7-17-2013

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Integrated Environmental Assessment and Management — Volume 9, Number 4—pp. 600–609


# Selecting Surrogate Endpoints for Estimating Pesticide Effects on Avian Reproductive Success

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*(Submitted 10 January 2013; Returned for Revision 8 February 2013; Accepted 17 July 2013)*



## ABSTRACT

A Markov chain nest productivity model (MCnest) has been developed for projecting the effects of a specific pesticide-use scenario on the annual reproductive success of avian species of concern. A critical element in MCnest is the use of surrogate endpoints, defined as measured endpoints from avian toxicity tests that represent specific types of effects possible in field populations at specific phases of a nesting attempt. In this article, we discuss the attributes of surrogate endpoints and provide guidance for selecting surrogates from existing avian laboratory tests as well as other possible sources. We also discuss some of the assumptions and uncertainties related to using surrogate endpoints to represent field effects. The process of explicitly considering how toxicity test results can be used to assess effects in the field helps identify uncertainties and data gaps that could be targeted in higher-tier risk assessments. *Integr Environ Assess Manag* 2013;9:600–609. © 2013 SETAC

**Keywords:** Surrogate endpoints Avian reproductive success Pesticides Simulation model Phase-specific risk quotients



## INTRODUCTION

Etterson and Bennett (this issue) presented a model for estimating the effects of pesticide applications on the annual reproductive success of avian populations. The model, known as the Markov chain nest productivity model or MCnest, is based on a conceptual approach outlined in Bennett et al. (2005) that integrates avian toxicity data with information on the temporal relationship between the timing of pesticide applications and the timing and duration of the nesting season of bird species (currently available at http://www.epa.gov/med/Prods_Pubs/mcnest.htm). MCnest simulates the nesting season of a population of females consisting of 1 or more nest attempts per female depending on the species of interest. Each MCnest simulation estimates the mean number of successfully fledged broods per female per year, which can be multiplied by the mean number of fledglings per successful nest to calculate the annual reproductive success (i.e., mean number of fledglings per female per year). More detail on the ecology underlying MCnest is provided in Etterson and Bennett (this issue). See also Bennett and Etterson (2006) and Etterson et al. (2011).

MCnest treats each nesting attempt as a series of discrete breeding phases, such as pair formation, egg laying, incubation, and nestling rearing. Within a MCnest simulation, all females in a population are not in the same breeding phase on a given day. Asynchrony in the population is introduced because females vary the date on which first nests are initiated, and asynchrony increases because all nest attempts are subject to a literature-derived, daily nest mortality rate based on ecological factors such as predation, parasitism, and weather. Pesticide effects are introduced by considering the various types of effects that may occur in the field during each breeding phase from exposure to a specific pesticide application and/or applications. In MCnest simulations, the model user defines the application rates and timing for 1 or more pesticide applications. During each breeding phase of each nest attempt, the expected pesticide exposure is compared to toxicity thresholds of specific measured endpoints in a series of decision points used to determine whether a pesticide application poses a risk to that nesting attempt. The specific measured endpoints used for comparison are referred to as surrogate endpoints because they are intended to act as surrogates for specific types of effects in the field.

MCnest is designed to draw surrogate endpoints from the 3 standardized avian toxicity tests (i.e., avian reproduction test, acute oral LD50 test, and 5-d dietary LC50 test) used by the US Environmental Protection Agency (USEPA 2012), but other types of studies may be suitable for providing additional or alternative surrogate endpoints. Traditionally, endpoints from these tests are compared to estimates of exposure for a specific pesticide-use scenario to produce risk quotients. To classify the potential for adverse risk to the species of concern, the value of a risk quotient is compared to a regulatory level of concern (LOC). If a risk quotient exceeds the LOC, additional assessment may be required for making a regulatory decision.

