USDA
United States Department of Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

Science, Technology,
and Analysis Services

Center for
Epidemiology and
Animal Health

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An Assessment of the Risk Associated with the Movement of Eggshells and Inedible Egg Product Into, Within, and Out of a Control Area During a Highly Pathogenic Avian Influenza Outbreak

March 2014 FINAL REVIEW AND CLEARANCE

Collaboration between the Egg Sector Working Group, the University of Minnesota's Center for Animal Health and Food Safety, and USDA:APHIS:VS:CEAH

Veterinary Services
Safeguarding Animal Health

UNIVERSITY OF MINNESOTA
CENTER FOR ANIMAL HEALTH
AND FOOD SAFETY

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Suggested bibliographic citation for this report:

Timothy Goldsmith, Carie Alexander, Christina Der, Susan Gale, David Halvorson, Shauna Voss, Todd Weaver, Sasidhar Malladi. **An Assessment of the Risk Associated with the Movement of Eggshells and Inedible Egg Product Into, Within, and Out of a Control Area During a Highly Pathogenic Avian Influenza Outbreak.** Collaborative agreement between USDA:APHIS:VS:CEAH and University of Minnesota Center for Animal Health and Food Safety, Fort Collins, CO. March 2014. 132 pgs. Retrieved from the University of Minnesota Digital Conservancy: https://hdl.handle.net/11299/176192

This document was developed through the Continuity of Business / Secure Food Supply Plans / Secure Poultry Supply project initiative. Related documents can be found at: https://www.aphis.usda.gov - search for "Continuity of Business”

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Acknowledgement:

We thank the Egg Sector Working Group and the United Egg Association for their support during the development of this risk assessment.

Keywords: avian influenza, continuity of business, HPAI, market, outbreak, risk, egg, chicken, poultry

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Contents

1. Abbreviations and Definitions

APHIS	Animal and Plant Health Inspection Service (USDA)
AI	Avian influenza
AMS	Agricultural Marketing Service
APPI	Animal Protein Products Industry
CEAH	Centers for Epidemiology and Animal Health
CFR	Code of Federal Regulations
C&D	Cleaning and Disinfection
EPA	Environmental Protection Agency
GMP	Good Manufacturing Practice
FSIS	Food Safety Inspection Service
HACCP	Hazard Analysis and Critical Control Point
HPAI	Highly pathogenic avian influenza
INEP	Inedible egg product
LPAI	Low pathogenic avian influenza
NA	Neuraminidase
NAHEMS	National Animal Health Emergency Management System
NPIP	National Poultry Improvement Plan
OIE	World Organization for Animal Health (formerly Office International des Epizooties)
P.I.	Probability interval
PPE	Personnel protective equipment
RRT-PCR	Real-time reverse transcription polymerase chain reaction
SES Plan	Secure Egg Supply Plan
U.S.	United States of America
USDA	United States Department of Agriculture
VS	Veterinary Services (USDA:APHIS:VS)

AERSCREEN

EPA's newest screening-level air quality model based on AERMOD.

AERMOD

A steady-state plume model that incorporates air dispersion based on planetary boundary layer turbulence structure and scaling concepts, including treatment of both surface and elevated sources, and both simple and complex terrain.

Buffer surveillance zone

The zone immediately surrounding the infected zone. The buffer surveillance zone and the infected zone comprise the control area.

Check

Check means an egg that has a broken shell or crack in the shell but has its shell membranes intact and its contents are not leaking (7CFR part 57.1, 9CFR950.5).

Continuous inspection

Continuous inspection requires the FSIS inspector to be on the premises of the egg products processing facility whenever pasteurization or egg-breaking equipment is operating.

Control area

A control area, consisting of an infected zone and a buffer surveillance zone, will be established to ensure the rapid and effective containment of the disease. The potential modes of transmission of HPAI is considered when determining the minimum size and shape of a control area. Movement control—through the use of permits—should be maintained until the disease is eradicated.

C&D Guidelines

These model procedures and guidelines have originally been proposed by the U.S. egg industry to support the permitted movement of egg industry products from monitored flocks. The procedures demonstrate how minimum biosecurity requirements can be met. However, to provide flexibility, individual companies or locations may adapt equivalent procedures to fit their particular needs while still meeting or exceeding the minimum criteria.

Dirty egg

Dirty egg or “dirties” are egg(s) that have an unbroken shell with adhering dirt or foreign material (7CFR part 57.1).

D_t-value

The time required to reduce a virus or bacterial concentration by 90 percent or 10^1 EID_{50}.

Dry eggshells

Eggshells dried in specialized equipment such as a rotary or belt dryer to a moisture content of approximately 4 percent.

Egg

The shell-egg of the domesticated chicken.

EID_{50}

50 percent egg infectious dose. One EID_{50} unit is the amount of virus that will infect 50 percent of inoculated eggs.

Inedible egg

Inedible means eggs of the following descriptions: black rots, yellow rots, white rots, mixed rots, sour eggs, eggs with green whites, eggs with stuck yolks, moldy eggs, musty eggs, eggs showing blood rings, and eggs containing embryo chicks (at or beyond the blood ring stage) (7CFR part 57.1, 9CFR950.5).

Inedible egg product (INEP)

Dried, frozen, or liquid egg products that are unfit for human consumption. Inedible egg products may be generated from: inedible and loss eggs including black rots, white rots, mixed rots, green whites, eggs with diffused blood in the albumen or on the yolk, crusted yolks, stuck yolks, developed embryos at or beyond the blood ring state, moldy eggs, sour eggs, any eggs that are adulterated and any other filthy and decomposed eggs. Inedible eggs include the following:

- Any egg with visible foreign matter other than removable blood and meat spots in the egg meat.
- Any egg with a portion of the shell and shell membranes missing and with egg meat adhering to, or in contact with, the outside of the shell.
- Any egg with dirt or foreign material adhering to the shell and with cracks in the shell and shell membranes.
- Liquid egg recovered from shell egg containers and leaker trays.
- Open leakers made in the washing operation.
- Any egg which shows evidence that the contents are, or have been, exuding prior to transfer from the case.

