

EPA/600/R-11/023  
ERASC-016 F  
February 2011

**ASSESSMENT OF METHODS FOR ESTIMATING RISK TO BIRDS FROM  
INGESTION OF CONTAMINATED GRIT PARTICLES**

by

Richard Bennett, Dale Hoff and Matthew Etersson  
U.S. Environmental Protection Agency  
Office of Research and Development  
National Health and Environmental Effects Research Laboratory  
Mid-continent Ecology Division  
Duluth, MN

Ecological Risk Assessment Support Center  
Office of Research and Development  
U.S. Environmental Protection Agency  
Cincinnati, OH

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Preferred Citation:

Bennett, R., D. Hoff and M. Etterson. 2011. Assessment of Methods for Estimating Risk to Birds from Ingestion of Contaminated Grit Particles. U.S. Environmental Protection Agency, Ecological Risk Assessment Support Center, Cincinnati, OH. EPA/600/R-11/023.

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## **AUTHORS, CONTRIBUTORS AND REVIEWERS**

### **AUTHORS**

Richard Bennett, Dale Hoff and Matthew Etterson  
U.S. Environmental Protection Agency  
Office of Research and Development  
National Health and Environmental Effects Research Laboratory  
Mid-continent Ecology Division  
Duluth, MN

### **CONTRIBUTORS**

Sharon Thoms  
U.S. Environmental Protection Agency  
Region 4  
Atlanta, GA

### **REVIEWERS**

Sherry K. Krest  
U.S. Fish and Wildlife Service  
Annapolis, MD

Robert Luttik  
National Institute for Public Health and the Environment (RIVM)  
Bilthoven, The Netherlands

Richard K. Peddicord  
Dick Peddicord & Company, Inc.  
Heathsville, VA

### **ACKNOWLEDGMENTS**

The first draft of this document was internally (within EPA) reviewed by Christopher Salice (EPA Office of Prevention, Pesticides and Toxic Substances) and Jill Awkerman (Office of Research and Development [ORD]/National Health and Environmental Effects Research Laboratory. Programmatic review was conducted by Katie Matta of EPA Region 3, a Trichair of EPA's Ecological Risk Assessment Forum.

## **INTRODUCTION**

In June 2006, the Ecological Risk Assessment Forum (ERAF) submitted a request to ORD's Ecological Risk Assessment Support Center (ERASC) to evaluate the effectiveness and utility of the European Plant Protection Organization (EPPO) Risk Assessment Scheme for Plant Protection Products (i.e., pesticides) for its ability to estimate the degree of exposure to songbirds from contaminants in soil. Specifically, are methods and information used for understanding avian exposure to pesticide granules useful for estimating the probability of ingesting other contaminated particles such as lead shot or fragments?

An important route of chemical exposure in many ecological risk assessments is the ingestion of chemicals on or in sediments, soil and small gravel (i.e., grit). Several approaches have been developed to address this route of exposure in risk assessments. The particular focus for this white paper is to evaluate approaches available for estimating the risk to birds from ingestion of lead shot or other lead fragments (hereafter referred to as lead particles). There are many similarities between lead particle ingestion and the ingestion of pesticide granules as grit or food items. More specifically, the paper focuses on two primary issues affecting avian exposure to lead—the rate of ingestion of lead particles by birds and the length of time lead particles are retained and eroded in avian gizzards to release a dose of lead to target organs. In this white paper, we briefly discuss soil and grit ingestion by birds, review several published approaches for estimating the rate of ingestion of lead particles or pesticide granules, and examine the important sources of uncertainty in parameter estimates for approaches to estimating exposure to lead particles. We recommend an approach for improving the estimation of lead particle exposure to birds in ecological risk assessments. This paper does not comprehensively review the toxicity of lead in birds, but does demonstrate an approach for estimating the risk of mortality from ingesting lead particles.

## **SOIL AND GRIT INGESTION BY BIRDS: WHO, WHY AND HOW MUCH?**

Many bird species ingest soil, sediment and small gravel while feeding—either inadvertently (e.g., sandpipers probing for invertebrates on mudflats or woodcock ingesting earthworms) or intentionally (e.g., as a source for minerals, to reduce gastric disturbances or gut acidity, or as grit for aiding digestion) (Beyer et al., 1994; Beyer and Fries, 2002; Gionfriddo and Best, 1999). The amount of soil and sediment ingestion can vary greatly among species and for some species can represent several percent of the total daily food ingestion rate (Beyer et al.,

1994; Beyer and Fries, 2002). The deliberate ingestion of small gravel and soil particles for use as grit also varies considerably among species depending on their diet composition and feeding behaviors. In some cases, hard seeds or pieces of insect exoskeletons serve as grit (Gionfriddo and Best, 1999). In addition to variation in the amount of grit ingested each day, species vary in their preferences based on grit size, shape, surface texture and color (Best and Fischer, 1992; Best and Gionfriddo, 1991, 1994; Gionfriddo and Best, 1996, 1999). For each species, grit selection also varies among individuals based on seasonal diet composition, age and nutritional and reproductive status (Gionfriddo and Best, 1999). Consequently, soil and grit ingestion rates are a function of many interacting factors.

It is very difficult to measure soil and grit ingestion rates directly in wild birds. However, soil and grit ingestion rates can be estimated from other available measurements. The most common published measurement of grit consumption by wild birds is a count of number of soil/grit particles in gizzards at the time of necropsy, often categorized by particle size (Best and Gionfriddo, 1991; Gionfriddo and Best, 1996; Luttik and de Snoo, 2004). Sometimes the amount of soil/grit in the gizzard is expressed as a measured mass or volume, rather than as a count of particles (Gionfriddo and Best, 1999). Another method of estimating the percentage of soil in the diet is calculating it as a function of the concentration of acid-insoluble ash in scat samples (Beyer et al., 1994; Beyer and Fries, 2002).

The amount of soil and grit in gizzard samples reflects a sample at one point in time, and the amount at any point in time is a function of the soil/grit ingestion rate and the amount of time soil/grit particles of different sizes are retained in the gizzard before being passed to the intestines. The two processes of soil/grit ingestion and retention in the gizzard are not independent. For grit particles in particular, several studies have shown that these two processes interact somehow to maintain a certain amount of grit in the gizzard, presumably in an attempt to maximize digestion efficiency (Best and Stafford, 2002). This is observed in controlled laboratory studies where, as the amount of grit ingested increases, so does the amount eliminated from the gizzard (i.e., retention time decreases). Conversely, if the amount of grit ingested is restricted, birds can retain grit in the gizzard for extended periods. Gionfriddo and Best (1999) present examples of extended grit retention times (up to 1 year) during periods of reduced grit availability. The exact mechanisms of grit retention in the gizzard are unknown, but several studies have made consistent observations about the relationship between ingestion rates and grit

retention times (Gionfriddo and Best, 1999). However, rather than a constant turnover of grit particle out of the gizzard, Trost (1981) observed in mallards (*Anas platyrhynchos*) that the majority of grit particles were contained in only 5% of the fecal pellets, indicating that the gizzards may periodically evacuate their contents. The high variability in gizzard grit counts among individuals within a species observed by Gionfriddo and Best (1996) may also indicate that the relationship between ingestion rates and grit retention times is quite dynamic.

### **ESTIMATING RISKS FROM SOIL AND GRIT INGESTION BY BIRDS**

Because of the intentional and unintentional ingestion of soil and small gravel particles, there is a risk of exposure to toxic chemicals, such as when ingesting soils containing chemical residues or mistakenly selecting pesticide granules or lead shot as grit or food (Gionfriddo and Best, 1999; Beyer and Fries, 2002). Several procedures have been developed to estimate the risk of chemical ingestion via these nonfood routes of exposure and will be discussed in this section. There are many similarities in the procedures developed to estimate the risk of ingesting particles, such as pesticide granules or lead shot, but there are also differences in how information (e.g., grit retention time) is used. Pesticide granules pose an acute toxicity risk, so the emphasis is on estimating the probability or possibility that enough granules could be consumed in a short period of time (i.e., one day) to be acutely lethal. For pesticides, grit retention time is only used in combination with gizzard count data to estimate the daily grit ingestion rate. Ingested lead shot or lead fragments, on the other hand, need to be eroded in the gizzard over a period of days or weeks to be toxic, so estimates of retention time are used not only in estimating particle ingestion rates, but also in assessing whether or not they will be retained long enough to pose a risk of toxicity.

#### ***EPPO scheme***

For pesticides the *Environmental Risk Assessment Scheme for Plant Protection Products. Chapter 11: Terrestrial Vertebrates*, published by the European and Mediterranean Plant Protection Organization (EPPO, 2003), provides a simple procedure for categorizing the risk to birds and mammals from ingestion of pesticides as granules, seed treatments and sprayed products. This scheme considers the ingestion of pesticide granules consumed accidentally as part of soil ingestion and intentionally as part of grit selection. The risk from various pathways of exposure is calculated using simple risk quotients based on an estimated exposure (i.e., the

daily dose expected as a function of application rate, soil or grit ingestion rates, etc.) divided by a toxicity value.

For granules ingested accidentally as soil, the EPPO scheme estimates risk based on the overall pesticide concentration in soil contributed by the presence of granules. The daily dry soil dose (DDSD) is calculated (using a look-up table) as a daily dose based on the pesticide concentration in soil as a function of application rate and the estimated percentage of the diet consisting of soil. The DDSD is divided by a toxicity value (e.g., LD<sub>50</sub> for short-term risk or lowest no-observed-effect dose [NOED] from a chronic reproduction test for long-term risk) to calculate an exposure-toxicity ratio (ETR). The magnitude of the ETR is used to categorize the risk as high, low, or uncertain.

For granules ingested intentionally as grit, the daily granule dose (*DGD*) is calculated as:

$$DGD \text{ (mg/kg body wt./d)} = DGI \times [G_{surface} / (SP_{surface} + G_{surface})] \times G_{loading} \quad (\text{Eq. 1})$$

where:

*DGI* = daily grit ingestion rate,

*G<sub>surface</sub>* = number of pesticide granules at surface per unit area,

*SP<sub>surface</sub>* = number of granule-sized soil particles at surface per unit area, and

*G<sub>loading</sub>* = the amount of active ingredient in one granule.

The procedure estimates exposure (i.e., *DGD*) under reasonable worst-case and most likely case scenarios. A key assumption in this model is that each pesticide granule and natural grit particle has an equal probability of being selected and ingested (i.e., there is neither preference for, nor avoidance of, lead particles). The *DGD* is divided by a toxicity value (e.g., LD<sub>50</sub> for short-term risk or lowest NOED from a chronic reproduction test for long-term risk) to calculate an ETR which also is used to categorize risk.

The risk quotient approach provides a means for classifying the risk of ingesting a toxic dose from pesticide granules ingested as grit. The risk classification is intended to be protective of all species. Because of the use of conservative assumptions, it is not intended for use in estimating the probability of ingesting a specific number of granules, although it is possible to obtain crude estimates. Removing the *G<sub>loading</sub>* term from the equation above provides a simple

estimate of the overall mean number of pesticide granules ingested each day based on the daily grit ingestion rate multiplied by the proportion of granule-sized particles that are pesticide granules. To accomplish this, the EPPO scheme estimates the daily grit ingestion rate (*DGI*) using estimates of the number of grit particles in gizzards of necropsied birds (geometric means of predominantly granivorous species or 90<sup>th</sup> percentile of distribution of species) multiplied by a conversion factor. A conversion factor of 4.2 was used and represented the inverse of the grit turnover rate (expressed in days), also known as grit retention time, from a study with house sparrows (*Passer domesticus*) fed diets containing various concentrations of grit particles (Fischer and Best, 1995). Their estimated grit retention time of 0.24 days represents one of the shortest reported retention times in the literature and possibly reflects the conditions of this specific experiment. Silica granules were mixed into a canned dog food (i.e., much softer than usual sparrow diet) at concentrations that would average from 4 to 64 granules per bird per day. Birds were necropsied over an 8-day period to determine the number of grit particles in the gizzard at death in relationship to estimated daily grit consumption. The cautions presented on the representativeness of this conversion factor are the same as presented in Stafford and Best (1999).

Best and Stafford (2002) expanded on the work of Fisher and Best (1995) by more precisely delivering specific doses of grit particles to both house sparrows and bobwhite (*Colinus virginianus*) fed either the same soft dog food diet or a grain diet. They concluded that the initial study in Fischer and Best (1995) “may not have accurately represented the relationship between grit consumption and grit retention” and stated that both species “eliminated large amounts of grit at the higher dose levels but retained most of the grit at lower levels, which suggests that birds need a certain amount of grit in their gizzard, perhaps to maximize efficiency of digestion.” Hence, the EPPO scheme uses a very short estimate of retention time which increases the estimated daily grit ingestion rate. The estimate of retention time in the paper by Fischer and Best (1995) is considerably shorter than information from other studies and reviews and results in a relatively conservative risk index for use in pesticide screening assessments.

When the chemical of concern may be ingested accidentally with soil as well as ingested intentionally as grit particles, these two routes of exposure may be independent and thus additive in terms of estimating a total exposure dose. However, birds may ingest a wide range of particle sizes from very fine soil particles to larger grit particles that may be 1 or more mm in size. It is

very difficult to separate accidental soil ingestion from intentional grit ingestion when there is overlap in the particle size ranges. In assessments of gizzard contents, Best and Gionfriddo (1991) and Luttik and de Snoo (2004) considered only particles >0.1 mm and >0.5 mm, respectively, as possible grit particles in their counts. However, in estimating percent soil ingestion, Beyer et al. (1994) used a method for calculating acid-insoluble ash content in feces that does not discriminate soil particle sizes. Consequently, estimates of percentage of soil in the diet may overlap somewhat with estimates of grit ingestion rates due to the differences in the methods used. Avian risk assessments of lead exposure will need to consider these two possible routes of exposure (i.e., accidental soil ingestion and intentional grit ingestion) in light of site-specific information on the nature of lead availability. In other words, is the lead available primarily as dissolved lead in soil, lead shot or fragments, or a combination?