Although the current avian reproduction test is not sufficient on its own to directly quantify effects on annual reproductive success (Mineau 2005; Bennett and Etterson 2006), the use of existing measured endpoints as surrogate indicators of specific types of effects potentially occurring in the field is critical to the functioning of MCnest. A review of avian reproduction tests by Mineau et al. (1994) used cluster analysis to segregate the test endpoints into 3 categories based on correlations of responses among endpoints. The categories reflected parental (especially

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Published online 3 August 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ieam.1478

maternal) toxicity, effects on eggshell quality, and developmental effects from in ovo exposure. They concluded that whereas the avian reproduction test is not a realistic simulation of the entire reproductive process of birds under a given pesticide exposure pattern, it does provide information on the types of reproductive effects potentially occurring at sublethal levels of exposure, especially when reproductive effects are observable at exposures below those causing observable parental effects. However, there are several types of potential effects in the field that are not represented explicitly in the reproduction test (Mineau et al. 1994; Mineau 2005; Bennett and Etterson 2006), although there are endpoints in the avian reproduction test that can act indirectly as surrogate endpoints for some of these effects. For other types of field effects there are no suitable surrogate endpoints available from the reproduction test. In such cases, surrogate endpoints may be available from other laboratory tests (i.e., acute LD50 or dietary LC50 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 nestlings from direct pesticide exposure, because juveniles are not fed pesticide-treated diets in the reproduction test.

The goal of this article is to more thoroughly discuss the process of selecting and using surrogate endpoints in a model such as MCnest for estimating avian reproductive success, toward the ultimate goal of improving our ability to extrapolate laboratory toxicity test results to field-based estimates of population risk. The first step is to consider the full range of potential pesticide effects on avian reproduction in wild birds. Next, for each of the potential effects on reproduction, we need to determine whether or not there is sufficient information for defining a surrogate endpoint that represents that effect. This article discusses potential surrogate endpoints from standardized avian toxicity tests and from other nontraditional tests. MCnest is designed to be flexible for changing and adding surrogate endpoints as appropriate for each assessment. Any potential field effect, for which there is an appropriate surrogate endpoint, can be integrated into the model as a decision point for estimating pesticide effects on annual reproductive success. Our goals in developing MCnest were to quantify, to the extent possible, the magnitude of pesticide effects on annual reproductive success using existing toxicity data and to highlight the information gaps where existing data alone leave us short of a complete understanding of the effects of pesticide use on annual reproductive success of populations. The identified gaps can guide the types of data collection needed in the future to improve risk assessments.

## TYPES OF PESTICIDE EFFECTS ON AVIAN REPRODUCTION

For assessing the population-level effects of pesticides on wildlife species, it is critical to quantify the relationship between specific measured effects of pesticides and their impact to the demographic rates (survival, growth, fecundity) of the species. In birds, the most important metric of effects on reproduction is a change in annual reproductive success, which we define in MCnest as the number of fledglings per adult female per year. Pesticides may cause a variety of proximate effects throughout all the breeding phases that may or may not result ultimately in a change in annual reproductive success. Also, there are several major categories of effects resulting from different pathways of exposure (Table 1). Bennett et al. (2005) described 3 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; 2) effects on juvenile growth and survival from external exposure; and 3) effects on juvenile growth and survival from in ovo exposure. Two additional categories involving indirect effects to adults and/or juveniles were not previously discussed, but they could be important to the assessment of overall effects on avian reproductive success (Campbell and Cooke 1997; Boatman et al. 2004; Poulin et al. 2010). Indirect effects on reproductive success may occur in a variety of forms, including reduced quantity or quality of food resources for adults or juveniles, changes in habitat quality, or changes is relationships with predators, competitors, or parasites.

During each breeding phase, 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, risk assessors are encouraged to identify all potential effects of a pesticide and, where data exists, 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 standardized laboratory tests because the avian reproduction test measures "a very unnatural and truncated reproductive performance" (Mineau et al. 1994).

## ATTRIBUTES OF SURROGATE ENDPOINTS

Surrogate endpoints must have certain attributes to be useful in models of reproductive effects such as those presented in Etterson and Bennett (this issue), 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. Below, we list and describe 4 important attributes of surrogate endpoints.

First, surrogate endpoints must be measurements of effect that can be linked to an exposure concentration or dose. Although 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, 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 lead to changes in reproductive success (See Table 1). For example, reduced hatching success can result directly in changes in reproductive success, and an observed reduction in hatching rate from in ovo exposure in a laboratory test may indicate that hatching rate would be reduced in the field from comparable pesticide concentrations in eggs, although Blus (1996) points out that even for extensively studied pesticides, like DDT and its derivatives, this relationship is difficult to establish, often because egg residues have not been determined. Measurement endpoints that are proposed as indirect indicators of effects on reproductive success (e.g., a change in a biochemical concentration or in a behavioral measurement) may require additional research to demonstrate their relationship to changes in reproductive success and 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 where there is no plausible

