weight basis than granivores. Based on the moisture content values used in T-REX, a non-granivore would consume 4.5 times more food on a wet weight basis than a granivore of the same body weight. Consequently, even though the residue concentrations on fruits and seeds are the same after application based on values in Table 3, the IDD for a frugivore would be 4.5 times higher than the IDD for a same-sized granivore.

Dose also varies as a function of body weight due to the allometric equation in Nagy (1987), resulting in the FIR/BW ratio increasing as body weight decreases. Consequently, regardless of the food type eaten, a 20-g or 100-g bird ingests a dose 3.9 or 2.2 times higher than a 1000-g bird, respectively, based on the calculations used in T-REX. Within a food type category, the specific food items selected by a 20-g bird vs a 1000-g bird likely differ, but there are insufficient data to determine how this might affect the relative difference in dose ingested between these two birds.

The consequences of expressing exposure as a dose instead of as a dietary concentration are that when the expected environmental exposure is near the toxicity threshold for sensitive surrogate endpoints, MCnest simulations may indicate a reduction in seasonal productivity for some species, even though the traditional risk quotient approach that considers only the dietary concentration does not indicate a risk of adverse effects to those species. This is primarily possible for smaller species and non-granivores. However, there are several points for model users to keep in mind when evaluating the output from MCnest in relation to assessments based on the traditional risk quotient approach for reproductive effects. At the screening level, risk quotients for reproductive effects (i.e., often referred to as "chronic risk quotients") are used to determine if there are values that exceed the established level of concern (LOC) so that the pesticide can be classified as to whether there is or is not a presumption of unacceptable risk. Although T-REX calculates reproductive risk quotients based on diets containing short or tall grass, broadleaf plants, fruits, seeds, or invertebrates, the risk conclusions are based on the highest quotients calculated, and the risk quotients for consumers of short grass are always 16 times higher than for granivores and 2.5 times higher than for insectivores (based on the maximum values in Table 3). Consequently, risk quotients are primarily designed to determine which pesticides do not need further assessment because even when using worst-case assumptions their quotients do not exceed LOCs, but risk quotients do not provide information on the probability or magnitude of risk and tell us little about which species or life-history strategies may be at risk.

MCnest is intended to quantify the effects to reproductive success for a range of avian species to provide more information on which species or life-history strategies are at greatest risk or which exposure scenarios produce the greatest effects. It does that, in part, by refining the exposure profile for each species by considering the diet composition during the breeding season and by refining the estimated daily dietary exposure by integrating information on the moisture content of foods in the diet and on the food ingestion rate for the body weight of each species. MCnest is based on the assumption that daily dose is a more ecologically-relevant expression of exposure than simply using residue concentrations on food types as the only factor. We also assume that MCnest is best suited to be used in higher tier risk assessments for pesticides where there is already a presumption of unacceptable risks based on screening-level assessments that requires further refinement and analysis. However, refining the estimates of daily dose for a series of species focuses attention on the empirical basis for the default assumptions used in those estimates. For example, how much variation is there in moisture content within food types and are the default moisture content values similarly representative of the moisture content of diets among species? Are the maximum and mean nomogram values (along with the standard deviations) for each food type presented in Table 3 representative of the distribution of residue values occurring on the foods consumed by each species? These default values may be the best information we have currently, but given their importance in the estimation of daily doses for avian species, additional research may be needed to improve the quality of dose estimates. While the basic version of MCnest uses default values from T-REX for moisture content and nomogram values, all of these input parameters are editable by the model user.

D. Using default species life-history profiles

To run simulations in MCnest for specific avian species, a suite of life-history parameters is required to describe the typical series of events during a breeding season. In developing

MCnest, we focused on a generalized model requiring a limited number of life-history parameters that can be applied to a broad range of species with limited life-history data. Also, the model treats avian nesting in a manner consistent with the way in which nesting parameters are typically estimated in the field, thus reducing the potential for currency mismatch between the model and available ecological data for the species of interest. Because MCnest runs on daily time steps, all life-history parameters expressed as durations (i.e., number of days) must be set as integer values.

The Basic Version of MCnest accesses a series of default life-history profiles for avian species associated with agroecosystems. These species profiles are draft versions for a range of life-history strategies and at this point are available for demonstration purposes. Once the Basic Version of MCnest is finalized for distribution and the exact structure for life-history profiles has been approved, the current series of life-history profiles will be finalized for peer review and work will begin on expanding the library of species, including variations in species profiles where important differences exist among geographical regions within a species range. The current species profiles include one example of this for the tree swallow, where two profiles represent northern and southern parts of their range.

Typical values for each of the following life-history parameters were harvested from the published literature. Each of the parameters is subject to variation among locations, within and among breeding seasons, and among techniques and study designs used to collect data. For most species, there is insufficient information to understand the extent to which these factors truly affect parameter estimates. For species with several published studies with consistent results, a mean value may be selected, while for other species the selected values may come from a single high quality study. Some life-history parameters are relatively easy to quantify and have a long history of being reported in the published literature (e.g., clutch size), while other parameters may be difficult to quantify or their quantification varies depending on the field methods employed (e.g., waiting periods from success or failure until first egg is laid in new nest). Consequently, when using the word "typical" in selecting values for each life-history parameter, the goal is to create a species profile that provides a reasonable representation of a species' breeding season across its entire range. It is recognized that the life-history parameters for many species with extensive ranges may vary among regions. This is especially true of the timing and duration of the egg-laying period. Future versions of the default species profiles may include region-specific species profiles for species with adequate data. Currently, model users can use the default life-history parameters or modify values to reflect regional variability.

Model users also may want to modify a species profile to represent a "reasonable worst-case" profile for a species in order to assure that model projections are adequately protective of all regional populations and subpopulations. While this may seem simple in concept, it must be approached with great caution in MCnest where temporal issues are extremely important. For example, because the start and end of egg laying and the length of the egg-laying period may vary from site to site, a model user may want to know what start and end egg-laying dates produce the greatest relative impact on reproductive success. However, the reality is that changing these dates may increase the apparent impact under some pesticide-use scenarios and decrease it under others. The bottom line is that no modifications to species life-history parameters will result in a relative impact that is universally a worst-case scenario. This is an issue that we will continue to explore as the species library expands and regional variations in

species profiles are considered. However, at this point model users are advised to use caution and explore changes in species profiles on a case by case basis.

1. **Species life-history parameters**

Each species life-history profile is defined by the following suite of parameters:

a. Initiation probability

Although species vary in the degree to which nest initiation is synchronized, there is variation in nest initiation dates among females in a population. For most species there is insufficient information to empirically define a distribution of first nest initiations, so the Basic Version of MCnest uses a geometric distribution defined by the value of the initiation probability to create a distribution of nest initiation dates for each simulation. Starting on the date of the first egg laid in the first nest of a season (T_1), the initiation probability defines the probability that a female that has not yet started laying will initiate the first nesting attempt. The default value for the initiation probability in each species life-history profile is 0.25 (i.e., each day 25% of the remaining females in the population initiate their first nest attempt). The initiation probability must be > 0 and ≤ 1. If the initiation probability is set equal to 1, all females initiate nests on the same date (i.e., the first day of egg laying, T_1). The lower the initiation probability, the broader the distribution of nest initiation dates.

Research will continue into methods for improving the definition for the start and end of the egg-laying period for females of each species, but those methods are not currently available for the Basic Version of MCnest. In the meantime, if a model user has data for a specific distribution of first nest start dates for a particular species, there is a way (albeit more cumbersome) to use that distribution instead of the default initiation probability. The model user will need to run a series of simulations by resetting the initiation probability to 1 and using a series of dates for T_1 that represent the range of first nest start dates. Next calculate the sum of number of successful broods per female on each date multiplied by the probability of nests starting on that date. This will provide an overall estimate of the number of successful nests per female based on the user-provided distribution of start dates.

b. Daily background nest failure rate during laying and incubation (m_1)

Nests fail due to a variety of non-pesticide causes, such as predation and adverse weather events. Many nesting studies report data on nest survival during laying and incubation as a daily nest survival rate using methods, such as those described by Mayfield (1961, 1975), to account for biases existing when the fate of each nest is not known throughout the entire period. The daily nest mortality rate (m_1) is 1 minus the daily nest survival rate. Other studies may report only the apparent nest survival or failure rate during the egg-laying and incubation periods (i.e., # successful or failed nests/total # nests). The apparent rates can be converted to the daily rate over the number of days for the egg-laying and incubation periods, as follows:

$$s_1 = \sqrt[a_1]{S_1}\,,$$

where,

s_1 is daily nest survival rate,

S_1 is overall survival rate for egg-laying and incubation periods, and

a_1 is the age, in days since the first egg was laid, at which nests typically hatch. Then:

$$m_1 = 1 - s_1.$$

However, this approach introduces bias by assuming knowledge of the fate of nests over the entire period when this is not the case. Apparent nest success rates overestimate the daily nest survival rates because some nests may fail before detection; however the degree of overestimation varies considerably due to the specific methods used in studies.

Some studies will report only an overall apparent nest survival rate for the entire nesting period (i.e., egg and nestling phases). As in other situations, overall nest survival rates can be converted to the daily rate over the number of days for the entire nest period (i.e., egg-laying, incubation, and nestling-rearing periods), with the same daily rate assigned to m_1 and m_2.

c. Daily background nest failure rate during nestling rearing (m_2)

Many nesting studies report data on daily nest survival rates during the nestling rearing phase. When only an apparent nest survival rate for the brood rearing phase is reported, it can be converted to a daily nest survival rate over the number of days in the brood rearing phase.

d. Date of first egg laid in first nest of season (T_1)

The length of the clutch initiation period is defined by the difference between the first egg in the first and last nests of the season (i.e., $T_{last} - T_1$). In the Basic Version of MCnest, new nests can only be initiated within this period. However, even though some literature sources report extreme egg-laying dates, the T_1 parameter is intended to represent when egg laying typically begins for the species of concern. Using extreme laying dates for T_1 and T_{last} may overestimate the length of the clutch initiation period for a species, resulting in more nest attempts than are commonly observed. The value for T_1 could represent the mean of several "first dates" reported from multiple studies or from a high quality study over multiple years.

e. Date of first egg laid in last nest of season (T_{last})

Similar to T_1, this represents the typical date for the first egg in the last nest of the season. Again, this is not intended to represent extreme egg-laying dates as that would serve to extend the simulated length of the breeding season and bias the model output.

f. Length of rapid follicle growth period in days for each egg (rfg)

Unlike fish, amphibians, and reptiles, birds do not lay their eggs in masses. Instead, most birds lay an egg each day until they complete a clutch, while some birds may lay an egg every other day or some other period longer than one day. The follicles that develop into egg yolks also start growing on a staggered schedule over a several day period, known as the rapid follicle growth (rfg) period. During the rfg period yolk material is deposited to the growing follicle until it reaches the size of a fully formed yolk just prior to ovulation.

Although estimates for the duration of the *rfg* period are available only for a subset of species, there is sufficient empirical information for estimating the *rfg* period for most species. Many song birds (i.e., passerines) have an *rfg* period of 3 to 4 days while in doves and pigeons the period is approximately 6 days. See Appendix A (Estimating the length of the rapid follicle growth period) for additional detail. The value selected for the duration of the *rfg* period must be an integer.

g. Length of eggshell formation period in days for each egg (ef)

After the yolky follicle is ovulated, it enters the oviduct for deposition of the albumin followed by formation of the egg membrane and shell. This process usually takes approximately 24 hours for most species. Consequently, the eggshell formation period is fixed at 1 day in the model, and thus does not show up in MCnest as one of the input parameters.

h. Mean clutch size (clutch)

Clutch size is one of the most commonly reported avian life-history parameters in the literature. Here again, the intent is to select a value representing the typical clutch size of a species rather than extreme values. The value selected for *mean clutch size* must be an integer.

i. Mean inter-egg laying interval in days (eli)

As mentioned above, most birds lay one egg each day, while other species may have a longer mean inter-egg laying interval. The value for *eli* can be any value ≥ 1 day and can be expressed as a decimal value.

j. Egg on which female typically begins incubation–penultimate vs ultimate (penult)

Those species beginning incubation after the last egg is laid are assigned a value for *penult* of 0, while those beginning with the penultimate egg are assigned a value of 1. For those species where both options are possible, a judgment is made as to which option is more typical for the species.

k. Duration in days from start of incubation to hatch (I)

The duration of the incubation period (*I*) also is a commonly reported life-history parameter in the literature. A typical value for the duration of the incubation period should be selected and it must be expressed as an integer. For pesticides that affect egg hatchability because of embryotoxicity due to *in ovo* exposure or infertility, there is a related parameter known as "doomed incubation" or *Id*. When pesticide exposure is high enough to cause embryotoxicity or reduced fertility, it is assumed the female does not become aware that these effects have occurred until the time at which eggs are expected to hatch. Consequently, the female continues to incubate the clutch of eggs that has failed a decision point and is considered to be "doomed," but the nest attempt does not actually fail until the end of incubation period when the eggs fail to hatch on schedule. In the Basic Version of MCnest the duration of the *Id* period is set to the same value as *I*.

l. Duration in days from hatch to fledging of nestlings (N)

The duration of the brood rearing period also is a commonly reported life-history parameter in the literature. A typical value for the duration of brood rearing should be selected and it must be expressed as an integer. Nestlings of some species can leave the nest early when stressed by predators or weather events such as floods. While many studies report a wide range of fledging durations that reflect that some nests fledge early under stress, the intent is to select a typical value for the brood rearing period reflective of non-stressed conditions (i.e., how long do nestlings typically remain in the nest if not stressed?).

m. Duration in days since nest failure due to non-pesticide reasons until female initiates new nest (W_e)

After a nest failure due to environmental causes such as predation or weather, females may attempt to renest after a period of recovery and reinitiation of the egg formation process. The value for W_e represents the duration from nest failure until the first egg is laid in a new nest and must be expressed as an integer. Many high quality studies have data on the duration of this period.

n. Duration in days since nest failure due to pesticides until female initiates new nest (W_p)

After a nest failure due to pesticide exposure, females also may attempt to renest after a period of recovery and reinitiation of the egg formation process. W_p represents the duration from nest failure until the first egg is laid in a new nest and must be expressed as an integer. Of all the life-history parameters, W_p may have the poorest amount of information for selecting a value. Pesticide field studies typically do not provide information on the probability or timing of renesting after a pesticide-related nest failure. Occasionally, laboratory reproduction studies are designed to include a period of untreated food at the end of the treatment period. These studies can provide information on the potential for egg production to increase or restart after treatment ends, though it is not clear if this is indicative of the potential for free-ranging birds to renest. Depending on the nature of the pesticide, the model user might assume that W_p equals W_e if birds recover quickly from an exposure. However, for chemicals with prolonged or delayed effects after exposure, a longer duration may be appropriate for W_p. A conservative assumption would be that females do not renest after pesticide failure (i.e., set W_p to a value larger than the length of the breeding season). However, suitable field examples of renesting periods after a pesticide-induced failure have not been found that provide a basis for additional guidance. The W_p cannot be shorter than W_e.

As a default in the Basic Version of MCnest, W_p is set equal to W_e. If a model user decides to change the value for W_p, it is unlikely that there would be sufficient information to set species-specific values, so the value for W_p is not located on the "Life History" window of each species. If the model user is running simulations on a single species, the value for W_p can be changed on the "Set Pesticide" window; however, if multiple species are being simulated using the "Batch mode," there is a toggle switch for overriding the W_p value used for all selected species. If a model user inserts a value that overrides the default W_p values, this value will be

used as the W_p for all species, except for species where the W_e value is larger, in which case the value of W_e is substituted for W_p.

o. Duration in days since successful fledging until female initiates new nest (W_f)

After successfully fledging an initial brood, some species will attempt one or more additional broods. W_f represents the duration from successful fledging until the first egg is laid in a new nest and must be expressed as an integer. In some species, fledglings become the responsibility of the male while the female immediately initiates a new clutch of eggs. In other species, both males and females continue to feed and care for fledglings until they become independent–a period of up to several weeks–after which the female may become available to start a new nest if time remains in the breeding season. When there is a period of female involvement in post-fledging care, the estimates of the period of time until the female renests found in the literature can be quite variable. It is not always clear if the shorter estimates reflect that some females renest relatively rapidly even if they still are assisting with fledgling care or that some females have lost their entire broods prior to becoming independent.

p. Mean female body weight and diet composition during breeding season

MCnest simulations involving a pesticide exposure require information on the mean female body weight (in grams) and diet composition, ideally representing weights and diet during the breeding season, as well as diet composition of juveniles prior to fledging. The body weight and diet information is used in converting application rates into the estimated daily dietary dose (in mg/kg body weight/day) for each species based on the algorithm used in OPP's T-REX model (http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm). The diet composition in MCnest species' profiles is expressed as the proportion of the diet in the six food categories presented in Table 3. It is intended that the proportions are based on the mass (wet weight) of each type, but in some species literature information on diet composition may only be expressed as volume or counts of food items.

q. Mean number of fledglings per successful nest

Each species profile contains an estimate of the mean number of fledglings per successful nest, which is multiplied by the number of successful nests per female per season (i.e., the primary output from MCnest simulations) to estimate the number of fledglings per female per season.

2. Representativeness of species profiles

In pesticide risk assessments, the number of successful broods produced under a scenario without pesticides is compared with results under a specific pesticide-use scenario to calculate the percent reduction in annual reproductive success due to pesticide exposure. The estimate of the number of successful broods also is used as a check of how well the default life-history profile of a species represents its breeding season output compared to available information in the literature. A comparison of MCnest output with literature-derived estimates of annual reproductive success is limited by the small number of studies designed specifically to monitor the cumulative production of juveniles throughout the breeding season in most species. Even though there are some studies that report estimates of annual reproductive success at a specific

place and time, it is often difficult to determine how representative those estimates are of the species in general.

To estimate annual reproductive success, the MCnest estimate of the number of successful broods per female was multiplied by an estimate of the number of fledglings per successful brood from the literature. The baseline estimates of the number of successful broods per female were based on MCnest simulations without a pesticide exposure using a population size of 100,000. Information on the mean number of fledglings per successful brood and sample sizes was gathered from literature sources. Although some sources report the number of fledglings produced per nesting attempt, we only used data based on the number of fledglings from successful nest attempts (i.e., one or more fledglings). Where there are multiple studies reporting mean numbers of fledglings, we based the estimate used in the species profile on the weighted mean number of fledglings per successful nest where sample sizes are reported or a simple arithmetic mean among studies where sample size information is lacking or incomplete.

Similarly, estimates of annual reproductive success were gleaned from the literature for 16 of the draft default species currently in the MCnest Species Library. This is limited by the small number of studies designed specifically to monitor the cumulative production of juveniles throughout the breeding season in most species. The methods for estimating reproductive success varied greatly among species–from models to empirical field estimates from marked populations. For eastern meadowlarks (Kershner et al. 2004) and dickcissels (Walk et al. 2004), field studies with radio-marked females documented annual reproductive success for marked populations. For many other species information about the number of fledglings per nest attempt or successful nest was integrated with information on the estimated number of broods per female to estimate annual reproductive success, though specific approaches varied by study. Also, Ricklefs and Bloom (1977) developed a simple model using length of breeding season, clutch size, nest success rate, and waiting periods following success or failure to estimate annual reproductive success for six species in the MCnest Species Library: horned lark, black-capped chickadee, eastern bluebird, American robin, northern mockingbird, and red-winged blackbird. Their model estimate was used for the chickadee and horned larks, but empirically-based estimates were used for the other species.

Based on the discussion in Etterson et al. (2009) on the use of a fixed breeding season length, we expected that MCnest-derived estimates of annual reproductive success may exceed estimates derived from field data. The comparison in Table 4 indicates that this is true in general. In two species where the literature-derived estimate was higher (i.e., horned lark and black-capped chickadee), these estimates came from the Ricklefs and Bloom (1977) model which also was based on a fixed-length breeding season. For many species, using a fixed-length breeding season will overestimate the number of nesting attempts because each female in the simulation continues nesting as long as there is still time in the breeding season, whereas in the field some individuals may quit earlier after a successful or failed nest attempt. This seems to explain the disparity observed for northern mockingbirds where the reported egg-laying dates span approximately 4 months, but in MCnest this results in a mean of over 5 nest attempts per female per year, which is much higher than the 2.7 nest attempts per female reported in Derrickson and Breitwisch (1992). The probability of quitting after a successful nest attempt may be relatively high in mockingbirds. In general, while there is anecdotal evidence that some females quit breeding early, there is little empirical evidence for quantifying quitting

probabilities. Additional research is needed on methods for improving the definition of breeding season for modeling annual reproductive success.

[NOTE: The comparisons of MCnest and literature-based estimates of annual reproductive success presented in Table 4 are preliminary because they are based on draft species profiles. Once the basic version of MCnest is finalized and the species profile database has been peer-reviewed and finalized, a more complete comparison of MCnest and literature estimates will be conducted. However, the preliminary comparisons are provided to help model users assess the representativeness of the MCnest species profiles.]

E. Modifying and creating species life-history profiles

Users may wish to create new species life-history profiles or modify existing default species profiles to fit a specific risk assessment application. For species with large geographic ranges, users may want to modify species profiles to reflect regional variability in key parameters, especially the starting and ending dates for egg laying.

To create a new species profile, species-specific estimates of each of the life-history parameters (described above) are needed. While there may be several studies published for a species that provide data on breeding season parameters, the challenge is in synthesis of available data because studies differ in their experimental designs, size and duration, techniques to collect data, methods for analyzing data, and formats for reporting data. Because of these differences, it is often difficult to determine realistic mean estimates of parameters across studies. As emphasized in the description of the life-history parameters, the goal is to select parameter estimates that are typical for the species that collectively provide a reasonable representation of the breeding season of the species.

Table 4. Calculated annual reproductive success (ARS) for each species compared to estimates derived from the literature.

Species	Successful broods/ female	Fledglings/ successful brood	Calculated ARS[1]	Literature reported ARS
Mallard	0.36	5.0	1.8	1.2
Mourning dove	2.79	1.85	5.2	3.6
Eastern phoebe	1.58	4.0	6.3	5.8
Horned lark	2.70	2.46	6.6	6.8[2]
Barn swallow	1.45	3.67	5.3	5.5
Black-capped chickadee	0.94	5.3	5.0	6.2[2]
Blue-gray gnatcatcher	0.7	3.0	2.1	1.8
Eastern bluebird	1.91	3.63	6.9	5.0
American robin	2.02	2.8	5.7	5.0
Northern mockingbird	2.07	2.8	5.8	2.5
Vesper sparrow	0.73	3.0	2.2	4.2
Dickcissel	0.62	2.9	1.8	1.2
Red-winged blackbird	0.72	1.86	1.3	1.3
Eastern meadowlark	1.08	3.46	3.7	2.6
American goldfinch	1.66	2.9	4.8	3.7
House sparrow	2.95	2.68	7.9	7.2

[1] Calculated ARS was derived by multiplying the MCnest estimate of the number of successful broods per female by the mean number of fledglings per successful nest from the literature.
[2] ARS based on model estimate in Ricklefs and Bloom 1977.

To modify an existing species profile, users can review the data and studies used in generating the default species profile and determine if modifying the selected parameter estimates is relevant and appropriate for their specific risk assessment application. For example, if a risk assessment is focused on a specific state or region, and there is a high quality breeding season study conducted in that area, the user may want to use that study to modify default parameters to be more reflective of that area. The timing of the breeding season, reflected in the starting and ending dates for egg laying, may be the parameters that are most variable among regions.