Infected zone

In an outbreak of HPAI, the infected zone will encompass the perimeter of all presumptive or confirmed positive premises (“infected premises”) and include as many “contact premises” as the situation requires logistically or epidemiologically. Activities in an infected zone include:

- Preventing products from birds and other susceptible animals from leaving the zone unless a risk assessment determines that such movement can be permitted.
- Preventing movement of vehicles, equipment, and non-susceptible animals out of the zone unless appropriate biosecurity procedures (as determined by a risk assessment) are followed.

Inline egg-breaking facility

A facility where shell-eggs for breaking are received from layer flocks present on the premises.

Batch

For the purposes of this risk assessment, we defined a batch of INEP or wet eggshells to be the quantity of INEP or wet eggshells produced by processing eggs from a single layer hen flock and included in the same truckload that is moved from the premises.

For wet eggshells from an inline facility, which are moved daily, the batch size is 1144.5 lb. This is the average estimated quantity of wet eggshells generated with breaking and processing of eggs laid by a 100, 000 hen table-egg layer flock in a day.

For inedible egg product from an inline facility that is moved twice per week, the batch size is 2654 lb. This is the average estimated quantity of inedible egg generated via breaking and processing of eggs laid by a 100,000 hen table-egg layer flock in 3.5 days. For inedible egg product moved once per week the corresponding batch size is 5308 lb.

Loss

An egg that is unfit for human food because it:

- is smashed or broken so that its contents are leaking,
- is overheated, frozen, or contaminated,
- is an incubator reject,
- contains a bloody white, large meat or blood spot, a large quantity of blood, or other foreign material (7CFR part 57.1, 9CFR950.5)

Movement permit

A VS Form 1-27, a State-issued permit, or a letter—customized to the applicant's situation—generated by the Permit Team and issued at the discretion of Incident Command to allow the movement of egg industry products from a premises or a geographic area described in a quarantine order.

Nest run egg

Eggs that have been packed, as they come from the production facilities, without having been washed, sized, and/or candled for quality—with the exception that some checks, dirties, or obvious under-grades may have been removed.

Restricted egg

Restricted egg means any check, dirty egg, incubator reject, inedible, leaker, or loss (7CFR part 57.1).

Standalone egg-breaking facility

Facilities that do not have live poultry on the premises and receive eggs from off-line farms.

Truck load

The quantity of wet eggshells and inedible egg product present in a single vehicle used for moving these by products (i.e. one or more batches).

Virus concentration

In this risk assessment, we use the infectivity with respect to chicken embryos as a proxy for the physical quantity of virus. Specifically, virus concentration is quantified in units of EID_{50} per gram (or EID_{50} per ml; or EID_{50}/cm^2) representing amount of HPAI virus infectivity in a substrate.

Wet eggshells	Eggshells that have undergone centrifugation or screening to remove adhering liquid inedible egg product, reducing the moisture level to about 16 percent. Wet eggshells have not undergone a thermal drying process.
Working face	Area of an open landfill cell where waste is dumped, compacted, and then covered with an appropriate material at the end of each operational day.
Z-value	Temperature increase necessary to reduce the D_t value by one log cycle.

2. Executive Summary

In the event of a highly pathogenic avian influenza (HPAI) outbreak in the U.S. poultry industry, local, State, and Federal authorities will implement a foreign animal disease emergency response. In these circumstances, permit requests to move poultry and poultry products must be supported by risk assessments, which demonstrate that the risk of HPAI spread associated with the movement of the product is acceptable. Performing the risk assessments prior to a HPAI outbreak can enhance emergency response and facilitate timely movement permitting decisions during an outbreak. Egg-breaking and egg-processing plants have limited holding capacity for eggshells (1 to 2 days) and liquid inedible egg product (INEP) (1 week). Consequently, extended movement restrictions may disrupt the operation of these facilities requiring expensive alternate disposal options. This risk assessment assesses the risk that the movement of eggshells and INEP from an egg-breaking plant that sources eggs from flocks located in a control area during an outbreak in the United States will result in HPAI spread to susceptible poultry.

Eggshells and INEP are generated as byproducts of egg-breaking, processing, and grading operations in the egg industry. The typical use or disposal options for eggshells include land application as a soil amendment, incorporation into poultry feed after drying, or disposal in a landfill. INEP is a valuable protein source and may be pasteurized and incorporated into pet food, furbearing animal feeds, or starter pig rations. The specific movements of eggshells and INEP addressed in this assessment are as follows:

- Movement of eggshells from an egg-breaking plant to:
    - A standalone egg-processing plant with a drier where wet eggshells are dried for incorporation into poultry feed
    - An agricultural land parcel for application as a soil amendment
    - A municipal solid waste landfill for disposal
    - A feed mill after drying
- Movement of liquid INEP from breaking plants to:
    - A pasteurization facility
    - A municipal solid waste landfill for disposal

This risk assessment is a joint effort between the table egg-layer industry, the University of Minnesota’s Center for Animal Health and Food Safety, and the United States Department of Agriculture (USDA) to support permits for moving eggshells and INEP during a HPAI outbreak. This assessment is applicable to egg-breaking and processing plants that participate in the USDA:APHIS:VS Secure Egg Supply Plan (SES Plan) in the event of a HPAI outbreak. The SES Plan provides science-based guidelines for permitting the movement of egg-industry products from operations in a HPAI Control Area.

This risk assessment identifies pathways of HPAI transmission associated with the movement of eggshells and INEP and assesses the risk of HPAI spread to susceptible poultry through these pathways despite implementation of all current preventive measures as well as future outbreak specific risk mitigation measures. The future outbreak specific risk-mitigation measures include those from the May 2012 Draft SES Plan as well as measures proposed by the egg-sector working group members for inclusion in the SES Plan. The following categories of outbreak specific risk-mitigation measures were considered in the evaluation:

- Active surveillance of egg-layer flocks in the control area as specified in the SES Plan.
- Vehicle cleaning and disinfection (C&D) and driver biosecurity measures.
- Process requirements for drying wet eggshells.
- Guidelines for land application of wet eggshells.
- Pasteurization of liquid INEP according to USDA requirements for whole egg or whole egg blends intended for human consumption as described in 9CFR590.570.