**Table 1**. Types of effects possible during each avian breeding phase by direct, indirect, or in ovo exposures to adults and juveniles

| Breeding phase | Adult direct | Juvenile in ovo | Juvenile direct | Adult indirect[a] | Juvenile indirect[a] |
|---|---|---|---|---|---|
| Pair formation and/or breeding site selection | Territory loss or nest abandonment due to sublethal effects or death | NA | NA | Territory loss or abandonment due to indirect effects | NA |
| Follicle growth and/or egg production | Reduced clutch size<br>Nest abandonment due to sublethal effects or death of adults or eggshell failures | NA | NA | Reduced clutch size<br>Nest abandonment or loss due to indirect effects | NA |
| Incubation to hatching | Nest abandonment due to sublethal effects or death of adults<br>Reduced hatch due to infertility | Embryotoxicity due to in ovo exposure | Embryotoxicity due to external eggshell exposure | Nest abandonment or loss due to indirect effects | NA |
| Nestling rearing to fledging | Brood abandonment due to sublethal effects or death<br>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 posthatch exposure | Brood abandonment or loss due to indirect effects<br>Reduced juvenile growth and/or survival due to reduced parental foraging success | Reduced juvenile growth and survival due to indirect effects |

NA = not applicable.
[a]Indirect effects may represent changes in the quantity and quality of food resources, nesting habitat quality, or a species' relationships to its predators, competitors, parasites, or disease agents.

linkage between the endpoint and effects on overall reproductive success. Establishing plausibility may require reliance on completely separate—and unrelated—studies or 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, each surrogate endpoint must be expressed as an exposure level below which the risk of adverse effects is considered acceptable. We define these exposure levels as toxicity thresholds. In the conceptual approach presented in Bennett et al. (2005) and applications of the approach (Roelofs et al. 2005; Bennett and Etterson 2007), the toxicity thresholds for surrogate endpoints from avian reproduction tests were defined as the no-observable-adverse-effect level (NOAEL) because experimental designs were based on hypothesis testing. This reflects the common practice of using the NOAEL of avian reproduction test endpoints for characterization of risks via risk quotients.

Fourth, although not strictly an attribute of the surrogate endpoint itself, the toxicity threshold of each surrogate endpoint needs to be compared to an estimate of environmental exposure during an appropriate exposure period. It is this comparison of estimated environmental exposures with the toxicity thresholds for surrogate endpoints that forms the basis of the decision points throughout the breeding period in models of reproductive success. The selection of appropriate exposure periods is discussed in greater detail below.

## SELECTING SURROGATE ENDPOINTS FROM EXISTING LABORATORY TESTS

The series of avian surrogate endpoints proposed by Bennett et al. (2005) are measurement endpoints from existing laboratory toxicity tests that represent many of the potential direct effects of a pesticide. The Basic Version of MCnest presented in Etterson and Bennett (this issue) makes several changes and additions to this suite of surrogate endpoints (Table 2). Depending on the nature of a particular chemical, additional surrogate endpoints may be appropriate for representing the same or other potential direct effects. This section provides additional discussion on the selection of appropriate surrogate endpoints from existing laboratory tests for use in models of avian reproductive success. These are divided into 3 categories of effects, following the first 3 columns of Table 1.

### *Effects from direct adult exposures*

Many of the pesticide-related responses potentially expressed by breeding adults in the field cannot be observed directly in the laboratory because of limitations in the design of the avian reproduction test (Bennett and Etterson 2006). In their analysis of avian reproduction tests, Mineau et al. (1994) found that the average adult body weight and the number of eggs laid during the test were significantly correlated in both mallard and bobwhite studies and concluded that they were representative of parental toxicity affecting well-being. Bennett