***Luttik and de Snoo (2004)***

As mentioned above, the EPPO scheme can be modified to produce a simple estimate of the overall mean number of pesticide granules ingested each day based on the daily grit ingestion rate multiplied by the proportion of granule-sized particles that are pesticide granules. Luttik and de Snoo (2004) present a model for estimating the probability of birds and mammals consuming 0, 1 or more pesticide granules per day while accidentally or intentionally ingesting soil particles. The model uses a binomial distribution and calculates the probability of selecting  $N$  pesticide granules based on a probability mass function. Estimates are required of the number of granule-sized particles ingested each day by a species and the proportion of granule-sized soil particles that consists of pesticide granules. As in the EPPO model, a key assumption is that each pesticide granule and natural grit particle has an equal probability of being ingested. The model can be simplified as follows to estimate the probability ( $Pr$ ) of selecting a specific number of pesticide granules in a day:

$$Pr (N) = \frac{T!}{N! \times (T - N)!} \times P^N \times (1 - P)^{T-N} \quad (\text{Eq. 2})$$

where

$N$  = the number of pesticide granules ingested per day,

$T$  = the total number of granule-sized particles ingested per day, and

$P$  = the proportion of the total number of granules and granule-sized soil particles at the surface that are pesticide granules.

[Note: The exclamation mark represents a factorial.  $N$  factorial, written as  $N!$ , equals the product of all positive integers from 1 to  $N$ . For example,  $3! = 1 \times 2 \times 3$ .]

Unlike the approach used in the EPPO scheme above, the method presented by Luttik and de Snoo (2004) calculates the probability of ingesting  $N$  or more granules each day. Because the focus of the Luttik and de Snoo (2004) approach is on the risk from acutely toxic pesticides in or on granule products, the approach does not consider grit retention time.

### ***Peddicord and LaKind (2000)***

The Peddicord and LaKind (2000) model was specifically developed to estimate the probability that birds at a recreational shooting range would ingest at least one lead shot/particle during their lifetime. Their model could be viewed as a simpler version of the binomial model of Luttik and de Snoo (2004), though there are three important differences in approach.

First, it estimates the proportion of grit-sized particles that consists of lead shot or fragments, both on the shooting range itself and in off-site habitat used by each species, with an estimate of fraction of foraging time spent on-site vs. off-site. Thus, it takes into account that an animal's foraging area may encompass more than just the site of concern, whether that is a shooting range or an agricultural field treated with pesticides.

Second, it purports to estimate the number of grit particles selected and retained in the gizzard in a lifetime. It does this using the following equation:

$$N = Y \left( D_e / D_p \right) \quad (\text{Eq. 3})$$

where

$N$  = number of grit particles selected and retained in the gizzard in a lifetime,

$Y$  = number of years a bird lives,

$D_e$  = number of days per year that a bird forages in the area, and

$D_p$  = retention time for a shot in the gizzard (days).

However, while the equation includes the life span in years and the number of days per year at the site of concern, it does not incorporate information on the number of particles ingested per day—only their retention time. By not including information on particle ingestion rates, the model presented in Peddicord and LaKind (2000) does not accomplish its stated goal of estimating the probability of a bird ingesting at least one lead particle in its lifetime. Consequently, the model, as presented, severely underestimates the number of particles selected and retained in the gizzard. The equation would accomplish its stated goal by adding a term for the mean number of particles in gizzards at time of necropsy. As noted previously, gizzard counts have been reported in the literature for many species. Dividing the mean number of particles in the gizzard by particle retention time provides a simple estimate of the number of particles ingested per day and results in the following modified equation:

$$N = Y \times D_e \times (G/D_p) \quad (\text{Eq. 4})$$

where

$G$  = mean number of particles in gizzard at time of necropsy.

Third, the Peddicord and LaKind (2000) model does not estimate the probability of ingesting exactly 1 or 2 or more lead particles, as in Luttik and de Snoo (2004). However, it estimates the probability that **none** of the ingested particles in a lifetime is a lead particle, and then estimates the probability of ingesting 1 or more lead particles as 1 minus the probability of ingesting no particles [i.e.,  $Pr(\geq 1) = 1 - Pr(0)$ ]. In this regard, using the same assumptions about the rate of grit ingestion and the proportion of particles that are lead, the Peddicord and LaKind (2000) and Luttik and de Snoo (2004) models calculate the same probability of ingesting 1 or more lead particles or pesticide granules.

If the Peddicord and LaKind (2000) model were modified, as in Eq. 4, to correctly estimate the number of particles selected and retained in the gizzard over a specified time period, there is another aspect of the model to consider in risk assessments. By attempting to estimate the probability that birds would ingest at least one lead shot/particle during their lifetime, where maximum life span is used ( $Y$ ), the model results in a conservative estimate of exposure for a simple screening process. However, a risk manager may need more discreet time frames in exposure estimates for developing risk mitigation strategies. By using the maximum life span,

the model attempts to calculate the risk over a period of time that is far longer than the life span of most individuals of the population. Consequently, the risk to most individuals is greatly overstated. Risks from ingesting lead particles would be more meaningful in risk assessment if expressed as a function of shorter time frames. For example, if the objective is to assess risks to populations, presenting risks on an annualized basis would be more effective since other inputs in a population model are typically expressed annually. In this case, that is easily accomplished by dropping the  $Y$  parameter from Eq. 4 to estimate the number of particles selected and retained in the gizzard per year. Shorter time frames also may be appropriate in meeting other management objectives, and this is discussed further below.

### **REVISED MODEL FOR ESTIMATING PROBABILITY OF INGESTING LEAD PARTICLES OR PESTICIDE GRANULES**

We developed a revised model for estimating the probability of ingesting lead particles that integrates some features of the Luttik and de Snoo (2004) and Peddicord and LaKind (2000) models, while addressing some of the issues discussed as well. Most importantly, it provides a means of estimating the probability of ingesting lead particles over any specified time frame. While the following section focuses on the probability of ingesting lead particles, the model also is useful for estimating the probability of ingesting pesticide granules when the same assumptions are met. The model runs in Microsoft<sup>®</sup> Excel. The input parameter cells are those with blue backgrounds. Other cells contain definitions or model calculations and should not be changed. An example set of calculations from the spreadsheet model is included in Appendix A.

For each species, an estimate of the number of grit particles ingested each day is required. This parameter is rarely measured directly. The most commonly available data on avian grit ingestion are typically counts of grit particles in the gizzard at necropsy, which represents a snapshot in time of grit usage. A very simple deterministic way to estimate overall grit ingestion rates is shown below as Eq. 5:

$$\text{Grit particles ingested/day} = \frac{\text{Mean \# grit-sized particles/gizzard}}{\text{Mean grit retention time (days)}} \quad (\text{Eq. 5})$$

Information in the literature concerning grit retention time is highly variable, so it may be appropriate to examine the responses under different estimated retention times.

The model is set up to examine the probability of ingesting lead particles during an exposure period that can range from 1 day (if interested in daily exposure probability) to the maximum number of days each year on the site of concern. In cases where the assumed retention time of individual lead particles may be too short to cause toxicity (or where more than one lead particle may be needed to cause toxicity), the revised lead model could be used to examine the probability of ingesting multiple lead particles over a specified exposure period. The total number of grit particles ingested per exposure period is calculated by multiplying the daily ingestion rate by the number of days selected. A rounding function is used to express the total number of grit particles per exposure period ( $T$ ) as a whole number.

As in the Peddicord and LaKind model, our revised model considers that the foraging range of a species may include both the contaminated area of interest (i.e., *on-site*), as well as surrounding uncontaminated or less contaminated habitat (i.e., *off-site*). Also, it uses the same simple estimate of the proportion of foraging activity on-site. The proportion of grit-sized particles from all foraging sites that is lead particles ( $P$ ) is calculated as:

$$P = P_{on-site} \times F + P_{off-site} \times (1 - F) \quad (\text{Eq. 6})$$

where

- $P_{on-site}$  = proportion of grit-sized particles available *on-site* that is lead particles,
- $P_{off-site}$  = proportion of grit-sized particles available *off-site* that is lead particles, and
- $F$  = proportion of foraging activity *on-site*.

Like the models discussed previously, the revised model uses a simple estimate of the proportion of available grit-sized particles consisting of lead particles (based on site-specific information) and assumes that each lead particle and natural grit particle has an equal probability of being ingested (i.e., there is neither preference for, nor avoidance of, lead particles). Best and Gionfriddo (1991, 1994) reported that particle characteristics such as shape, texture and color can affect avian preferences for various types of grit particles. We found no information suggesting that avian species might prefer or avoid lead shot or lead fragments relative to other grit types. The assumption of equal acceptance among particle types remains a source of

uncertainty in all of these models estimating the probability of ingesting lead or pesticide particles.

The model then calculates the probability ( $Pr$ ) of ingesting  $N$  lead particles during an exposure period. Probabilities are calculated for  $N = 0$  through 8 particles using the same probability mass function presented in Luttik and de Snoo (2004):

$$Pr(N) = \frac{T!}{N! \times (T-N)!} \times P^N \times (1-P)^{T-N} \quad (\text{Eq. 7})$$

where

- $N$  = the number of lead particles ingested during exposure period,
- $T$  = the total number of grit-sized particles ingested during exposure period, and
- $P$  = the proportion of the total number of lead and grit-sized soil particles at the surface that are lead particles.

Just as in Peddicord and LaKind (2000), the revised model calculates the probability of ingesting one or more lead particles by essentially calculating 1 minus the probability that none of the particles ingested is lead [i.e.,  $Pr(\geq 1) = 1 - Pr(0)$ ].

## **THE IMPORTANCE OF ESTIMATES OF GRIT RETENTION TIME**

The estimates used for grit retention time, whether based on assumptions or measurements in published studies, have a great influence on the revised model estimates of the probability of ingesting lead particles. Since  $T$ , the number of particles ingested in a specified time period, is part of an exponent in the model, the model results are very sensitive to variations in  $T$ . Also, the daily grit ingestion rate is inversely related to input parameter estimates for grit retention time. Consequently, the estimates for grit retention time indirectly have considerable influence on model outcomes of the probability of ingesting lead particles. The significance of estimates of grit retention time is an artifact of not having direct measurements of grit ingestion rates for wild birds.

Estimates for retention time also are very important in the assessment of the degree of risk posed by lead particles to various species. The toxicity of lead particles is related to the length of time the particles are retained and eroded in the gizzard. McConnell (1967) found that

lead shot retained in the gizzards of bobwhite quail or mourning doves (*Zenaidura macroura*) were eroded to a small fraction of their original size within 2 to 3 weeks. Bellrose (1959) set a 20-day limit for waterfowl to void ingested lead particles or die. Consequently, under conditions where grit is retained only briefly (e.g., less than a few days), the risks of lead poisoning may be reduced, but these risks increase with increasing estimates of the retention time of lead particles because more of the particle is eroded and taken up in the blood. In this case, estimates of retention time have a direct bearing on estimates of the internal dose of lead from eroded particles and the overall characterization of risk from lead poisoning.

These two issues related to retention time (i.e., estimating ingestion rates and internal dose) are not independent and are discussed further in the following sections.

#### ***Use of grit retention time in estimating grit ingestion rates***

As mentioned above, grit retention time is highly variable and depends on a number of factors including the rate of grit ingestion, the characteristics of the diet, and the nature of the grit particles themselves (see review by Gionfriddo and Best, 1999). Even within an individual bird, when a group of identical grit particles is inserted into the gastrointestinal tract at one time, they are not all retained for the same period of time and then voided. Initially particles are eliminated very rapidly, but then the elimination of the remaining particles slows significantly (Fischer and Best, 1995). For example, house sparrows voided 50% of particles in the first 24 hours, but 32% of particles remained for more than 13 days. In red-winged blackbirds (*Agelaius phoeniceus*), 90% of particles were voided in the first 24 hours. In other experiments house sparrows with unlimited access to quartz grit were switched from quartz to feldspar. Six hours after the switch, sparrow gizzards contained 40% feldspar/60% quartz, and after 24 hours contained 88% feldspar/12% quartz (Gionfriddo and Best, 1995). However, several of the quartz particles still remaining after 24 hours were retained for up to 30 days. Trost (1981) and King and Bendell-Young (2000) reported a similar pattern in mallards and described the grit retention rates as exponential functions. Consequently, while the models described above for estimating the probability of ingesting particles use a point estimate for grit retention time, the fate of any particular particle in the gizzard can be highly variable (e.g., voided almost immediately or retained for days or weeks). In reviewing the literature for information on grit retention time it also is important to determine if the authors have reported an estimate of the retention time for a

group of particles as the median (i.e., time until 50% of particles are voided), mean, maximum (i.e., time until all particles are voided), or some other type of expression of retention time. Estimates of mean grit retention time are most appropriate for estimating grit ingestion rates, and this is discussed further below.

As mentioned previously, one of the shortest grit retention times reported in the literature is 0.24 days for house sparrows (Fischer and Best, 1995). Although this is expressed essentially as a mean retention time, it is based on a study with a high rate of grit ingestion and an atypically soft diet for the species. The results with sparrows and red-winged blackbirds presented by Fischer and Best (1995) indicate that birds consuming soft diets may not need much, if any, grit to aid digestion. Consequently, grit particles that are ingested by these birds are eliminated rapidly from the gizzard. The relatively low particle counts in gizzards of insectivorous birds (see Species Prioritization section below) may be a function of this short retention time as well as a lesser incentive for ingesting grit in the first place.

At the other extreme, Gionfriddo and Best (1999) discuss that during periods of reduced grit availability (e.g., snow cover during winter), birds have the ability to retain grit in their gizzards for extended periods of time. Many of the examples discussed are used to illustrate that it is possible for birds to maintain grit in the gizzard for digestion even when grit is limited in availability, but these examples tell us little about more typical conditions when grit availability is not a limiting factor. One of the longest examples (9 months) in Gionfriddo and Best (1999) is based on a study by Robel and Bisset (1979) where bobwhite quail were maintained in environmental chambers for 9 months without access to grit, but at necropsy birds still had some of the original grit from an earlier housing arrangement retained in their gizzards. Most of these examples are not a reflection of mean grit retention time, and several reflect experimental manipulation of grit availability.

There are few actual estimates of median or mean grit retention time in birds. Mateo and Guitart (2000) estimated the half-life (i.e., median retention time) for calcareous grit in mallards as 1.4 days. They also used information from Trost (1981) to estimate the half-life for quartzite grit as 3.1 days in mallards. Experiments with house sparrows dosed with quartz grit suggest a median grit retention time of approximately 0.5 day (Gionfriddo and Best, 1995). Best and Stafford (2002) found that on a more typical seed diet the grit retention time of sparrows and bobwhite quail decreased as the number of silica grit particles in the gavage dose increased. For

daily doses between 72 to 144 silica particles per day, sparrows and bobwhite on seed diets had mean grit retention times of approximately 1 day. Lower doses resulted in mean retention times of >1 day. The weight of grit consumed each day by rufous-collared sparrows (*Zonotrichia capensis*) on a seed diet was approximately the same as the weight of grit in gizzards of field-collected sparrows, suggesting a mean grit retention time of approximately 1 day (Lopez-Calleja et al., 2000).