F. Running MCnest: Understanding the phase-specific decision points

When running a simulation on MCnest, each female will have one or more nesting attempts during a breeding season. During each breeding phase of the nesting attempt (see Table 2) there is a series of decision points related to possible responses to chemical exposures. This section discusses the decisions to be made during each of the breeding phases and the response

of the female if the estimated exposure is higher than the toxicity threshold values for the surrogate endpoints. This decision framework is diagrammed in Figure 1.

During the pair formation phase, there are two surrogate endpoints (i.e., the NOAEL of the pre-laying adult body weight and 1/10 of the LD50) for effects on adult well-being.

- Both surrogate endpoints are compared each day of the pair formation phase to the estimated daily dose for that day. On any day that the estimated exposure dose is greater than either of the toxicity thresholds for the two surrogate endpoints, the initiation of egg laying is delayed until the estimated exposure dose decreases below the toxicity threshold for both surrogate endpoints, at which point the female would move into rapid follicle growth for the first egg.

During the follicle growth and egg production phase, there are two surrogate endpoints (i.e., the NOAELs for the number of eggs laid per hen and mean eggshell thickness per hen) for effects on adult well-being.

- The toxicity threshold for the number of eggs laid is compared to the estimated daily doses during each day of the follicle growth and egg production phase. On any day during the phase that the estimated exposure dose is greater than the toxicity threshold for the number of eggs laid, the nest attempt is considered failed and the female enters the waiting period after pesticide failure.

- The toxicity threshold for eggshell thickness is compared to the estimated daily doses during each day of shell formation. On the day of shell formation for each egg laid if the estimated exposure dose is greater than the toxicity threshold for mean eggshell thickness, the nest attempt is considered failed and the female enters the waiting period after pesticide failure.

During the incubation phase, there are three surrogate endpoints (i.e., the NOAEL of the pre-laying adult body weight, 1/10 of the LD50, and the NOAEL for proportion of eggs set that contain viable embryos) for effects on adult well-being and one surrogate endpoint (i.e. the NOAEL for the percentage of viable embryos producing hatchlings) for effects on egg hatchability from *in ovo* exposure.

- Two of the surrogate endpoints for adult well-being (i.e., the NOAEL of the pre-laying adult body weight and 1/10 of the LD50) are compared each day to the estimated daily dose for that day. On any day that the estimated exposure dose is greater than either of the toxicity thresholds for these surrogate endpoints the nest attempt is considered failed, and the female enters the waiting period after pesticide failure.

- The third surrogate endpoint for adult well-being (i.e., the NOAEL for proportion of eggs set that contain viable embryos) is compared to the estimated daily dose on each day of the follicle growth and egg production phase. If the estimated daily dose during any day of the egg-laying period exceeds the toxicity threshold, the clutch is completed and enters a doomed incubation state, which means the female incubates the clutch, but the nest

attempt is considered failed on the date the eggs should have hatched, and the female enters the waiting period after pesticide failure.

- The toxicity threshold for egg hatchability endpoint is compared with a time-weighted average (TWA) for exposure during the rapid follicle growth and shell formation period of each egg in a clutch. When the TWA exposure for one or more eggs exceeds the toxicity threshold for hatchability, the clutch is completed and enters a doomed incubation state, the nest attempt is considered failed on the date the eggs should have hatched, and the female enters the waiting period after pesticide failure.

During the nestling rearing phase, there are two surrogate endpoints (i.e., the NOAEL of the pre-laying adult body weight and 1/10 of the LD50) for effects on adult well-being and three surrogate endpoints for nestling survival–one from *in ovo* exposure (i.e., the NOAEL for the percentage of hatchlings surviving to 14 days of age) and two from dietary exposure (i.e., fraction of the LC50 and 1/10 of the LD50).

- Both surrogate endpoints for adult well-being are compared each day to the 2-day TWA exposure (i.e., on each day of the phase the average exposure dose on that day and the preceding day is calculated). On any day that the 2-day TWA exposure is greater than either toxicity threshold the nest attempt is considered failed, and the female enters the waiting period after pesticide failure.

- The nestling survival endpoint from *in ovo* exposure is compared with time-weighted average (TWA) for exposure during the rapid follicle growth and shell formation period of each egg in a clutch. When the TWA exposure for one or more eggs exceeds the toxicity threshold for nestling survival, the clutch is completed and enters a "doomed" incubation state even though it is assumed that at least some of the nestlings will hatch. Although study reports give the proportion of hatchlings that survive to 14 days of age, they may not report the distribution of nestling deaths during the 14-day period. However, since the *in ovo* exposure may cause mortality in hatchlings soon after they hatch, the nest attempt then is considered failed on the date the nestlings hatched, and the female enters the waiting period after pesticide failure.

- The two surrogate endpoints for nestling survival from dietary exposure are compared to different measures of exposure. The fraction of the LC50 endpoint is compared with the 5-day TWA for dietary exposure to nestlings (i.e., average of nestling exposure doses on each day and the preceding 4 days). Consequently, comparisons between the toxicity threshold and the 5-day TWA begin once nestlings have had 5 days of dietary exposure (i.e., at beginning of 6th day since hatching) and continue each day until fledging. With this surrogate endpoint, nest failure does not occur until nestlings are older than 5 days. The 1/10 of the LD50 endpoint is compared each day of the nestling rearing phase to the estimated daily dose for that day based on the nestling diet. If either one of the measures of nestling exposure exceeds the corresponding toxicity threshold on one or more days, the nest attempt is considered failed immediately, and the female enters the waiting period after pesticide failure.

There is also a probability of nest failure due to ecological causes (e.g., nest predation, adverse weather, etc.) during each day of the egg-laying, incubation and nestling-rearing phases. If a nest fails due to ecological causes, the female enters the waiting period after ecological failures. If a nest attempt proceeds through each breeding stage without a failure due either to an ecological cause or a pesticide exposure that exceeds a toxicity threshold, the nest attempt is considered to have been successful, and the female enters the waiting period after fledging success. This waiting period may be very brief for species where the female is not involved with post-fledging juvenile care or may be an extended period for species where the female cares for juveniles until they become more independent.

At the end of the waiting periods after success or failure from either pesticides or ecological causes, the female would return to egg production in a new nest attempt if there is time remaining in the breeding season and the estimated daily exposure dose is below the toxicity thresholds for both the NOAEL of the pre-laying adult body weight and 1/10 of the LD50. If the estimated daily exposure dose is higher than either of these toxicity thresholds, the female remains in that waiting period until the exposure dose falls below both toxicity thresholds, at which time a new nest attempt begins if time remains in the breeding season. If, on the other hand, the end of a waiting period comes after the date set for the first egg in the last nest of the season (i.e., T_{last}), the female quits breeding for the season.

G. Model assumptions and uncertainties

Many of the assumptions and uncertainties associated with using the MCnest model have been mentioned in the previous sections. This section is intended to present a more thorough discussion of model assumptions and uncertainties and their possible consequences on the model outcomes. Also, where appropriate, suggestions will be provided for reducing uncertainties and lessening the reliance on assumptions.

1. Assumptions reflecting limitations of toxicity testing

By necessity, laboratory toxicity tests simplify the way test organisms are exposed to test substances and limit the range of possible responses to them. Several types of potential field effects either cannot be observed in the laboratory test (e.g., parental behavioral effects during incubation or nestling rearing) or, if observable, the response may be restricted by the limitations of laboratory test systems (e.g., may see a decrease in the rate of egg production with no information about how this could relate to changes in clutch size in the field). Also, most reproductive toxicity tests, including those for birds, were not originally designed to quantify the magnitude of effects on reproductive success, so there is limited information for quantitatively describing dose-response relationships. To address these limitations, a key assumption in MCnest is that the surrogate endpoints selected from available toxicity tests are suitable indicators for the possible field effects due to pesticide exposure. As mentioned above, surrogate endpoints vary in the degree to which they directly represent the field effect of concern. Even for relatively indirect surrogate endpoints such as a change in body weight during the pre-laying period, it is assumed that a pesticide-related reduction in body weight in the laboratory is indicative of other adverse effects on adult well-being in the field that could lead to nest failure.

A related assumption is that the NOAEL for surrogate endpoints from the avian reproduction test, as well as the fractions of the LD50 and LC50, are adequate approximations of the exposure dose below which unreasonable adverse effects are not expected. Conversely, when exposure exceeds these toxicity thresholds it is assumed that adverse effects are possible, though because of the lack of quantitative dose-response information, it often is not possible to estimate the probability or magnitude of adverse effects. Consequently, when estimated exposure doses exceed the toxicity thresholds for surrogate endpoints, it is assumed that the most severe effect is that the nest attempt would fail, with the female having the opportunity to renest *if* the exposure dose drops below the toxicity threshold for effects on adult behavior *and* there is time remaining in the breeding season. In reality, it is unlikely that all exceedances of the toxicity threshold in the field would lead to a nest failure, but without additional information it is not possible to determine the probability of nest failure. By assuming that all exceedances of toxicity thresholds lead to nest failure, MCnest outputs may represent a conservative estimate of potential pesticide effects on reproductive success (i.e., may overestimate the effects of the pesticide on overall reproductive success).

The estimation of pesticide effects on overall reproductive success in MCnest could be improved if avian reproduction tests were designed to quantitatively describe dose-response relationships for sensitive endpoints using regression analysis. This has been discussed in several research papers (Bennett et al. 1990, Stromborg 1986a, b) and review papers (Bennett and Etterson 2006, Bennett and Ganio 1991, OECD 1996). However, this alone does not address the fact that many of the measured test endpoints used as surrogates are indirect measures of the field effect of concern. We also would need to understand the functional relationship between the surrogate endpoint and the response of the field effect. For example, if the avian reproduction test were designed to quantify the dose-response relationship for changes in pre-laying body weight, we would need to understand how that relates to changes in adult well-being that could lead to nest failure in order to improve the estimation of the percent change in reproductive success from pesticide exposure. Alternatively, development of an avian reproduction test using natural incubation has been discussed (Bennett and Ganio 1991, Mineau 2005), which, if available, would reduce the need for indirect surrogate endpoints by more directly measuring the types of effects possibly occurring in the field. While these issues have been in discussion for many years, the current avian reproduction test protocol will be the standard used for the foreseeable future. Consequently, the Basic Version of MCnest focuses on how to make the greatest use of the existing testing data in improving the quality of risk assessments, while helping to illuminate testing deficiencies that could be improved upon in the future.

2. <u>Assumptions related to parameterizing the exposure profile</u>

When data are lacking or highly variable, assumptions are made about exposure parameter values such as the half-life of pesticide residues on various food types, initial residue concentrations on food types, dietary composition of each species, and the proportion of the diet derived from treated fields. In screening-level risk assessments, conservative default assumptions are usually used for residue half-life estimates or initial residue concentrations to examine a worst-case exposure scenario. However, beyond screening-level assessments, if the goal is to more realistically estimate the magnitude of effect on reproductive success in a population, the use of conservative exposure assumptions may not accomplish this goal because

not only can they overestimate a pesticide's effect on avian reproductive success under a specific use scenario, but they also can distort what is learned from comparisons of the relative effects among species or application dates. To more realistically estimate the magnitude of effects, values for exposure parameters should be as representative as possible of empirical observations from the field. Admittedly, this is difficult because often there are limited empirical data on which to base a parameter estimate. For example, there may be data on the degradation half-life of a pesticide only on plant leaves, so can one assume the same half-life is appropriate for seeds and insects? Even when empirical data exist, it is often not possible to determine how representative the data are of field scenarios. For example, while Fletcher et al (1994) summarized pesticide residue concentrations on various plant food types from a large number of existing studies, these studies were not conducted from the perspective of describing the distribution of residues on foods of wildlife species, so it is unclear how well the reported mean and standard deviation of pesticide residues on each food type represent the distribution of residues in the diets of a population a particular species. These uncertainties due to data limitations need to be considered in light of the management questions being addressed when the model user is setting exposure parameters.

Dietary composition also varies among individuals with populations and among seasons. The goal in establishing the default values for diet composition in each species' profile was to estimate the typical proportion consumed in each of the six diet categories from Table 3 by females during the breeding season based on available diet information. For species that are purely granivorous or insectivorous, this is straightforward even though there may be tremendous variability among individuals or regions in the species of seeds or invertebrates consumed. For omnivores consuming foods from multiple categories it is more difficult to estimate proportions consumed from each not only because of the variability among individuals and regions, but also because of the paucity of quantitative analysis available for some species. The values selected for each diet category affect the calculation of daily ingested dose because of differences in the nomogram values and estimates of percent moisture for each diet category. The Basic Version of MCnest uses nomogram values and percent moisture estimates from OPP's T-REX model. However, because a model user may want to modify these values with scenario-specific information, all nomogram values, dietary composition estimates and percent moisture estimates are editable by the model user. It is the responsibility of the model user to justify changes to these default values.

Finally, while the food ingestion rate per body weight (FIR/BW) ratio used in calculating the daily dietary dose is based on species-specific data for adults, this calculation is much more difficult in nestlings for a couple of reasons. First, nestling body weights and their daily ingestion rates are changing rapidly from hatching to fledging. Second, while daily growth of nestlings has been documented in some species, daily estimates of the food ingestion rate in nestlings are uncommon. The Basic Version of MCnest uses an estimate of nestling FIR/BW ratio from an analysis discussed in Section C above where the FIR/BW was calculated each day of the nestling period for an insect diet (EFSA 2009). By choosing the day with the highest FIR/BW ratio, this approach is intended to represent a conservative estimate of the FIR/BW ratio throughout the nestling rearing period. This is an exposure parameter endpoint that could be improved by additional research, but until better information is available the default values for the FIR/BW ratio in the Basic Version of MCnest are 1.08 for the non-seed portion of the

nestling diet and 0.24 for the seed portion. The model user can use these default values or modify the values if suitable data are available.

3. <u>Assumptions related to modeling avian breeding seasons</u>

In developing the MCnest model we tried to minimize the number of life-history parameters required and to rely on parameters that are typically estimated in field studies and reported in the literature. Our goal is a model that is applicable to a broad range of species with limited life-history data. In general, we assume that the species profiles developed are adequate representations of breeding seasons for those species. It is difficult to verify MCnest estimates of reproductive success against field estimates because very few field studies are designed specifically to measure the annual reproductive success of a population or the number of successful broods per female per year. Even when some field studies do estimate these parameters, they may reflect the results at a specific place and time, but it is unclear how representative those results are for the species in general. We develop the most realistic representation we can of the breeding season of each species, but in risk assessment applications we are not relying on the absolute estimate of a chemical's effect on reproductive success because the MCnest model results will be used primarily to estimate the relative difference between chemical and no-chemical scenarios. However, there still are assumptions made in modeling a species' breeding season that could bias the relative effects. Most notable among these assumptions is that a fixed length breeding season is an appropriate description for the start and end of breeding activity in a population of birds. Under this assumption, all females renest after both nest successes and failures as long as there is still time remaining in the breeding season, but all re-nesting ends after a fixed end date. In reality, for many species there is evidence that the probability that a female will quit breeding for the season increases over time until finally all females have quit, but there is little data to quantitatively describe these time-dependent quitting probabilities (Etterson et al. 2009). In the Basic Version of MCnest when a fixed end to the breeding season is assumed, there may be more females in the simulation nesting late in the breeding season than occurs in the field. Consequently, if a pesticide is applied late in a species' breeding season, more nesting attempts may be at risk in the model simulation than might occur in the field at that time. Research continues on methods for improving the definition of the length of the breeding season. Similarly, other life-history parameters are assumed to be constant throughout the breeding season in the Basic Version of MCnest, such as the daily rate of nest mortality and clutch size. However, for many species these parameters also may vary over time though there is often not sufficient data available to quantify these temporal relationships.

In MCnest simulations involving a pesticide application(s), every female in the population is assumed to be exposed to the pesticide. However, given the spatially heterogeneous use of a pesticide in the field, it is unlikely that all females of a population would be exposed to a specific pesticide except for possibly locally-defined populations. The results of MCnest can be used in conjunction with spatially-explicit or spatially-implicit modeling approaches to simulate the proportion of a population expected to be exposed given the use patterns of a pesticide, but the Basic Version of MCnest does not incorporate this capability. Similarly, MCnest simulations assume that each female in the population is an active breeder, whereas populations of many species may include a proportion of males and females that are not reproductively active (i.e., non-breeding floaters). To the extent that information exists for quantifying the proportion of non-breeders in a population, it would be best to use results of

MCnest in a population modeling framework that can address the issue of floaters, especially since the proportion of non-breeders each year may be a density-dependent function.

H. Interpreting MCnest outputs

The interpretation of MCnest outputs occurs at two levels. First, the model user needs to understand the information presented in the series of outputs from each model simulation. Second, the model user needs to understand how the outputs from a series of MCnest simulations can be used in ecological risk assessments, including understanding how uncertainties in the model affect the interpretation of results.

1. <u>Interpreting outputs from individual MCnest simulations</u>

At the completion of each model simulation, several outputs are available. The most basic output is displayed on the MCnest main window and includes the mean number of successful broods per female in the population, the 95% confidence interval (CI), and the number of pesticide applications. The full table of results also can be displayed and includes the mean number of nest attempts (plus 95% CI), the overall nesting success (plus 95% CI), the date(s) and application rate(s) for each application, and the initial exposure dose for adults and nestlings after each application.

The confidence intervals for each of the main output parameters reflect the variability among the replicate populations used in the simulation. For example, if a simulation is setup to run 10 replicate populations of 100 females, for a total population size of 1000, the confidence intervals represent the variability observed among the 10 replicates. The variability among populations is due to the fact that the temporal pattern of nesting activity for each female varies due to different dates for the start of the first nest attempt (determined by the initiation probability) and differences in the timing of nest failures from ecological causes (determined by m_1 and m_2). Variability among populations also results when using the lognormal distribution option for creating the exposure profile. Consequently, for simulations that hold pesticide exposure constant for all females (i.e., using maximum or mean nomogram values), if the initiation probability for a species is set to 1 and m_1 and m_2 are set to 0, the responses of all females and all replicates are identical. Since there are other important sources of uncertainty in the model that are not quantified (e.g., inability to adequately quantify dose-response relationships, field effects that are not observed in laboratory test, simple assumptions about the length of the breeding season), the confidence intervals should not be interpreted as reflection of the variability around the estimate of the true response of the population, but rather as a measure of how well the model has converged upon the projected average number of successful broods per female, given the number of females simulated.

The model user also can display the "Phase diagram" for each simulation (see example in Figure 3) which shows the proportion of females in each of 11 states (see Section D for definitions) throughout the entire breeding season, including:

1. Pair formation phase (*PF*)
2. Rapid follicle growth period (*rfg*)
3. Overlap of rapid follicle growth & eggshell formation periods (*rfg/ef*)
4. Eggshell formation period (*ef*)
5. Incubation phase (*I*)
6. Nestling rearing phase (*N*)
7. Doomed incubation phase (*Id*)
8. Waiting period after pesticide failure (*W_p*)
9. Waiting period after ecological failure (*W_e*)
10. Waiting period after success (*W_f*)
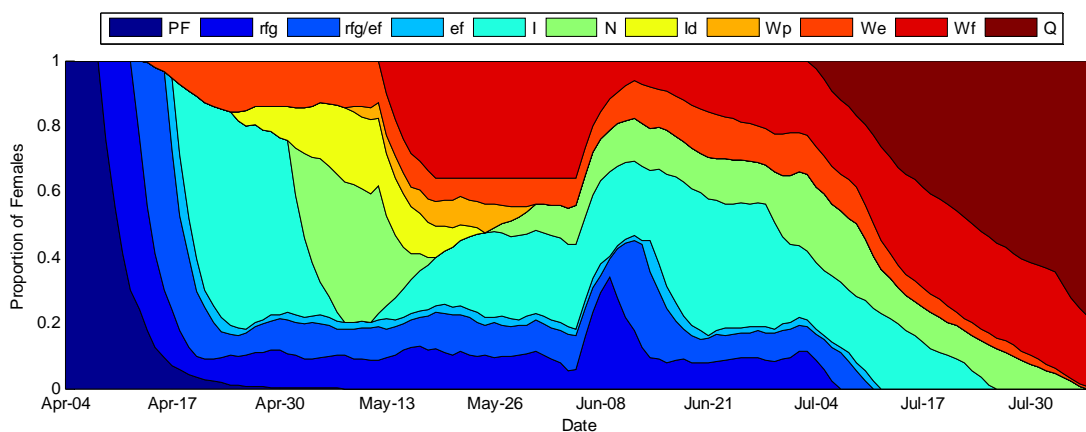11. Quit breeding (*Q*)

Figure 3. Example of phase diagram for eastern meadowlark.

The "Phase diagram" is used to visualize the response of the population to a pesticide exposure scenario over time, and Section F above discusses several of the transitions between breeding phases resulting from pesticide-related nest failures. The next few paragraphs discuss some additional aspects about phase transitions in MCnest that help in the interpretation of the "Phase diagrams."

At the beginning of the breeding season, the "Phase diagram" shows all females starting in the pair formation phase before transitioning into egg laying based on a geometric distribution where 25% of the remaining females each day initiate their first clutch (i.e., initiation probability of p = 0.25). Three of the diagram states (i.e., 2, 3, and 4) represent the egg-laying period. Each egg goes through a several day period of rapid follicle growth until the egg yolk is ready for ovulation and a period of approximately one day from ovulation to laying where the egg albumin is deposited and the eggshell is formed (See Figure 3 above.). Because birds lay eggs one at a

time, during the formation of a clutch of eggs there is an initial period where only rapid follicle growth (RFG) is occurring for the first egg (i.e., state 2), followed by a period where both RFG for subsequent eggs and eggshell formation (EF) is occurring (i.e., state 3). Finally, there is a one-day period of EF for the final egg laid (i.e., state 4).

All of the waiting periods are defined as the duration from a nest failure or success until laying the first egg in a new nest. Because there is also a period of rapid follicle growth for the first egg of the new nest, the waiting periods, as visualized in the ''Phase diagram,'' may seem shorter than they are defined for each species because on the days where the waiting period and rapid follicle growth period overlap, the birds are shown as being in the rapid follicle growth period (i.e., state 2) in the ''Phase diagram.'' For example, if the waiting period after success is 7 d and the rapid follicle growth period is 4 d, a female will only be in state 10 (i.e., Waiting period after success) for 3 d before transitioning to state 2. This is especially noticeable in species where the duration of the waiting periods and rapid follicle growth period are similar (e.g., mourning dove). The exception is when pesticide residue concentrations on food remain higher than the toxicity threshold values for adult behavioral surrogate endpoints, because when this occurs the birds will remain in the waiting period until residue concentrations fall below these thresholds.

The MCnest model also produces a ''Brood histogram'' that plots a histogram based on the frequency distribution of the number of successful broods per female and an ''Exposure series'' that plots the daily exposure dose over time for both adults and juveniles. When the model user chooses to draw calculated doses from a distribution, the ''Exposure series'' also plots the 25th and 75th percentile from that distribution for both adults and juveniles. Finally, the model produces a ''Log file'' that documents the results of a simulation run as well as all of the input parameters. This provides the model user with all the information required to exactly recreate a model simulation if need be.

After each model simulation, the model user can save a specific simulation or, if multiple simulations have been run, the entire table of simulations can be saved. When a single simulation is saved, MCnest automatically names the file with the following format: species name, date of the run (yyyy.mmm.dd), and time of the run (hh.mm.ss AM/PM). An example file name is "dickcissel.2011.Jun.20.12.30.45 PM." By using the exact date and time in the file name, there is no possibility of overwriting previously saved files. By saving a model simulation, the model user has access to all of the model output discussed above. When an entire table is saved, MCnest prompts the user to supply a file name.