The risk evaluation was performed in two parts: an entry assessment and an exposure assessment. The entry assessment section evaluates the likelihood of HPAI virus being transmitted onto a susceptible poultry premises via contaminated eggshells, INEP, or vehicle or driver transporting product. Simulation models of within flock disease transmission—active surveillance protocols (SES Plan)—were used to estimate the likelihood of HPAI virus being present in eggshells and INEP moved from a breaking facility. The impact of the drying process on inactivating HPAI virus was considered for evaluating the likelihood of entry via dry eggshells. The key results from the entry assessment section are:

- Only a small fraction of wet eggshells, moved from a breaking plant that receives eggs from an HPAI infected but undetected flock in the control area, is expected to be contaminated (7.6×10^{-5}, 90% P.I., 0- 2.4×10^{-4}) when dilution of the virus concentration – due to mixing of contaminated and virus free eggshells –is minimal.
- The mean HPAI virus concentration of liquid INEP moved from a breaking facility that receives eggs from flocks in the control area is estimated to be less than 1.5 EID_{50}/ml.
- The likelihood of HPAI contaminated dry eggshells being moved from an inline breaking facility is *negligible.*

The exposure assessment section evaluates the likelihood of susceptible poultry being exposed to HPAI virus associated with the movement of eggshells and INEP. Exposure pathways involving aerosol, fly, and wild mammal transmission were considered for evaluating the risk associated with the movement of wet eggshells to a land application site. The risk evaluation for the movement of wet eggshells to another processing facility for drying, considered pathways for cross-contamination of finished product, vehicles, and drivers transporting dry eggshells. Finally, pasteurization according to USDA FSIS standards for whole egg or whole-egg blends was considered for evaluating the risk associated with the movement of liquid INEP to a pasteurization facility. Evaluating the component risks associated with movement of eggshells or INEP to specific end-use or disposal locations gave the following results:

- The risk of movement of wet eggshells to an agricultural land application site resulting in HPAI spread to susceptible poultry is *negligible.*
- The risk of movement of dry eggshells to a poultry feed mill resulting in HPAI spread to susceptible poultry is *negligible.*
- The risk of the movement of wet eggshells to a standalone egg breaking plant for drying resulting in HPAI spread to a susceptible poultry flock is *low.*
- The risk of the movement of wet eggshells or liquid INEP for disposal in a landfill resulting in HPAI spread to a susceptible poultry flock is *negligible.*

- The risk of the movement of liquid INEP in tankers to a pasteurization facility resulting in HPAI spread to susceptible poultry is *negligible.*
- The risk of the movement of liquid INEP in carboys to a pasteurization facility resulting in HPAI spread to susceptible poultry is *low.*

This document is an evolving product-specific risk assessment that will be reviewed and updated as necessary (before and during an outbreak) to incorporate the latest scientific information and preventive measures. If the Incident Command System is activated in response to a HPAI outbreak, APHIS (and Incident Command staff) will review this risk assessment with respect to the situation in order to assess industry requests for movement of eggshells or INEP.

Overall Finding and Conclusion

The risk that movement of eggshells and INEP into, within, and outside of a control area during a HPAI outbreak results in the infection of susceptible poultry is *negligible* to *low* if the applicable outbreak specific risk mitigation measures from the SES Plan—and the measures proposed by the egg-sector working group evaluated in this risk assessment—are strictly followed.

3. Background

In the event of a HPAI outbreak in the U.S. poultry industry, local, State, and Federal authorities will implement a foreign animal disease emergency response. This response consists of a control and eradication strategy that will utilize depopulation, quarantine, and movement control measures to prevent further spread of HPAI virus. State and/or Federal authorities will also issue official permits to allow movement of live poultry and their products from premises identified in a quarantine order during an outbreak. A request for a movement permit must be supported by a risk assessment (or some scientifically based, logical argument) to demonstrate that the risk of HPAI spread associated with the movement of the product in question is acceptable. Completing these types of risk assessments, in a timely manner, during an outbreak can be challenging.

Eggshells and INEP are byproducts of egg-breaking, processing, and grading operations in the egg industry. The typical use or disposal options for wet eggshells include land application as a soil amendment, or incorporation into poultry feed after drying, or disposal in a landfill. INEP is a valuable protein source and may be pasteurized and incorporated into pet food, furbearing animal feeds, or starter-pig rations. The available storage capacity for holding byproducts from egg-breaking operations during an outbreak, while a risk assessment is being completed, may not be adequate. Breaking plants typically do not have the capacity to hold wet eggshells for more than a day, or inedible liquid for more than a few days (a week at most), and may have to divert this material to a landfill for disposal if a longer holding time is required. Proactive risk assessment identifies areas of risk and incorporates risk mitigation steps proposed by internal and external stakeholders that minimize the spread of HPAI virus to susceptible poultry. Evaluating risk before an outbreak occurs facilitates timely emergency response and movement permitting decisions, and promotes continuity of the food supply.

In previous risk assessments, discrete event simulation models of disease transmission, and active surveillance protocols, were developed for use in the series of proactive assessments for the risk of HPAI spread via movement of egg industry products. We used these simulation models to evaluate the likelihood of HPAI contaminated wet eggshells and INEP being moved from a breaking plant, sourcing eggs from a HPAI infected but undetected flock. The SES Plan identifies product-specific biosecurity protocols and C&D guidelines for the movement of various types of eggs, egg-industry products, and egg-handling materials during a HPAI outbreak. The plan also specifies an active surveillance protocol to be implemented by industry in conjunction with APHIS during an outbreak, as well as a two-day on-farm holding time prior to the movement of nest-run or shell-eggs from an offline farm to the egg-breaking plant. The active surveillance protocol—in conjunction with the two-day time interval before the movement of eggs—reduces the likelihood of moving contaminated eggs before HPAI infection is detected. We note that a similar holding time would not apply for wet eggshells generated at an inline breaking facility because there is no practical way to hold eggs before breaking.

This risk assessment does not guarantee that movement will be permitted during a HPAI outbreak. Rather, it provides the framework necessary for decision makers to assess the effectiveness of preventive measures as they pertain specifically to the handling and movement of eggshells and INEP.

4. Scope

4.1 Facilities Covered Under this Risk Assessment

This risk assessment is applicable to egg-breaking and egg-processing premises that generate wet and dry eggshells and inedible egg product and are under continuous inspection by FSIS as specified in 9CFR590 and abide by the USDA:APHIS:VS Secure Egg Supply Plan in the event of a HPAI outbreak.