If we assume that the retention of ingested grit particles in avian gizzards can be modeled as a negative exponential function, as reported in Trost (1981) and King and Bendell-Young (2000), then observations from various studies of the proportion of grit particles remaining at specific time periods can be used to estimate the mean and median grit retention times. As new grit particles are ingested each day, some of the particles are passed through the gizzard relatively rapidly while the rate of passage for the remainder of the particles slows considerably. By describing the retention time as an exponential function, as illustrated in Figure 1, a rate parameter ( $\lambda$ ) is calculated for each distribution that can be used to estimate the mean and median retention time. Mean retention time equals  $1/\lambda$ , while the median equals  $\ln(2)/\lambda$  or  $0.693/\lambda$ . For a more thorough discussion of the mathematical relationships of exponential functions and examples of their use, see Appendix B. For an example of how this may be applied to reported observations, consider a study by Fischer and Best (1995) where wild house sparrows were captured, dosed with 5 silica particles each, and released to the wild. These sparrows were collected 1, 4, 7, 10 and 13 days postdosing to count the number of silica particles retained in the gizzards. They observed that 50% of the silica particles were remaining after 24 hours. If the retention of these grit particles follows an exponential function, the rate parameter ( $\lambda$ ) equals  $0.693 \text{ days}^{-1}$ , resulting in a mean retention time of 1.44 days and a median of 1 day. Additional estimates of mean and median particle retention times based on published observations are presented in Table 1.

A critical aspect of this assumption is that each ingested particle has an equal probability of being voided from the gizzard at any particular time. When ingested particles vary in their physical characteristics (i.e., size, shape, texture), the gizzard may selectively retain certain particles over others. Selective retention becomes an issue in studies of grit retention times because investigators need to be able to identify the grit particles consumed (or dosed) at a particular point in time from those particles consumed later. To do this, investigators may

change the type of particle or some other particle characteristic (e.g., color) used so that they are recognizable. In some cases, however, the recognizable characteristics of particles also can affect their retention probability, and thus change the pattern of retention over time from that estimated using an exponential function. For example, while the house sparrows dosed with silica particles (Fischer and Best, 1995) voided 50% of silica particles in the first 24 hours, sparrows collected on days 4, 7, 10 and 13 postdosing all retained about 1/3 of the dosed silica particles (Figure 1). An exponential function with a rate parameter of  $0.693 \text{ day}^{-1}$  indicates that virtually all the dosed particles would have been voided by 7 days postdosing. These study results suggest that even if silica particle retention initially followed an exponential function, the remaining silica particles were selectively retained by the gizzard over other naturally available grit particles for an extended period. Fischer and Best (1995) similarly observed that while dosed red-winged blackbirds voided 90% of silica particles in the first 24 hours, the remaining 10% was retained in the gizzard for up to 13 days (i.e., no additional loss of silica particles observed after 24 hours). The extent to which gizzards are selective in retaining individual grit particles and how it occurs are unclear (Gionfriddo and Best, 1999).

A consistent observation amongst researchers studying grit ingestion rates and grit retention times is that these parameters are highly variable among birds and within a bird over time due to a variety of factors discussed above. In general, when grit availability is not limited, birds are routinely replenishing grit and mean grit retention times may range from less than 1 day to several days, though this observation is based on limited studies with only a few species. As mean grit retention times become shorter, daily grit ingestion rates increase, resulting in an increased probability of ingesting a lead particle (or other type of toxic particle). However, as retention times increase, even though the probability of ingesting a lead particle decreases, the estimated internal dose from an ingested lead particle may increase.

### ***Use of grit retention time to estimate internal dose***

Once a lead particle is ingested, it must be eroded in the gizzard before the lead can be taken up in the blood. As mentioned above, the complete erosion of a lead particle may take up to several weeks in some species. If grit retention times are relatively short and an ingested lead particle is voided within hours of ingestion, the risk may be small. However, even when the median grit retention time is relatively short, some fraction of ingested particles has been

observed to remain in the gizzard for many weeks (Gionfriddo and Best, 1995; Trost, 1981). Consequently, even if all ingested particles have an equal probability of being retained, any individual ingested lead particle has a small probability of being retained significantly longer than the mean retention time. The probability that a particle is retained for a specified period of time can be calculated using the exponential functions illustrated in Figure 1. If the mean grit retention time is assumed to be 1.44 days ( $\lambda = 0.693$ ), each particle has a probability of 0.5 of being retained each day and one can calculate the probability that a particular particle could be retained for more than X days. For example, there is a 0.125 probability that a particle will be retained 3 days (proportion of particles remaining at 3 days = 0.125) and a 0.0078 probability at 7 days. If the mean retention time is assumed to be 4 days ( $\lambda = 0.25$ ), the probability that a particle would be retained for  $\geq 3$  or  $\geq 7$  days is 0.47 and 0.17, respectively. However, if gizzards selectively retain lead particles longer than other types of natural grit particles, lead particles may have an even greater probability of being retained long enough to pose a risk to birds, even if mean grit retention times in general are relatively short. This section examines the literature on retention time of lead particles in gizzards.

McConnell (1967) reported that bobwhite quail and mourning doves dosed with lead shot expelled shot in their feces at regular intervals starting 3 days after dosing and continuing up to 22 days. In the shot excreted by bobwhite, there was no noticeable deterioration in the first week, but by 10 days shot were eroded to 1/2 of their original size and to 1/6 original size by day 22. In doves, shot excreted after 6 days were eroded to 1/2 their original size and to 1/6 original size after day 17.

Mourning doves dosed with 4 No. 8 lead shot had an average of 2.3 shot remaining in the gizzard at necropsy 34 days later (Buerger et al., 1986). These doves were fed a diet of 95% cracked corn, 5% commercial pigeon diet, and <1% oyster shell grit. Kendall et al. (1982) developed a prediction equation to estimate the percent of lead shot remaining (i.e., not yet eroded) in the gizzard of ringed turtle doves (*Streptopelia risoria*) over time (i.e., percent of lead shot remaining =  $97.8 - 4.24 \times \text{time (days)}$ ,  $R^2 = 0.88$ ). They observed that approximately 70% of a 110 mg No. 6 lead shot was eroded in a dove's gizzard in 14 days.

Acutely dosed mallards and black ducks (*Anas rubripes*) voided or completely eroded all ingested lead shot within 21 days after dosage (Chasko et al., 1984). Mortality was directly related to the length of lead particle retention time, and lead retention times were highly variable

among individuals receiving the same dose. Lead particle retention times were shortest for asymptomatic birds, intermediate for birds showing signs of lead poisoning, and longest for birds that eventually died (Chasko et al., 1984). During the experiment, ducks were fed a diet of natural foods including seeds, aquatic plants, small fish and aquatic invertebrates, including small snails, crabs and mussels. There is no mention of additional grit sources.

Vyas et al. (2001) reported considerably shorter lead shot retention times in brown-headed cowbirds (*Molothrus ater*). Seven of 10 cowbirds on a predominantly seed diet survived being dosed with a single No. 7½ lead shot, with six birds excreting the shot within 24 hours and one within 48 hours. Three of 10 cowbirds died within 24 hours. All three birds exhibited signs of lead poisoning and retained the dosed shot either in their gizzard or intestines.

As little as a single lead shot may also cause mortality in other avian species. Pain and Barnett (1988) observed 60% mortality in black ducks dosed with a single No. 4 lead shot. All birds died within 6 days and retained the dosed shot in their gizzard. No mortality was observed in mallards dosed with a single No. 4 shot (Pain and Barnett, 1988). Four of 27 ring-necked ducks (*Aythya collaris*) dosed with a single No. 4 lead shot died 14 to 21 days after dosing, with peak blood lead levels observed one week after dosing. Mourning doves dosed with a single No. 8 lead shot suffered 24% mortality over a 34-day period (Buerger et al., 1986), compared to 60% and 52% mortality in doves receiving two or four shot, respectively.

The trend observed in the above papers reporting on the retention of lead shot in avian gizzards suggests that lead particles are typically retained for at least several days in many species and as long as three weeks. It is very difficult to directly compare this information with the information on mean and median grit retention times discussed in the previous section because of the differences in experimental procedures and species tested. In most of the literature on lead exposure there is no mention of access to alternative sources of grit. Consequently, the retention of lead shots may or may not be extended in these studies due to lack of access to other sources of grit. Conversely, hard grit particles have been shown to increase the rate of lead shot erosion. Also, the rate of lead particle erosion is faster for individuals consuming a hard diet (e.g., cereal grains) than a soft diet (e.g., varied plant and animal diet) (Chasko et al., 1984).

Three studies with adult mallards can be combined to provide a comparison of the retention times of lead versus more natural grit types—calcareous grit (Mateo and Guitart, 2000),

quartzite grit (Trost, 1981) and lead shot (Chasko et al., 1984). Mateo and Guitart (2000) and Trost (1981) quantitatively described the retention of grit particles as exponential functions with mean retention times of 2.04 and 4.46 days, respectively (Figure 2). Chasko et al. (1984) dosed mallards with five No. 6 lead shot. Of the six dosed mallards, four survived without signs of toxicity while two died. The birds that died retained all the dosed shot for at least a week and retained 60% of shot at death. The retention time of lead shot in asymptomatic birds was somewhat longer than was observed with calcareous and quartzite grit, at least initially (Figure 2). If we were to assume that retention of lead shot in the asymptomatic mallards follows an exponential function, we could calculate the rate parameter for observations at day 3, 7, 10 and 14. If retention followed an exponential function the estimated  $\lambda$  values at the four observations would be approximately equal. However, the calculated rate parameters increased over time (i.e.,  $\lambda = 0.10, 0.11, 0.16$  and  $0.21$  at 3, 7, 10 and 14 days, respectively), indicating that the exponential function was not an adequate description of the lead particle retention because these  $\lambda$  values suggest retention times were decreasing. One possible explanation is that as lead shot are eroded and become smaller, their probability of being retained in the gizzard decreases. This explanation needs to be tempered by the small sample sizes and the comparisons among dissimilar studies. However, the consistent trend from this comparison and other studies cited above is that lead shot may be retained in gizzards longer than other types of grit and that birds that die of lead poisoning may retain lead shot longer than those that survive. *Consequently, assuming that the retention times for lead particles are equal to those of other grit particles may lead to an underestimation of risk.*

Sanderson and Irwin (1976; as cited in Trost [1981]) found that steel shot was retained significantly longer than lead shot. While steel shot is retained longer than lead shot and natural grit, it does not necessarily follow that grit and lead shot are retained at the same rate. The difficulty in comparing the retention times of grit and lead shot relates to the toxic effects caused by lead when sufficient lead shot are ingested to study retention quantitatively.

### ***Summary on the use of grit retention time***

While grit retention time is an important parameter for calculating the amount of grit consumed daily and for estimating the risk to birds from ingestion of lead particles, there are few studies that directly measure mean or median grit retention time. For purposes of using estimates

of grit retention time to calculate grit ingestion rates in birds, a review of published literature indicates that grit retention time can be relatively short (i.e., <1 day to a few days) when grit availability is not limited and relatively long (e.g., up to a year) if grit availability is very limited. The shorter the retention time, the higher the grit ingestion rate and, thus, the higher the probability of ingesting a lead particle. However, once a lead particle is in the gizzard, the literature indicates that in many species lead particles can be retained for days to weeks and can be almost completely eroded in less than 3 weeks. Whether or not lead particles are preferentially retained in gizzards over natural grit is difficult to determine. However, several studies indicate that birds dosed with lead shot retained the shot in their gizzards long enough to cause toxicity and death. Even if lead particles are not preferentially retained, the skewed distribution of retention times for individual particles (i.e., Trost, 1981), described as a negative exponential function, suggests that an individual lead particle may be voided very quickly or could be retained long enough to cause toxicity, even if the median grit retention time is relatively short.

### **SPECIES PRIORITIZATION IN ASSESSING RISK TO BIRDS FROM CONTAMINATED PARTICLES**

A key component of ecological risk assessment's (ERA) problem formulation is the development of a conceptual site model (CSM) (U.S. EPA, 1997). In developing the CSM, investigators identify pathways of exposure linking contaminated media to receptors representing foraging guilds (e.g., insectivorous passerine) and then choose specific receptors of concern (ROC) within each foraging guild in order to develop exposure models. When ingestion of contaminated particles is a consideration within the CSM, it is important to note that the number and size of grit particles will vary by species and foraging guild. This will influence the probability of avian species ingesting contaminated particles. By comparing the foraging and grit use habits of birds with the physical characteristics of the contaminated particles, it may be possible to focus quantitative risk characterization on a fewer number of species to decrease overall uncertainty in the assessment. Gionfriddo and Best (1996) examined the gizzards of 35 different species (with 5 or more gizzards per species) from the midwestern United States and documented the frequency of occurrence of grit within each set of gizzards, the mean and median number of particles ( $\geq 0.1$  mm), and the particle size (Table 2). To illustrate the variation in grit use frequency and number of particles, consider that within this data set, occurrence of grit

ranged from 0 to 100% while mean grit counts per gizzard ranged from 0-281. Similarly, Luttik and de Snoo (2004) presented the frequency of grit occurrence, mean number of grit particles, and particle size for 27 avian species in The Netherlands (Table 3). Due to differences in the methods used in these two studies it is difficult to merge the databases, but data from these studies will be summarized to help investigators prioritize ROCs when considering the importance of contaminated particles ingested as grit for representative receptors in the CSM.

### ***Dietary class***

Dietary class (or foraging guild) can have a significant influence on the occurrence and numbers of grit found in the gizzards of bird species (Gionfriddo and Best, 1996). The most common proposed function of grit within the gizzards of birds is for the mechanical grinding of food (Gionfriddo and Best, 1999). Therefore, birds in avian trophic guilds consuming hard plant (e.g., seeds) and animal material tend to have a higher frequency of grit usage relative to birds that have a soft diet. Gionfriddo and Best (1996) found grit in nearly all of the gizzards (94%) from six granivorous species, while Luttik and de Snoo observed grit in 100% of gizzards from seven granivores (Table 2). Similarly, grit was observed in most of the gizzards collected from omnivorous species in both studies, compared to only 40% in nine insectivorous species (Gionfriddo and Best (1996). The nongranivore dietary class in Luttik and de Snoo (2004) represents a diverse collection of species with the waterfowl species ingesting significantly more grit particles than other species in the class. Gionfriddo and Best (1996) reported that the number of grit particles was significantly different among dietary classes (ANOVA,  $p < 0.001$ , see Table 4).