**Table 2**. Phase-specific effects, related types of exposure, 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 exposure | Test endpoint used as surrogate | Comparable exposure period for phase-specific RQ |
|---|---|---|---|---|
| Pair formation and/or breeding site selection | Adult behavioral effects leading to territory abandonment or delayed breeding | Adult direct | 1/10 of LD50 | 1-day EDD |
| | | Adult direct | NOAEL for adult body wt prelaying | 1-day EDD |
| Follicle development and egg laying | Adult behavioral effects leading to abandonment of nest attempt | Adult direct | NOAEL for the number of eggs laid per hen | 1-day EDD |
| | Reduced eggshell quality leading to abandonment of nest attempt | Adult direct | NOAEL for mean eggshell thickness | 1-day EDD |
| | Reduced clutch size | Adult direct | NONE | NONE |
| Incubation and hatching | Adult behavioral effects leading to abandonment of nesting attempt or reduced nest attentiveness | Adult direct | 1/10 of LD50 | 1-day EDD |
| | | | NOAEL for adult body wt prelaying | 1-day EDD |
| | Reduced fertility | Adult direct | NOAEL for proportion of viable eggs per eggs set per hen | 1-day EDD during egg laying |
| | Embryotoxicity from in ovo exposure leading to reduced hatchability | Juvenile in ovo | NOAEL for proportion of hatchlings per viable eggs per hen[a] | Follicle development TWA |
| | Embryotoxicity from external eggshell exposure leading to reduced hatchability | Juvenile direct | NONE | NONE |
| Nestling rearing until fledging | Adult behavioral effects leading to brood abandonment or abnormal parental care | Adult direct | 1/10 of LD50 | 2-day TWA |
| | | | NOAEL for adult body wt prelaying | 2-day TWA |
| | Reduced nestling survival from direct exposure | Juvenile direct | 1/10 of LD50 | 1-day EDD (juvenile diet) |
| | | | Fraction of 5-d LC50 | 5-day TWA (juvenile diet) |
| | Reduced nestling survival and growth from in ovo exposure | Juvenile in ovo | NOAEL for proportion of 14-day-old juveniles per hatchling per hen | Follicle development TWA |

RQ = risk quotient; EDD = estimated daily dose; MCnest = Markov chain nest productivity model; NOAEL = no observed adverse effect level; TWA = time-weighted average.

[a]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.

et al. (2005) proposed using a change in adult body weight during the prelaying period of the avian reproduction test as a surrogate endpoint for parental well-being during all breeding phases from pair formation to fledging, except the egg-laying phase, but they did not specify the timing of changes even though adult body weights are measured at least every 2 weeks during the approximately 10-week prelaying period. In MCnest this proposal has been refined to focus on changes in body weight observed in the first 2 weeks after the onset of treatment because it is intended to be a surrogate for physiological or behavioral responses occurring rapidly after an initial pesticide exposure, such as the nest or brood abandonment observed shortly after pesticide application by Busby et al. (1990) and Brewer et al. (1988).

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 the pair formation and incubation phases (i.e., 1-day estimated daily dose [EDD]) or a 2-day time-weighted average (TWA) during the nestling rearing phase. During discussions on revisions to the European Union Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2008), these 1- and 2-day exposure periods were criticized as being too short given that changes in body weight may not be observed for weeks. However, 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. 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 foods or feeding sites. However, if the laboratory birds show a significant change in body weight in the first 2 weeks of exposure, possibly linked to reduced food consumption or metabolic efficiency, it is likely that other sublethal effects that may threaten the success of the nest attempt began shortly after the initial exposure (Bennett and Etterson 2006).

A rapid reduction in adult body weight in the laboratory is an indirect surrogate for possible behavioral responses in the field that affect the success of a nest attempt. For some pesticides, this may lead to a very conservative decision point if the NOAEL for change in body weight is considerably lower than the dose required to produce behavioral effects leading to a nest failure. However, in most cases, we would have no knowledge of such a relationship. Other types of pesticides may cause effects on adult behavior and well-being 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.

Because of the concern about using prelaying body weight as a surrogate endpoint for adult well-being, 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 (LD50) test. Ideally, the LD50 test would provide information on the single oral dose below which mortality and/or overt signs of poisoning are not observed. However, many LD50 tests produce mortality and other signs of toxicity at each dose tested and do not adequately document the presence and severity of sublethal signs of poisoning. A review of LD50 studies showed that severe signs of toxicity likely to lead to deficits interfering with a bird's normal activities tend to be recorded at dosing levels greater than 1/10 of the LD50 (Callahan and Mineau 2008). On the basis of this work, it was proposed that 1/10 of the LD50 be used as a surrogate endpoint for effects on adult behavior leading to disruption of 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.