4.2 Types of Movements Addressed Under this Risk Assessment

This risk assessment addresses the following movements into, within, and out of the control area during a HPAI outbreak:

a) Movement of wet eggshells from an egg-breaking plant to:
  - A standalone egg-processing plant with a drier, where wet eggshells are dried for incorporation into poultry feed
  - An agricultural land parcel for application as a soil amendment
  - A municipal solid waste landfill for disposal

b) Movement of dry eggshells to a feed mill

c) Movement of liquid INEP from breaking plants to:
  - A pasteurization facility
  - A municipal solid waste landfill for disposal

5. Overview of Data Analysis Approaches

5.1 Risk Assessment Overview

The assessment follows the general qualitative risk assessment principles recommended by the OIE import risk analysis guidelines. However, the risk assessment organization has been modified from that proposed in the OIE import risk analysis handbook as appropriate for the movement of eggshells and inedible egg product [2]. The risk assessment is comprised of hazard identification and two evaluation steps: (1) entry assessment (entry of HPAI virus onto a susceptible poultry premises through the movement of a commodity); and (2) exposure assessment (exposure of susceptible animals). If the entry assessment demonstrates a negligible likelihood of the commodity, associated vehicle, driver or handling materials being contaminated with HPAI virus, the risk assessment may be concluded. However, if the likelihood is estimated to be greater than *negligible*, the next step in the risk assessment is the exposure assessment, which would assess the likelihood that susceptible poultry will be infected by HPAI virus through the commodity in question.

The assessment utilizes a qualitative evaluation approach where the likelihoods of individual events in the pathway were rated according to a qualitative scale (see **Table 1**). The qualitative ratings for the events in the pathway were determined using multiple data sources and evaluation approaches such as literature review, expert opinion, quantitative simulation model predictions, and past outbreak experiences. The quantitative simulation model results from previously completed proactive risk assessments were used to estimate the number of HPAI contaminated eggs produced from potentially infected, but undetected table-egg layer flocks supplying eggs to a breaking facility. Steady state aerosol dispersion models recommended by the EPA were utilized to partly inform the risk of aerosol spread associated with land application of wet eggshells along with other approaches. The likelihood for main steps in each pathway was assessed and categorized using the descriptive scale described in **Table 1**.

5.2 Likelihood and Risk Ratings

The likelihood for main steps in each pathway was assessed and categorized using the descriptive scale in **Table 1**.

Table 1. Descriptive scale used to estimate the likelihood for an event to occur.

Likelihood Rating	Description
Extremely High	The event is almost certain to occur
High	There is more than an even chance that the event will occur
Moderate	The event is unlikely but does occur
Low	It is very unlikely that the event will occur
Negligible	The likelihood that the event will occur is insignificant, not worth considering

The risk estimate in each pathway in the exposure assessment was determined by combining the likelihoods of the individual events. For determining the overall risk rating for pathways involving a chain of events which all have to occur for the pathway to be completed, relatively more weight was given to events with the lowest likelihood in the chain. The risk rating scale used in this assessment is provided below.

> Negligible risk: The spread of HPAI infection to susceptible poultry through the risk pathway is insignificant or not worth considering.
>
> Low risk: The spread of HPAI infection to susceptible poultry through the risk pathway is very unlikely.
>
> Moderate Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is unlikely to but does occur.
>
> High Risk: There is more than an even chance that the spread of HPAI infection to susceptible poultry through the risk pathway will occur.
>
> Extremely High Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is almost certain to occur.

5.3 Uncertainty Estimation

The uncertainty of the likelihood/risk estimation was assessed by using a range defined by the terms in the descriptive rating scale provided in **Table 1**. For example, a risk estimate of *negligible* to *low* encloses the true risk, which is not deterministically known, where the interval between the two ratings represents the uncertainty in the analysis.

6. Key Assumptions Used in the Risk Assessment

This assessment is proactive in nature and cannot address the specific circumstances surrounding an outbreak in detail. Therefore, we are making some assumptions to establish context and applicability. These assumptions are:

a) That a HPAI outbreak has been detected, APHIS is implementing the HPAI Response Plan; and, some degree of planning has taken place at other levels. The APHIS HPAI Response Plan is intended to complement regional, State, and industry plans and APHIS recommends their continued development.

b) Every table-egg layer flock (birds in one house) from which eggs are sourced for the breaking operations has HPAI infection but has not yet been detected. Given that it is unlikely for all layer flocks within a Control Area to be infected with HPAI virus, this is a conservative assumption which over estimates the risk. If there were absolute certainty that HPAI infection was absent, there would be no risk. If HPAI infection has been detected, it is assumed that Incident Command would shut down the premises and movement of eggs for breaking, as well as eggshells and INEP, would not be allowed.

c) That all relevant preventive measures from the SES Plan, and associated C&D guidelines, are strictly followed. The assessment does not evaluate the risk that the preventive measures in the SES Plan are incorrectly implemented—either intentionally or unintentionally.

d) The adverse consequences of spread of HPAI virus to a different premises housing susceptible poultry were assumed to be extremely high. Given this assumption, a consequence assessment was not included in the risk estimation.

e) Because the adverse consequences of HPAI virus spread are assumed to be very high, the risk rating was determined based on the likelihood of HPAI spread to the destination premises and the consequences of the event were not evaluated.

f) Side loading is the practice of processing eggs from an offline layer flock, at an inline egg-processing facility, with poultry on the premises. In the event of a HPAI outbreak, this practice is prohibited in the SES Plan and is outside the scope of the assessment. Similarly, this assessment does not include the processing of wet eggshells at another inline egg processing facility.

g) The HPAI virus strain causes clinical infection and increased mortality in infected chickens. The risk assessment may not apply to strains that do not cause clinical signs representative of HPAI infection (i.e. are classified as highly pathogenic on a molecular basis only). For such strains, this risk assessment would have to be revised to reflect the biological characteristics of the virus.