### ***Size of particles***

Mean body size has a significant influence on the size of grit particles that avian species ingest (Gionfriddo and Best, 1996; Figure 3,  $p < 0.0001$ ,  $R^2 = 0.66$ ). Luttik and de Snoo (2004) reported that the mean size of grit particles increased with mean body size for the granivorous species, but not among the nongranivorous species. By quantifying the size of contaminated grit particles, investigators can further focus the assessment and measurement endpoints on specific avian species. Of the 35 species sampled by Gionfriddo and Best (1996), 89% of the species had mean particle sizes less than 1.9 mm (Table 2, Figure 4). In his masters thesis, Edwards (2002, Virginia Tech) sifted several soil samples from a public shooting range near Blacksburg Virginia

in the George Washington-Thomas Jefferson National Forest and found that more than 90% of the shot found was number 6 to number 8 pellets (2-3 mm). In this example, an investigator would focus the species of concern towards species most likely to ingest this size of particles (Figure 4): killdeer, mourning dove, rock dove, ring-necked pheasant and American crow. Conversely, Edwards also noted that within 30 meters of the firing line at the range there was a higher incidence of lead fragments ranging down in size to 0.01 mm. The firing line area was a small fraction of the range as a whole, but to the extent that it would be occupied by other species of smaller size, those species may also be of concern.

### ***Number of particles***

As a result of factors discussed in earlier sections of this document, the number of particles ingested by different species and individuals within species can vary considerably at any given time. From the Gionfriddo and Best (1996) data set (replicated in Table 2) it is noteworthy to view the standard deviations around the mean number of particles found in the gizzards of the various avian species. In most instances, the standard deviation is larger than the mean value (e.g., house sparrow, mean number of particles = 281, standard deviation = 476,  $n = 146$ ). Based on the species from Gionfriddo and Best (1996), regressing body mass with mean number of particles yields no significant or identifiable relationship (Figure 5). Although no discernable relationship between body mass and number of particles could be found, an obvious cluster of points is observed in bird species with masses between 10 and 100 grams having less than an average of 50 particles in their gizzards (Figure 5). This data translated into histogram format (Figure 6) illustrates that only five of 35 species in the data set were found to have an average number of particles greater than 50 in their gizzards. Those species were rock dove, fox sparrow, ring-necked pheasant, American tree sparrow and house sparrow.

Conversely, Luttik and de Snoo (2004) reported that the mean number of grit particles was related to body weight ( $\log_{10}$  particle number =  $1.21 * \log_{10}$  (body wt.),  $R^2 = 0.66$ ,  $n = 27$ ). However, this analysis included all particles, including fine soil particles. Also, this relationship was dominated by the species of the diverse nongranivore category that included several waterfowl species that consumed large amounts of fine soil or sediment material. This illustrates that across a diverse set of species, the ingestion of particles ranges from those that intentionally ingest grit particles to those that ingest particles incidental to their feeding activity.

Consequently, the number of grit particles ingested is influenced by multiple factors, including diet composition, body weight, and foraging behavior.

### ***Qualitative considerations across species prioritization categories***

Considering across these various factors (dietary class, contaminated particle size and average number of particles ingested) influencing the probability of species ingesting contaminated particles, certain inferences can be drawn depending on the habitat setting and CSM involved. For example, if the particle contamination is primarily lead shot (which is typically larger than 2 mm) in upland settings, one would want to choose a granivorous species like a rock dove and/or ring-necked pheasant. Nearly all the individuals within these species will use grit, the grit size found in their gizzards is consistent with contaminated particle size, and their gizzards typically contain a high number of particles. This rationale is one that would be used when a risk assessor is seeking a species with a relatively high probability of being exposed to a contaminated particle. The habitat setting, physical characteristics of the contaminated particles, and other aspects of the problem formulation in the risk assessment (e.g., assessment and measurement endpoints) will influence how one might use these factors in prioritizing species of concern when assessing the risk of contaminated particles to avian species.

## **USING GIONFRIDDO AND BEST (1996) DATA SET TO PARAMETERIZE MODEL**

### ***Estimating mean number of particles ingested per day***

In the revised lead model (Eq. 7), the number of particles ingested per unit time ( $T$ ) is a quotient of the mean number of particles per gizzard divided by the mean grit retention time (Eq. 5). Although the Gionfriddo and Best data set does not attempt to represent the mean number of particles found in their gizzard samples as a particle ingestion rate, we feel that this robust data set of randomly collected individuals per species is a reasonable source of information identifying the number of particles at any given time that may be in the gizzards of birds. If we assume that that number (although variable) could be replicated at another point in time, an ingestion rate can be estimated.

Given the large variation observed in gizzard counts among individuals, the use of point estimates for the number of gizzard grit particles in model calculations is intended to reflect the probability of ingesting lead particles across a large population of birds and is less useful for estimating the probability that individuals may ingest a lead particle. A prudent adjustment of

the average number of particles found in the gizzards of individuals in the Gionfriddo and Best (1996) data set would be to calculate a 95% Upper Confidence Limit (*UCL*) of the mean from the mean and standard deviation values given in Table 2. This calculation was completed by using the following equation taken from a U.S. EPA OSWER guidance document (U.S. EPA, 2002):

$$95\% \text{ } UCL_{1 - \alpha} = \textit{mean} + T_{\textit{val}} \times (\sigma/\sqrt{n}) \quad (\text{Eq. 8})$$

where

*mean* = mean number of particles/gizzard at time of necropsy

$T_{\textit{val}}$  = from a table of critical values of the Student's *t* distribution. The  $(1 - \alpha)^{\text{th}}$  percentile of the Student's *t* distribution with  $n - 1$  degrees of freedom

$\sigma$  = standard deviation and

$\sqrt{n}$  = the square root of the number of observations.

In the example cited earlier for house sparrow, the mean number of grit particles found in the gizzards of 146 individuals was 281, and the standard deviation was 476. When this information is used to parameterize Eq. 8, the following result is yielded:

$$95\% \text{ } UCL = 281 + 1.6554 \times (476/12.08) = 346 \text{ particles} \quad (\text{Eq. 9})$$

This computation assumes a normal distribution of the number of grit particles found in the gizzards of a number of individuals per species. Little information is available to determine the robustness of this assumption. Table 5 shows the upper 95% *UCL* calculated in this manner for all species in the Gionfriddo and Best (1996) data set. These values may be substituted for the mean number of particles ingested in Eq. 5. Using these estimates, and a range of retentions times as discussed in previous sections, reasonable daily particle ingestion rates can be calculated to parameterize Eq. 7.

## **AN APPROACH FOR ESTIMATING RISK OF MORTALITY FROM INGESTING LEAD PARTICLES**

Thus far this paper has focused on approaches for estimating exposure, i.e., the probability of ingesting toxic particles such as lead shot or pesticide granules. In the case of lead particles, the probability of mortality due to particle ingestion is a function of both the rate of lead particles ingested and their cumulative retention time in the gizzard. This may occur if a single lead shot is retained long enough to cause death or if the cumulative exposure from multiple ingested shot within a certain time period is sufficient to result in death.

One approach for estimating the risk of mortality from ingesting lead particles is to estimate the cumulative exposure to lead particles in the gizzard for a specific time period and to compare the estimate to a critical value indicative of death. To do this we can calculate the number of particle-exposure-days, which is defined as the cumulative number of days that one or more particles is retained in the gizzard within a specified period of days. For example, one lead particle retained for 6 days and three particles each retained for 2 days within a 6-day period both represent six particle-exposure-days. A death occurs when the number of particle-exposure-days exceeds some critical amount, expressed as  $\alpha$  particle-exposure-days, within a specified time period of  $w$  days.

To model this process we developed a simulation strategy in three broad steps (described in more detail in Appendix C). The first step is to develop a probability model for the number of lead particles in the gizzard of a bird on any given day, conditional on the number of lead particles in the gizzard the previous day. Development of the probability model requires six assumptions:

1. Gizzard load of grit particles is fixed,
2. Birds pass any given grit particle from the gizzard with a fixed daily probability,
3. Lead particles are passed at the same rate as other natural grit particles,
4. Gizzard grit contents are replenished to a fixed load of grit particles daily,
5. Lead particles are acquired during replenishing in proportion to their concentration in the environment (i.e., there is neither preference for, nor avoidance of, lead particles), and
6. Particle-exposure-day is an adequate expression of cumulative exposure over a specified period of time.

In reality, the gizzard load varies due to factors that are not well understood, and it is not known if lead particles are selected in proportion to their occurrence and retained at the same rate as

natural grit particles, but these assumptions are made to provide a starting point for estimating potential exposure. With the above assumptions we formulate a stochastic matrix ( $M$ ) giving the probability distribution for the number of lead particles in the gizzard on any given day, conditional on the number of particles that were in the gizzard the previous day (Appendix C).

The second step is to use the probability model above to simulate a time-series of grit particles in the gizzards of individual birds within an exposed population over an ecologically relevant time period. For example, the time period may range from a few days for some migrant species passing through a site to all or most of the year for year-long resident species at a site. This requires an assumption about the distribution of lead particles in the gizzard at the start of the time series. In this version of the model we assume that there are no lead particles in gizzards at the start of the time series (i.e.,  $n_1 = 0$ ).

The simulation model has eight inputs, of which seven are required ( $q$  and  $RT$  are redundant):

- $n_d$  = number of lead particles in the gizzard at the start of day  $d$ , assuming birds arrive at a site with no lead particles in their gizzard, i.e.,  $n_1 = 0$ ,
- $G$  = mean gizzard load expressed as the total number of particles in the gizzard at a time,
- $RT$  = mean retention time (in days) for a particle in the gizzard,
- $q$  =  $1/RT$  = daily probability that a particle is voided,
- $(\alpha, w)$  = ordered pair representing a fatal dose of toxic particles, where a bird will die if it experiences at least  $\alpha$  particle-exposure-days within a period of  $w$  days
- $S$  = the length of time (in days) that birds use the habitat, and
- $B$  = the number of birds to simulate.

The third step is to track the number of simulated birds for which the number of particle-exposure-days exceeds some critical amount within a specified window of time. In this step, each particle is assumed to reside in the gizzard in units of a full day (an exposure day) and a running total of particle-exposure-days is tracked within a moving window of  $w$  days beginning on day  $d - w$ . On each day, any female that exceeds a total of  $\alpha$  particle-exposure-days within  $w$  days is assumed to have died of lead poisoning and is removed from the simulation. If  $d < w$  then exposure days are tracked back to day 1 only. The number of dead birds at the end of the time series divided by the number birds simulated ( $B$ ) is the probability of mortality during the full time period ( $S$ ). The value selected for  $B$  does not necessarily reflect the population size at a

particular site. The higher the value of  $B$  used in the simulations, the more precise the estimated probability of mortality will be.

To illustrate the simulation model, we provide examples of model output using northern bobwhite (*Colinus virginianus*) based on information in McConnell (1967) and brown-headed cowbird (*Molothrus ater*) based on information in Vyas et al. (2001). Input data for both simulations are found in Table 6.

In McConnell (1967) northern bobwhite died when 52 particle-exposure-days had been accumulated in a 20 day period. For bobwhite foraging on a contaminated site for 90 days where lead particles accounted for 2% of total particles, the probability of death by the end of the season was estimated to be 7.6% (Figure 7). In other words, 7.6% of the bobwhite feeding at this site for 90 days would be expected to have died due to lead exposure. The longer birds remain on a contaminated site, the higher their overall probability of achieving a lethal exposure. Brown-headed cowbirds died after one particle-exposure-day in a one-day period (Vyas et al., 2001). For cowbirds foraging at a contaminated site for 45 days with lead accounting for 0.1% of the total particles, the estimated probability of death was substantially higher (36%) even with a much lower concentration of lead in the environment (i.e., 1 in 1000 particles) and a shorter total period of exposure (Figure 8). This is due to the lower critical threshold for cowbirds (1 particle-exposure-day over a 1-day window), but also due to the faster gizzard turnover rate. In its current form, the model also outputs a graph showing the cumulative probability of death as a function of time at the contaminated site (Figures 7 and 8).

The primary limitation to the use of this simulation model is that several input parameters are highly variable or there is little empirical data on which to base parameter estimates. In the model, the terms for mean gizzard load ( $G$ ) and mean retention time ( $RT$ ) are the same as in the revised model for estimating the probability of ingesting lead particles discussed above. Evidence presented above suggests that both model inputs may vary greatly due to factors such as diet composition, availability of grit, and grit characteristics. Also, very few studies have empirically measured mean retention time, and those studies have been under laboratory conditions (Table 1), so there is considerable uncertainty in deriving estimates of mean retention time for field scenarios. Additionally, although examples exist for parameterizing  $\alpha$  (particle-exposure-days) and  $w$  (critical time period of exposure), these parameters are not reported in studies and must be estimated from available information. Also, the critical value  $\alpha$  probably

varies as a function of  $w$ , but we have insufficient data to speculate on the form of those relationships. Thus the number of species for which this model can be parameterized directly with empirical data is limited. Because of the uncertainty surrounding several of the input parameters, the simulation model currently is best used as a tool to explore the relationships among input parameters and to evaluate model outputs under a variety of assumptions about input parameters. Additional research would be needed to understand how model mortality estimates are influenced by bias in input parameter estimates.

The model above has three other important limitations. First, simulations are only feasible with gizzard loads up to about 250 particles due to limitations in MATLAB software on the size of matrices. Thus the model cannot be applied to species such as waterfowl in its current form because of their ingestion of larger amounts of grit particles. Second, the model does not track additional mortality that would occur due to particles persisting in the gizzard after the end of the season. Thus the mortality estimates are biased low, especially for birds with long gizzard residence times. The first two limitations could be addressed through revisions to the model if necessary. Third, in reality, the rate of particle erosion is a function of their size and shape, yet at this point the model does not consider particle size and there is little data on which to incorporate size-related erosion rates into the model. Additional research would be required to address this limitation.