2. <u>Interpreting MCnest outputs in ecological risk assessment</u>

As stated in the introductory overview, to put the output from a single MCnest simulation involving a pesticide exposure into perspective, it is compared to the output of a simulation(s) without pesticides. Calculating the relative difference between scenarios with and without pesticide exposure gives the model user an estimate of the proportional reduction in annual reproductive success due to the specific pesticide-use scenario simulated. In ecological risk assessments, this estimate should stimulate at least two additional questions. First, how reflective is the estimate of the response expected in the field? Second, what does the estimated reduction in annual reproductive success mean to the sustainability of the population?

While there is no way of knowing exactly how well the model estimate of reduced annual reproductive success reflects the potential field response, the model user can examine how assumptions made in valuation of parameter inputs affect the model outcomes. As is stated in earlier sections of the Technical Manual, using conservative assumptions about the value of input parameters, instead of more empirically-based values reflecting observed field data, can lead to model outcomes that overestimate the proportional reduction in reproductive success. Often it is necessary to use conservative assumptions in risk assessment when the needed data are absent or of poor quality. However, when there are data for a specific parameter, even if not considered adequate for use in risk assessment, the model user can run simulations to examine to what extent a conservative assumption for a specific parameter estimate affects the overall results compared to the empirically-based data. This provides some insight into the degree to which conservative assumptions overestimate the proportional reduction in reproductive success.

There are a couple of conservative assumptions (discussed in previous sections) that are built into the Basic Version of the MCnest model, and model users cannot examine alternatives. First, when the estimated exposure exceeds the toxicity threshold for each decision point, the nest attempt is considered to have failed. If avian reproduction tests were designed to quantify dose-response relationships for surrogate endpoints **AND** if we knew the quantitative relationship between the laboratory-measured surrogate endpoints and the field effects they represent, decision points could be based on proportional responses rather than the current success/failure dichotomy, and model users would have the ability to explore alternative assumptions about these quantitative relationships. There is much about these relationships that is poorly understood, but the architecture of MCnest already can incorporate dose-response relationships. The options available in MCnest can expand as our knowledge of these relationships grows and can be formalized. Second, the length of the egg-laying period for each species is defined by a start and end date (i.e., $T_{last} - T_1$) and all females are assumed to continue making nest attempts until reaching the end date.   Research continues on alternative methods for defining the length of the egg-laying period for each female. These methods incorporate knowledge that after each nest success or failure, a portion of the population will quit breeding for the year, but those alternatives currently are not available in the Basic Version of MCnest.

The second question relevant to risk assessments addresses our understanding of how changes in one demographic parameter–in this case the fecundity rate–affect the population level. This question is often simplified to ask how much is too much? The answer to this question is outside the scope of MCnest and is better addressed through population modeling. However, MCnest plays a role by providing population modelers with better quantitative estimates of the change in annual reproductive success from a specific pesticide-use scenario.

I. Quality Assurance: Verifying that the model performs as intended

Beyond the model outputs discussed in the previous section, each simulation produces several additional outputs that are not available currently in the compiled version of MCnest for use on computers without Matlab software. However, these output files are used when running the model in Matlab primarily for diagnostic purposes to verify that the model is performing as intended. All of the transitions from one state to another (discussed in Sections F and G) can be verified for each female in the simulation by using a series of output files. The "StateMatrix" is the primary diagnostic tool for determining if the transitions among states are occurring as

intended in the model, especially at each of the decision points. For each female in a simulation, it reports which of the 11 states a female is in on each day of the breeding season. To verify model performance a series of simulations are run to isolate specific decision points or specific types of effects, and the "StateMatrix" is reviewed to evaluate if transitions are occurring as expected. For example, a series of simulations can be set up where only a single surrogate endpoint is triggered by a brief period of exposure exceedance to isolate specific decision points. The "StateMatrix" also can be compared with a file called "Endpoints" that calculates the exposure dose used for each of the MCnest model decision points on each day of the breeding season. This is the exposure dose that is compared with the toxicity threshold for surrogate endpoints to determine whether or not there is an exceedance that would lead to a nest failure or the female can return to egg laying from a waiting period. A related file called "Exceedances" compares the daily exposure value from the "Endpoints" file with the toxicity threshold values for each decision point and displays a "1" if exposure exceeds the toxicity threshold or "0" if not. These three files can be used to verify that all decision points in the simulation are functioning as intended. Whenever changes are made to the code of a version of MCnest, assessments are conducted to verify that all transitions continue to function as intended.

We also need to verify that the model is correctly calculating the mean response for endpoints from all the individual bird responses, as well as the algorithms for calculating the 95% confidence intervals. During each simulation in MCnest, a file called "Broods" is created that tabulates the number of successful broods, the number of nest attempts, and the proportion of nest attempts that are successful for each female in the population. For example, if the simulation used a population size of 1000, the "Broods" file is a matrix of three columns and 1000 rows. To verify that MCnest has correctly calculated the mean number of successful nests and nest attempts, this matrix can be copied and pasted into Excel and the mean function can be used for columns 1 (number of successful broods) and 2 (number of nest attempts). To verify the proportion of nest attempts that are successful for the population, we divide the mean number of successful nests by the mean number of nest attempts. Simply taking the mean of column 3 (i.e., ratio of successful nest/total nests for each female) will not correctly estimate the proportion of nest attempts that are successful for the population because individual birds differ in the number of nests attempted. The 95% confidence intervals can be verified using another file created with each MCnest simulation called "BroodReps." When setting up a simulation in MCnest, the user chooses the number of population replications and the number of individual females in each population replicate. For example, the default when MCnest opens is set to 10 replicates of 100 females for a total population size of 1000 females. MCnest captures the mean number of successful broods, the mean number of nest attempts, and the mean proportion of nest attempts that are successful for each replicate in the "BroodReps" file as a matrix with three columns and one row for each replicate. MCnest calculates the 95% confidence interval for these three output parameters as the mean $\pm$ (1.96 * std dev). The calculations are verified by copying the "BroodReps" matrix into Excel to calculate the mean, standard deviation, and upper and lower 95% confidence limit using Excel functions.

There also is a need to verify that the calculations in MCnest of the exposure doses at application are functioning as intended. Currently, MCnest uses the same approach as OPP EFED's T-REX model for converting an application rate, expressed as pounds per acre, into an initial daily dietary dose, expressed as mg/kg body weight/day, for each species. MCnest uses the same formulas for integrating information on diet composition and body weight to perform this

conversion, although MCnest is estimating the exposure for specific species rather than generalized species used in T-REX. For quality assurance purposes, an Excel spreadsheet has been developed that calculates the initial dietary dose for the specific body weights and diet compositions of the species currently used in the species life-history database at a specified application rate using the T-REX formulas. These calculations are compared to the initial dose calculations used in MCnest simulations to verify that MCnest is calculating initial daily exposure doses exactly the same as the approach used in T-REX. This spreadsheet currently exists only as an internal quality assurance tool. Once the species library is expanded and finalized, the spreadsheet may be available as part of the species library documentation.

J. Analysis of model sensitivity to variation in input parameters

Sensitivity analysis is the study of how variation in the model input parameters affects model outputs. In MCnest, sensitivity analysis is used to determine how changes in input parameters affect the estimated number of successful broods per female in the simulated populations–the primary output parameter from MCnest. The first step is to determine which of the input parameters to consider in the sensitivity analysis. As mentioned in Section A (Overview of the conceptual approach used in MCnest), there are three categories of input parameters: 1) species life-history parameters, 2) pesticide application scenarios, and 3) toxicity threshold values for surrogate endpoints.

1. Approach for a sensitivity analysis of life-history parameters

Species life-history profiles used in MCnest are based on a series of parameters gleaned from a variety of literature sources including journal articles, books, and reports. Many of the life-history parameters reported in the literature may vary considerably within and among studies. The variability evident within studies may be from year-to-year differences or differences among sites with different habitat quality or weather-related parameters. Variability among studies may be evident because studies were conducted at different times and/or different locations or because of differences in experimental methods. Selection of typical values for life-history parameters is made more complicated when information from the literature is variable, but the factors responsible for that variability are poorly understood or described. A sensitivity analysis of the effects of variation in life-history parameters on MCnest outputs would identify which parameters have the greatest effect on changing MCnest outputs and would be useful in the development of species life-history profiles.

To determine which parameters have the greatest impact on MCnest output, input parameters can be varied by the same relative amount (e.g., each input parameter varied by plus or minus a specific percentage of the estimate to examine the relative change in model output relative to a baseline simulation). First a baseline simulation was run for a specific suite of life-history parameters. Next, a series of simulations was run modifying one parameter at a time by either increasing or decreasing the baseline value by 20%. The difference in response due to variation of each life-history parameter was calculated as the proportional change in the number of successful broods per female relative to the baseline value, i.e., (modified – baseline)/baseline.

The life-history parameters considered were: 1) nest initiation probability, 2) daily nest mortality rates during egg laying/incubation (m_1) and during nestling rearing (m_2), 3) duration of

the breeding season (*T*), 4) length of the rapid follicle growth period for each egg (*rfg*), 5) mean clutch size (*clutch*), 6) duration of incubation period (*I*), 7) duration of the nestling rearing period (*N*), 8) duration of the waiting period after ecological failure (W_e), and 9) duration of the waiting period after success (W_f). Because daily nest mortality rates for the two periods (i.e., egg-laying/incubation and nestling-rearing) are often similar, simulations examined variation in these rates individually and combined (i.e., varied both m_1 and m_2 by same amount). The length of the breeding season was defined here as the difference between the dates of the first egg in the first nest (T_1) and the first egg in the last nest (T_{last}). All durations were expressed in days.

Some of the life history parameters are similar among species, whereas others are quite different–reflecting the diversity of breeding season strategies among species. Consequently, there is no single baseline simulation that can be used for exploring variation in input parameters that would be representative of all species. A series of baseline simulations was used that reflected the variation observed in three of the most dynamic parameters, while holding the other parameters constant. The first is the duration of the breeding season (*T*) to represent short, medium, and long breeding seasons. Durations of 60, 90, and 120 days were used for *T* in baseline simulations. The second in the combined daily nest mortality rates (m_1 & m_2) to represent low (0.015 d^{-1}), medium (0.03 d^{-1}), and high (0.045 d^{-1}) daily nest mortality rates. The third is the waiting period after fledging (W_f) to represent short (10 d), medium (20 d), and long (40 d) periods of post-fledging juvenile care by the female prior to initiating a new clutch of eggs. The three levels of three parameters result in 27 combinations of input parameters to define the suite of baseline simulations. All other input parameters were held constant for the 27 baseline simulations, including: 1) length of the rapid follicle growth period (5 d), 2) mean clutch size (5 eggs), 3) duration of incubation period (10 d), 4) duration of the nestling rearing period (10 d), and 5) duration of the waiting period after ecological failure (10 d). Also, all simulations set the nest initiation probability at 0.25, used an egg-laying interval of 1 day, and set incubation to start with the laying of the last egg.

For each of the 27 baseline simulations, 20 additional simulations were run by either increasing or decreasing the baseline value for each life history parameter by 20%. For m_1 and m_2, simulations were run on each parameter separately and combined (i.e., a 20% increase or decrease during both m_1 and m_2). Each simulation was run using 10 replicates of 1000 females for a total population size of 10,000 females.

2. <u>Results of sensitivity analysis of life history parameters</u>

An immediate pattern that emerged is that variation in the initiation probability and the duration of the rapid follicle growth period had no effect on MCnest outputs. Changing the initiation probability by 20% resulted in only small changes to the distribution of first nest attempts and had no observable impact on overall MCnest outputs. The lack of effect of changes in the rapid follicle growth period is because the first egg overlaps entirely with the waiting period after either success or failure of the previous nest attempt. Waiting periods are defined as the duration between a nest's success or failure (from any cause) until the first egg is laid in the subsequent nest and are almost always longer than the rapid follicle growth period. Consequently, varying the rapid follicle growth period has virtually no influence on the number of nest attempts possible within the breeding season. The results for simulations varying the length of the follicle growth period or initiation probability are not discussed further.

Among the 27 baseline simulations the number of successful broods per female ranged from 0.71 (short season, high nest mortality rates, long W_f) to 2.96 (long season, low nest mortality rates, short W_f) (Table 5). Not surprisingly, as the length of the breeding season T increases, the number of successful broods also increases. Also, the number of successful broods increases as the daily nest mortality rate decreases and the duration of the waiting period after success W_f decreases. For each simulation, the number of successful broods per female is reported in Table 5 and the proportional changes from the baseline value are reported in Table 6. Bar graphs for visualizing the relative sensitivity of variation in life history parameters for each of the 27 baseline simulation are presented in Appendix A.

Across all baseline scenarios, the largest proportional changes in the number of successful broods per female were due to variation in T. A 20% decrease in T resulted in a 3% to 19% decrease in the number of successful broods, while a 20% increase in T resulted in an 11% to 45% increase (Table 6). For most of the baseline scenarios, the proportional increase and decrease for each baseline scenario are relatively similar, but one set of conditions stand out as different. For baseline scenarios where the duration of a complete successful nesting attempt (including the duration of the waiting period after success W_f) is slightly longer than T, a 20% increase in T provides sufficient time for many of the females to attempt an additional nest. The most extreme example of this is for the baseline scenario defined as $T = 60$ d, $m_1 = m_2 = 0.015$ d^{-1}, and $W_f = 40$ d (Table 6), where a 20% increase in T resulted in a 45% increase in the number of successful nests, while a 20% decrease resulted in only a 3% decrease in the number of successful broods. A similar result was observed for the baseline scenario defined as $T = 60$ d, $m_1 = m_2 = 0.03$ d^{-1}, and $W_f = 40$ d. There are additional baseline scenarios where a 20% increase in T resulted in a noticeable increase in the number of successful broods per female because the additional time allowed for one more nesting attempt than was observed in the baseline simulation (e.g., $T = 60$ d, $m_1 = m_2 = 0.045$ d^{-1}, and $W_f = 40$ d and $T = 120$ d, $m_1 = m_2 = 0.015$ d^{-1}, and $W_f = 40$ d).

Variation in combined daily nest mortality rates (i.e., m_1 and m_2) also resulted in large proportional changes in the number of successful broods per females when the baseline level of daily nest mortality was high (i.e., 0.045 d^{-1}). The largest impact is for the baseline scenario defined as $T = 60$ d, $m_1 = m_2 = 0.045$ d^{-1}, and $W_f = 10$ d (Table 6), where a 20% increase in m_1 and m_2 resulted in an 18% decrease in the number of successful nests, while a 20% decrease resulted in 22% increase in the number of successful broods. The significance of variation in m_1 and m_2 combined decreased as the baseline level for m_1 & m_2 decreased. When considering variation in m_1 and m_2 separately, the vast majority of simulations resulted in a less than 10% change in the number of successful broods per female.

Variation in the waiting period after success W_f resulted in a pattern of responses across all 27 baseline scenarios that was similar to the pattern observed for T. In general variation in W_f resulted in less than a 10% change in the number of successful broods (Table 6). However, for baseline scenarios where the duration of a complete successful nesting attempt (including the duration of W_f) is slightly longer than T, a 20% decrease in W_f provides sufficient time for many of the females to attempt an additional nest. Consequently, the largest increases (up to 32%) in the number of successful broods due to decreases in W_f occurred for the same baseline scenarios as responded to increases in T (Table 6). This can be visualized in Appendix A where the MCnest output is skewed to the right for T and W_f for those simulations where sufficient time

exists for an additional nesting attempt relative to the baseline simulation (e.g., $T = 60$, $m_1 = m_2 = 0.015$, $W_f = 40$).

Variation in other life-history parameters, such as clutch size (*clutch*), duration of the incubation (I) and nestling rearing (N) periods, and the waiting period after environmental failure (W_e), resulted in smaller impacts on the number of successful broods per female, and impacts were more consistent across the 27 baseline scenarios. Varying clutch size by 20% resulted in changes in the number of successful broods from 0% to 9% (Table 6), while variation in I or N resulted in 0% to 13% change in number of broods. Variation in W_e had a small (1% to 7%) impact on the number of successful broods per female.

Unfortunately, the life-history parameters that had the greatest impact on the MCnest outputs (i.e., T, m_1, m_2, and W_f) are also among the most variable parameters in the literature. The length of the breeding season, as well as the typical starting and ending dates for egg laying, vary considerably among geographical regions and in the way they are reported in the literature. As a life-history profile is developed for a species, considerable thought needs to be given during evaluation of available literature for selecting dates to represent the start and end of egg laying that produce an overall species profile that is reflective of that species. Also, for risk assessments focused on a particular region, it may be appropriate to select dates for the start and end of egg laying that are representative of that region. Variation in m_1 and m_2 in the literature reflects that nest success varies due to factors such as habitat quality, weather patterns, and the abundance of nest predators and parasites. This analysis indicates that it is important to document the rationale for selecting typical values of m_1 and m_2 in the development of a species profile because of the impact these values can have on MCnest output. Although the duration of the waiting period after fledging (W_f) differs greatly among species because of differences in the role of females in post-fledging juvenile care, there also is considerable variation within some species probably reflecting differences among individual females in how rapidly they can liberate themselves from fledgling care for renesting. This parameter also suffers because few studies follow females throughout the breeding season to actually document W_f. Consequently, much of the information in the literature relevant to W_f is anecdotal or based on small sample sizes. Also, some estimates may reflect females that renest after losing fledged juveniles that had not completely reached independence. The waiting period after ecological failure (W_e) is similar to W_f in that few studies follow females throughout the breeding season to actually document W_e. However, the degree of independence of juveniles is not a factor in W_e, but the length of time for rapid follicle growth and/or to build a new nest does affect W_e. The manner in which a female loses a nest attempt may also influence W_e, but few studies provide insight into these factors.

The avian literature contains much more information on clutch size and the duration of incubation and nestling rearing phases, so establishing typical values for these parameters is relatively easy for most species. Given the results of this analysis, variation in selecting typical values for these parameters will have minor impacts on MCnest outputs.

Table 5. Changes in the number of successful broods per female due to variation in life history parameters from 27 baseline species profiles.

| Baseline | | | | Mean number of successful broods per female for life history parameters increased (▲) or decreased (▼) by 20% | | | | | | | | | | | | | | | | | |
| | | | | m1 | | m2 | | m1&2 | | T | | clutch | | I | | N | | We | | Wf | |
T	m1&m2	Wf	# broods	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲
60	0.015	10	1.59	1.62	1.57	1.63	1.55	1.69	1.52	1.42	1.94	1.62	1.55	1.67	1.52	1.66	1.52	1.62	1.56	1.62	1.57
		20	1.47	1.51	1.42	1.50	1.43	1.55	1.39	1.26	1.63	1.51	1.44	1.53	1.40	1.53	1.41	1.49	1.44	1.51	1.42
		40	0.97	0.97	0.96	0.97	0.96	0.98	0.95	0.94	1.41	0.97	0.96	0.98	0.95	0.97	0.95	0.98	0.96	1.28	0.96
	0.03	10	1.21	1.27	1.14	1.29	1.14	1.37	1.07	1.03	1.43	1.27	1.16	1.30	1.12	1.32	1.12	1.27	1.18	1.23	1.19
		20	1.11	1.18	1.04	1.16	1.05	1.24	0.99	0.94	1.27	1.15	1.07	1.19	1.03	1.19	1.02	1.16	1.07	1.14	1.07
		40	0.86	0.89	0.83	0.89	0.83	0.91	0.80	0.80	1.10	0.88	0.84	0.88	0.83	0.88	0.82	0.88	0.84	1.02	0.86
	0.045	10	0.89	0.98	0.81	0.98	0.82	1.09	0.73	0.75	1.07	0.93	0.85	0.98	0.80	0.99	0.80	0.94	0.85	0.91	0.88
		20	0.83	0.92	0.76	0.90	0.76	0.98	0.70	0.71	0.97	0.87	0.80	0.93	0.76	0.92	0.76	0.89	0.80	0.86	0.81
		40	0.71	0.75	0.67	0.76	0.66	0.81	0.62	0.63	0.85	0.73	0.69	0.76	0.66	0.76	0.67	0.74	0.68	0.78	0.71
90	0.015	10	2.30	2.36	2.25	2.38	2.24	2.43	2.18	1.98	2.78	2.38	2.25	2.42	2.20	2.44	2.18	2.35	2.26	2.37	2.26
		20	1.97	2.02	1.92	2.02	1.93	2.06	1.88	1.64	2.32	2.09	1.80	2.16	1.78	2.16	1.78	1.99	1.93	2.14	1.81
		40	1.63	1.65	1.60	1.68	1.59	1.70	1.56	1.41	1.82	1.67	1.59	1.70	1.57	1.71	1.57	1.67	1.60	1.74	1.54
	0.03	10	1.75	1.84	1.64	1.84	1.67	1.95	1.55	1.47	2.07	1.82	1.67	1.90	1.60	1.90	1.60	1.81	1.67	1.79	1.70
		20	1.54	1.63	1.45	1.63	1.46	1.70	1.38	1.28	1.81	1.63	1.45	1.68	1.41	1.70	1.40	1.59	1.51	1.61	1.46
		40	1.31	1.36	1.24	1.38	1.24	1.43	1.19	1.10	1.50	1.35	1.27	1.39	1.23	1.39	1.22	1.35	1.27	1.40	1.23
	0.045	10	1.28	1.41	1.17	1.40	1.19	1.54	1.07	1.07	1.51	1.35	1.21	1.44	1.15	1.45	1.15	1.35	1.22	1.32	1.26
		20	1.18	1.29	1.08	1.29	1.09	1.40	1.01	0.97	1.38	1.24	1.12	1.32	1.06	1.31	1.06	1.23	1.14	1.22	1.14
		40	1.03	1.11	0.95	1.11	0.96	1.18	0.88	0.87	1.19	1.08	0.99	1.12	0.94	1.13	0.94	1.06	0.98	1.08	0.97
120	0.015	10	2.96	3.05	2.88	3.06	2.89	3.15	2.81	2.41	3.54	3.07	2.87	3.16	2.80	3.17	2.78	3.04	2.90	3.07	2.89
		20	2.52	2.46	2.56	2.45	2.58	2.40	2.63	2.15	2.99	2.58	2.45	2.63	2.38	2.64	2.40	2.56	2.46	2.61	2.39
		40	1.88	1.90	1.86	1.91	1.86	1.92	1.84	1.71	2.31	1.90	1.86	1.92	1.84	1.92	1.84	1.91	1.86	2.20	1.84
	0.03	10	2.26	2.38	2.11	2.38	2.12	2.53	1.98	1.85	2.66	2.35	2.16	2.47	2.08	2.45	2.06	2.33	2.17	2.31	2.20
		20	1.98	1.88	2.08	1.89	2.10	1.79	2.18	1.65	2.32	2.04	1.90	2.13	1.82	2.13	1.84	2.04	1.91	2.08	1.90
		40	1.61	1.67	1.55	1.66	1.54	1.73	1.48	1.38	1.88	1.65	1.57	1.69	1.53	1.69	1.51	1.65	1.57	1.76	1.55
	0.045	10	1.67	1.82	1.51	1.83	1.53	1.99	1.38	1.35	1.95	1.76	1.57	1.86	1.47	1.87	1.48	1.76	1.59	1.72	1.62
		20	1.51	1.65	1.37	1.64	1.39	1.77	1.29	1.25	1.79	1.59	1.44	1.67	1.35	1.67	1.36	1.58	1.46	1.56	1.45
		40	1.30	1.39	1.19	1.39	1.21	1.48	1.12	1.09	1.52	1.36	1.25	1.40	1.20	1.40	1.20	1.36	1.25	1.38	1.25

Table 6. Proportional change from baseline value due to variation in life history parameters for 27 baseline species profiles, with proportions ≥ 0.15 highlighted.