7. Hazard Identification - HPAI Overview

Hazard identification consists of listing the pathogenic agents associated with the species from which a commodity is derived and whether the agents can be classified as hazards for further consideration in the risk assessment[2]. For movement of eggshells and INEP, the pathogenic agent of concern in this assessment is HPAI virus. Properties of HPAI viruses including environmental persistence, transmission characteristics, and physical and chemical inactivation have been extensively reviewed in comprehensive texts[3]. This section is a brief summary of the key properties of HPAI viruses from published literature and expert opinion, with emphasis on the variability between HPAI strains and transmission characteristics in chickens.

7.1 Agent and Host Range

Avian influenza viruses are negative sense, segmented, ribonucleic acid viruses of the family *Orthomyxoviridae*. The *Orthomyxoviridae* family includes several segmented viruses including the Type A, B and C influenza viruses. The Type A influenza viruses, which include all AI viruses, can infect a wide variety of animals including wild ducks, chickens, turkeys, pigs, horses, mink, seals and humans. The type B and C viruses primarily infect only humans and occasionally pigs [3, 4] .

Two surface glycoproteins of the influenza A virus, hemagglutinin (HA) and neuraminidase (NA), are the most important antigenic sites for the production of protective immunity in the host; however, these proteins also have the greatest variation. There are sixteen different subtypes of HA (H1 to H16), nine different subtypes of NA (N1 to N9) and 144 different HA:NA combinations. [3] Although relatively few of the 144 subtype combinations have been isolated from mammalian species, all subtypes, in the majority of combinations, have been isolated from avian species.

7.1.1 Definition of Highly Pathogenic Notifiable Avian Influenza

For the purpose of disease control programs and international trade in domestic poultry products, HPAI is defined in the Code of Federal Regulations, Title 9, Section 53.1 as:

> a) Any influenza virus that kills at least 75 percent of eight, 4- to 6-week-old susceptible chickens, within ten days following intravenous inoculation with 0.2 ml of a 1:10 dilution of a bacteria-free, infectious allantoic fluid.
>
> b) Any H5 or H7 virus that does not meet the criteria in paragraph (a) of this definition, but has an amino acid sequence at the hemagglutinin cleavage site that is compatible with HPAI viruses.
>
> c) Any influenza virus that is not a H5 or H7 subtype and that kills one to five chickens and grows in cell culture in the absence of trypsin.

All H5 or H7 isolates of both low and high pathogenicity and all HPAI isolates regardless of subtype are reportable to State and national veterinary authorities and to the OIE. [5] Although other LPAI viruses may cause considerable morbidity and production losses, they are not reportable diseases to the OIE (but may be reportable in some States).

7.1.2 Host Range

Wild waterbirds are considered the natural reservoirs of low pathogenic avian influenza (LPAI) viruses, but most highly pathogenic avian influenza (HPAI) viruses responsible for high mortality in domestic birds do not have recognized wild bird reservoirs[6].

The phrase 'highly pathogenic for chickens' does not indicate or imply that the AI virus strain is highly pathogenic (HP) for other bird species, especially wild ducks or geese (Anseriformes). However, if a virus is highly pathogenic for chickens, the virus will usually be HP for other birds within the order Galliformes, family Phasianidae, such as turkeys and Japanese quail. To date, most HPAI viruses for chickens are generally non-pathogenic for ducks and geese in experimental studies [4]. However, lethality of HPAI viruses in ducks has changed with the re-emergence of H5N1 HPAI viruses in Hong-Kong in 2002, as some strains have become highly lethal in some naturally and experimentally infected ducks.[6]

HPAI strains are known to emerge in poultry after the introduction of LPAI viruses from wild birds, and after circulation of virus for varying lengths of time in domestic poultry [7]. Recent identification of a H5N2 virus with a HPAI genotype, with evidence of non-lethal infection in wild waterfowl, and without evidence of prior extensive circulation in domestic poultry, suggests that some AI strains with a potential high pathogenicity for poultry could be maintained in a wild waterfowl community prior to introduction[6].

Host adaptation is a key determinant in the ability to maintain transmission of a HPAI virus within domestic poultry. Once adapted to gallinaceous poultry, HPAI viruses are unlikely to return back to circulate among wild birds because they are adapted to poultry.[8] However, the emergence of Asian HPAI H5N1 strains have led to increased uncertainty regarding the role of wild birds as reservoirs in the maintenance of HPAI viruses in nature [9]. Prior to the outbreak of HPAI H5N1 virus in Europe, Asia, and Africa, HPAI viruses had only rarely been isolated from wild birds, usually associated with outbreaks in domestic poultry with one exception.[10] An outbreak of HPAI H5N3 in South Africa in 1961 was observed in a population of terns. Asian HPAI H5N1 strains; however, have been isolated from multiple species of wild birds[11]. Both these H5N3 and H5N1 HPAI viruses were isolated in sick, moribund or dead wild birds. Despite extensive global wildlife surveillance efforts, infection with H5N1 HPAI viruses was not detected in healthy wild birds, except for a few isolated cases. Therefore, the significance of wild birds as a source of infection and their influence on the epidemiology of H5N1 HPAI viruses is yet to be fully established [6].

Experimental studies have shown that various LPAI viruses can replicate in pigs, ferrets, and cats to levels comparable to human influenza viruses [12]. An outbreak of an avian-derived H10N4 within several mink (Mustela *vison*) farms in southern Sweden caused 100% mortality in these mammals, resulting in approximately 3000 deaths [13]. A survey of wild raccoons in the United States found a prevalence of 2.4 percent for AI antibodies [14]. Asian HPAI H5N1 strains have a wider host range and have been isolated from up to 176 species including wild birds and mammals [15].

7.2 Geographic Distribution of H5N1 HPAI

- The most current H5N1 HPAI global overview and worldwide situation report can be viewed at www.fao.org.
- The current list of all confirmed affected countries with H5 or H7 infection in animals is maintained by the OIE at www.oie.int.

7.3 Virus Shedding

HPAI viruses have been isolated from respiratory secretions, feces, and feathers, as well as the eggshell surface, albumen, yolk and meat from infected poultry. Estimates of HPAI virus fecal concentrations in chicken feces mostly ranged between 10^3 to10^7 EID_{50}/gram although concentrations as high as 10^9 EID_{50}/gram have been observed in some cases[16-18].