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. A reduction in eggshell thickness is a surrogate for nest failures related to cracked and broken eggs with reduced eggshell quality. Adverse effects of reduced eggshell thickness in the field, such as egg breaking or punctures, may be expressed during either the egg-laying or incubation phases, but in MCnest it is used as a surrogate endpoint during egg laying—the earliest breeding phase where it could 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. (1994) showed that these 2 endpoints segregated into different categories of responses observed in avian reproduction tests, and both are needed as surrogate endpoints to represent a 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 (Haywood 1993; Sharp et al. 1998). 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, Dominguez et al. 1990; Bennett, Bentley et al. 1990; Bennett et al. 1991), 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 time-weighted average may be appropriate. However, 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 prelaying exposure.

Bennett et al. (2005) also proposed that the percentage of viable (fertile) eggs of all eggs set per hen be used in decisions during the egg-laying phase. However, adverse effects on egg viability usually would not be detected by the parent until late in incubation, making it more appropriately a surrogate endpoint at the end of the incubation phase (Table 2). Also,

egg viability is determined in the reproduction test by candling bobwhite eggs at 11 days of incubation and mallards at 14 days (USEPA 2012), and although this endpoint is intended to be a surrogate measure for pesticide effects on fertility of adults (i.e., production of infertile eggs), it is very difficult to separate infertility due to parental exposure from early embryo mortality resulting from in ovo exposure. Consequently, without detailed analysis of failed eggs, this endpoint potentially represents a combination of infertility and early embryo death.

### *Effects from in ovo exposures*

Two surrogate endpoints for effects on juveniles resulting from in ovo exposure are appropriate for all or most pesticides (Table 2). Bennett et al. (2005) proposed that the proportion of hatchlings per number of eggs set per hen be used during the incubation phase as a surrogate for effects on hatchability from in ovo exposure. In MCnest this endpoint has been modified to be the proportion of hatchlings per number of viable eggs per hen to separate the effects of in ovo exposure on late embryotoxicity from the combined effects of reduced fertility and early embryo mortality. Because the avian reproduction test does not expose chicks to pesticide-treated diets, observed treatment-related effects on juvenile survival in the test must result from in ovo exposures. Therefore, the proportion of 14-day-old chicks per number of hatchlings per hen is a surrogate for effects to nestling survival until fledging due to in ovo exposure.

### *Effects from direct juvenile exposures*

Because juveniles are raised on untreated diets in the avian reproduction test, the results do not provide information on juvenile sensitivity to direct pesticide exposures after hatching. As a surrogate endpoint for direct pesticide exposure to nestlings, Bennett et al. (2005) proposed using a dietary exposure level derived from the 5-day dietary toxicity (LC50) test with juveniles that would not result in adverse effects—essentially an effects threshold. This surrogate endpoint would be compared to the 5-day TWA for the juvenile diet. However, 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 thresholds of effect or no observed adverse effect concentrations (NOAECs) 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, a fraction of the LC50 is 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 3 LOCs related to the 5-day toxicity test are 0.5 LC50 for acute risk, 0.2 LC50 for acute restricted use risk, and 0.1 LC50 for acute endangered species risk (see discussion of LOCs at http://www.epa.gov/oppefed1/ecorisk_ders/toera_risk.htm [cited 2013 June 11]). The model user is responsible for using the fraction of the LC50 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 (Mineau et al. 1994; Hill 1995). 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 for each pesticide.

The European Union (EU) Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2008) alternatively proposed to use 1/10 of the adult LD50 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 (Grue and Shipley 1984; 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 LD50 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).

## SELECTING ADDITIONAL SURROGATE ENDPOINTS FROM OTHER SOURCES

Many other types of potential pesticide-related effects in Table 1 are not addressed by the suite of surrogate endpoints proposed by Bennett et al. (2005) or used in the Basic Version of MCnest (Etterson and Bennett, this issue). Although these effects may not have appropriate surrogates from standardized avian toxicity tests, there may be measurements from nontraditional test sources that can serve as surrogate endpoints for these effects. Nontraditional tests may be conducted infrequently, and the exact experimental design and measurement of endpoints may vary among tests and chemicals. However, nontraditional tests may provide information concerning types of effects possible in the field that are not addressed by the standardized tests. Because of the varied nature of nontraditional tests, this section discusses some additional types of effects for which surrogate endpoints could be considered in reproductive success models when information is available, but cannot define new surrogate endpoints precisely.