Baseline				Proportional change from baseline for life history parameters increased (▲) or decreased (▼) by 20%																	
				m1		m2		m1&2		T		clutch		I		N		We		Wf	
T	m1&m2	Wf	# broods	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲	▼	▲
60	0.015	10	1.59	0.02	-0.01	0.03	-0.03	0.06	-0.04	-0.11	0.22	0.02	-0.03	0.05	-0.04	0.04	-0.04	0.02	-0.02	0.02	-0.01
		20	1.47	0.03	-0.03	0.02	-0.03	0.05	-0.05	-0.14	0.11	0.03	-0.02	0.04	-0.05	0.04	-0.04	0.01	-0.02	0.03	-0.03
		40	0.97	0.00	-0.01	0.00	-0.01	0.01	-0.02	-0.03	0.45	0.00	-0.01	0.01	-0.02	0.00	-0.02	0.01	-0.01	0.32	-0.01
	0.03	10	1.21	0.05	-0.06	0.07	-0.06	0.13	-0.12	-0.15	0.18	0.05	-0.04	0.07	-0.07	0.09	-0.07	0.05	-0.02	0.02	-0.02
		20	1.11	0.06	-0.06	0.05	-0.05	0.12	-0.11	-0.15	0.14	0.04	-0.04	0.07	-0.07	0.07	-0.08	0.05	-0.04	0.03	-0.04
		40	0.86	0.03	-0.03	0.03	-0.03	0.06	-0.07	-0.07	0.28	0.02	-0.02	0.02	-0.03	0.02	-0.05	0.02	-0.02	0.19	0.00
	0.045	10	0.89	0.10	-0.09	0.10	-0.08	0.22	-0.18	-0.16	0.20	0.04	-0.04	0.10	-0.10	0.11	-0.10	0.06	-0.04	0.02	-0.01
		20	0.83	0.11	-0.08	0.08	-0.08	0.18	-0.16	-0.14	0.17	0.05	-0.04	0.12	-0.08	0.11	-0.08	0.07	-0.04	0.04	-0.02
		40	0.71	0.06	-0.06	0.07	-0.07	0.14	-0.13	-0.11	0.20	0.03	-0.03	0.07	-0.07	0.07	-0.06	0.04	-0.04	0.10	0.00
90	0.015	10	2.30	0.03	-0.02	0.03	-0.03	0.06	-0.05	-0.14	0.21	0.03	-0.02	0.05	-0.04	0.06	-0.05	0.02	-0.02	0.03	-0.02
		20	1.97	0.03	-0.03	0.03	-0.02	0.05	-0.05	-0.17	0.18	0.06	-0.09	0.10	-0.10	0.10	-0.10	0.01	-0.02	0.09	-0.08
		40	1.63	0.01	-0.02	0.03	-0.02	0.04	-0.04	-0.13	0.12	0.02	-0.02	0.04	-0.04	0.05	-0.04	0.02	-0.02	0.07	-0.06
	0.03	10	1.75	0.05	-0.06	0.05	-0.05	0.11	-0.11	-0.16	0.18	0.04	-0.05	0.09	-0.09	0.09	-0.09	0.03	-0.05	0.02	-0.03
		20	1.54	0.06	-0.06	0.06	-0.05	0.10	-0.10	-0.17	0.18	0.06	-0.06	0.09	-0.08	0.10	-0.09	0.03	-0.02	0.05	-0.05
		40	1.31	0.04	-0.05	0.05	-0.05	0.09	-0.09	-0.16	0.15	0.03	-0.03	0.06	-0.06	0.06	-0.07	0.03	-0.03	0.07	-0.06
	0.045	10	1.28	0.10	-0.09	0.09	-0.07	0.20	-0.16	-0.16	0.18	0.05	-0.05	0.13	-0.10	0.13	-0.10	0.05	-0.05	0.03	-0.02
		20	1.18	0.09	-0.08	0.09	-0.08	0.19	-0.14	-0.18	0.17	0.05	-0.05	0.12	-0.10	0.11	-0.10	0.04	-0.03	0.03	-0.03
		40	1.03	0.08	-0.08	0.08	-0.07	0.15	-0.15	-0.16	0.16	0.05	-0.04	0.09	-0.09	0.10	-0.09	0.03	-0.05	0.05	-0.06
120	0.015	10	2.96	0.03	-0.03	0.03	-0.02	0.06	-0.05	-0.19	0.20	0.04	-0.03	0.07	-0.05	0.07	-0.06	0.03	-0.02	0.04	-0.02
		20	2.52	0.02	-0.02	0.02	-0.03	0.04	-0.05	-0.15	0.19	0.02	-0.03	0.04	-0.06	0.05	-0.05	0.02	-0.02	0.04	-0.05
		40	1.88	0.01	-0.01	0.02	-0.01	0.02	-0.02	-0.09	0.23	0.01	-0.01	0.02	-0.02	0.02	-0.02	0.02	-0.01	0.17	-0.02
	0.03	10	2.26	0.05	-0.07	0.05	-0.06	0.12	-0.12	-0.18	0.18	0.04	-0.04	0.09	-0.08	0.08	-0.09	0.03	-0.04	0.02	-0.03
		20	1.98	0.05	-0.05	0.06	-0.05	0.10	-0.10	-0.17	0.17	0.03	-0.04	0.08	-0.08	0.08	-0.07	0.03	-0.04	0.05	-0.04
		40	1.61	0.04	-0.04	0.03	-0.04	0.07	-0.08	-0.14	0.17	0.02	-0.02	0.05	-0.05	0.05	-0.06	0.02	-0.02	0.09	-0.04
	0.045	10	1.67	0.09	-0.10	0.10	-0.08	0.19	-0.17	-0.19	0.17	0.05	-0.06	0.11	-0.12	0.12	-0.11	0.05	-0.05	0.03	-0.03
		20	1.51	0.09	-0.09	0.09	-0.08	0.17	-0.15	-0.17	0.19	0.05	-0.05	0.11	-0.11	0.11	-0.10	0.05	-0.03	0.03	-0.04
		40	1.30	0.07	-0.08	0.07	-0.07	0.14	-0.14	-0.16	0.17	0.05	-0.04	0.08	-0.08	0.08	-0.08	0.05	-0.04	0.06	-0.04

3. **Approach for examining model sensitivity to changes in pesticide-related parameters**

The other two categories of input parameters are specific to the pesticide and pesticide-use scenario being evaluated. Toxicity threshold values are determined from avian toxicity tests. The pesticide-use scenario parameters such as the application rate are specified on the pesticide label, while the residue half-life parameter often is determined from registrant-submitted fate studies. The range of application dates may not be specified on the label because applications are tied to the conditions (i.e., weather, pest levels) in each region, but information about typical application dates may be available from registrants or extension agents.

A specific pesticide-use scenario for a particular pesticide is defined by this suite of parameters including toxicity threshold values, applications dates(s) and rate(s), and residue half-life estimates. Variation in any of the toxicity threshold values or pesticide-use scenario parameters may or may not affect the MCnest output, depending on the specific scenario being considered. For example, the timing of a pesticide application relative to the timing of the breeding season of a species is critically important to the magnitude of effect observed. In fact, because there is virtually an infinite number of combinations of toxicity threshold values and pesticide application scenarios that could be considered, it is very difficult to generalize how variation in any particular parameter will affect the MCnest output. For example, increasing or decreasing a toxicity threshold value by a specific amount will significantly change the MCnest output results under some pesticide application scenarios (especially when the toxicity threshold value is close to the estimated dietary exposure at application), but have virtually no effect under other scenarios (e.g., if a pesticide application causes nest attempts to fail near the end of the egg-laying window).

Because the response of the MCnest output parameter is so dependent on the unique toxicity characteristics of each pesticide and on the specific pesticide application scenario for the simulation (especially timing of the application), a complete sensitivity analysis based on all combinations of toxicity threshold values and pesticide-use scenario parameters is not possible. However, once a specific pesticide is identified and the toxicity thresholds and application scenario parameters are determined, the model user may want to examine variation in certain parameters, especially the application date. As mentioned above, MCnest runs simulations with each pesticide application occurring on a single date, but within a geographic area the pesticide may be used over a range of dates. To examine the differences in model response to pesticide applications on different dates, it is suggested that the model user run a series of simulations for various possible application dates. This is simple to do using the batch options and provides considerable insight into the effect of application timing on avian reproductive success. Similarly, although a specific pesticide use may have a proposed application rate, the model user may want to examine how risk to reproductive success changes when changing the proposed application rate. There may be additional reasons why a model user may want to examine variation in the toxicity threshold values, application dates, or the estimated residue half-life values on a pesticide-by-pesticide basis, but this was not done as part of a formal sensitivity analysis because of the difficulty of generalizing how variation in any particular pesticide parameter will affect the MCnest output. However, the sensitivity of changes in two parameters –residue half-life and application date–will be addressed in the remainder of this section to illustrate how variable the response may be depending on the values chosen for other parameters.

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The quality of information on the half-life of pesticide residues on avian food types varies among pesticides; i.e., some pesticides may have specific measured degradation rate data for food types such as seeds or fruits while others may have no degradation data specifically on avian foods. The Office of Pesticide Programs uses a default degradation half-life on food types of 35 days based on the work by Willis and McDowell (1987) that reported available foliar residue half-lives for approximately 80 pesticides, with a maximum value of 36.9 days. They reported an average half-life (± standard deviation) across all formulations and extraction methods for organochlorine, pyrethroid, organophosphorus, and carbamate insecticides was 5.0 ± 4.6 d, 5.3 ± 3.6 d, 3.0 ± 2.7 d, and 2.4 ± 2.0 d, respectively. Since the MCnest outputs can be very sensitive to changes in the residue degradation half-life, the default value may significantly overestimate the magnitude of effects on reproduction if the actual pesticide half-life on foods is much shorter. When information on the half-life of residues on foods is uncertain, the model user can examine the effects of changes in the degradation half-life on MCnest outputs to understand the implication of input parameter selections.

To evaluate the role of changes to the residue half-life value, a series of simulations was conducted using a portion of the baseline profiles used above for evaluating life history parameters. Twelve profiles were developed based on four durations for the egg-laying period (i.e., 30, 60, 90, and 120 days) and three combined daily nest mortality rates (m_1 & m_2) to represent low (0.015 d^{-1}), medium (0.03 d^{-1}), and high (0.045 d^{-1}) daily nest mortality rates. In this series of simulations the waiting period after fledging (W_f) was held constant at 20 d. All other life history input parameters were held constant for the 12 baseline simulations, including: 1) length of the rapid follicle growth period (5 d), 2) mean clutch size (5 eggs), 3) duration of incubation period (10 d), 4) duration of the nestling rearing period (10 d), and 5) duration of the waiting period after ecological failure (10 d). Also, all simulations used an egg-laying interval of 1 day and set incubation to start with the laying of the last egg.

Compared to the simulations above evaluating the sensitivity of life-history parameters, additional input parameters must be defined. Because these simulations focus on the effect of a pesticide on estimated annual reproductive success, the timing of the pesticide application relative to the timing of the breeding season is critical and the body weight and diet composition information are needed to calculate the initial daily dose. Consequently, in addition to defining the duration of the egg-laying period (T), the dates for T_1 and T_{last} must be defined. All 12 baseline profiles used a midpoint date for T of June 15, resulting in dates for T_1 and T_{last} of May 31-June 30, May 16-July 15, May 1-July 30, and April 16-August 14 for the 30, 60, 90, and 120 d durations, respectively. The application date used for all simulations was June 15–midway through the window for egg laying.

Also, all simulations were based on a 20 g insectivore (i.e., 100% of diet for adults and juveniles was invertebrates). Using an application rate of 1 lb/acre results in an initial daily dose for adults of 107 mg/kg/d. The simulated pesticide was defined to primarily affect egg hatchability via *in ovo* exposure, and the toxicity threshold used in all simulations was 10.7 mg/kg/d (i.e., equivalent to a risk quotient of 10). To examine the effect of changes in residue half-life on the model results, each of the 12 baseline profiles was run using the default 35-d half-live as well as half-lives of 10 d and 3.5 d. All simulations were run using 10 replicates of 1000 females for a total population size of 10,000 females.

Simulation results indicate that the residue half-life estimate had little impact on seasonal productivity for birds with short egg-laying durations (i.e., 30 d), but at longer durations the percent reduction in productivity is greater using a 35 d half-life than one of 3.5 d (Figure 4). For birds with the longest egg-laying duration (i.e., 120 d), the percent reduction is greater using a 35 d half-life than for both 3.5 d and 10 d. If a model user wanted to compare the effects of the pesticide across a series of species defined by the characteristics of these 12 profiles, using a 35-d residue half-life the conclusion would be that species with short egg-laying durations (i.e., 30 d) are at less risk than species with longer egg-laying durations (Figure 4). However, if the actual residue half-life of the pesticide was 3.5 d, the conclusion would be that species with short egg-laying durations are at greatest risk.
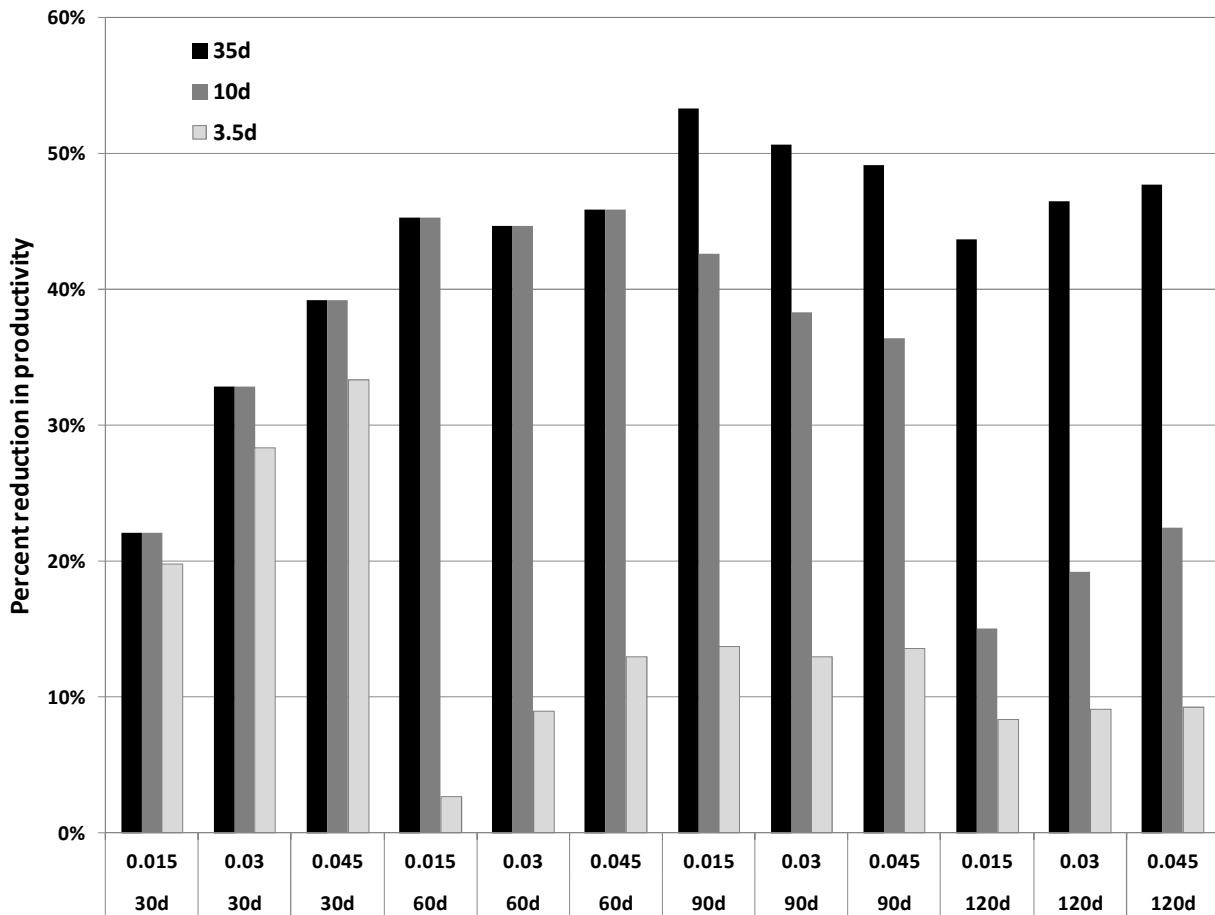
Figure 4. The effect of a single pesticide application on June 15, using three estimates of its residue half-life (i.e., 3.5, 10, and 35 d), on the percent reduction in seasonal productivity for 12 species profiles based on three daily nest mortality rates (i.e., 0.015, 0.03, and 0.045 d^{-1}) and four egg-laying durations (i.e., 30, 60, 90, and 120 d) with a midpoint date of June 15.

If the series of simulations in Figure 4 is repeated with only a single change in the input parameters–the application date is changed to May 15 instead of June 15–the results change significantly, primarily for profiles with shorter egg-laying durations. Species profiles with the

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shortest egg-laying periods (i.e., 30 d) experience virtually no impact if the residue half-life is set at 3.5 d, but a 100% reduction in productivity when the half-life is set at 35 d. With a May 15 application date, the pesticide residues would have decreased below the toxicity threshold by May 31 (i.e., the first day of egg laying for the profiles with 30-d egg-laying durations) if the residue half-life was 3.5 d, but would have remained about the toxicity threshold throughout the egg-laying period if the half-life was set at 35 d.
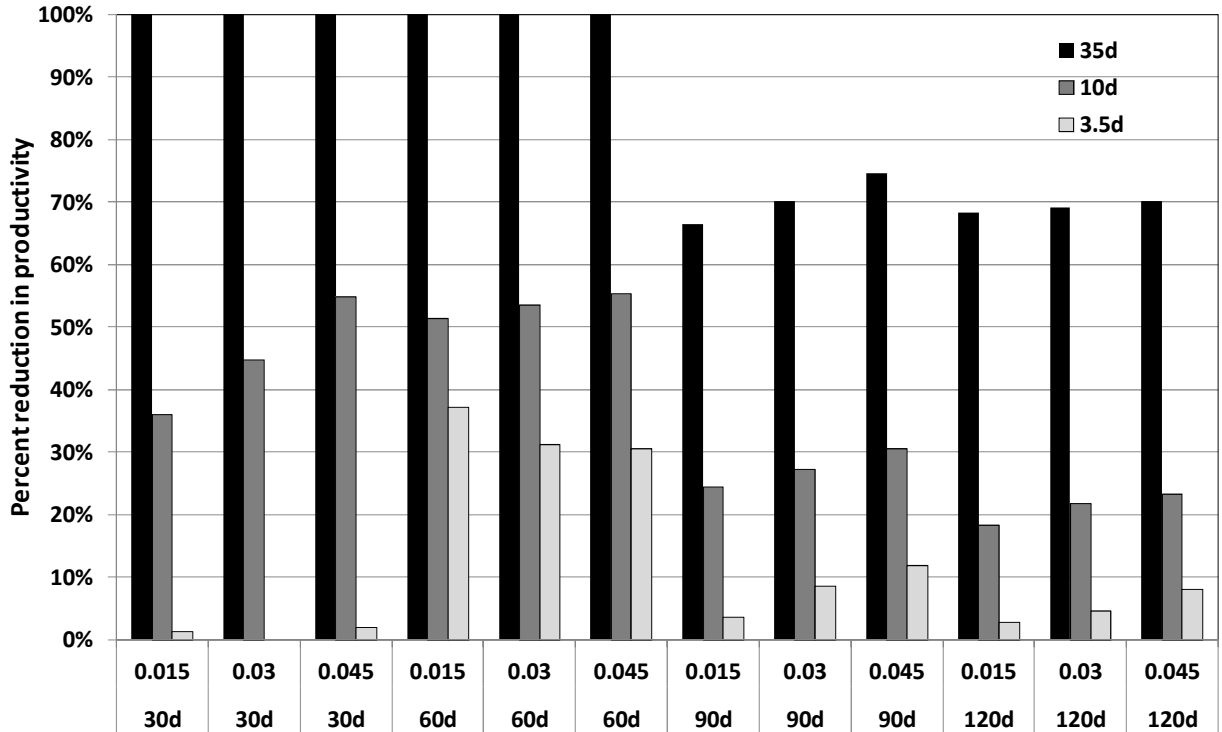
Figure 5. The effect of a single pesticide application on May 15, using three estimates of its residue half-life (i.e., 3.5, 10, and 35 d), on the percent reduction in seasonal productivity for 12 species profiles based on three daily nest mortality rates (i.e., 0.015, 0.03, and 0.045 d^{-1}) and four egg-laying durations (i.e., 30, 60, 90, and 120 d) with a midpoint date of June 15.

When information on a pesticide's residue half-life on avian foods is lacking or inadequate, the model user may need to use a conservative assumption about the half-life value, such as OPP's default of 35 d. However, these two series of simulations illustrate that for some species and pesticide-use scenarios the selection of an estimate for the residue half-life has little or no influence on the results for that species, but for other species or scenarios the selection of a half-life can significantly affect not only the absolute magnitude of the estimated effect, but the relative response among species. Model users should be aware of this potential outcome when interpreting model results.

The above series of simulations also illustrates the importance of the timing of the pesticide application relative to the time of a species' breeding season. As mentioned above, although each pesticide application in MCnest simulations occurs on a single date, pesticide

applications in a certain geographical region may occur over a range of possible dates depending on factors such as the status of the crop and insect population levels. Since the model response can vary among application dates, the model user is encouraged to examine pesticide effects on seasonal productivity over a range of possible application dates. If the model user has information on the distribution of application dates, the cumulative response could be estimated as the weighted average of responses from a series of application dates.

To examine the reproductive response to changes in application dates, a series of model simulations was conducted using four profiles based on four durations for the egg-laying period (i.e., 30, 60, 90, and 120 d). In this series of simulations, the waiting period after fledging (W_f) was held constant at 20 d and the daily nest mortality rate was held constant at 0.03 d^{-1}. All other life-history input parameters were held constant for the 4 baseline simulations, including: 1) length of the rapid follicle growth period (5 d), 2) mean clutch size (5 eggs), 3) duration of incubation period (10 d), 4) duration of the nestling rearing period (10 d), and 5) duration of the waiting period after ecological failure (10 d). Also, all simulations used an egg-laying interval of 1 day and set incubation to start with the laying of the last egg.

The dates for T_1 and T_{last} remain the same as in the previous simulations, i.e., May 31-June 30, May 16-July 15, May 1-July 30, and April 16-August 14 for the 30, 60, 60, and 120d durations, respectively. The application dates occurred on March 1 or on dates at 15-d intervals thereafter through the growing season.

Like above, all simulations were based on a 20 g insectivore. Using an application rate of 1 lb/acre results in an initial daily dose for adults of 107 mg/kg/d. The simulated pesticide was defined to primarily affect egg hatchability via *in ovo* exposure, and the toxicity threshold used in all simulations was 10.7 mg/kg/d. In this series of simulation the pesticide residue half-life was set at 10 d. All simulations were run using 10 replicates of 1000 females for a total population size of 10,000 females.