### **RANGE OF PARTICLE SIZES: IMPLICATIONS TO RISK ASSESSMENTS**

Contaminants in soils exist in a wide variety of particle sizes and more often exist as part of the soil matrix. To reiterate a point discussed earlier regarding the EPP0 scheme models for soil and grit ingestion, when the chemical of concern may be ingested accidentally with soil as well as ingested intentionally as grit particles, these two routes of exposure may be independent and thus additive in terms of estimating a total exposure dose. However, because birds may ingest a wide range of particle sizes, from very fine soil particles to larger grit particles (i.e., >1 mm in size), it is very difficult to separate accidental soil ingestion from intentional grit ingestion when there is overlap in the particle size ranges. The soil ingestion estimates by Beyer et al. (1994) are based on a method where the size range of particles included an overlap with the information of grit size preferences by Best and Gionfriddo (1991) and Luttik and de Snoo (2004). Consequently, estimates of percentage of soil in the diet may overlap somewhat with estimates of grit ingestion rates due to the differences in methods used to calculate the estimates.

Each site-specific risk assessment of lead should consider these two possible routes of exposure in light of site-specific information on the nature of lead availability (i.e., does the lead exist primarily as dissolved lead in soil, lead shot and fragments, or a combination?). At firing ranges where larger lead particles are more common, the probability that some species are ingesting lead particles as grit increases. To use models for estimating the probability of ingesting lead particles as grit, we need measurements of the proportion of grit-sized particles on the surface made up of lead particles or a means of estimating this proportion. When information is available on the lead particle size distribution at a site, this can be compared to the distributions of preferred grit particles for various avian species to determine which species have the greatest overlap. For example, if the lead at a site is predominately in the form of No. 8 lead shot (~2.3 mm), this helps focus on the subset of species typically ingesting grit particles in this range; many of these species would not be inadvertently ingesting particles of this size as soil, but would be more likely to intentionally select these particles as grit. In this case, the models for estimating the probability of ingesting lead particles as grit may be the most appropriate method for estimating exposure. On the other hand, if the lead particles at a site are variable in size and predominantly smaller (say <1.5 mm), it would be difficult to separate lead ingested through accidental soil ingestion from intentional grit ingestion. In this case, estimating exposure via the grit ingestion models would likely overlap to some extent with estimates based on soil ingestion methods, and investigators would need to develop a weight-of-the-evidence case, using available site-specific information, in order to estimate exposure potential. When using grit ingestion models, investigators need to consider the nature of the contamination in the soil matrix within the conceptual site model.

## **CONCLUSIONS**

In this paper we reviewed several approaches for estimating the probability of ingesting contaminated particles such as pesticide granules or lead particles (i.e., shot or bullet fragments), and all of these approaches are strongly influenced by the estimate of daily grit ingestion rate, which is not measured directly. The daily grit ingestion rate can be estimated using the number of grit particles found in gizzards at a point in time and an estimate of the grit retention time (i.e., length of time that grit particles are retained in the gizzard). However, grit retention times have only been measured in a few controlled studies with a few species, and empirical evidence

indicates that grit retention times can vary considerably due to factors such as diet composition, availability of grit, and grit characteristics. Consequently, estimating the probability of ingesting contaminated particles depends heavily on assumptions made about grit retention times. Also, all of these approaches assume that contaminated particles, whether they are pesticide granules or lead fragments, are ingested in proportion to their availability in the environment (i.e., there is neither preference for, nor avoidance of, contaminated particles), but there is little empirical information about whether birds actively prefer or avoid contaminated particles, which could result in an underestimation or overestimation, respectively, of the probability of exposure.

In addition to approaches for estimating the probability of ingesting contaminated particles, we presented an approach for using this information to estimate the probability of mortality from the ingestion of lead particles. Mortality from the erosion of lead particles is a function of the number of lead particles ingested and the length of time each particle is retained in the gizzard. In the approach presented, the estimate of the grit retention time is used not only to estimate the daily grit ingestion rate of a species, but also to estimate the length of time ingested particles are retained and eroded in the gizzard. A model was developed to determine if the cumulative number of days that one or more particles was retained in the gizzard was sufficient to cause mortality. However, several input parameters are highly variable or there is little empirical data on which to base parameter estimates. Consequently, the model currently is best used as a tool to explore the relationships among input parameters and to evaluate model outputs under a variety of assumptions about input parameters. However, additional research would be needed to understand how uncertainty in input parameters affects mortality estimates from the model.

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**Table 1. Studies of Avian Grit Retention in Gizzards with Estimates of Mean and Median Retention Times.**

Study Description and Reference	Species	Time Since Dose (days)	Proportion of Initial Dose Remaining	Rate Parameter or $\lambda$ (days <sup>-1</sup> )	Mean Retention Time (days)	Median Retention Time (days)
Birds were dosed with 5 silica particles, released to the wild, and collected at various time intervals postdosing (Fischer and Best, 1995).	House sparrow	1	0.50	0.693	1.44	1.00
	Red-winged blackbird	1	0.1	2.303	0.43	0.30
Birds with access to quartz grit were switched to feldspar grit and euthanized at specific time intervals. The proportion of two grit types in gizzards was recorded (Gionfriddo and Best, 1995).	House sparrow	0.25	0.6	2.043	0.49	0.34
		1	0.12	2.120	0.47	0.33
Birds were dosed with 25 green quartzite particles, and particles passed in feces were counted over a 24-day period (Trost, 1981).	Mallard	Multiple measurements		0.224 <sup>a</sup>	4.46	3.09
Ducklings with access to one color of grit were switched to another color and euthanized at specific time intervals. The weight of original color grit was recorded (King and Bendell-Young, 2000).	Mallard	Multiple measurements		0.48 <sup>b</sup>	2.08	1.44
Birds with access to calcareous grit were switched to siliceous grit and euthanized at specific time intervals. The weight of calcareous grit remaining was recorded (Mateo and Guitart, 2000).	Mallard	Multiple measurements		0.49 <sup>c</sup>	2.04	1.42

<sup>a</sup> Trost (1981) expressed retention of quartzite particles as a negative exponential function with a 4-day interval retention rate of 0.408, which is equivalent to a daily rate parameter ( $\lambda$ ) of 0.224 days<sup>-1</sup>.

<sup>b</sup> King and Bendell-Young (2000) expressed retention of grit particles as an exponential function with “grit turnover rate” of 0.02/hr, which is equivalent to a daily rate parameter of 0.48 days<sup>-1</sup>.

<sup>c</sup> Mateo and Guitart (2000) expressed retention of calcareous grit as an exponential function with a daily rate parameter of 0.49 days<sup>-1</sup>.

**Table 2. Data Summary Table of Dietary Classes, Mean Particle Counts, Mean Particle Size, and Body Masses of Avian Species Sampled for Gizzard Analyses in Gionfriddo and Best (1996).**

Species	Dietary Class	Percent Occurrence	Number Sampled	Average # of Particles	sd	Median # of Particles	Mean Size mm.	Average Mass (g)				Rank	Percentage of Species
								Females	Males	Non-specified	Combined		
Northern Oriole	Omnivore	0	5	0	0	0	0			33.6	33.6	1	3%
Yellow-rumped Warbler	Insectivore	0	5	0	0	0	0	12.2	12.9		12.55	2	6%
House Wren	Insectivore	57	7	3	5	1	0.3			10.9	10.9	3	9%
Cedar Waxwing	Frugivore	20	20	9	40	0	0.3	33.1	30.6		31.85	4	11%
Hermit Thrush	Omnivore	83	6	14	17	6	0.3			31	31	5	14%
Common Yellowthroat	Insectivore	21	14	21	1	0	0.3	9.9	10.3		10.1	6	17%
American Tree Sparrow	Granivore	100	8	267	212	203	0.4			20.1	20.1	7	20%
Fox Sparrow	Granivore	100	5	102	88	93	0.5			32.3	32.3	8	23%
Lark Sparrow	Omnivore	100	5	42	50	22	0.6			29	29	9	26%
House Sparrow	Granivore	98	146	281	476	97	0.7	27.4	28		27.7	10	29%
Dickcissel	Omnivore	25	28	4	18	0	0.8	24.6	29.3		26.95	11	31%
American Robin	Omnivore	44	43	12	26	0	0.8			77.3	77.3	12	34%
Song Sparrow	Omnivore	93	14	14	15	8	0.8	20.5	21		20.75	13	37%
Common Grackle	Omnivore	57	47	10	21	1	0.9	100	127		113.5	14	40%
Chipping Sparrow	Omnivore	95	20	12	16	7	0.9			12.3	12.3	15	43%
Vesper Sparrow	Omnivore	86	125	12	14	8	0.9	24.9	26.5		25.7	16	46%
Red-Headed Woodpecker	Insectivore	57	27	31	59	2	0.9			71.6	71.6	17	49%
Indigo Bunting	Omnivore	81	21	35	91	4	0.9	14.1	14.9		14.5	18	51%
Brown-headed Cowbird	Omnivore	52	175	10	30	1	1	38.8	49		43.9	19	54%
European Starling	Omnivore	36	56	21	85	0	1	79.9	84.7		82.3	20	57%

**Table 2. cont.**

Species	Dietary Class	Percent Occurrence	Number Sampled	Mean # of Particles	sd	Median # of Particles	Mean Size mm.	Average Mass (g)				Rank	Percentage of Species
								Females	Males	Non-specified	Combined		
Brown-headed Cowbird	Omnivore	52	175	10	30	1	1	38.8	49		43.9	19	54%
European Starling	Omnivore	36	56	21	85	0	1	79.9	84.7		82.3	20	57%
Northern Cardinal	Omnivore	73	22	40	82	6	1	43.9	45.4		44.65	21	60%
Savannah Sparrow	Granivore	100	21	41	102	20	1	19.5	20.6		20.05	22	63%
Red-winged Blackbird	Omnivore	73	82	17	61	4	1.1	41.5	63.6		52.55	23	66%
Barn Swallow	Insectivore	22	23	1	4	0	1.2			18.6	18.6	24	69%
Horned Lark	Omnivore	99	69	11	13	8	1.2	30.8	31.9		31.35	25	71%
Eastern Kingbird	Insectivore	24	29	1	4	0	1.3			39.5	39.5	26	74%
Western Meadowlark	Insectivore	44	9	2	3	0	1.4	89.4	106		97.7	27	77%
Yellow-billed Cuckoo	Insectivore	44	9	3	6	0	1.6			64	64	28	80%
Blue Jay	Omnivore	85	20	35	42	15	1.6			86.8	86.8	29	83%
Northern Bobwhite	Omnivore	90	75	49	106	12	1.8			178	178	30	86%
Killdeer	Insectivore	93	28	8	9	5	1.9	101	92.1		96.55	31	89%
Mourning Dove	Granivore	68	40	10	16	3	2.1	115	123		119	32	91%
Rock Dove	Omnivore	100	15	69	43	60	2.3			542	542	33	94%
Ring-necked Pheasant	Granivore	100	37	151	190	88	2.3	953	1317		1135	34	97%
American Crow	Omnivore	53	64	49	156	2	2.9	438	458		448	35	100%

sd = standard deviation.

**Table 3. Data Summary Table of Dietary Classes, Percent Occurrence of Grit, and Mean Grit Particle Counts by Size Class of Avian Species Sampled for Gizzard Analyses by Luttk and de Snoo (2004).**

Species	Scientific Name	Dietary Class	Number Sampled	Percent Occurrence	Mean Number of Grit Particles Per Bird			
					<0.25 mm	0.25–0.5 mm	0.5–0.75 mm	>0.75 mm
Linnet	<i>Carduelis cannabina</i>	Granivore	2	100	0	11	7	93
Goldfinch	<i>Carduelis carduelis</i>	Granivore	1	100	0	20	6	37
Twite	<i>Carduelis flavirostris</i>	Granivore	1	100	0	0	1	121
Greenfinch	<i>Chloris chloris</i>	Granivore	8	100	14	47	10	85
Woodpigeon	<i>Columba palumbus</i>	Granivore	20	100	13	5	1	207
Chaffinch	<i>Fringilla coelebs</i>	Granivore	8	100	19	26	4	61
Brambling	<i>Fringilla montifringilla</i>	Granivore	1	100	0	9	1	187
House sparrow	<i>Passer domesticus</i>	Omnivore	11	100	519	218	32	147
Tree sparrow	<i>Passer montanus</i>	Omnivore	1	100	127	44	6	71
Grey partridge	<i>Perdix perdix</i>	Omnivore	25	100	1,566	378	37	639
Pheasant	<i>Phasianus colchicus</i>	Omnivore	16	100	408	98	10	204
Skylark	<i>Alauda arvensis</i>	Nongranivore	6	100	1,610	423	45	172
Mallard	<i>Anas platyrhynchos</i>	Nongranivore	11	100	50,250	15,903	1,827	1,959
White-fronted goose	<i>Anser albifrons</i>	Nongranivore	1	100	77,419	24,707	2,974	5,348
Greylag goose	<i>Anser anser</i>	Nongranivore	5	100	144,889	73,931	9,999	11,202
Bean goose	<i>Anser fabalis</i>	Nongranivore	1	100	85,216	40,827	5,462	5,395
Meadow pipit	<i>Anthus pratensis</i>	Nongranivore	9	22	106	38	5	1
Carrion crow	<i>Corvus corone</i>	Nongranivore	13	92	13,348	4,268	497	214
Jackdaw	<i>Corvus monedula</i>	Nongranivore	1	100	25,340	7,425	823	482

**Table 3. cont.**

Species	Scientific Name	Dietary Class	Number Sampled	Percent Occurrence	Mean Number of Grit Particles Per Bird			
					<0.25 mm	0.25–0.5 mm	0.5–0.75 mm	>0.75 mm
Reed bunting	<i>Emberiza schoeniclus</i>	Nongranivore	3	33	42	11	1	0
Black-headed gull	<i>Larus ridibundus</i>	Nongranivore	8	75	1,134	419	54	42
White wagtail	<i>Motacilla alba</i>	Nongranivore	3	33	0	8	2	1
Magpie	<i>Pica pica</i>	Nongranivore	11	64	1,969	615	71	26
Starling	<i>Sturnus vulgaris</i>	Nongranivore	10	20	1,301	342	35	3
Blackbird	<i>Turdus merula</i>	Nongranivore	10	80	33,629	6,094	448	32
Song thrush	<i>Turdus philomelos</i>	Nongranivore	10	90	833	236	26	17
Lapwing	<i>Vanellus vanellus</i>	Nongranivore	2	100	3,877	919	89	32

**Table 4. Mean ( $\pm$  Standard Deviation) of Percent Occurrence of Grit and Number of Grit Particles Found in Gizzards at Necropsy by Dietary Class from Gionfriddo and Best (1996) and Luttkik and de Snoo (2004).**

Dietary Class	$N^a$	% Occurrence of Grit	Mean # of Grit Particles in Gizzard <sup>b</sup>
Gionfriddo and Best (1996)			
Granivore	6	94 $\pm$ 13	142 $\pm$ 113
Insectivore	9	40 $\pm$ 27	7.8 $\pm$ 10.9
Omnivore	19	70 $\pm$ 28	24 $\pm$ 19
Luttkik and de Snoo (2004)			
Granivore	7	100	134 $\pm$ 55
Omnivore	4	100	471 $\pm$ 405
Nongranivore	16	76 $\pm$ 31	13,966 $\pm$ 26,110

<sup>a</sup>  $N$  represents number of species in each class.