### *Embryotoxicity because of external eggshell exposure*

In addition to in ovo exposure, developing embryos may be exposed to pesticides via the eggshell surface resulting in reduced hatchability in the field (Rondeau and Desgranges 1995). Embryotoxicity resulting from external eggshell exposure is not part of standardized laboratory tests, but it has been measured in some nontraditional laboratory tests for pesticides and other chemicals (Hoffman 1978, 1979; Hoffman and Albers 1984). These tests typically have been conducted to address concerns about direct overspray of nests or egg contamination from the plumage and/or feet of incubating parents (Mineau 2005). Many of the laboratory tests of external eggshell exposure report the volume or mass of a chemical

applied to the egg surface, but one of the greatest challenges to using this information as a surrogate endpoint is to relate the application rate of a pesticide to an environmentally realistic dose at the shell surface, especially because variation in degree of parental contamination or protective cover of nest may be more important in determining the dose to eggshells than application rate. Factors such as nest type (e.g., cavity vs open), nest height (e.g., ground vs tree or shrub canopy), degree of nest concealment, and response of the incubating parent to application equipment (e.g., flush vs remain on nest) could result in differences among species in the amount of egg exposure from an application, but currently there is insufficient information from which to develop a generalized approach for estimating the degree of eggshell exposure for all species from application rates alone. Hoffman and Eastin (1981) linked eggshell exposure to application rate by dipping intact eggs for 30 s in a solution equivalent to the tank mixture prepared for a specific application rate. This may represent a worst case relationship because most eggs would likely receive less exposure from the same application under field conditions.

Another important factor is that nonpesticidal constituents of formulated products also may be embryotoxic (e.g., light petroleum solvents used in pyrethroid formulations) (Lutz-Ostertag and Lutz 1970). Consequently, testing of toxicity from eggshell exposure should be done with the same product that is the focus of the risk assessment.

### *Abnormal sexual maturation because of in ovo and direct exposure*

The current avian reproduction test raises hatchlings to 14 days of age and records the number of surviving chicks and their body weight. Certain modes of action may cause effects from in ovo exposure that do not become apparent until the chicks reach breeding age, so they are unobservable in the current test. Although a multi-generational test guideline that could identify abnormal sexual maturation and transgenerational effects in birds is in development (see status of guidelines at http://www.epa.gov/scipoly/oscpendo/pubs/assayvalidation/status.htm [cited 2013 June 25]), it is not clear if the final test design and suite of endpoints will provide information for a surrogate endpoint for effects on sexual maturation. Depending on how the test is designed, we may or may not be able to separate effects via in ovo exposure from effects via direct dietary exposure of chicks. Determining if and how information on abnormal sexual maturation could be integrated into MCnest will depend on the specific test that is developed and on the management questions being addressed.

### *Indirect effects on adults and juveniles*

Certain effects on avian reproductive success may result indirectly from the action of pesticides by reducing food availability or changing the amount or quality of nesting habitat (Campbell and Cooke 1997; Boatman et al. 2004). These indirect effects are not detectable from laboratory avian toxicity tests. Information on the indirect effects of pesticide use on avian reproductive success most likely would be derived from field studies. Ideally, studies would be designed to separate indirect effects from direct toxicological effects, although with acutely toxic insecticides, this may be difficult.

Possibly the greatest challenge for effectively developing a surrogate endpoint for indirect effects is determining if there is a consistent relationship between an application rate and effects on adults or chicks when so many co-occurring factors are involved that affect this relationship. For example, even if a particular study could demonstrate a relationship between the application rate and a decrease in juvenile survival via a reduction in food abundance, it is not clear to what extent those findings can be extrapolated to other levels of invertebrate abundance or other locations, crops, and species. Similarly, if a study of an insecticide does not detect evidence of indirect effects in birds under a specific set of conditions, it is not possible to conclude that adverse indirect effects would not be observed for all species under all conditions. For example, *Bacillus thuringiensis* (*Bt*) is considered nontoxic to birds and mammals, but several studies have been conducted to examine the potential indirect effects of reduced lepidopteran populations on avian reproduction. No significant effects on reproductive success were detected by Hanowski et al. (1997), Holmes (1998), or Sopuck et al. (2002), but Poulin et al. (2010) reported that clutch size and fledgling survival of house martins (*Delichon urbicum*) were significantly lower at treated sites relative to control sites. Although we know indirect effects can be important ecologically, it is very difficult to predict the set of conditions under which the indirect effects of pesticide applications would adversely affect avian reproductive success. It is possible that indirect effects could be incorporated into modeling approaches like MCnest on a case-by-case basis, but at this time there is not a generalizable approach for the inclusion of indirect effects.