Simulation results indicate that depending on the application date, the effect on seasonal productivity can range from no impact (i.e., 0% reduction) to complete failure (i.e., 100% reduction). The greatest impacts are observed for species with short breeding seasons (i.e., 30 d) where the pesticide is applied just prior to the onset of egg laying, but applications before and after egg laying had little impact. For long breeding seasons the magnitude of effect was lower (i.e., $\leq 33\%$), but effects in the range of 20 to 30% were observed following applications ranging over a four-month period. Consequently, determining the species at greatest risk is dependent on the application date or range of dates used in the simulations.
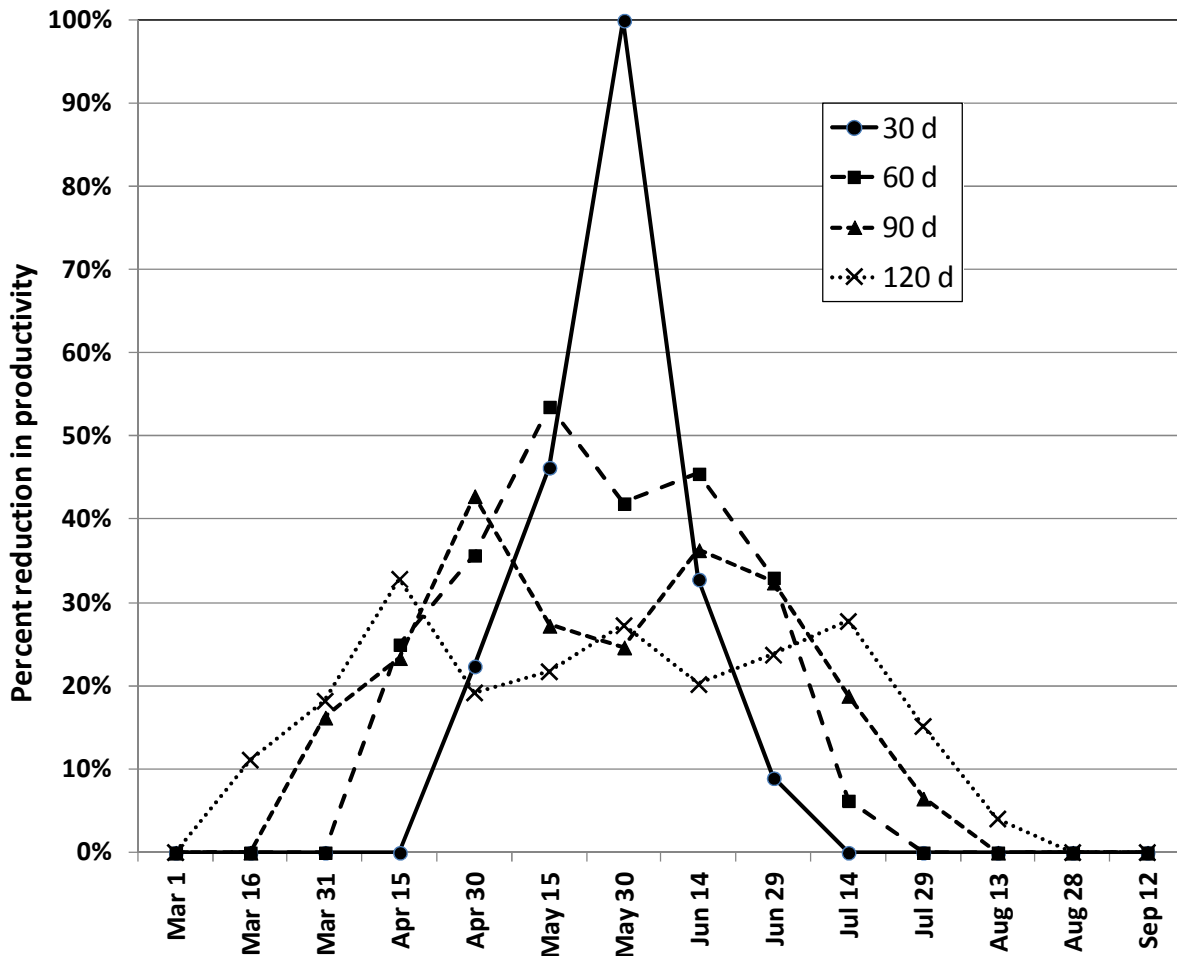
Figure 6. The effect of a single pesticide application on each of a series of application dates on the percent reduction in seasonal productivity for 4 species profiles based on 4 egg-laying durations (i.e., 30, 60, 90, and 120 d) with a midpoint date of June 15.

K. Example of how MCnest could be used in a tiered risk assessment process

Throughout the Technical Manual we have identified uncertainties that exist in the toxicity data and knowledge of species life history and have discussed how uncertainties in ecological risk assessments are often addressed by making conservative assumptions about parameter values. This is consistent with a tiered risk assessment process that starts with a simple screening level based on conservative, worst-case assumptions about exposure and toxicity values. If the screening level assessment indicates that unacceptable adverse effects are possible, higher tier assessments are performed that refine these assumptions with more realistic values when acceptable data are available while retaining conservative values where uncertainties remain that cannot be resolved due to lack of information. The use of conservative

assumptions at any tier in the risk assessment process is intended to avoid wrongly concluding that there is no unacceptable risk when, in fact, there is.

MCnest can be used in a tiered risk assessment to explore the change in model outcomes as input parameters are refined from conservative default values to values more reflective of field conditions. While it is possible to use MCnest as a screening tool, it is more informative when used in higher tier assessments with the best data available for the input parameters, especially when the risk management questions involve comparing the magnitude of effects among species or among application dates. This section presents an example of how MCnest might be used in a tiered process using a hypothetical pesticide that primarily affects the hatchability of eggs and its effects on American robins.

Based on the default species profile, robins begin laying eggs on April 12 and the first egg in the last nest of the season is laid on July 22. Robins weigh approximately 77 g and during the breeding season consume approximately 72% invertebrates and 28% fruit. Without pesticides, MCnest estimates that robins make an average of 4.1 nest attempts per female and produce 2.02 successful broods per female per year for an overall nest success rate of 49%. The mean number of fledglings per successful nest is 2.8, so the annual reproductive success for robins is 5.7 fledglings per female per year without pesticide exposure.

In this example, we assume the pesticide is applied at 1 pound active ingredient per acre and that the typical date of application is May 15. Based on the avian reproduction test, we assume that the most sensitive endpoint is the proportion of hatchlings per viable eggs and that the NOAEL, when transformed from a dietary concentration to a daily dose, is 10 mg/kg body wt/day. Initially, the model uses the default 35-d half-life for residue degradation and uses the maximum nomogram values for calculating initial daily dose. Under this scenario, an application on May 15 would not affect the first nest attempt of robins, but would cause all subsequent nest attempts during the breeding season to fail (Figure 7), resulting in 0.71 successful broods per female (i.e., a 65% reduction in productivity).
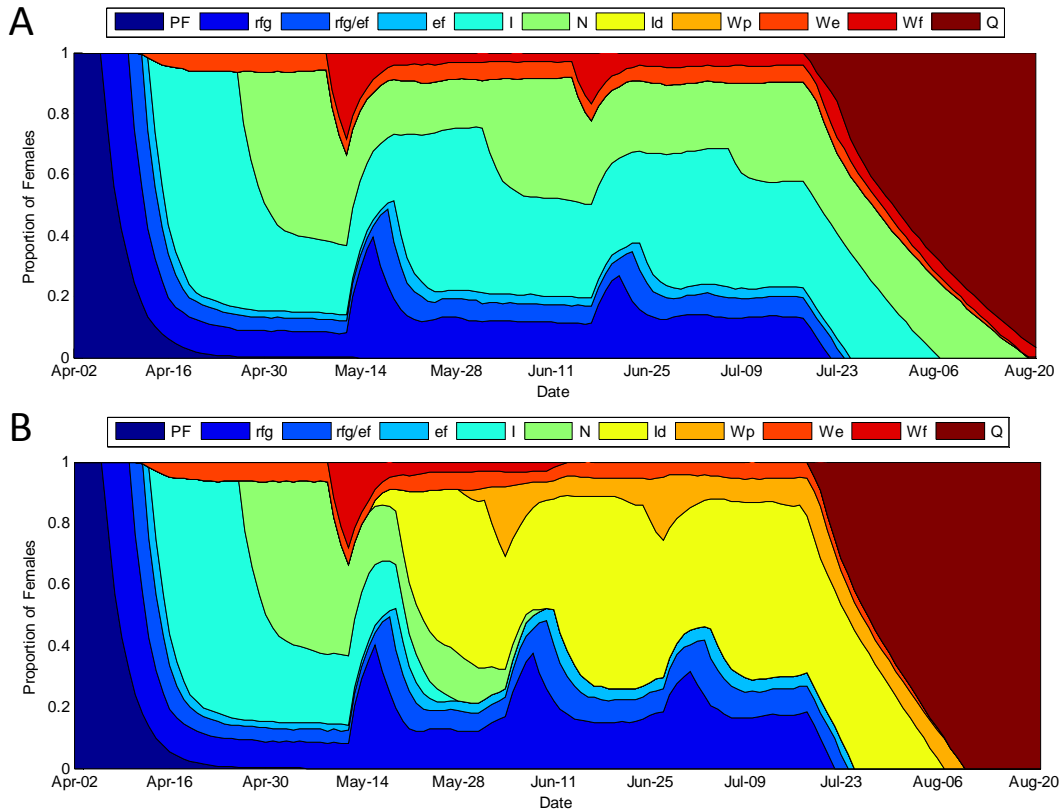
Figure 7. Phase diagrams for American robins A) without pesticide exposure and B) with exposure to a pesticide application on May 15 assuming maximum nomogram residue values for diet and a 35-d half-life for residue degradation.

It is unlikely that all of the females in a population would be exposed to diets with the maximum nomogram residue concentrations. If we rerun the simulation using a lognormal distribution of initial residue concentrations based the mean and standard deviation for residues on each food type in the diet, the robin population produces a mean of 1.29 successful broods per female per year (i.e., 36% reduction in productivity compared to control). Again, the pesticide application does not affect the first nest attempt (Figure 8), but in subsequent nest attempts only those females randomly assigned to the higher concentrations from the lognormal distribution experience lost nest attempts.
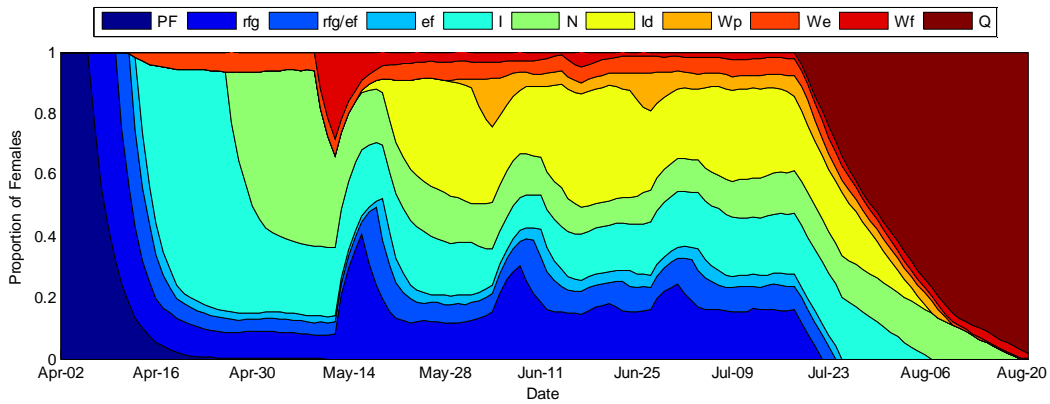
Figure 8. Phase diagram for American robins with exposure to a pesticide application on May 15 assuming a lognormal distribution of initial residue values for diet and a 35-d half-life for residue degradation.

If additional information was available that demonstrated that this pesticide's residue half-life was 7 d, instead of the default 35 d, the rerun simulation indicates that the robin population produces 1.77 successful broods per female per year (i.e., 12% reduction in productivity from control). With the shorter residue half-life, the period of time where exposure doses exceed the toxicity thresholds is greatly reduced, resulting in fewer nest failures near the end of the breeding season (Figure 9).
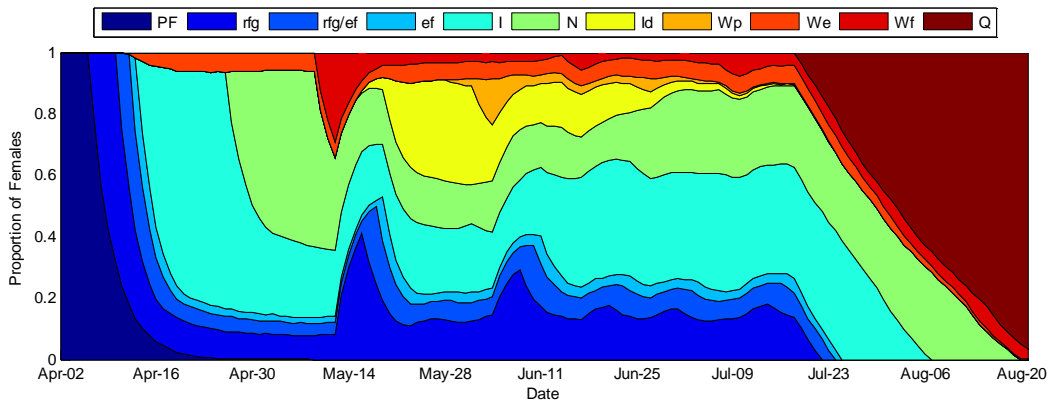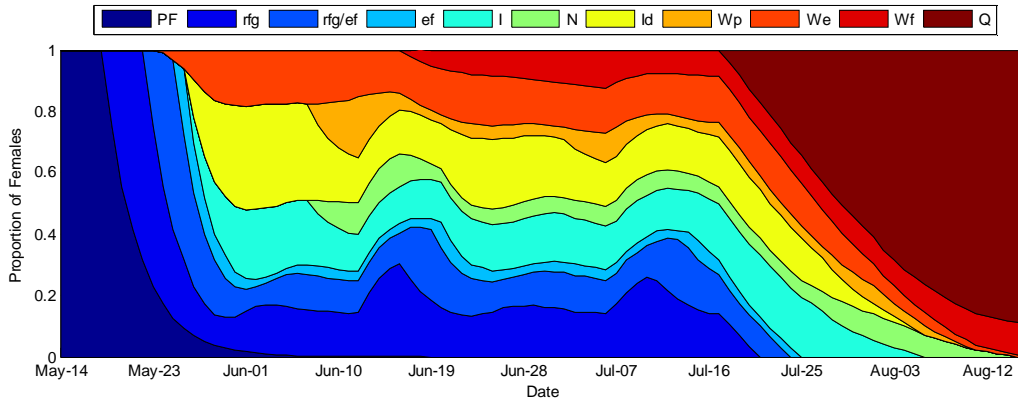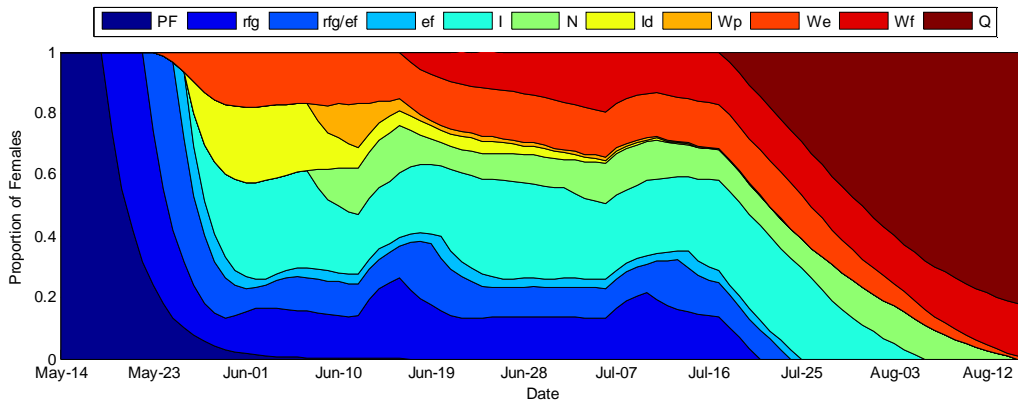

Figure 9. Phase diagram for American robins with exposure to a pesticide application on May 15 assuming a lognormal distribution of initial residue values for diet and a 7-d half-life for residue degradation.

Although May 15 was identified as the typical date for application of this pesticide, we may have information that it could be applied any time in May. For simplicity if we assume that there is an equal probability that the pesticide is applied on each day in May, we can use the batch function in MCnest to rerun the simulation for the 31 days in May and average the results to get an estimate of the overall effect for the application of this pesticide during a typical season. If empirical data exist, any other distribution of application days could be assumed in deriving an overall estimate. Taking the mean of simulations over the month of May indicates the robin population produces 1.81 successful broods per female per year (i.e., a 10% reduction in productivity compared to control), which is only slightly different than the response on May 15 because there was relatively little change in response over this one-month period (Figure 10). However, the timing of pesticide applications, for example May 1 vs May 31, produced failures of nest attempts at different portions of the breeding season (Figure 11).
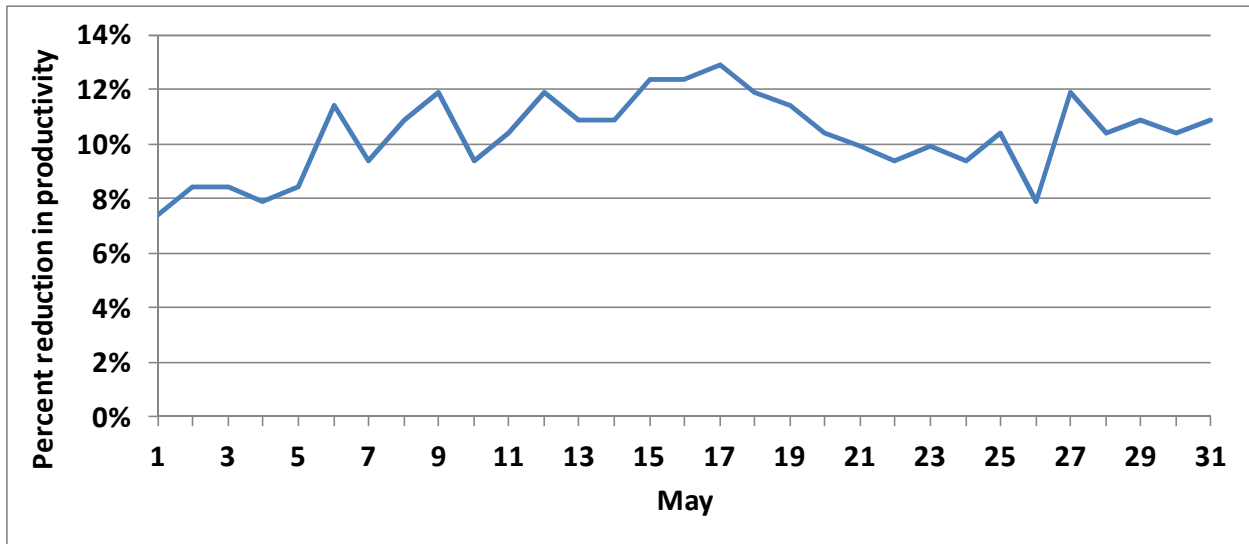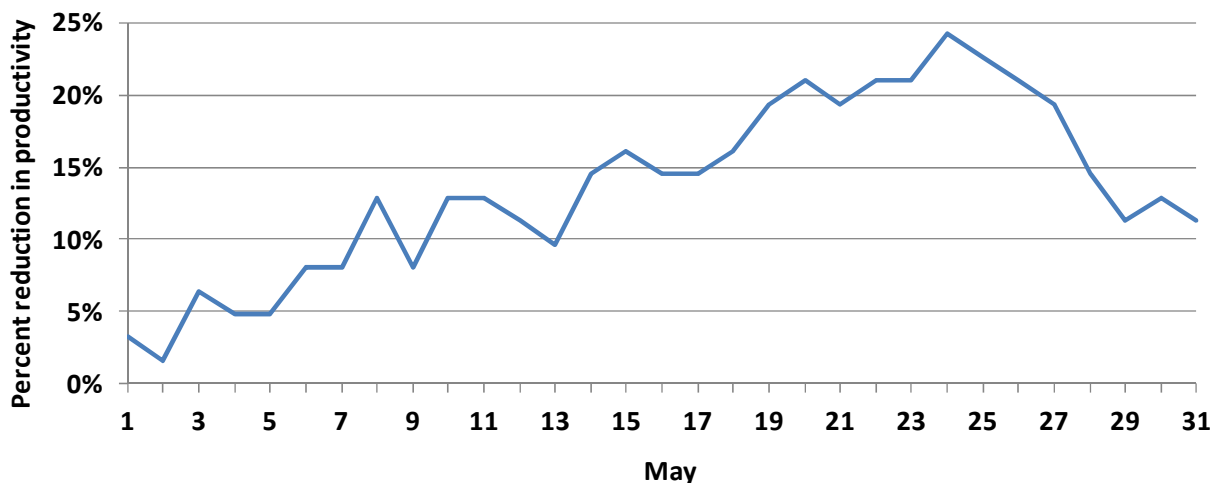

Figure 10. The percent reduction in number of successful broods per female per year for a single application if it occurred on a specific day in May.
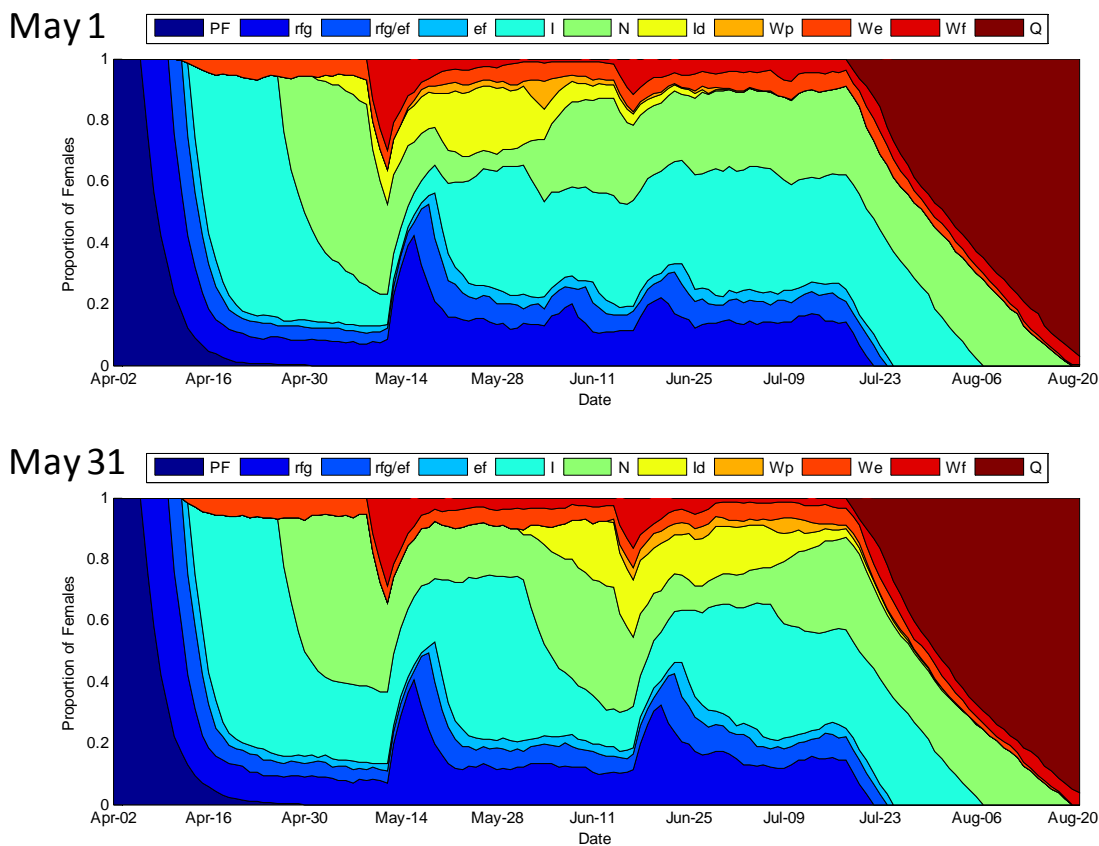
Figure 11. Phase diagrams for American robins with exposure to a pesticide application on May 1 or May 31 assuming a lognormal distribution of initial residue values for diet and a 7-d half-life for residue degradation.