<sup>b</sup> In Luttkik and de Snoo mean number of particles represent the total particles  $\geq 0.25$  mm.

**Table 5. 95% Upper Confidence Limit of the Mean Estimates for Number of Particles Found in the Gizzards of Avian Species.**

Species	Number Sampled	Mean # of Particles	sd	T-value	95% UCL
Northern Oriole	5	0	0	2.1318	0
Yellow-rumped Warbler	5	0	0	2.1318	0
Barn Swallow	23	1	4	1.7171	2
Eastern Kingbird	29	1	4	1.7011	2
Western Meadowlark	9	2	3	1.8595	4
House Wren	7	3	5	1.9432	7
Yellow-billed Cuckoo	9	3	6	1.8595	7
Dickcissel	28	4	18	1.7033	10
Killdeer	28	8	9	1.7033	11
Cedar Waxwing	20	9	40	1.7291	24
Brown-headed Cowbird	175	10	30	1.6537	14
Common Grackle	47	10	21	1.6787	15
Mourning Dove	40	10	16	1.6849	14
Horned Lark	69	11	13	1.6676	14
American Robin	43	12	26	1.6820	19
Chipping Sparrow	20	12	16	1.7291	18
Vesper Sparrow	125	12	14	1.6572	14
Hermit Thrush	6	14	17	2.0150	28
Song Sparrow	14	14	15	1.7709	21
Red-winged Blackbird	82	17	61	1.6639	28
Common Yellowthroat	14	21	1	1.7709	21
European Starling	56	21	85	1.6730	40
Red-Headed Woodpecker	27	31	59	1.7056	50
Blue Jay	20	35	42	1.7291	51
Indigo Bunting	21	35	91	1.7247	69
Northern Cardinal	22	40	82	1.7207	70
Savannah Sparrow	21	41	102	1.7247	79
Lark Sparrow	5	42	50	2.1318	90

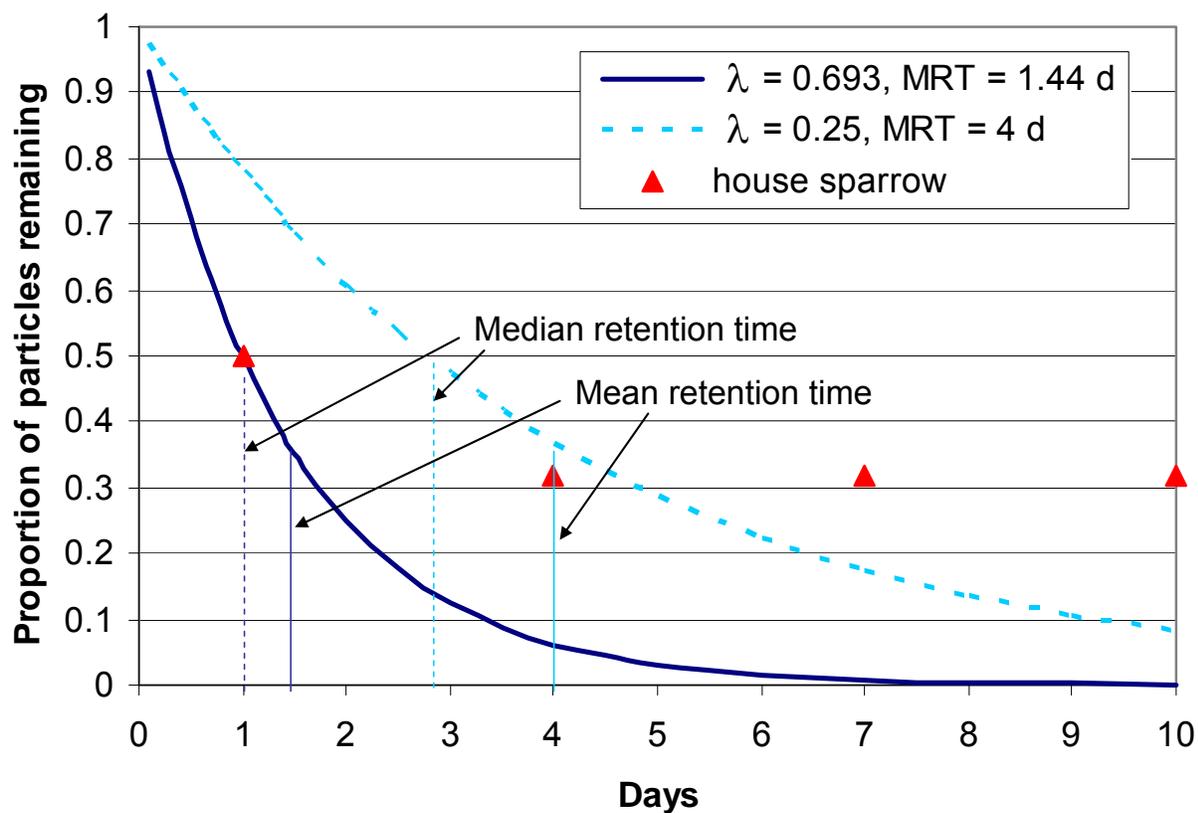
**Table 5 cont.**

Species	Number Sampled	Mean # of Particles	sd	T-value	95% UCL
American Crow	64	49	156	1.6694	82
Northern Bobwhite	75	49	106	1.6657	69
Rock Dove	15	69	43	1.7613	89
Fox Sparrow	5	102	88	2.1318	186
Ring-necked Pheasant	37	151	190	1.6883	204
American Tree Sparrow	8	267	212	1.8946	409
House Sparrow	146	281	476	1.6554	346

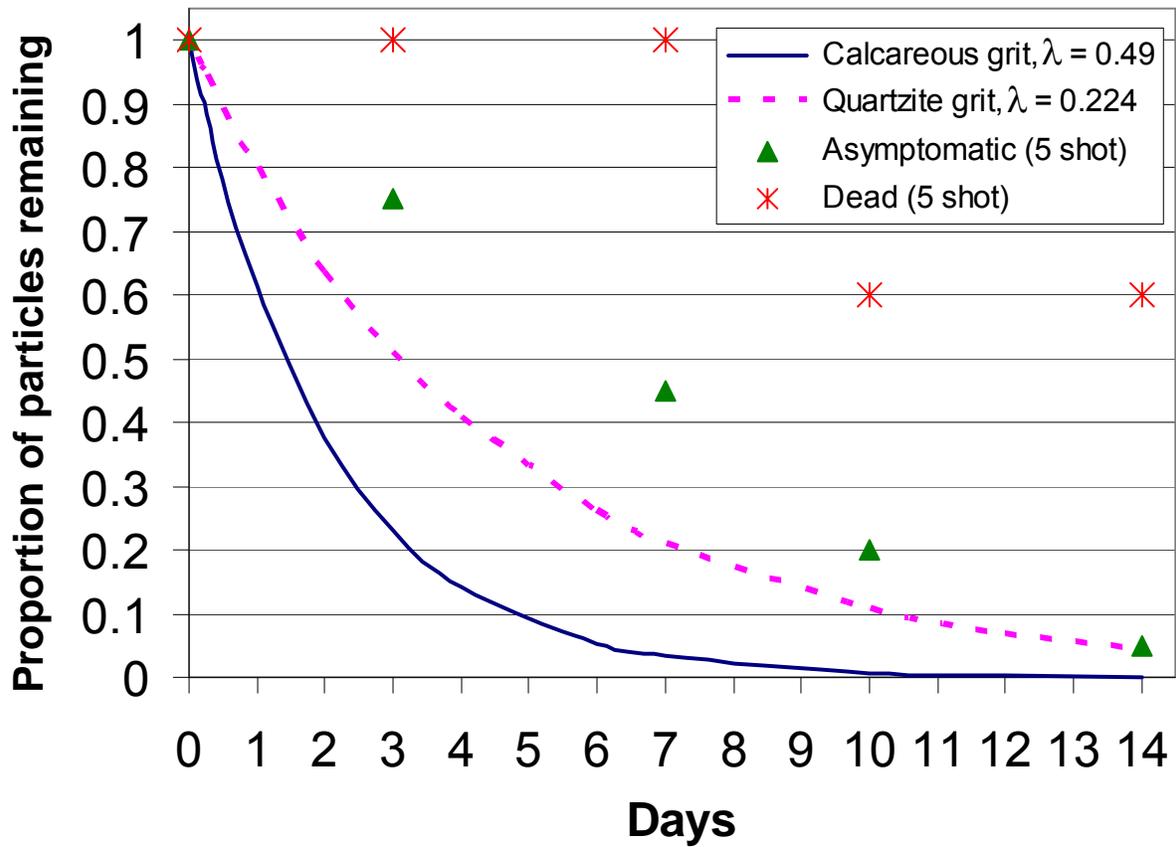
sd = standard deviation.

**Table 6. Input Parameters for Simulation Model.**

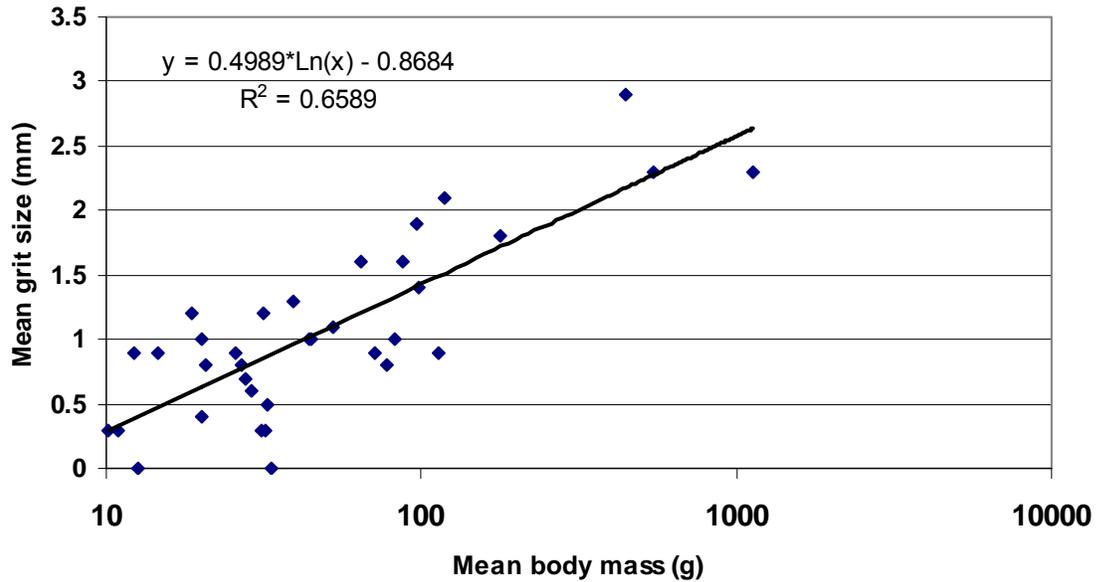
Parameter	Definition	Northern Bobwhite	Brown-headed Cowbird
$G$	Gizzard load (i.e., number of grit particles in gizzard)	49	10
$P$	Proportion of grit-sized lead particles in environment	0.02	0.001
$RT$	Mean retention time for grit in gizzard ( $= 1/q$ )	4 days	1 day
$q$	Daily probability that a grit particle is removed from the gizzard ( $= 1/RT$ )	0.25	1
$\alpha$	Critical number of particle-exposure-days	52	1
$w$	Critical time window for experiencing $\alpha$ exposure days	20	1
$S$	Length of season that bird uses the contaminated environment	90 days	45 days
$B$	Number of birds simulated	10,000	10,000



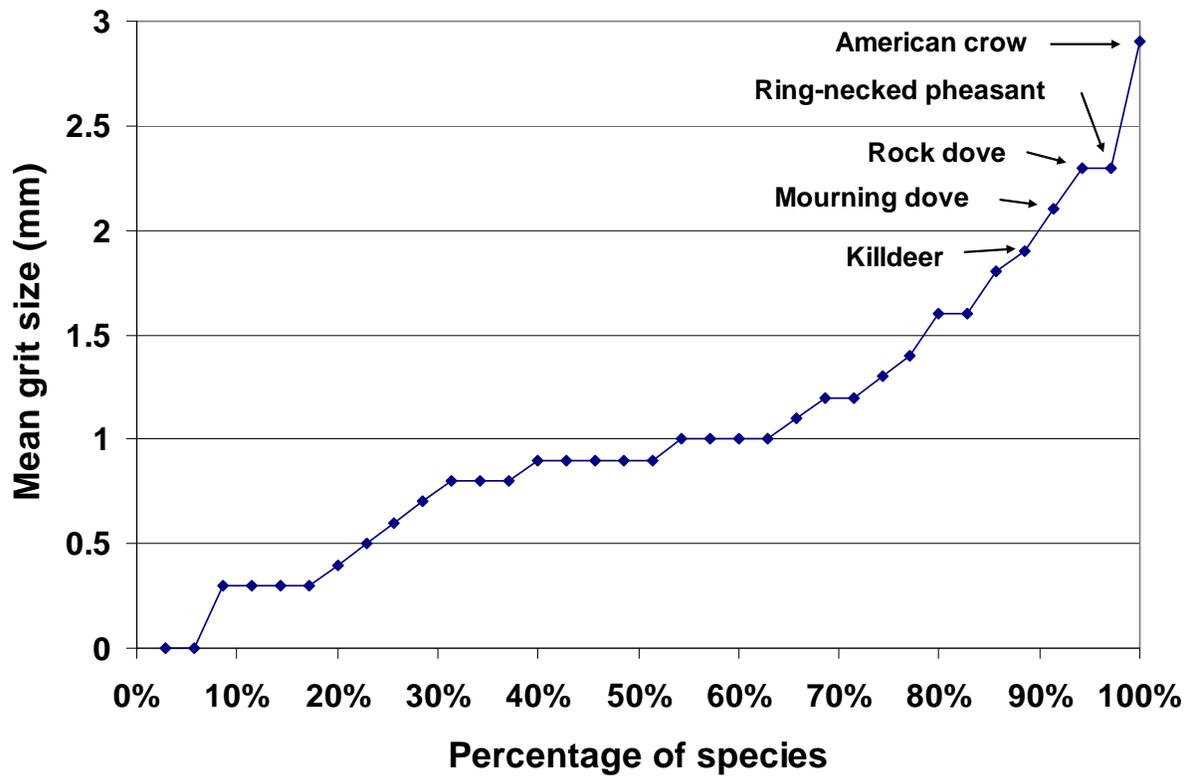
**Figure 1. Illustration of the Mean and Median Particle Retention Times Based on Two Exponential Functions with Rate Parameters of 0.693 and 0.25.** MRT = mean retention time. Red triangles represent the observed proportion of silica grit remaining in house sparrow gizzards as a function of time postdosing (Fischer and Best, 1995).