H5N2 HPAI viruses have been isolated from the eggshell surface, yolk and albumen of eggs laid by experimentally inoculated hens[19]. In experimental studies, H5N2 HPAI viruses were not recovered from eggs laid on the first day post inoculation of hens. This may have been due to the developing egg being protected from exposure in the shell gland (uterus) during the later stages of eggshell formation (about 15 hours), in combination with the latently infected period of at least 6 hours in individual birds in this study. In contrast, HPAI virus was recovered from the yolk and albumen of eggs forming in the oviduct of dead chickens at postmortem, 35 to 37 hours after being experimentally infected with a HPAI virus strain isolated from chickens [20]. Italian HPAI H7N1 viruses have also been isolated from eggs laid by infected hens [21].

In an experimental study, the concentration of H5N2 HPAI virus ranged from 0.97 to $10^{5.9}$ EID_{50}/eggshell and from 0.97 to $10^{6.1}$ EID_{50}/ml in albumin and from 0.93 to $10^{4.8}$ EID_{50}/ml in yolk of eggs laid by infected hens [19].

7.4 Chemical and Physical Inactivation

AI viruses are inactivated by physical factors such as heat, extremes of pH, hyper-isotonic conditions, and dryness; however, their infectivity can be maintained for several weeks under moist, low temperature conditions.

Due to their lipid envelope, AI viruses are relatively sensitive to disinfection agents and inactivation by lipid solvents such as detergents. The Environmental Protection Agency (EPA) maintains a list of disinfectants with label claims for avian influenza viruses. These products include halogens, aldehydes, quaternary ammoniums, phenols, alcohols, peroxides and some detergents [22-24]. To ensure effective disinfection, appropriate operational conditions as recommended by the manufacturer have to be maintained. Operational conditions such as disinfectant concentration, temperature, contact time, pH and organic load may impact the degree of inactivation.

7.5 Persistence of HPAI in Manure and Other Media

Persistence of AI viruses in the environment in different media is summarized in **Table 2**. The HPAI virus shed by infected birds may be protected environmentally by accompanying organic material that shields the virus particles from physical and chemical inactivation. Specific environmental conditions such as cool and moist conditions increase survival times in organic media and on surfaces. For example, H5N2 HPAI viruses have remained viable in liquid poultry manure for 105 days in winter under freezing conditions, 30-35 days at 4º C, and seven days at 20º C, and H5N1 HPAI for four days at 25 to 32 ºC when kept out of direct sunlight.

Table 2. Persistence of avian influenza viruses in the environment in different media under various environmental conditions.

Virus strain	Media	Conditions	Survival duration and temperature	Reference
Duck influenza viruses (H7N2 and H3N6)	Untreated Mississippi river water	Un- chlorinated water	4 days 22 º C; 20 days 0 º C	Webster *et. al.* (1978) [25]
	Duck feces	Relatively high humidity (sealed in a vial)	30 days 4 º C; 7 days 20 º C	
Pennsylvania HPAI H5N2	Wet manure	In a barn (winter under freezing conditions)	105 days after depopulation	Fichtner *et. al.* (1987) [26]
2 Clades of HPAI H5N1	Duck feathers	Relatively high humidity (sealed in a vial)	160 days 4 º C; 15 days 20 º C; Titers of $10^{4.3}$ EID_{50}/ml for 120 days at 4 C	Yamamoto *et. al.* (2010) [27]
	Drinking water (commercial mineral water)	Collected at 3 days post infection and stored at 4 C or 20 C	Inconsistently isolated from water stored at 4 º C over a 30 day period; no virus isolated from drinking water at 20 C after 3 days	
H7N2 LPAI	10^{7-8} EID_{50} mixed with chicken manure from 3 sources	2 sources were chickens housed in BSL2 facilities; 1 source commercial layers	Inactivated in commercial layer manure after 6 days at 15 to 20 º C; 2 days at 28 to 30 º C	Lu *et. al.* (2003) [28]
8 wild type LPAI viruses, H5 and H7 subtypes; and 2 HPAI H5N1 subtypes	0, 15, and 30 parts per thousand salinity; 17 and 28 C	Simulated winter and summer coastal marshland temperatures in LA	H5N1 had shorter environmental times survival compared to wild-type LPAI viruses; 2 clades persisted for 94 to 158 days at 17 º C	Brown *et. al.* (2007) [29]

Virus strain	**Media**	**Conditions**	**Survival duration and temperature**	**Reference**
HPAI H5N1 from chickens in central Thailand	0.2ml $10^{6.3}$ EID_{50}/ml in allantoic fluid, feces, one cubic inch of meat or eggs	Virus added to allantoic fluid or feces	In *shade* (25-32 °C): survived for 10 days in allantoic fluid; for 4 days in feces; for 3 days in water from a rice field In *sunshine* (32-35 °C): killed within 30 minutes after placing the sample in sunlight	Songserm *et. al.* (2006) [30]
		Virus added to meat or eggs	Killed if cooked for > 3 minutes at 70 °C	
3 isolates from hunter killed ducks from varied waterfowl habitats in Louisiana (H6N2, H4N6, H10N7)	Distilled water adjusted to pH (6.2, 7.2, 8.2); (0, 20 ppt); (17 C and 28 C); and surface water from a rice field and two marshes	Salinity for fresh and brackish sea water; mean winter and summer temps for coastal Louisiana	Survival in surface water ranged from 9 to 55 days; persistence in simulated water samples ranged from 9 to 100 days	Stallknecht *et. al.* (1990) [31]
5 HA subtypes from hunter killed ducks in Louisiana (H3N8, H4N6, H6N2, H12N5, H10N7)	Distilled water at 17 and 28 C	Mean winter and summer temps for coastal Louisiana	Survival for 207 days at 17 C; and 102 days at 28 °C depending on subtype	Stallknecht *et. al.* (1990) [32]
H5N1 HPAI	$2.38 \times 10^{5.25}$ ELD_{50}	Virus added to normal chicken manure; pH 9.23; 13.7% moisture (dry manure)*	No virus recovered after 24 hrs. at 25 C; or 15 minutes at 40 C. Virus recovered after 4 hrs of UV exposure at room temperature (25 °C)	Chumpolbanchorn *et. al.*(2006) [33]