We can run this same series of pesticide-use scenarios with dickcissels that typically produce only one brood per year. Based on the default species profile, dickcissels begin laying eggs on May 24 and the first egg in the last nest of the season is laid on July 21. Dickcissels weigh approximately 25 g and during the breeding season consume approximately 70% invertebrates and 30% seeds. Without pesticides, MCnest estimates that dickcissels make an average of 2.8 nest attempts per female and produce 0.62 successful broods per female per year for an overall nest success rate of 22%. The mean number of fledglings per successful nest is 2.9, so the annual reproductive success for dickcissels is 1.8 fledglings per female per year without pesticide exposure.

Again, we assume the pesticide is applied at 1 pound active ingredient per acre and that the typical date of application is May 15. Based on the avian reproduction test, we assume that the most sensitive endpoint is the proportion of hatchlings per viable eggs and that the NOAEL, when transformed from a dietary concentration to a daily dose, is 10 mg/kg body wt/day. Initially, the model uses the default 35-d half-life for residue degradation and uses the maximum nomogram values for calculating initial daily dose. Under this scenario, an application on May 15 would result in the failure of all nest attempts until the very end of the breeding season

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(Figure 12), resulting in 0.02 successful broods per female (i.e., a 97% reduction in productivity compared to control).
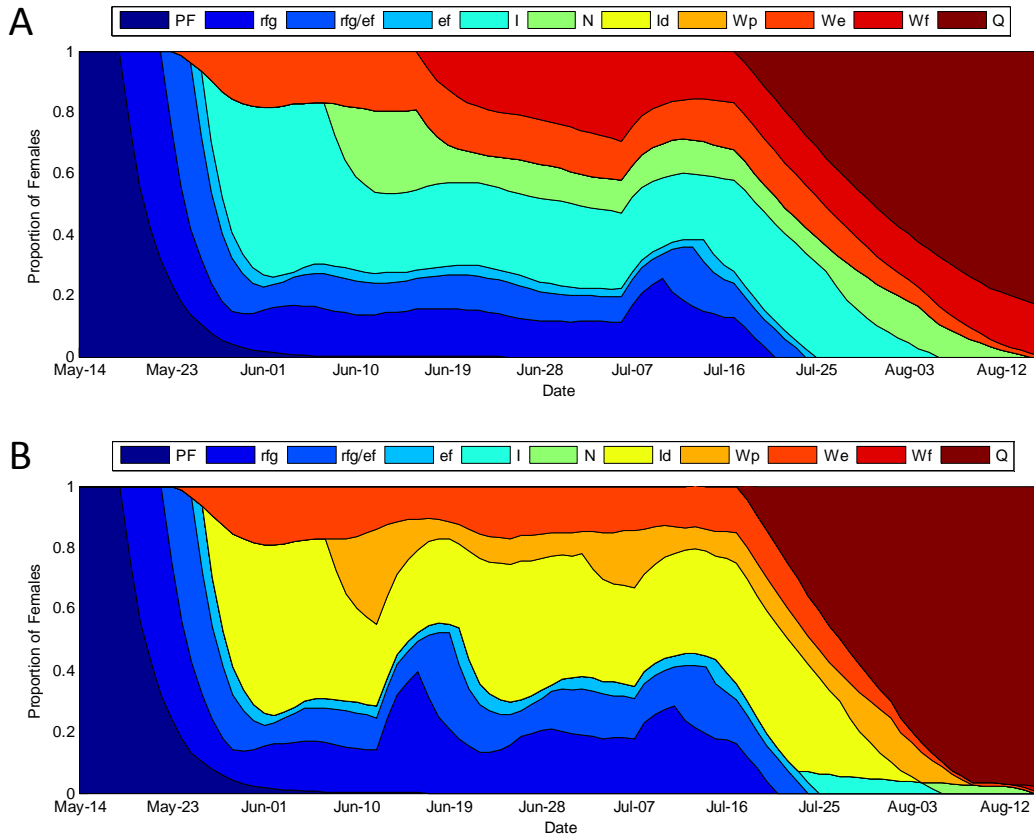
Figure 12. Phase diagrams for dickcissels A) without pesticide exposure and B) with exposure to a pesticide application on May 15 assuming maximum nomogram residue values for diet and a 35-d half-life for residue degradation.

If we rerun the simulation using a lognormal distribution of initial residue concentrations based the mean and standard deviation for residues on each food type in the diet, the dickcissel population produces a mean of 0.32 successful broods per female per year (i.e., a 48% reduction in productivity compared to control). Under this scenario, the pesticide application does affect a portion of the nest attempts throughout the breeding season (Figure 13), but a portion of the nest attempts by females randomly assigned to the lower concentrations from the lognormal distribution are successful.

Figure 13. Phase diagram for dickcissels with exposure to a pesticide application on May 15 assuming a lognormal distribution of initial residue values for diet and a 35-d half-life for residue degradation.

Again, if additional information is used to decrease the pesticide's residue half-life to 7 d, the rerun simulation indicates that the dickcissel population produces 0.54 successful broods per female per year (i.e., 13% reduction in productivity from control). With the shorter residue half-life, the period of time where exposure doses exceed the toxicity thresholds is greatly reduced, resulting in fewer nest failures near the end of the breeding season (Figure 14).

Figure 14. Phase diagram for dickcissels with exposure to a pesticide application on May 15 assuming a lognormal distribution of initial residue values for diet and a 7-d half-life for residue degradation.

Again, if we assume that the pesticide could be applied anytime in May and that there is an equal probability that the pesticide is applied on each day in May, the batch function can be used to run a series of simulations resulting in a mean of 0.54 successful broods per female per year (i.e., a 13% reduction in productivity compared to control). While this is the same overall

result as was observed on May 15, there was considerable change in the response over time during the month (Figure 15), ranging from a 5% reduction or less at the beginning of the month to 24% reduction on May 24, before dropping again by the end of the month.

Figure 15. The percent reduction in number of successful broods per female per year for a single application if it occurred on a specific day in May.

These examples illustrate some ways that MCnest could be used in a tiered risk assessment process to refine the description of the risk posed by a specific pesticide-use scenario. They also illustrate the degree to which refinement of input parameters can change the outcome of the model and help identify where additional data may provide the greatest improvement in model performance. The model user is free to use additional data from specific locations or from other sources of information of pesticide toxicity or use characteristics to refine to refine the model inputs further. Also, as mentioned above, research continues on alternative methods for defining avian breeding seasons or improving the exposure profile that will offer users more options for refining the risk description in future versions of MCnest.

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Appendix A. Estimating the length of the rapid follicle growth (RFG) period

Estimating the length of the RFG period for each species is important because it is used in defining the exposure period for comparing with the surrogate endpoints for hatchability and nestling survival based on *in ovo* exposure. The time-weighted average for exposure during the RFG period for each egg is used as the exposure measure in these decision points. Because the Basic Version of MCnest focuses on exposures that occur during rapid follicle growth, it is best suited for rapidly metabolized chemicals where deposition to egg yolks is primarily from recently consumed foods. It may not be well suited for bioaccumulative chemicals where the chemical deposition to the egg comes primarily from tissue residue stores accumulated over a longer period of time.

As mentioned above, since birds lay one egg at a time, the ovarian follicles that develop into egg yolks start growing on a staggered schedule about 1 d apart or longer. Each follicle grows over a several day period, known as the RFG period. During the RFG period yolk material in deposited to the growing follicle until it reaches the size of a fully formed yolk just prior to ovulation. The rate of follicle growth is approximately sigmoidal, and the energy requirement for each developing follicle over time approximates a bell-shaped curve (King 1973). After ovulation, the yolky follicle enters the oviduct for about 1 d where albumin is deposited and the eggshell is formed. In many species there is an overlap in the RFG and eggshell formation (EF) periods where eggs are being laid at the same time that follicles of subsequent eggs are growing (Figure A1).

Species vary in the reported length of their RFG period (Table A1). Alisauskas and Ankney (1992) show that the duration of the RFG period is related to the egg mass. The general relationship for all birds between RFG (days) and egg mass (E), measured in grams, is:

$$RFG = 2.852 * E^{0.31} \ (r^2 = 0.62).$$

Follicle growth rates for waterfowl are faster relative to egg mass, so the waterfowl-specific relationship is:

$$RFG = 1.273 * E^{0.43} \ (r^2 = 0.71).$$
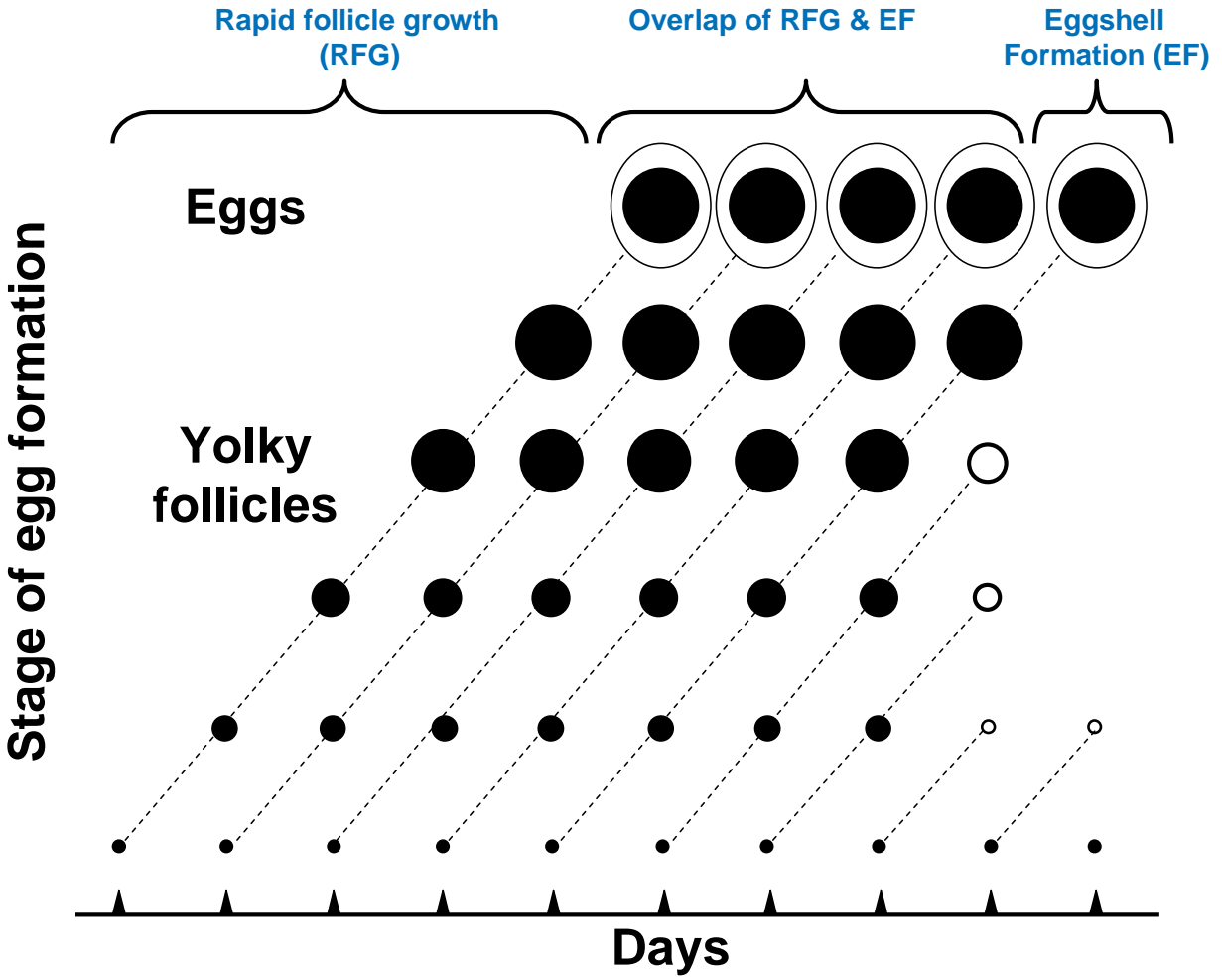
Figure A1. Example of the rapid follicle growth (RFG) and eggshell formation (EF) periods for a clutch of five eggs (adapted from an illustration in Haywood 1993).

Order	Species	RFG period (days)	References
Anseriformes	Mute swan (*Cygnus olor*)	12	Alisauskas & Ankney 1992
	Giant Canada goose (*Branta canadensis maxima*)	13	Alisauskas & Ankney 1992
	Cackling goose (*Branta canadensis minima*)	12	Alisauskas & Ankney 1992
	Domestic duck	6-7	King 1973
	Mallard (*Anas platyrhychos*)	6	Alisauskas & Ankney 1992
	Northern pintail (*Anas acuta*)	4.2	Esler 1994
	American wigeon (*Anas americana*)	5.1	Esler 1994
	Northern shoveler (*Anas clypeata*)	5	Alisauskas & Ankney 1992
	Wood duck (*Aix sponsa*)	7	Drobney 1980
	Canvasback (*Aythya valisineria*)	7	Barzen & Serie 1990
	Redhead (*Aythya americana*)	7	Alisauskas & Ankney 1992
	Lesser scaup (*Aythya affinis*)	6	Alisauskas & Ankney 1992
	Lesser scaup (*Aythya affinis*)	5	Esler 1994
	Greater scaup (*Aythya marila*)	5.2	Gorman et al. 2007
	Ring-necked duck (*Aythya collaris*)	6	Alisauskas & Ankney 1992
	Common goldeneye (*Bucephala clangula*)	8	Alisauskas & Ankney 1992
	Common eider (*Somateria mollissima dresseri*)	6	Alisauskas & Ankney 1992
	White-winged scoter (*Melanitta fusca*)	6	Alisauskas & Ankney 1992
	Ruddy duck (*Oxyura jamaicensis*)	5-6	Alisauskas & Ankney 1994
	Common merganser (*Mergus merganser*)	9	Alisauskas & Ankney 1992
Falconiformes	Eurasian kestrels (*Falco tinnunculus*)	7	Meijer et al. 1989
Galliformes	Domestic chicken	7-8	King 1973
	Chukar (*Alectoris graeca*)	~6	King 1973
	California quail (*Lophortyx californicus*)	6-7	King 1973
	Japanese quail (*Coturnix coturnix*)	5	Sonoda et al. 1996
Gruiformes	American coots (*Fulica americana*)	7	Alisauskas & Ankney 1985
Charadriiformes	Bar-tailed godwit (*Limosa lapponica*)	8-12	Roudybush et al. 1979
	Ruddy turnstone (*Arenaria interpres*)	5-6	Roudybush et al. 1979
	Western sandpiper (*Calidris mauri*)	5-8	Roudybush et al. 1979
	Red phalarope (*Phalaropus fulicarius*)	4-5	Roudybush et al. 1979
	Northern phalarope (*Phalaropus lobatus*)	6-7	Roudybush et al. 1979
	Herring gull (*Larus argentatus*)	9-10	King 1973
	Herring gull (*Larus argentatus*)	11-13	Roudybush et al. 1979
	Glaucous gull (*Larus hyperboreus*)	12	Roudybush et al. 1979
	Great black-backed gull (*Larus marinus*)	13	Roudybush et al. 1979
	Glaucous-winged gull (*Larus glaucescens*)	12	Roudybush et al. 1979
	Western gull (*Larus occidentalis*)	10-11	Roudybush et al. 1979
	Ring-billed gull (*Larus delawarensis*)	12	Roudybush et al. 1979
	Mew gull (*Larus canus*)	5-8	Roudybush et al. 1979
	Black-legged kittiwake (*Rissa tridactyla*)	9	Roudybush et al. 1979
	Sabine's gull (*Xema sabini*)	7-8	Roudybush et al. 1979
	Arctic tern (*Sterna paradisaea*)	6	Roudybush et al. 1979
	Common murre (*Uria aalge*)	12-18	Roudybush et al. 1979
	Black guillemot (*Cepphus grylle*)	8	Roudybush et al. 1979

Table A1. Estimated duration of rapid follicle growth (RFG) period for avian species.

	Pigeon guillemot (*Cepphus columba*)	10	Roudybush et al. 1979
	Cassin's auklet (*Ptychoramphus aleuticus*)	8	Roudybush et al. 1979
	Tufted puffin (*Lunda cirrhata*)	12-13	Roudybush et al. 1979
Columbiformes	Domestic pigeon	5-8	King 1973
	Ring dove (*Streptopelia sp.*)	5-7	King 1973
Passeriformes	Jackdaw (*Corvus monedula*)	~5	King 1973
	Starling (*Sturnus vulgaris*)	3	Ricklefs 1976
	Great tit (*Parus major*)	3-4	King 1973
	Hermit warbler (*Dendroica occidentalis*)	3	Pearson & Rohwer 1998
	Townsend's warbler (*Dendroica townsendi*)	3	Pearson & Rohwer 1998
	House sparrow (*Passer domesticus*)	4	Krementz & Ankney 1986
	Eastern meadowlark (*Sturnella magna*)	4	Pearson & Rohwer 1998
	Western meadowlark (*Sturnella neglecta*)	4	Pearson & Rohwer 1998
	Tricolored blackbird (*Agelaius tricolor*)	3-4	King 1973
	Brown-headed cowbird (*Molothrus ater*)	~3	Scott 1978
	White-crowned sparrow (*Zonotrichia leucophrys*)	~4	King 1973
	Song sparrow (*Melospiza melodia*)	~4	King 1973
	Zebra finch (*Taeniopygia guttata*)	4	Haywood 1993

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Appendix B. Bar graphs representing sensitivity analysis of life history parameters

For each of the 27 baseline scenarios, a bar graph is presented to help visualize how increasing or decreasing each life history parameter by 20% affects the MCnest output (as summarized in Table 6). The MCnest output is represented on the x-axis of each graph and is expressed as the proportional change in the number of successful broods per female relative to the baseline scenario (i.e., (modified–baseline)/baseline). The blue bar represents the proportional change in broods due to a 20% increase in the specific parameter value, while the red bar represents a 20% reduction in parameter value.

At the top of each figure is the identifier for each baseline scenario, such as $T = 60$, $m_1 = m_2 = 0.015$, $W_f = 10$, which indicates that for this particular baseline simulation the length of the breeding season (T) was set at 60 d, the daily nest mortality rates during egg laying, incubation, and nestling rearing (m_1 and m_2) was 0.015 d^{-1}, and the waiting period after nest success (W_f) was 10 d. All other input parameters were held constant for the 27 baseline simulations, including: 1) length of the rapid follicle growth period (rfg; 5 d), 2) mean clutch size ($clutch$; 5 eggs), 3) duration of incubation period (I; 10 d), 4) duration of the nestling rearing period (N; 10 d), and 5) duration of the waiting period after ecological failure (W_e; 10 d). Also, all simulations used an egg-laying interval (eli) of 1 d and set incubation to start with the laying of the last egg.