**Figure 2. Comparison of the Proportion of Particles Retained in Mallard Gizzards Over Time as Observed for Calcareous Grit (solid line), Quartzite Grit (dashed line), and Doses of Five No. 6 Lead Shot in Surviving Birds Without Signs of Lead Poisoning (solid triangles,  $n = 4$ ) and in Birds that Died (six-point stars,  $n = 2$ ).**



**Figure 3. Relationship Between Mean Grit Size and Mean Body Mass.** Body masses were obtained from Dunning (1993). Graph replicated based on analyses in Gionfriddo and Best (1996). Each point represents the average mass of individual species in which at least 5 gizzards were sampled.



**Figure 4. Distribution of Mean Size of Grit Particles Found in the Gizzards of Avian Species from Gionfriddo and Best (1996).**

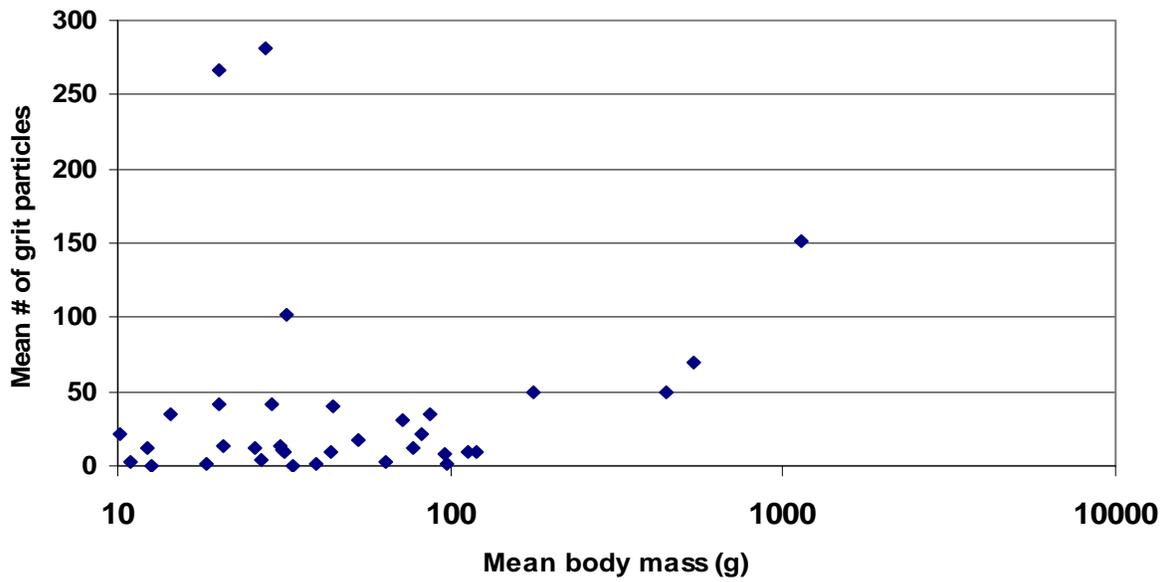


Figure 5. Relationship Between Mean Number of Grit Particles Found in the Gizzards of Avian Species and Their Body Mass.

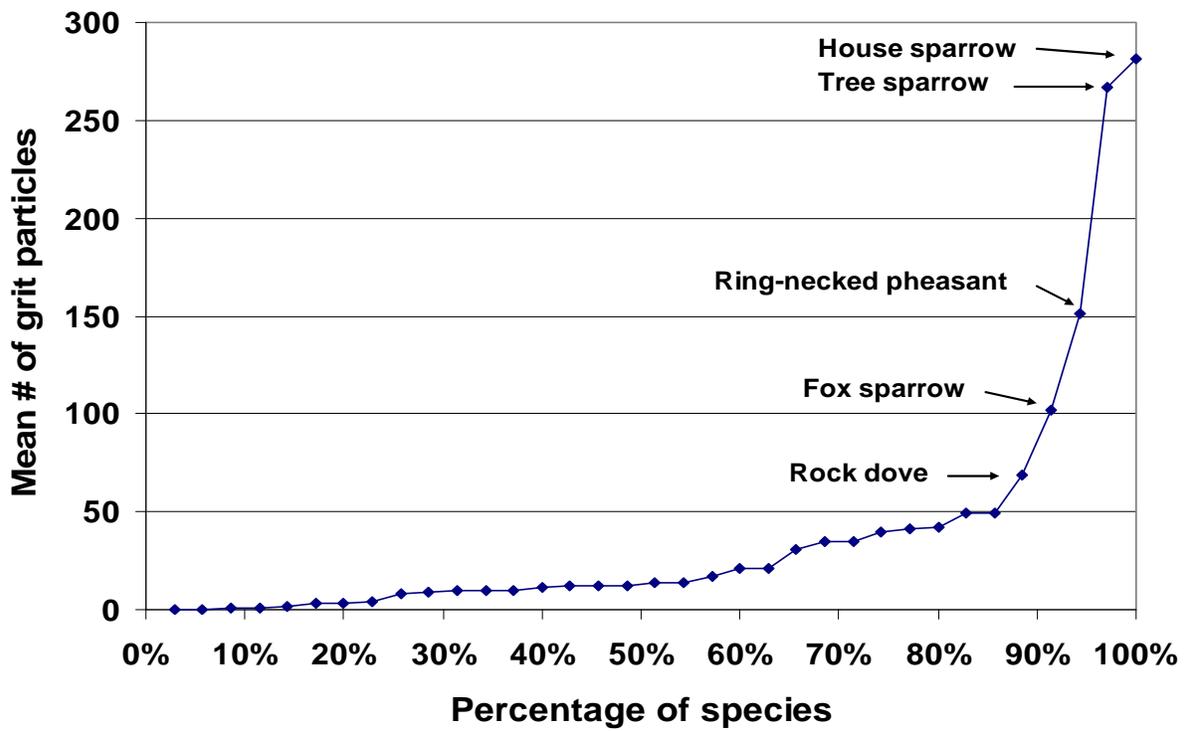


Figure 6. Distribution of Mean Number of Grit Particles Found in the Gizzards of Avian Species.

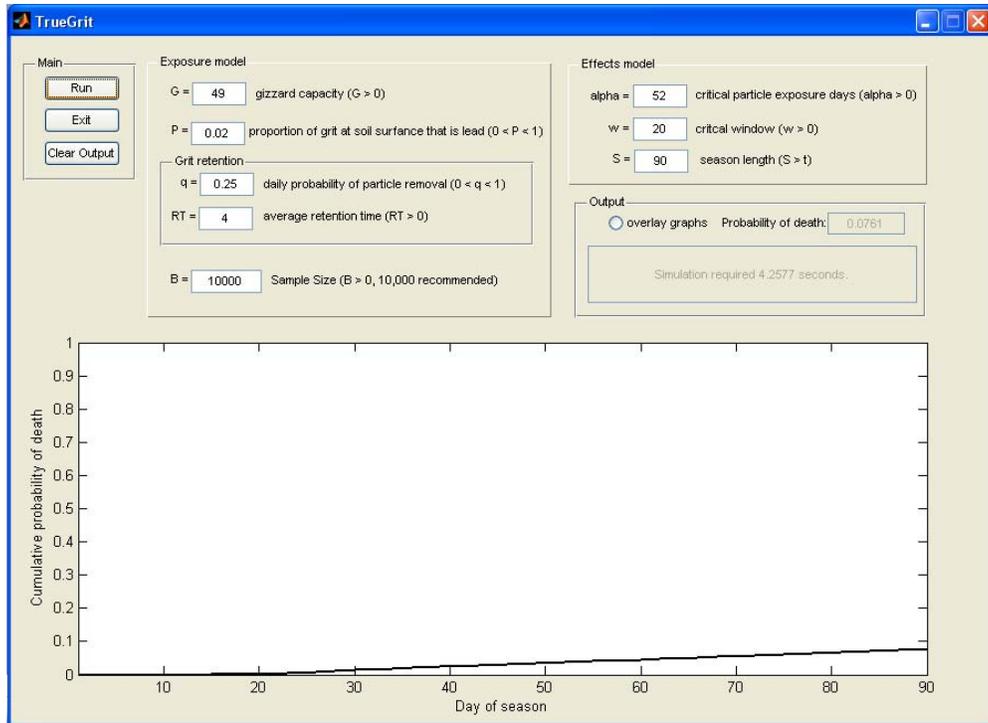


Figure 7. Sample Model Output for Northern Bobwhite Using Parameters in Table 6.

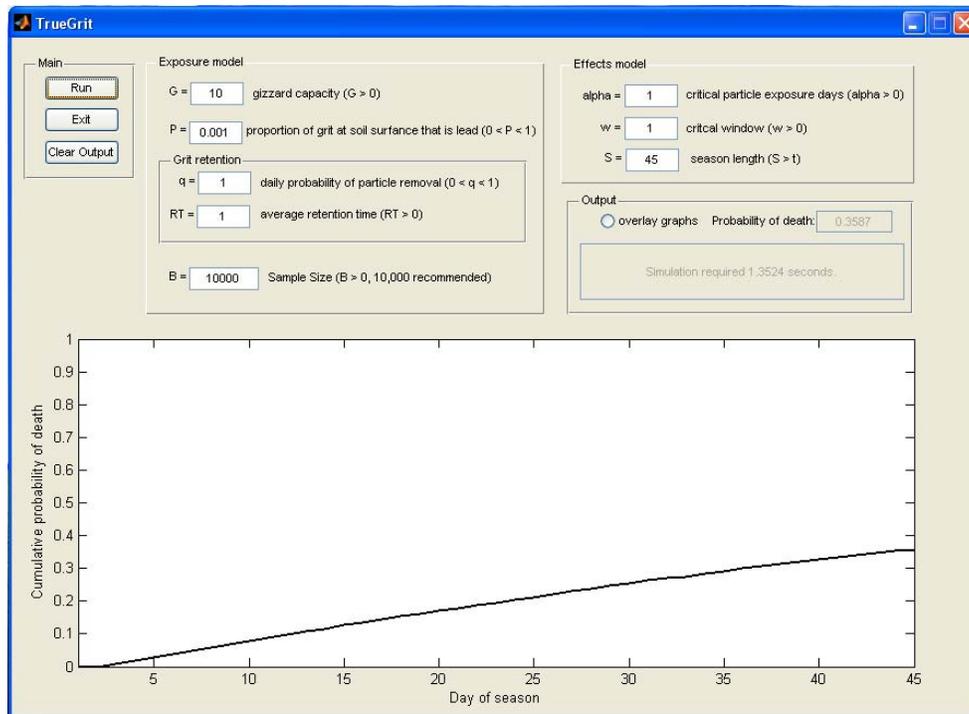


Figure 8. Sample Model Output for Brown-headed Cowbird Using Parameters in Table 6.

## **APPENDIX A. EXAMPLE SPREADSHEET CALCULATIONS USING THE REVISED MODEL FOR ESTIMATING PROBABILITY OF INGESTING LEAD PARTICLES**

The probability of ingesting  $N$  lead particles is calculated for a scenario based on northern bobwhite with an overall average number of grit particles in the gizzard of 49 and a mean grit retention time of 1.4 days. The scenario is based on feeding 100% of the time during a 1-day period on a contaminated site where 1% of the available grit-sized particles is a lead particle.

Under this scenario, there is a 70% probability that no lead particles would be ingested during the 1-day period and a 30% probability of ingesting 1 or more lead particles, including a 25% probability of ingesting 1 lead particle and a 4% probability of ingesting 2 particles.

The spreadsheet model also includes a Factorial Worksheet to explain the factorial calculations, especially for scenarios where the number of grit particles ingested is very high.



**Factorial Worksheet**

The probability mass function includes factorials in a term expressed as  $T!/(N! \times (T - N)!)$

where:

$T$  = the total number of grit-sized particles ingested during the specified exposure period, and

$N$  = the number of lead particles ingested during the specified exposure period.

Excel only calculates factorials up to 170!

For species with high grit ingestion and exposure periods of many days,  $T$  can easily exceed 170.

The formulas for  $N = 0$  through 8 have been simplified because algebraically many of the values in the numerator and denominator cancel.

48

$N$	$T!/(N! \times (T - N)!)$
0	$T!/(0! \times (T - 0)!) = T!/(1 \times T!) = 1$ <span style="float: right;">Note: <math>0! = 1</math></span>
1	$T!/(1! \times (T - 1)!) = T/1 = T$
2	$T!/(2! \times (T - 2)!) = (T \times (T - 1))/(1 \times 2)$
3	$T!/(3! \times (T - 3)!) = (T \times (T - 1) \times (T - 2))/(1 \times 2 \times 3)$
4	$T!/(4! \times (T - 4)!) = (T \times (T - 1) \times (T - 2) \times (T - 3))/(1 \times 2 \times 3 \times 4)$
5	$T!/(5! \times (T - 5)!) = (T \times (T - 1) \times (T - 2) \times (T - 3) \times (T - 4))/(1 \times 2 \times 3 \times 4 \times 5)$
6	$T!/(6! \times (T - 6)!) = (T \times (T - 1) \times (T - 2) \times (T - 3) \times (T - 4) \times (T - 5))/(1 \times 2 \times 3 \times 4 \times 5 \times 6)$
7	$T!/(7! \times (T - 7)!) = (T \times (T - 1) \times (T - 2) \times (T - 3) \times (T - 4) \times (T - 5) \times (T - 6))/(1 \times 2 \times 3 \times 4 \times 5 \times 6 \times 7)$
8	$T!/(8! \times (T - 8)!) = (T \times (T - 1) \times (T - 2) \times (T - 3) \times (T - 4) \times (T - 5) \times (T - 6) \times (T - 7))/(1 \times 2 \times 3 \times 4 \times 5 \times 6 \times 7 \times 8)$

Example if  $T = 20$ :

$N$	$T!/(N! \times (T - N)!)$
0	$20!/(0! \times (20)!) = 1$
1	$20!/(1! \times (19)!) = 20/1 = 20$
2	$20!/(2! \times (18)!) = (20 \times 19)/2 = 190$
3	$20!/(3! \times (17)!) = (20 \times 19 \times 18)/6 = 1140$
4	$20!/(4! \times (16)!) = (20 \times 19 \times 18 \times 17)/24 = 4845$
5	$20!/(5! \times (15)!) = (20 \times 19 \times 18 \times 17 \times 16)/120 = 15504$
6	$20!/(6! \times (14)!) = (20 \times 19 \times 18 \times 17 \times 16 \times 15)/720 = 38760$
7	$20!/(7! \times (13)!) = (20 \times 19 \times 18 \times 17 \times 16 \times 15 \times 14)/5040 = 77520$
8	$20!/(8! \times (12)!) = (20 \times 19 \times 18 \times 17 \times 16 \times 15 \times 14 \times 13)/40320 = 125970$

## APPENDIX B. GRIT RETENTION TIME EXPRESSED AS AN EXPONENTIAL FUNCTION

Trost (1981) and King and Bendell-Young (2000) both described the probability of grit particles being retained in a gizzard as an exponential function. If we assume that an exponential function generally describes grit retention times in birds, we can use observations of the proportion of grit particles retained in the gizzard after specific periods of time to estimate mean and median grit retention times for a species.