Virus strain	Media	Conditions	Survival duration and temperature	Reference
3 LPAI viruses (H4N6, H5N1, H6N8)	Starting titers ranged from $10^{4.14}$/ml – $10^{5.14}$/l	3 different water types: DW distilled water (pH 7.8); NS normal saline 0.9% (pH 7.2); SW surface water from Lake Constance. Incubated at -10, 0, 10, 20, and 30 ° C.	Viruses remained infective the longest in DW, followed by SW. Detectable in SW at all temps: H4N6 182 days; H5N1 182 days; H6N8 224 days; persistence inversely proportional to water temperature	Nazir *et. al.* (2010) [34]
3 LPAI viruses (H4N6, H5N1, H6N8), and H1N1	Lake sediment, duck feces, and duck meat	$10^{6.25}$ $TCID_{50}$/ml virus loaded onto germ carriers incubated at 30, 20, 20, and 0 ° C	Persistence highest in lake sediment (5 to 394 days), feces (1 to 75 days), meat (1 to 81 days)	Nazir *et. al.*(2011) [35]
H7N1 LPAI; H7N1 HPAI	HPAI: Breast and thigh meat from chickens, turkeys and ducks infected oro-nasally, collected 3 days post infection	Homogenized meat samples were held at 4 ° C	Infectivity in meat at 4 ° C: 135 days in chicken meat; 90 days in turkey meat; 75 days in duck meat	Beato *et. al.* (2009) [36]
	HPAI and LPAI: virus inoculated into allantoic fluid	Infectivity assayed after holding allantoic fluid samples at 4 ° C and 20 ° C	Infectivity in allantoic fluid: HPAI up to 210 days at 4 ° C; LPAI up to 270 days at 4 ° C; HPAI not detectable at 60 days at 20 ° C, LPAI 2.9 log EID_{50} at 60 days at 20 ° C.	
		Infectivity assayed after holding allantoic fluid samples at 4 ° C and 20 ° C with pH adjusted to 5 and 7	Persistence time higher for viruses at pH 7 than for pH 5; HPAI more persistent at pH7; LPAI more persistent at pH5	

Virus strain	Media	Conditions	Survival duration and temperature	Reference
H13N7 LAPI	10 µl of 6.3 X 10^6 $TCID_{50}$/ml	Steel, wood, tile, tire, gumboot, feather, egg shell, egg tray (cardboard), plastic, latex, cotton fabrics, polyester fabric; placed in sealed tubes and stored in a drawer at room temperature	Survival up to 72 hrs. on most surfaces; 24 hrs. on cotton; 6 days on latex; 6 days on feathers; 2 days on wood; 1 day on egg tray; 3 days on truck tires. Survival appeared to be less on porous vs. non-porous surfaces	Tiwari *et. al.* (2006) [37]
H6N2	3.4 x 10^8 EID_{50}	Treatments: Virus in allantoic fluid mixed with chicken manure, used litter, and feed; homogenized embryonated chicken egg in corn silage. Specimens: held in mesh bags buried in compost; vials of allantoic fluid buried in compost; Controls: held in sealed vials at ambient temperature (23-26 ° C)	Treatments: Virus in all mesh bag specimens inactivated at 40-50 ° C by day 3 except for one manure sample at 40 ° C; Viable virus from allantoic fluid in vials on day 3 (46- 43 ° C); day 7 (55.5 ° C); and day 10 (62.2 ° C) Controls: Viable virus at 21 days (22.7 – 25.7 °C)	Guan *et. al.* (2008) [38]

*Chicken fecal moisture may be as high as 60%

7.6 Transmission

Contact with migratory waterfowl, sea birds, or shore birds is a risk factor for introduction of AI virus into domestic poultry populations.[39] Because AI virus can be isolated in large quantities from feces and respiratory secretions of infected birds, an important mode of transmission is the mechanical transfer of infective feces[3]. Once introduced into a flock, AI virus can spread from flock to flock by direct movement of infected birds and indirect movement of contaminated equipment, egg flats, feed trucks, and service crews, or other means. Windborne transmission may occur when farms are closely situated and appropriate air movement exists. [40, 41]

7.7 Dose Response

7.7.1 Dose Response in Chickens

Most experimental studies in chickens used intranasal inoculation as an entry point. For the intranasal route, the 50 percent chicken infectious dose (CID_{50}) for 10 HPAI strains varied between $10^{1.2}$ to $10^{4.7}$ EID_{50} with a geometric mean of $10^{2.82}$ EID_{50}.[42] Most strains in this study had a mean CID_{50} above 10^2 EID_{50} except for the HPAI H7N1. Other studies have also found similar estimates for the CID_{50} through the intranasal route. [43]

Single hit dose response models (e.g., exponential) have been used for HPAI virus in chickens and mammals. [44] These models assume that each virion has the capacity to independently act and cause infection in the host. Dose response models enable us to estimate the probability of infection when a bird is exposed to a dose different from the 50% infectious dose. For example, given a CID_{50} less than$10^{2.82}$ EID_{50}, a chicken exposed to 10 EID_{50} would have a 1% chance of infection according to the single hit exponential dose response model.

Given limited data, there is a greater uncertainty regarding the infectious dose for other routes such as oral consumption of infected material. Swayne and Kwon (2010) found a substantially higher 50% infectious dose for HPAI H5N1 via oral consumption of chicken meat (10^7 EID_{50}) or drinking of contaminated water$10^{6.7}$ EID_{50}.[45] However, in this study, a group of 3 to 5 chickens were fed contaminated meat with a single virus concentration and details regarding the uncertainty in the estimates were not provided. The study also found higher infectious doses for the intra-gastric inoculation route by gavage ($10^{6.2}$ EID_{50} for liquid and $10^{7.4}$ for meat EID_{50}) compared to the intranasal route. In Swayne and Beck (2005), feeding of finely chopped meat from chickens infected with H5N1 HPAI viruses at higher doses ($10^{7.8}$ EID_{50}/bird), resulted in transmission of H5N1 HPAI virus[46]. However feeding of HPAI H5N2 infected chicken breast or thigh meat to SPF chickens ($10^{3.5–3.6}$$EID_{50}$/bird) did not produce infection. The authors reasoned that lack of direct exposure of respiratory tract (i.e. minced meat likely did not pass through the choanal cleft and contact nasal surfaces) could explain the lack of infection in H5N2 trials with lower doses. Moreover, a reference is made to a feeding trial by Purchase *et.al.* (1931), where 0.5g of blood fed to chickens resulted in HPAI transmission whereas feeding 5 g of meat did not, suggesting that transmission is more likely if a feedstuff is conducive to passage into the nasal cavity[47]. However, in this study, the HPAI concentration in blood was not estimated and it may have been sufficient to cause infection via intra-gastric route.