T = 60, *m*₁ = *m*₂ = 0.015, *W_f* = 10
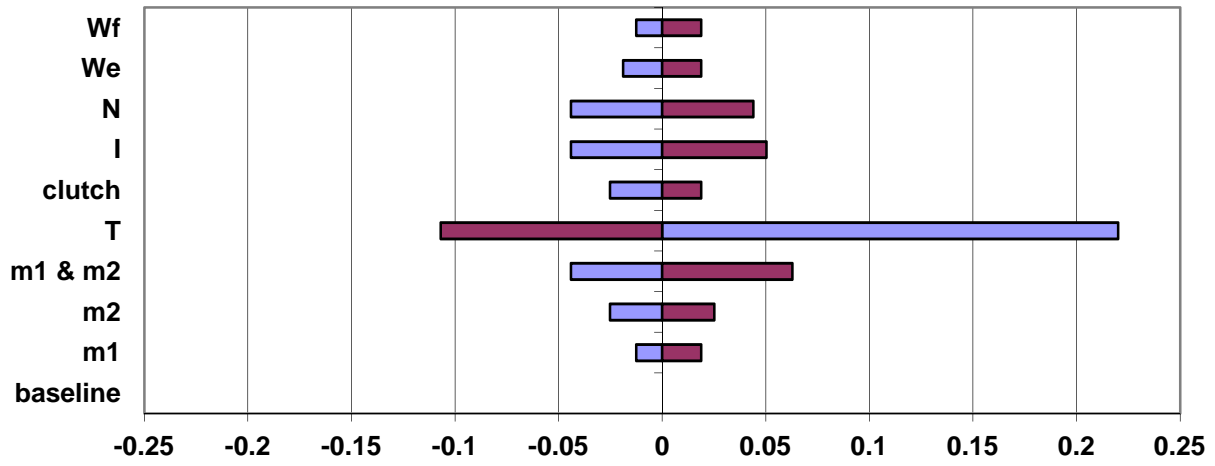
T = 60, *m*₁ = *m*₂ = 0.015, *W_f* = 20
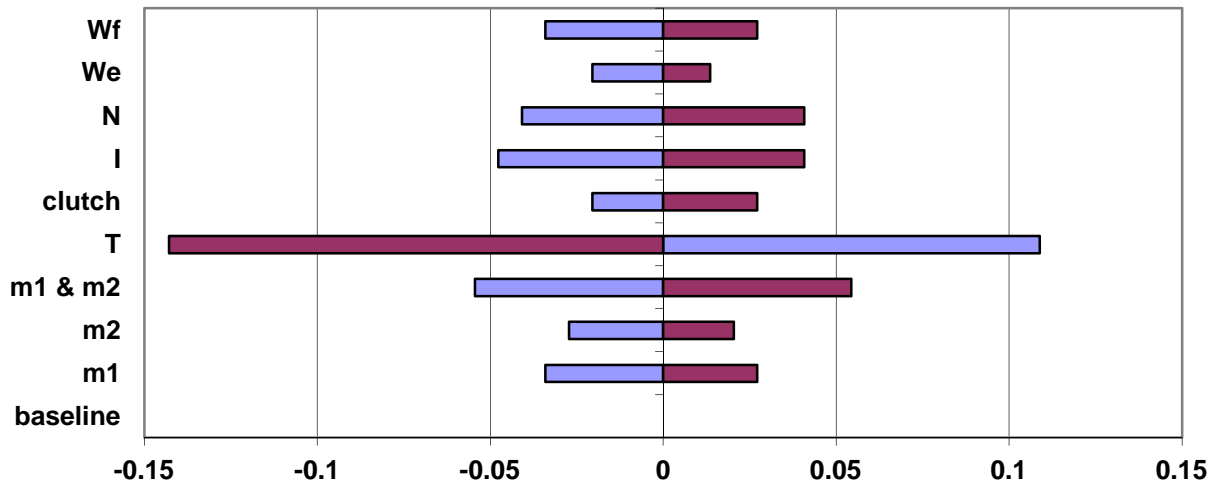
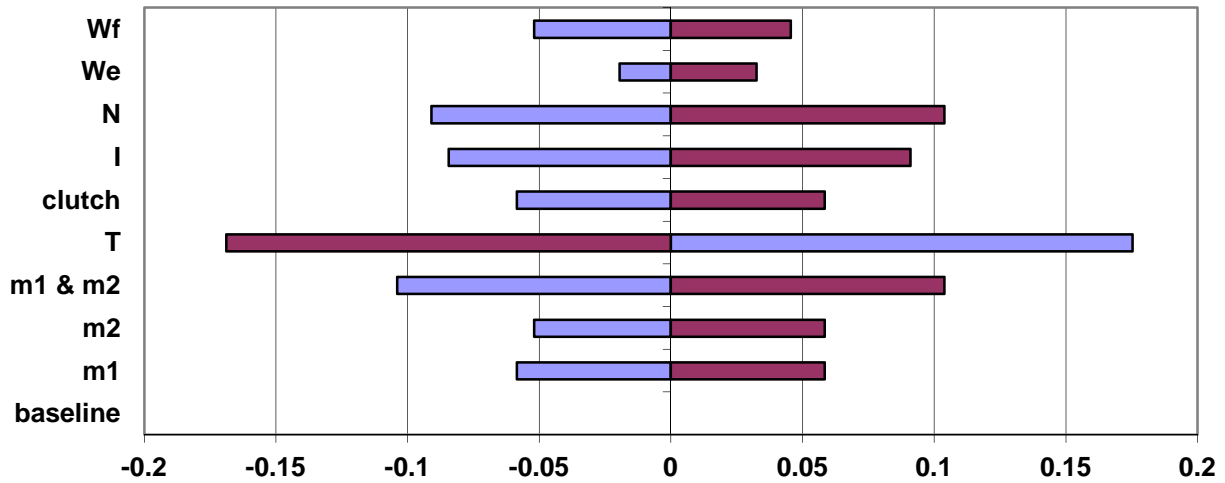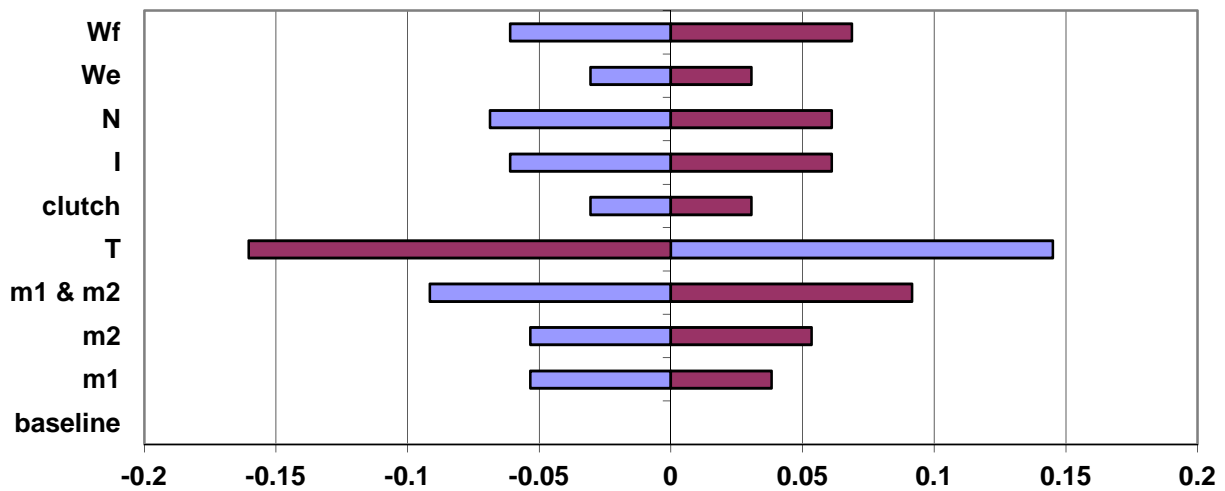*T* = 60, *m*₁ = *m*₂ = 0.015, *W_f* = 40
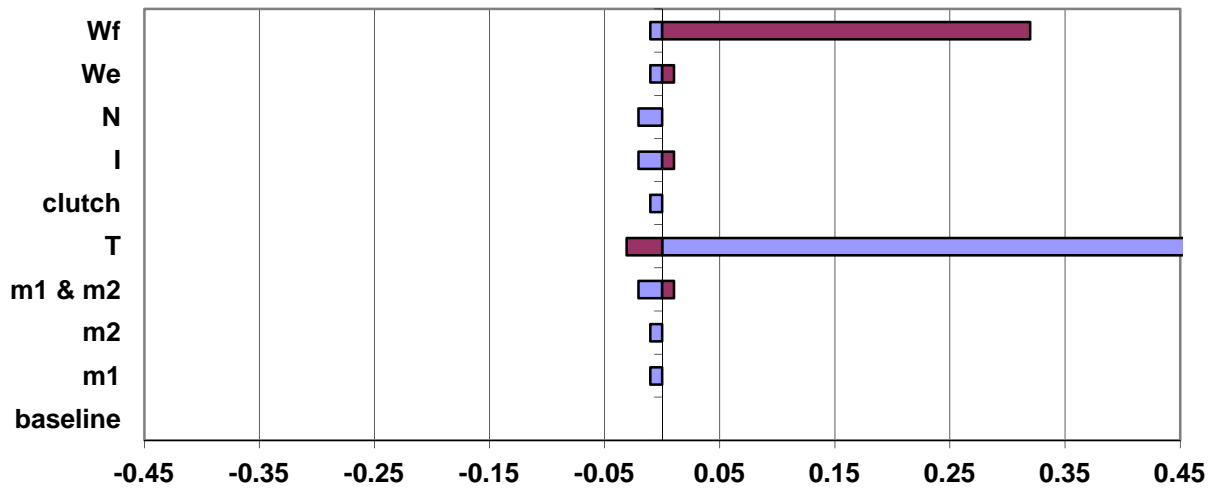
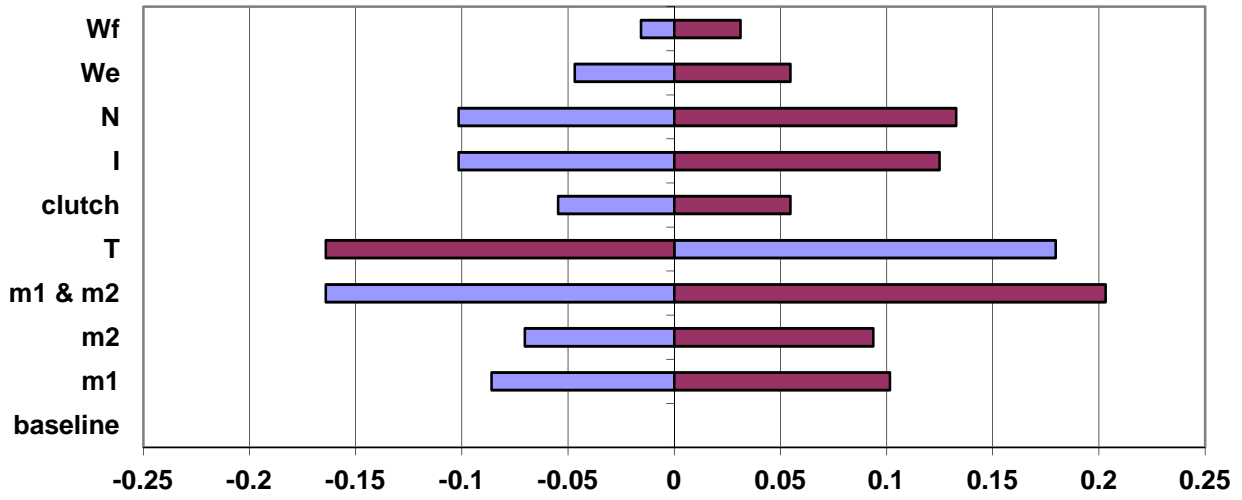$T = 60$, $m_1 = m_2 = 0.03$, $W_f = 10$
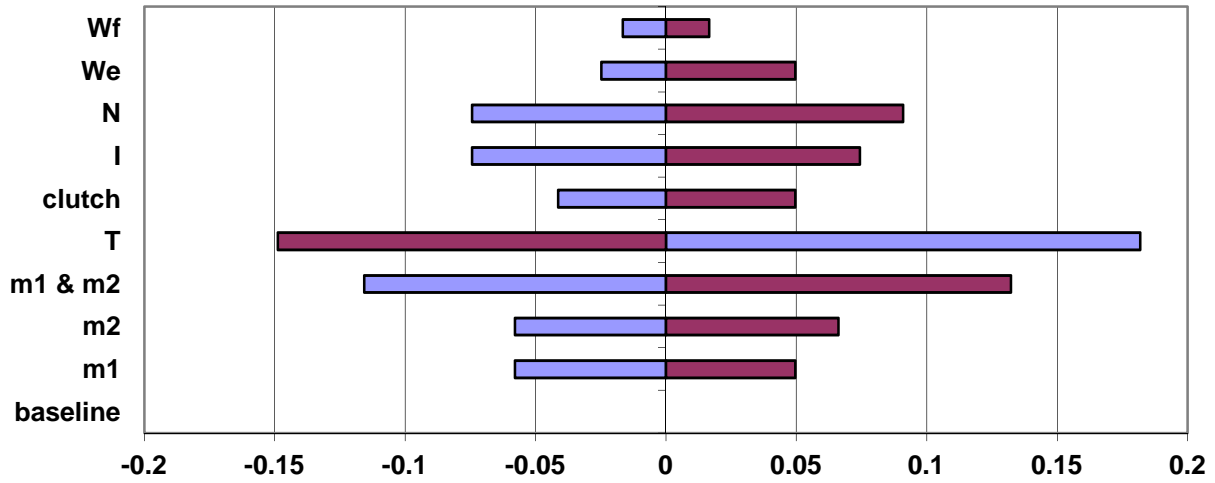
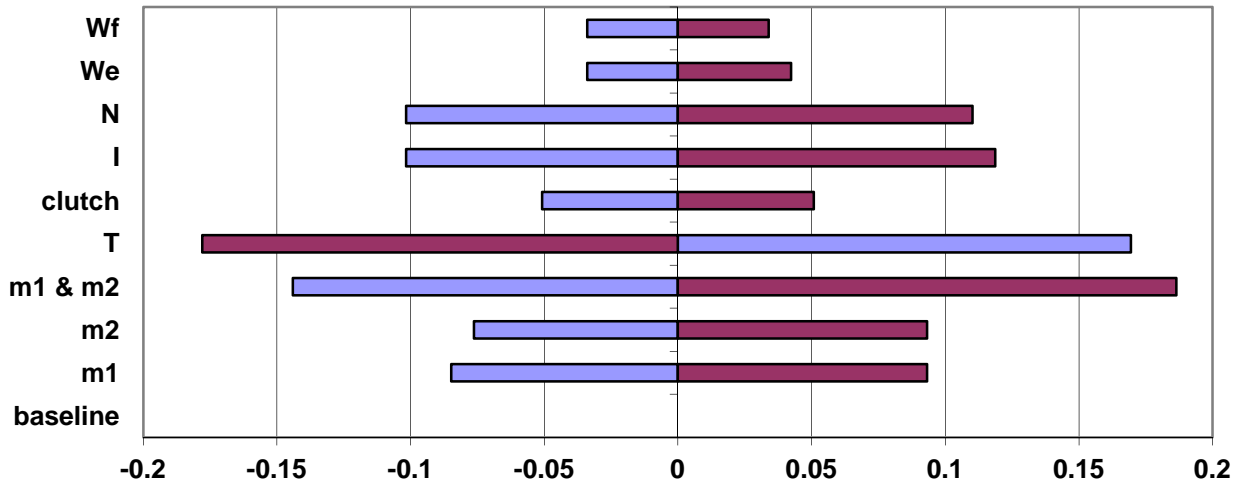$T = 60$, $m_1 = m_2 = 0.03$, $W_f = 20$
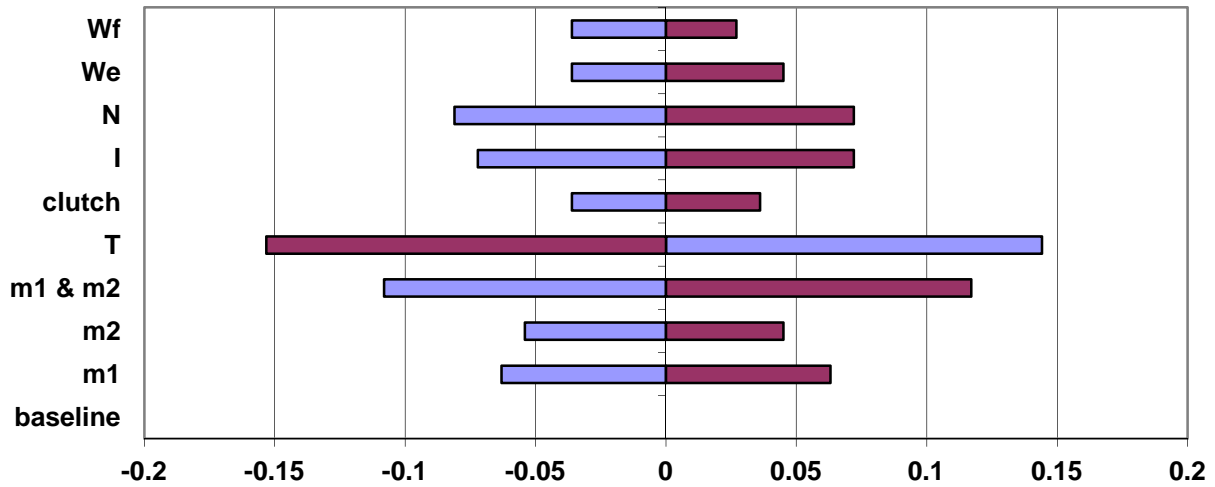
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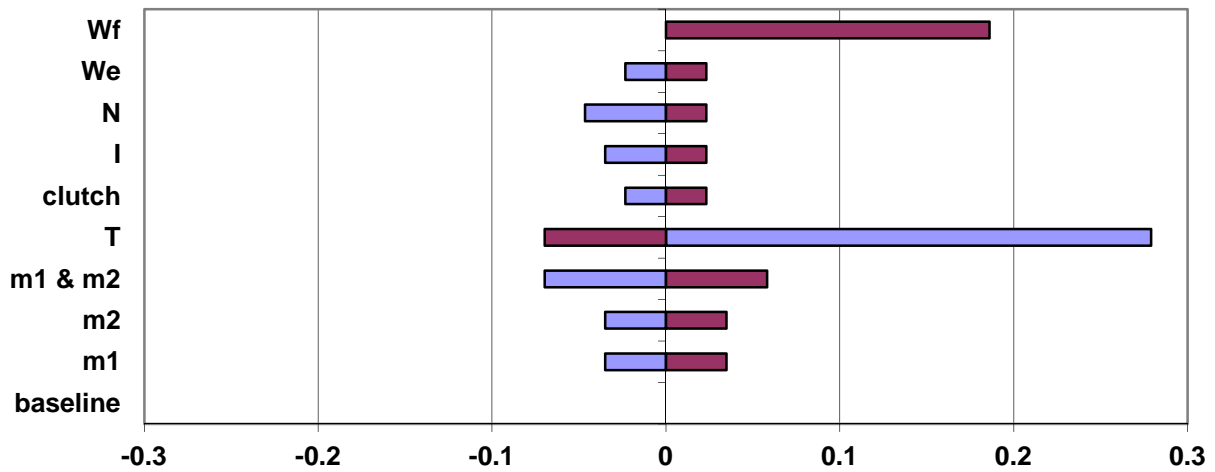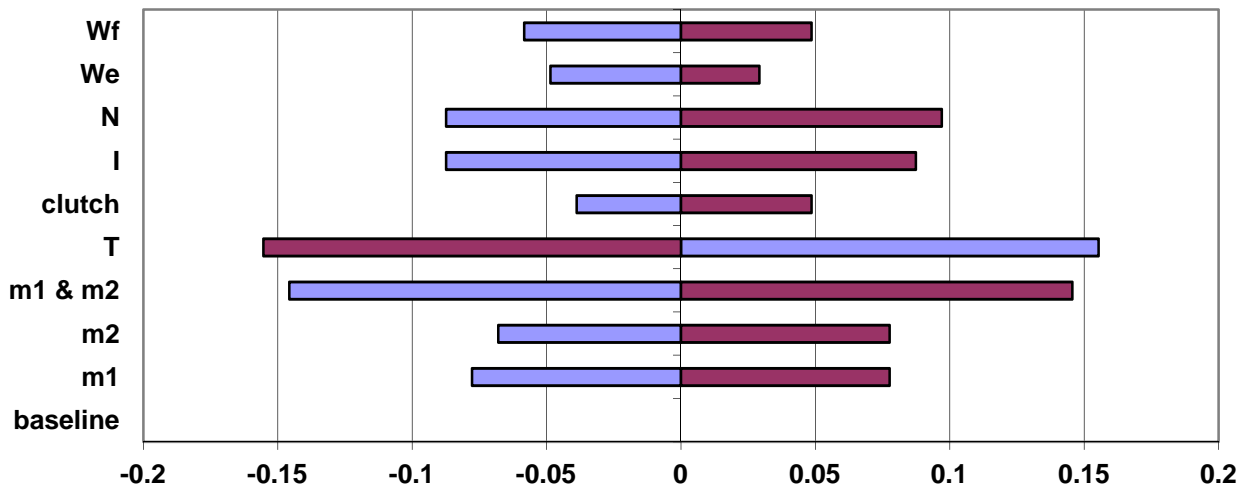
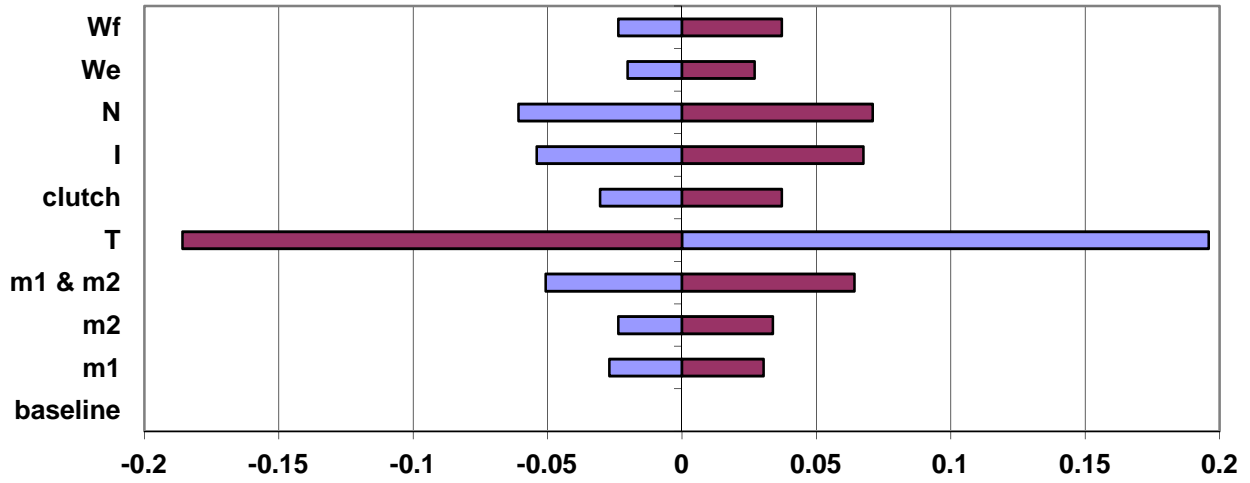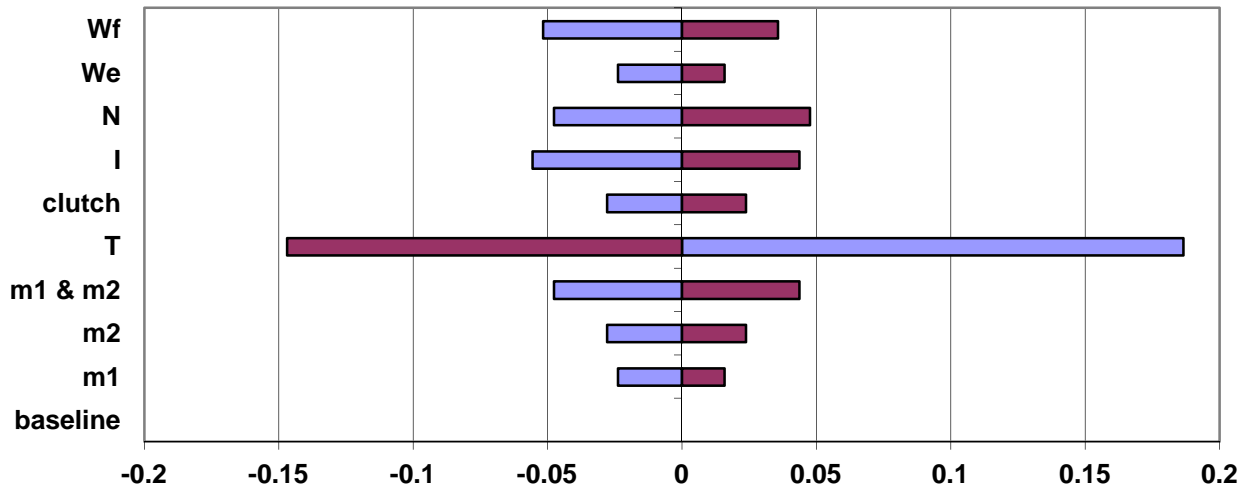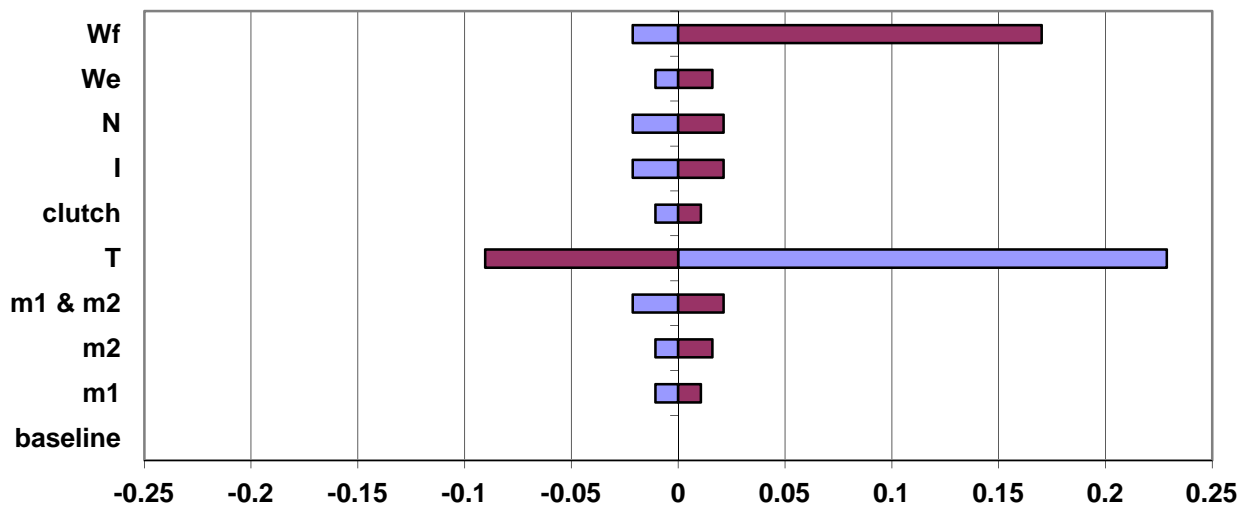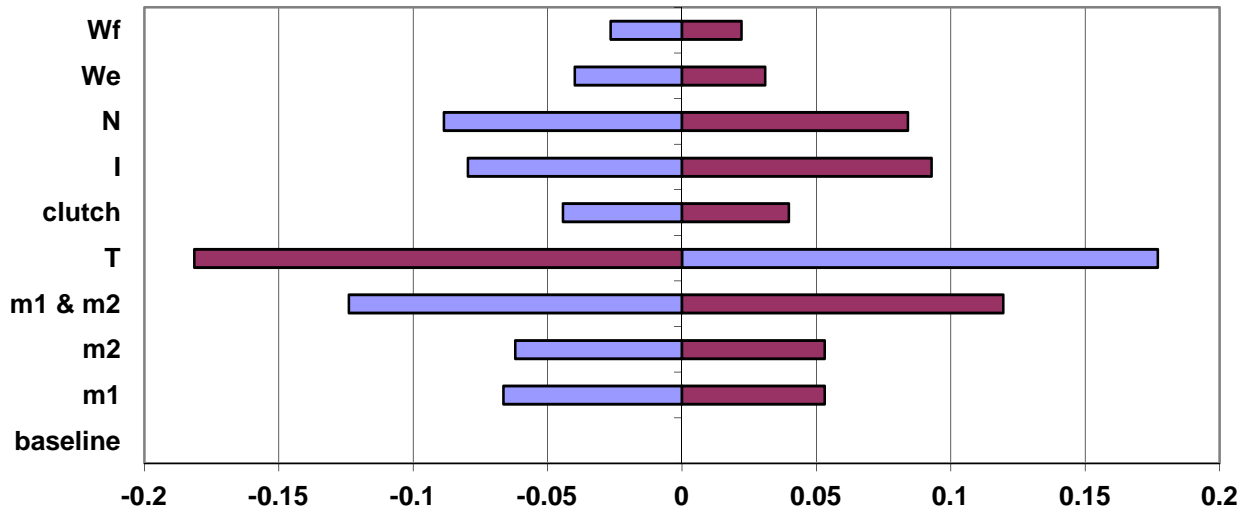$T = 60$, $m_1 = m_2 = 0.045$, $W_f = 10$
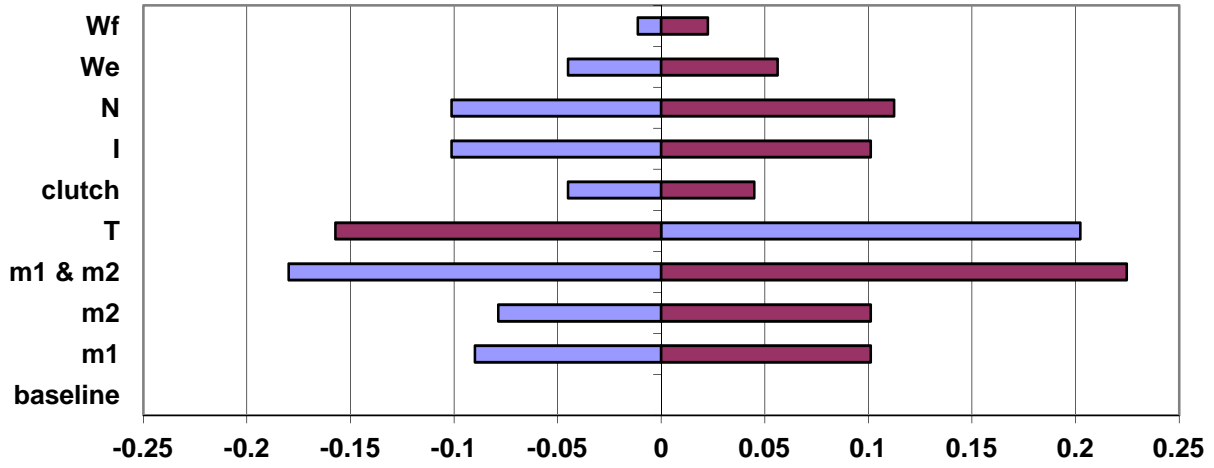
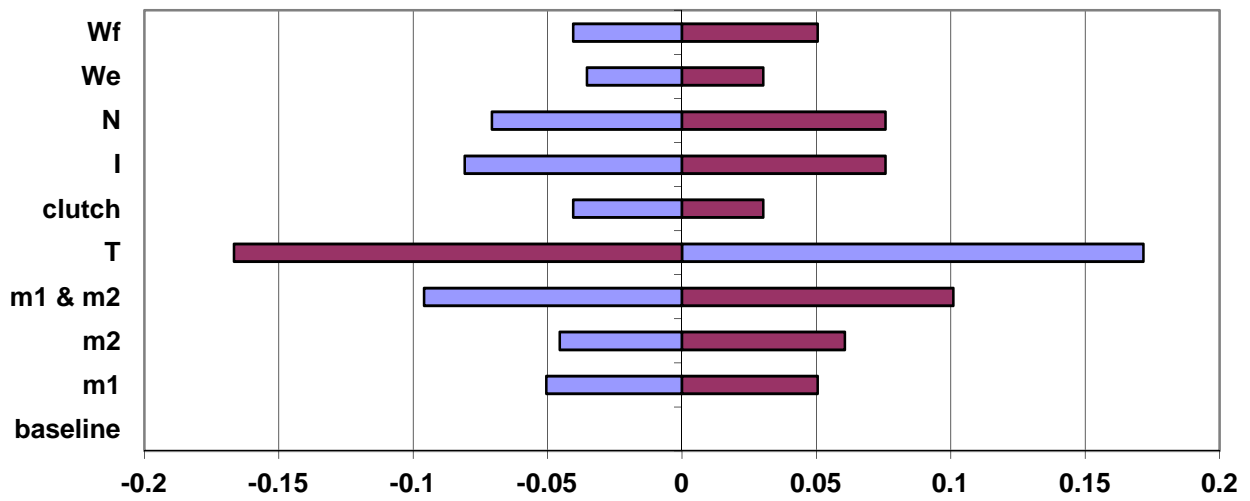$T = 60$, $m_1 = m_2 = 0.045$, $W_f = 20$
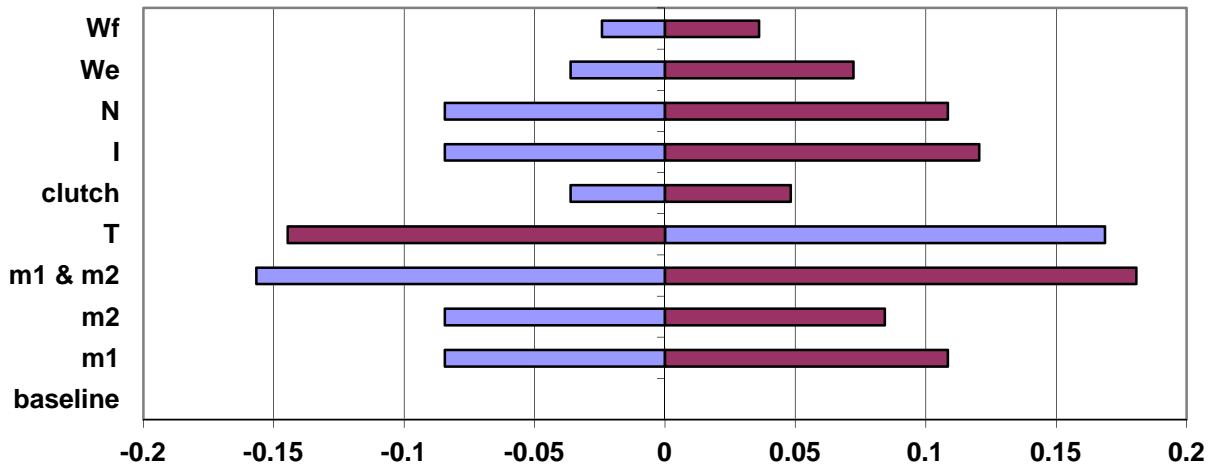
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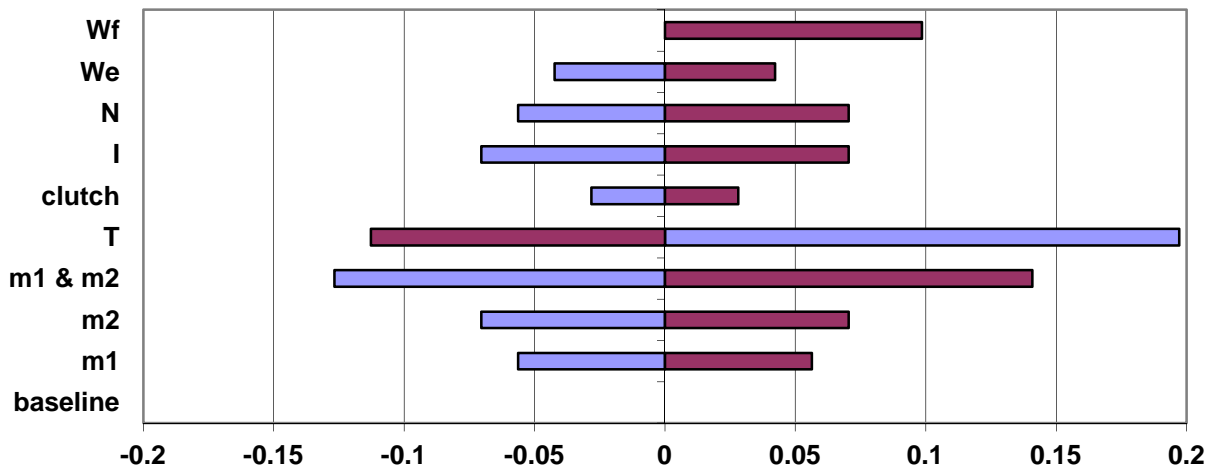
$T = 90$, $m_1 = m_2 = 0.015$, $W_f = 10$
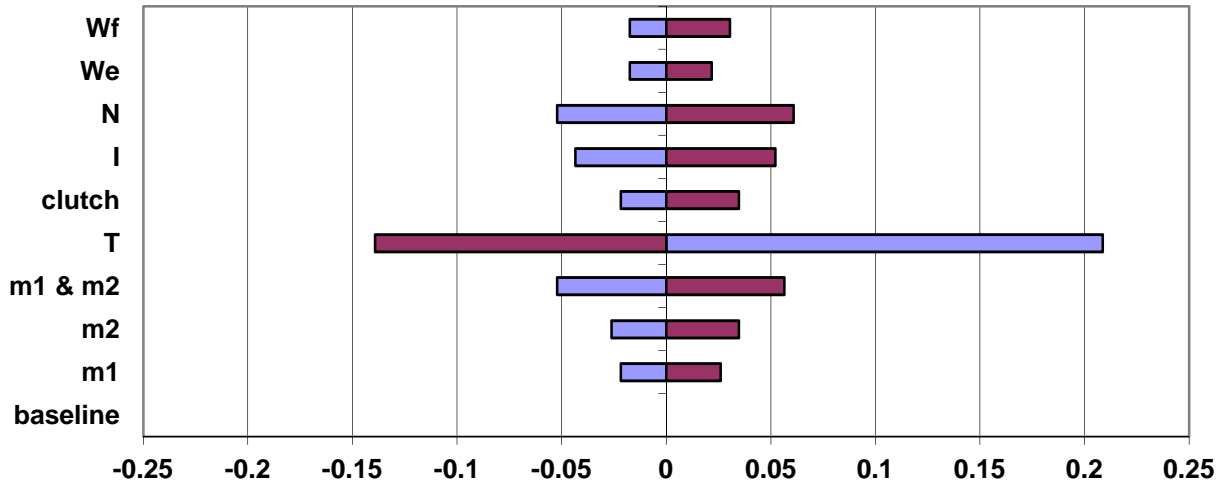
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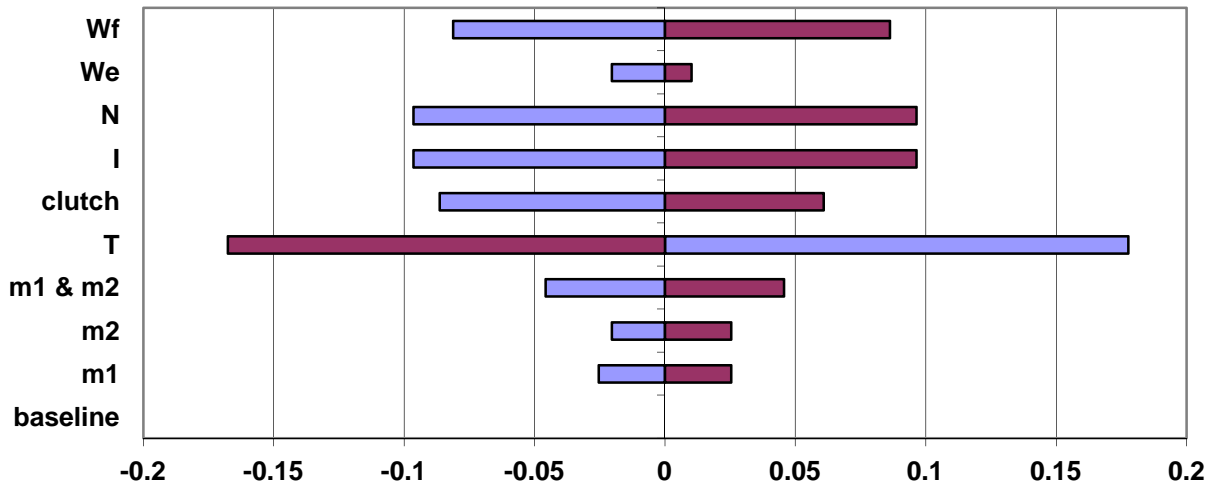
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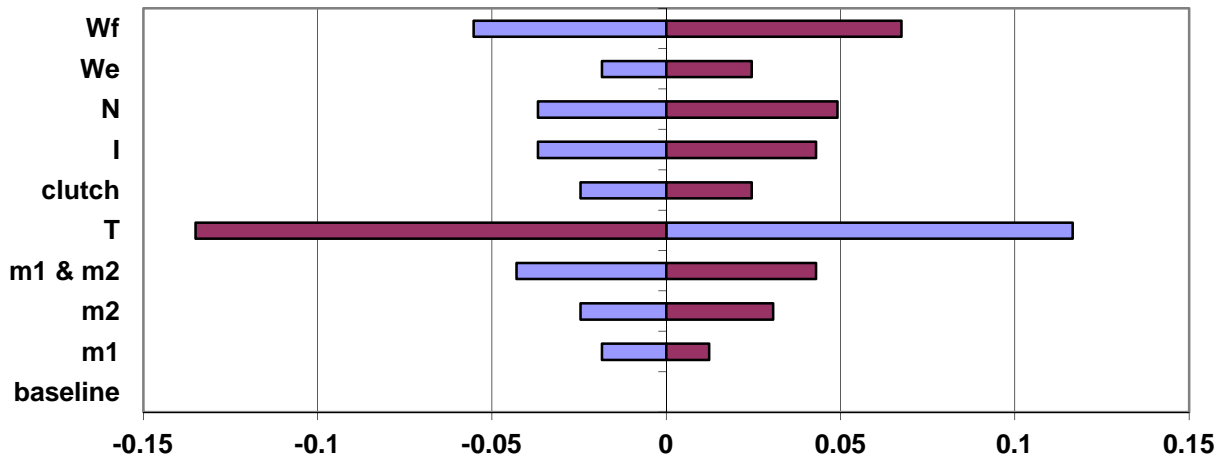
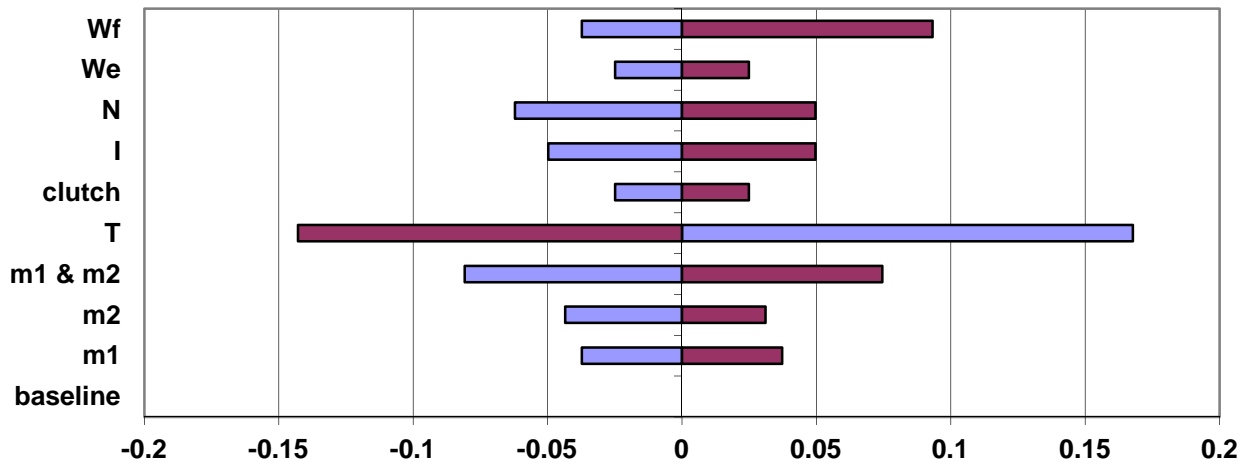*T* = 90, *m*₁ = *m*₂ = 0.03, *W*𝒻 = 10
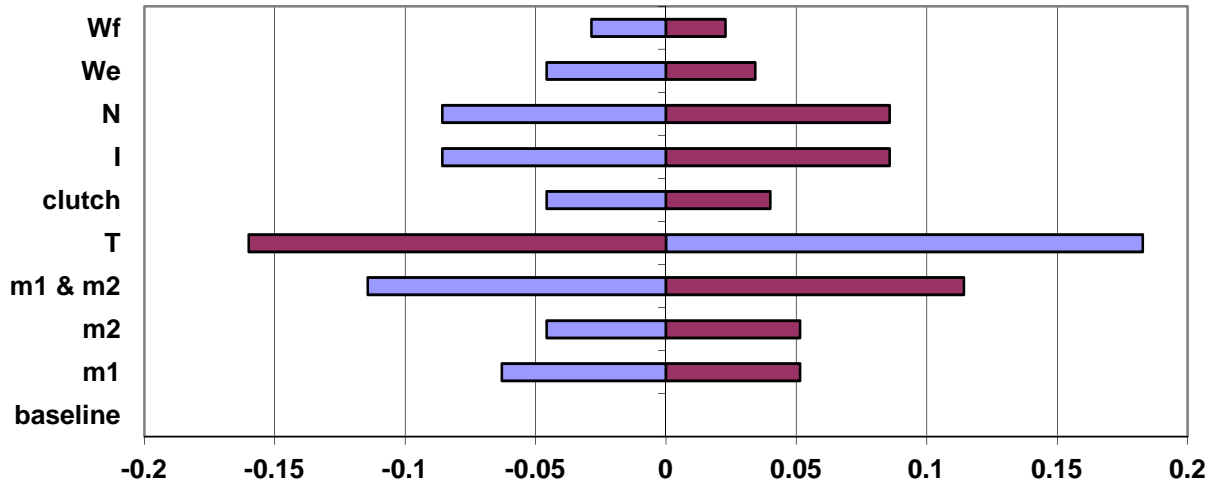
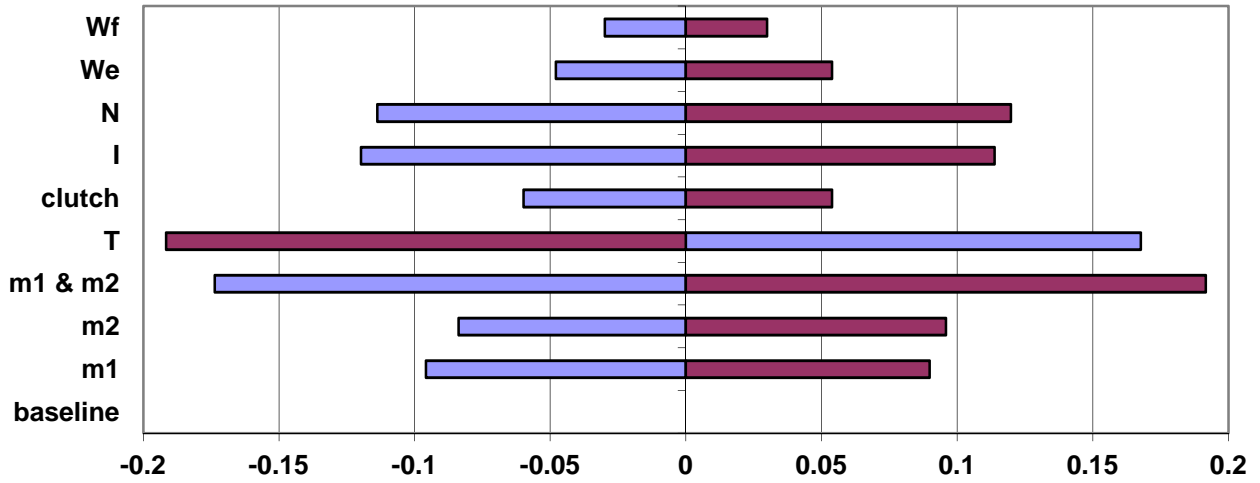$T = 90$, $m_1 = m_2 = 0.03$, $W_f = 10$