## SELECTING AN EXPOSURE PERIOD FOR EACH SURROGATE ENDPOINT

Currently within the USEPA, the characterization of pesticide risks to avian reproduction compares the lowest NOAEL from the reproduction test with the maximum estimated exposure, which in most cases represents the exposure estimated on the day of application. In the EU, screening-level risk assessments compare the lowest NOAEL from the reproduction test to estimated exposures based on a 21-day time-weighted average (TWA) assuming a 10-day residue half-life on food (EFSA 2009). However, in the breeding phase-specific approach, Bennett et al. (2005) proposed that the duration of the exposure period to compare with each surrogate endpoint should be based on an assessment of how quickly a given effect may occur in the field. For each surrogate endpoint, the proposed exposure periods were expressed as single-day estimated dietary doses or as TWAs of dietary doses over periods of 2 or more days. For example, surrogate endpoints for effects on juvenile survival and growth resulting from in ovo exposure were compared to a TWA of exposures during the period of rapid follicle growth, which varies from 3 to 10 days among many upland species (King 1973; Pearson and Rohwer 1998). The case studies by Shore et al. (2005) and Roelofs et al. (2005) with skylarks used a 3-day TWA. Consequently, the critical exposure during egg formation occurs many days before effects on hatchability or juvenile survival would be detected by the parent and could affect the status of a nesting attempt.

There is a disconnect between the exposure scenario used in the current avian reproduction test and the temporal pattern of exposures observed for today's pesticides. The laboratory reproduction test was designed using constant dietary concentrations over a long prelaying period and extended laying period for testing bioaccumulative chemicals with slow degradation rates in the field. Most pesticides today have much shorter degradation half-lives on plant parts (Willis and

McDowell 1987), creating a situation where the test results based on a long constant concentration exposure are used to assess risks for pesticides with much shorter periods of exposure in the field. The laboratory reproduction test provides no information about how rapidly effects may be expressed after an initial exposure, and information concerning time to effect is derived from nontraditional tests or field observations (see review by Mineau 2005). Bennett, Bentley et al. (1990) used northern bobwhite (*Colinus virginianus*) fed methyl parathion to compare results of the current 20-week exposure test with a test with a 3-week exposure period starting during egg laying. They concluded that all dose-related effects observed in the long-term exposure test also were observed in the short-term test, except for the number of adult mortalities. Several additional reproduction tests with shorter exposure periods beginning during egg laying have shown that many of the effects observed in the 20-week test also can be observed from much shorter exposures, sometimes within days of initiation of exposure (Stromborg 1981, 1986a, 1986b; Bennett et al. 1991).

In determining whether it is appropriate to use a single-day estimated dietary dose or a multiple-day TWA for comparing with a surrogate endpoint, several factors need to be considered. As stated above, the exposure period should reflect how quickly a given effect may occur in the field. This may be different from the response time of the laboratory effect used as a surrogate for the field effect. For effects on juvenile survival and growth from chemicals deposited into egg yolks, a TWA during the period of rapid follicle growth may be appropriate.

Another important factor in deciding on the use of a TWA is the degradation half-life of a pesticide on food types. Consider the use of a default 21-day TWA in the EU. For pesticides with long residue half-lives, the estimated dose on any particular day is not considerably different than the 21-day TWA. However, as half-lives decrease in length, the peak dose at application can be several times the 21-day TWA (e.g., peak dose for a pesticide with a 1-day half-life is over 10 times the 21-day TWA). For those effects that can occur very rapidly after the initiation of exposure, the peak dose at application may be much more relevant to understanding the potential risk than a TWA over a longer time period, so it may be most appropriate to compare the estimated dietary dose on each day to the toxicity threshold value. Some effects may require longer periods of exposure before being expressed, however, because of the long prelaying exposure period, the current avian reproduction test provides no information for determining the appropriate length of a TWA for comparing with reproductive endpoints. This information may need to come from other nontraditional tests or field observations. Although default values for exposure periods can be established at lower tiers of the risk assessment process, risk assessors should evaluate the appropriateness of the duration of exposure windows associated with each surrogate endpoint on a species-specific and chemical-specific basis, especially at higher risk assessment tiers.