For the first example, consider the house sparrows dosed with five silica grit particles and released by Fischer and Best (1995). The sparrows on average voided 50% of ingested particles in the first 24 hours (i.e., 50% probability that the time for a particle to be voided will be between 0 and 24 hours).

Consider the exponential random variable. Let  $P$  be the probability. The parameter  $X$  is the random variable corresponding to a specific elapsed time before a particle that we are interested in is voided (days). Lower case  $x$  represents the many possible choices for  $X$  that are defined by the probability mass function,  $f(x)$ .

The exponential random variable is a continuous random variable defined by the parameter  $\lambda > 0$ .

$$f(x) = \begin{cases} \lambda e^{-\lambda x} & \text{if } x \geq 0 \\ 0 & \text{if } x < 0 \end{cases}$$

Assuming the time to void the particle is distributed as an exponential random variable,

$$\begin{aligned} P \{X \leq a\} &= \int_0^a \lambda e^{-\lambda x} dx \\ &= -e^{-\lambda x} \Big|_0^a \\ &= 1 - e^{-\lambda a} \quad a \geq 0 \end{aligned}$$

Taking the first example of the 50% probability that the time for a particle to be voided will be between 0 and 24 hours (1 day), we can solve for the rate parameter  $\lambda$ .

$$0.5 = P \{X \leq 1\}$$

$$0.5 = \int_0^1 \lambda e^{-\lambda x} dx = -e^{-\lambda x} \Big|_0^1 = 1 - e^{-\lambda}$$

$$e^{-\lambda} = 0.5$$

$$\lambda = 0.693 \text{ day}^{-1}$$

The expectation of the exponential random variable is  $1/\lambda$ . The expectation is the expected value or a weighted value that considers the magnitude of the possible values for the retention time multiplied by the probability that the retention time assumes that value. The expectation can give an idea of what the mean retention time might be. In the case of the sparrow the mean retention time was 1.44 ( $1/0.693$ ) days. The median retention time is equal to  $\ln(2)/\lambda$  or 1.0 day.

For a second example, consider the house sparrows whose grit access was switched from quartz to feldspar (Gionfriddo and Best, 1995). There was a

1. 40% probability that the quartzite grit particle was voided within the first 6 hours.
2. 88% probability that the quartzite grit particle was voided within the first 24 hours.

Solving again for  $\lambda$  for each of the measures of retention time,

$$0.4 = P \{X \leq 0.25\} = \int_0^{0.25} \lambda e^{-\lambda x} dx = 1 - e^{-0.25\lambda}$$

$$0.6 = e^{-0.25\lambda}$$

$$\lambda = 2.04 \text{ day}^{-1}$$

$$0.88 = P \{X \leq 1\} = \int_0^1 \lambda e^{-\lambda x} dx = 1 - e^{-\lambda}$$

$$0.12 = e^{-\lambda}$$

$$\lambda = 2.12 \text{ day}^{-1}$$

The mean retention time measured in the house sparrow study using the quartz/feldspar was about 0.5 days. The differences in the estimates for the two house sparrow studies could reflect the conditions of the experiment or the types of grit used.

Sometimes, when there are multiple observations of the proportion of grit retained at specific time periods, the calculated rate parameters ( $\lambda$ ) differ considerably—suggesting that the exponential function may not appropriately describe the temporal pattern of grit retention over all time periods. This may occur when the probability of particle retention changes because the gizzard selectively retains or passes certain types of particles. An example of this occurred in the house sparrow experiment by Fischer and Best (1995). In addition to reporting that 50% of the silica particles remained after 1 day, the authors observed that 32% of the particles were retained 13 days postdosing in sparrow gizzards.

$$0.32 = P \{X > 13\}$$

$$0.32 = \int_{13}^{\infty} \lambda e^{-\lambda x} dx = -e^{-\lambda x} \Big|_{13}^{\infty} = e^{-13\lambda}$$

$$0.32 = e^{-13\lambda}$$

$$\lambda = 0.0876 \text{ day}^{-1}$$

This observation produces a much lower estimated rate parameter (0.0876 day<sup>-1</sup> vs. 0.693 days<sup>-1</sup>) and, consequently, a higher estimate of mean retention time (11.4 days vs. 1.44 days). Since Fischer and Best (1995) observed the same percentage of silica grit retained

from day 4 to day 13, an exponential function is not a good description of the process of grit retention during this period.

A similar pattern was observed by Fischer and Best (1995) for red-winged blackbirds. In the blackbirds 90% of the dosed silica particles were voided within the first 24 hours, but the remaining 10% of the particles were retained through day 13 postdosing.

$$0.9 = P \{X \leq 1\} = \int_0^1 \lambda e^{-\lambda x} dx = 1 - e^{-\lambda}$$

$$e^{-\lambda} = 0.1$$

$$\lambda = 2.3 \text{ day}^{-1}$$

$$0.1 = P \{X > 13\} = \int_{13}^{\infty} \lambda e^{-\lambda x} dx = e^{-13\lambda}$$

$$0.1 = e^{-13\lambda}$$

$$\lambda = 0.177 \text{ day}^{-1}$$

The two estimates of  $\lambda$  are not very close for the red-winged blackbird, probably reflecting that after an initial period where the dosed silica particles were rapidly voided from blackbird gizzards, a small proportion of the silica particles were selectively retained. Consequently, the exponential function seems to provide a useful description of grit retention rates under conditions when each particle has an equal probability of being voided from the gizzard at any particular time period. However, when the physical characteristics of a particular grit particle (or type of particles) lead to selective retention within the gizzard, the exponential function may underestimate the retention time at certain time periods.

If one assumes that all ingested particles have an equal probability of being retained, including lead particles, an exponential function can be used to estimate the probability that an ingested lead particle is retained for a specific period of time. To do this we define the critical number of days required for a lead particle to be retained to cause mortality of a bird as  $C$ . For a given  $\lambda$  we can estimate the probability that a particle is retained for  $C$  or more days as:

$$P \{X > C\} = \int_C^{\infty} \lambda e^{-\lambda x} dx = e^{-C\lambda}$$

For example, consider that a bird has a mean grit retention time of 1.4 days:

$$\lambda = 1/1.4 \text{ days} = 0.7143 \text{ day}^{-1}$$

Assume  $C$  is 7 days, i.e., it takes 7 days for a particle to be eroded enough to cause harm.

$$P \{X \geq 7\} = \int_7^{\infty} \lambda e^{-\lambda x} dx = e^{-7 \times 0.7143} = 0.0067$$

Consequently, if lead particles have an equal probability of being retained and grit retention time is relative short (i.e., 1.4 days), then there is <1% probability that the lead particle is retained in the gizzard for 7 or more days.

## APPENDIX C. DESCRIPTION OF THE SIMULATION MODEL $Pr(n_{d+1} | n_d)$

Let:

- $n_d$  = number of lead particles in the gizzard at the start of day  $d$ . By convention,  $n_1 = 0$ .
- $y$  = number of particles removed from the gizzard during one time-step (i.e., 1 day).
- $x$  = number of lead particles remaining after  $y$  total particles are removed during one time-step ( $x = n_d - y$ ).
- $z$  = number of lead particles added back to the gizzard during one time-step.
- $G$  = gizzard capacity expressed as the total number of particles in the gizzard at a time.
- $p_i$  =  $Pr(n = i - 1)$ , thus  $p_i$  is the probability of a given value for  $n$ , where  $1 \leq i \leq G + 1$ .
- $N_d$  =  $\{Pr(n_d = i - 1)\}$ , where  $i$  indexes the columns of  $N_d$ . Thus,  $N_d$  is a row-vector with  $G + 1$  columns. By convention,  $N_1$  has a 1 in the first column and zeros elsewhere.

We wish to use information about  $n_d, y, z$  and  $G$  to predict the number of lead particles in the gizzard from one day to the next. In other words, we want a probability model of the form  $P(n_{d+1} | n_d)$  or, equivalently  $N_{d+1} = f(N_d)$ . We make the following assumptions about processes:

1. Birds replenish their full gizzard daily and gizzard capacity is fixed.
2. Birds void particles daily with probability  $q$ . Thus,  $y \sim binomial(G, q)$
3. Toxic particles are equally likely to be voided as nontoxic particles. Thus,  $x \sim hypergeometric(n_d, n_d - y, G)$ .
4. Particles are picked up proportionally to their concentration in the environment. Thus,  $z \sim binomial(y, P)$ , where  $P$  is the proportion of grit-sized particles that are toxic.

To anticipate problems with computer time, we will develop the model in matrix notation to take advantage of MATLAB. In the development below, we condition on  $y$ , then marginalize over its distribution.

First, we need a matrix  $X_y = Pr(x | n_d, y, G)$  that gives the conditional probabilities for having  $x$  lead particles remaining in the gizzard after a day given that  $y$  total particles were removed, of which  $n_d - x$  were lead.

What does  $\mathbf{X}_y$  look like?

1. Its rows correspond to different values for  $n_d$ .
2. Its columns correspond to different values for  $x$ .
3. The cells give the transition probabilities from  $n_d$  to  $x$  conditional on  $G$  and  $y$ .

Therefore, row  $i$  of  $\mathbf{X}_y$  gives the hypergeometric distribution for having  $x = j - 1$  toxic particles remaining in the gizzard conditional on starting with  $n_d = i - 1$  particles. More generally:

$$\mathbf{X}_y = \left\{ Pr(x = j - 1 \mid n_d = i - 1, y, G) = \frac{\binom{i - 1}{i - 1 - x} \binom{G - i + 1}{y - i + j}}{\binom{G}{y}} \right\} \quad (\text{Eq. C1})$$

where

$$Pr(x = j - 1 \mid n_d = i - 1, y, G) \equiv 0 \text{ if } \begin{cases} x > \min(n_d, y, G) \\ x < 0 \end{cases}$$

Now let us define another matrix  $\mathbf{Z}_y = Pr(n_{d+1} \mid x, y, r)$  that gives the conditional probabilities for having  $n_{d+1}$  lead particles in the gizzard after picking up  $y$  particles given that the bird was left with  $x$  particles after the removal step.

What does  $\mathbf{Z}_y$  look like?

1. The rows of  $\mathbf{Z}_y$  correspond to the starting points  $x$ .
2. Columns of  $\mathbf{Z}_y$  correspond to the ending points  $n_{d+1}$ .
3. The cells give the transition probabilities from  $x$  to  $n_{d+1}$  conditional on  $y$ .

Therefore, row  $i$  of  $\mathbf{Z}_y$  gives the binomial distribution for picking up  $i = n_{d+1} - x$  toxic particles out of  $y$  particles picked up. More generally:

$$\mathbf{Z}_y = \left\{ Pr \left( n_{d+1} = j-1 \mid x = i-1, y, P \right) = \binom{y}{j-1} P^{(j-i)} (1-P)^{(y-j+i)} \right\} \text{ (Eq. C2)}$$

where

$$Pr \left( n_{d+1} = j-1 \mid x = i-1, y, P \right) \equiv 0 \text{ if } \begin{cases} n_{d+1} > y+x \\ n_{d+1} < x \end{cases}$$

Finally, we marginalize over  $y$ :

$$Pr \left( n_{d+1} \mid n_d \right) = \mathbf{M} = \sum_y \mathbf{X}_y \mathbf{Z}_y Pr \left( y \mid G, q \right) \text{ (Eq. C3)}$$

To estimate the number of lead particles in the gizzard on an arbitrary day  $d > 1$ , we can use powers of  $\mathbf{M}$ :

$$Pr \left( n_d \mid n_1 \right) = \mathbf{M}^d \text{ (Eq. C4)}$$

### *Particle-Exposure-Days*

The model (Eq. C4) alone gives only the daily probability of uptake, elimination and retention of lead particles. It does not attempt to estimate the probability of death associated with a given time-series of gizzard contents. However, we can use  $\mathbf{M}$  (Eq. C4) to estimate the probability that a bird ingests a fatal dose of toxic particles, given some information on what constitutes a lethal exposure. Below we develop a simulation model of fatality due to ingested particles.

The model uses  $\mathbf{M}$ , together with a random number generator, to simulate the progress of birds picking up and ejecting lead particles over the course of a season. To do so, we define an exposure day to have occurred when a single particle resides in the gizzard for a day. The basic strategy of the simulation model is to track the total number of exposure days that occur within a fixed period of time. All parameters for the model are as previously defined, except:

1.  $(\alpha, w)$  = ordered pair representing a fatal dose of toxic particles. Thus a bird will die if it experiences at least  $\alpha$  exposure days within a period of  $w$  days.
2.  $S$  = the length of time that birds use the habitat.
3.  $B$  = the number of birds to simulate.

Assuming that birds enter the habitat on day 1 with no lead particles in their gizzard, the matrix  $M$  is used to generate a time series of particle counts in the gizzard over the full  $S$ -day season. As the simulation proceeds, the cumulative number of exposure days (in the previous  $w$  days) is tracked, and simulated birds are assumed killed if their exposure exceeds  $(\alpha, w)$ .