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$T = 90$, $m_1 = m_2 = 0.045$, $W_f = 40$

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$T = 120$, $m_1 = m_2 = 0.015$, $W_f = 20$

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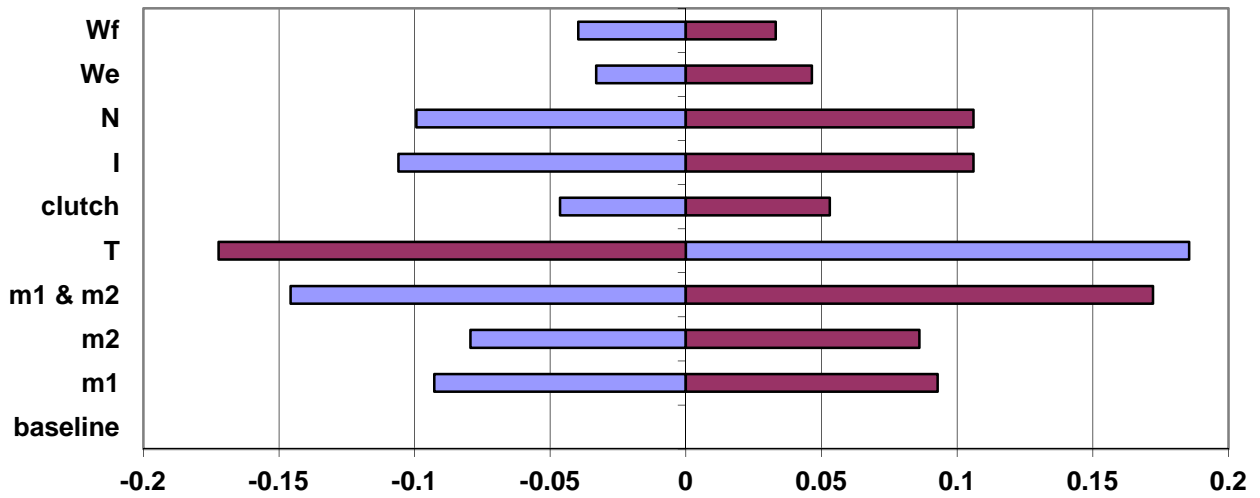$T = 120$, $m_1 = m_2 = 0.03$, $W_f = 20$

$T = 120$, $m_1 = m_2 = 0.03$, $W_f = 40$

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$T = 120$, $m_1 = m_2 = 0.045$, $W_f = 20$

$T = 120$, $m_1 = m_2 = 0.045$, $W_f = 40$