## DISCUSSION

The breeding phase-specific approach described in Bennett et al. (2005) reframes the problem of assessing chemical risks to avian reproductive success by encouraging risk assessors to consider all of the types of effects that a chemical could cause during a breeding season and to gather evidence on the potential for those effects from various data sources. The approach integrates available information about potential chemical effects, along with information on life history and timing of pesticide applications, into a decision process for estimating effects on reproductive success over the entire breeding season. This is different from the more traditional approach where the results of the 3 standardized avian toxicity tests have been considered separately in risk assessments, with each used to address the risks of different types of effects.

For many pesticides, information about effects on reproduction still may come primarily from the standardized avian reproduction test. However, where additional information exists from other sources, such as data from other laboratory tests or field studies that can be used as a surrogate endpoint for a potential field effect, it can be incorporated into the decision process of the phase-specific approach. This article broadens the discussion of surrogate endpoints started in Bennett et al. (2005) to include data from sources other than the reproduction test.

During the evaluation of a chemical, it may be concluded that there are types of potential effects on reproduction for which there is not sufficient information to support a useful surrogate endpoint in the decision process. For example, an insecticide may be suspected of causing indirect effects on juvenile survival because of its efficacy in killing insect foods, but there may not be empirical evidence to indicate that juvenile survival is reduced at or above a specific application rate. When specific types of effects cannot be integrated into the decision process, models of reproductive success may be biased toward underestimating the overall effects on reproductive success, especially if those effects could have been observed at lower exposure levels than other effects being evaluated.

Even when surrogate endpoints can be defined for potential field effects, the relationship between the measured endpoint and the field effect of concern can be quite uncertain. One source of uncertainty comes from the indirect relationship between some of the measured surrogate endpoints and the field effects they represent (Bennett et al. 2005; Mineau 2005; Bennett and Etterson 2006). This is particularly true of several of the effects resulting from direct parental exposure because they cannot be observed directly in the avian reproduction test, and little is known about the actual relationship between these measured endpoints and the field effect.

Another important source of uncertainty comes from the experimental design of the avian reproduction test based on hypothesis testing that uses a small number of treatment groups and often wide spacing between dietary concentrations. Consequently, because the determination of NOAELs is affected by the dietary concentrations selected for testing and the statistical power of the test, NOAELs are an inconsistent, potentially misleading, estimate of a no-effect level (Chapman et al. 1996; Landis and Chapman 2011). An alternative is to use regression analysis to quantify the dose-response relationship and estimate the concentrations associated with defined levels of effect, but for many endpoints the small number of treatment groups in the current test provides little insight into the location and shape of the dose-response relationship. Although the current test design may have been appropriate for the chemicals and issues being considered at the time of test's development, it is poorly suited for the questions today related to magnitude of effect and significance at the population level.

One of the most striking findings of the analysis of avian reproduction tests by Mineau et al. (1994) was the lack of correspondence of responses between mallards and bobwhites. For the majority of pesticides examined, developmental effects

were observed in 1 species but not the other. It is unclear if this reflects a true difference in the types of responses to pesticide exposure between 2 species or is an artifact of the test design and low statistical power for many endpoints. In either case, it raises questions about the extent to which information on specific effects from the 2 tested species can be used to represent the responses of all untested species. This is particularly important because the 2 tested species have precocial young that hatch with their eyes open and are capable of leaving the nest within a day whereas many of the species of concern in risk assessments have altricial young that are hatched with eyes closed, often with little or no down, and unable to leave the nest for many days.

Despite the uncertainties involved in using surrogate endpoints to represent potential field effects, the phase-specific approach provides a more thorough assessment of the overall risks to reproductive success compared to the current assessment approach by integrating toxicity information on the types of effects possible throughout a nesting attempt with information on life history and the temporal relationship between nesting activity and pesticide use (Etterson and Bennett, this issue). A key part of the approach is a thorough evaluation of available evidence for selecting surrogate endpoints. Even when potential field effects cannot be incorporated or are represented by indirect surrogate endpoints, the process of considering evidence for all potential effects on reproduction should lead to a more explicit description of the remaining uncertainties in a risk assessment and identification of critical data gaps where additional testing could improve the quality of the assessment.

*Acknowledgment*—We thank Dr. Pierre Mineau for review and discussion on an earlier draft of the manuscript and Kristin Garber for critical review of the manuscript. This is EPA contribution number MED-12-5741 of the USEPA, Mid-continent Ecology Division.

*Disclaimer*—Although the present article has been reviewed and approved in accordance with USEPA requirements and approved for publication, its contents do not necessarily reflect USEPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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