Sargeev *et al*., (2012) found a CID_{50} of $10^{3.9}$ EID_{50} and $10^{5.2}$ EID_{50} for oral inoculation and intra-gastric inoculation via gavage tube respectively.[48] The authors suggested contamination of the nasal mucosal membranes from the oral cavity via choanal slit as a possible internal mechanism for transmission via the fecal oral route.

There is considerable uncertainty regarding the infectious dose via the aerosol route. Direct aerosol data from Spekreijse *et al.* (2012) suggests very low transmission rates even at a duration of 24 hours exposure to more than 10^3 EID50/m^3 of H5N1 HPAI virus concentration in air coming from a room housing infectious chickens. [49] We fit exponential and logistic dose response models to data from Spekreijse *et al.*(2012) and maximum likelihood estimation suggested a CID_{50} for the aerosol route between 5 to 6 log EID_{50}.[49] An estimate of 5 to 6 log EID_{50} is more consistent with the lower transmission rates for AI observed between chickens housed in adjacent cages in most studies.[50]

Sergeev *et al*., (2012) found considerably lower CID_{50} estimates (approximately 1 log EID_{50}) for various HPAI H5N1 strains when susceptible chickens were exposed to 0.5 to 2 µm diameter aerosols generated from liquid contents of HPAI infected embryonating eggs. [48] The results from this paper are not consistent with other studies that indicate lower aerosol transmission between infected and susceptible chickens housed in adjacent cages and are also not consistent with data published in Spekreijse *et al*. (2012).[49] A possible explanation for the differences between this study and Spekreijse *et al.* (2012) is that the characteristics of 0.5 to 2µm diameter contaminated aerosols generated by nebulizing embryonating egg contents are different from naturally contaminated aerosols emanating from a chamber with infectious chickens.[49]

7.7.2 Route of Entry and 50 Percent Infectious Dose Estimate Used in this Assessment

In the chicken, the choanal cleft (palatine fissure) - located on the roof of the mouth - is a papillae lined, narrow slit that connects the oral and nasal cavities. During the process of mastication or drinking, contents of the oral cavity may pass through this slit and contact the mucosal surfaces lining the nasal cavity.

Because of the variability in the susceptibility of different tissues for infection with HPAI virus (intranasal vs. intragastric) observed in laboratory inoculation and experimental feeding trials, there is considerable uncertainty as to the infectious dose that is appropriate for natural exposure via feeding of contaminated materials. The route of entry used impacts the dose response parameters in the exposure assessment.

We asked experts for their opinion regarding the appropriate infectious dose (intranasal or intragastric) that best represents oral exposure in chickens given the limited data on this aspect. Experts stated that it is reasonable to assume that transmission may occur if contaminated food or water were to pass through the choanal cleft into the nasal cavity. Therefore, due to the limited studies on exposure via natural feeding of contaminated materials and the associated uncertainty, we conservatively assumed that transmission of HPAI viruses through consumption of contaminated materials might occur with exposure to doses infectious for the intranasal route.

7.7.3 Dose Response in Mammals

Several species of mammals have been shown to become infected with HPAI via intranasal inoculation or through feeding of infected materials. For example, feeding a dose of 10^{6-10} EID_{50} via HPAI H5N1 infected meat produced infection in ferrets, cats and red foxes[7, 51 9, 52].

Intra-gastric inoculation of mice with 10^3 EID_{50} via gavage tube did not initiate infection with 3 of the 4 strains of HPAI H5N1 tested [51]. For intranasal inoculation, experimental studies have suggested a 50% lethal dose between 2-3 log EID_{50} in mice and ferrets [53].

7.8 Latently Infected and Infectious Periods

Table 3 summarizes the estimated latently infected period, infectious period, and mean time to death of various HPAI viruses from laboratory inoculation and field studies in individual birds. At the individual bird level, the incubation period is dependent on the dose, route of exposure, and individual host susceptibility. At the flock level, detection is highly dependent on the performance of clinical signs and the ability of the grower to detect them [54]. So for trade purposes, the OIE defines the flock incubation period as 21 days.[4]

Table 3. Estimates of latent and infectious periods from literature review of laboratory and field studies for different HPAI virus strains in chickens.

Strain	Mean time to death	Latent period	Infectious period	Study
HPAI H5N1	-	0.24 days (0.099-0.48)*	2.1 days (1.8-2.3)	Bouma *et. al.* (2009) [55]
HPAI H5N1	36-48 hours			Pfeiffer *et. al.* (2009)[56]
HPAI H7N7	-	Between 1 and 2 days	6.3 days (3.9-8.7)[#]	Vandergoot *et. al.* (2005) [57]; Bos *et. al.* (2007) [58]
LPAI H5N2	-		Inoculated chickens, 4.8 days (CV 9%); contact animals, 4.25 days (2.57-5.93, 95% CI)	Vandergoot *et. al.* (2003) [59]
HPAI H5N2			Contact chickens, 6.8 days (4.91-8.69, 95% CI)	

*95% credible interval; [#] 95% confidence interval; [±] Coefficient of variation

7.9 Clinical Signs

The presence and severity of clinical signs of HPAI infection depends on the type of bird species affected[8]. Infected wild and domestic ducks may be asymptomatic, whereas clinical signs in gallinaceous poultry are usually severe, resulting in high mortality. In poultry (chickens and turkeys), the clinical signs associated with HPAI infection include marked depression with ruffled feathers, lack of appetite, excessive thirst, decreased egg production, soft-shelled or misshaped eggs, respiratory signs (coughing and sneezing), watery diarrhea or sudden, unexpected death. Mature chickens frequently have swollen, cyanotic combs and wattles, and edema surrounding the eyes. The mortality rate in an infected flock can reach 100 percent.[4]

In mature birds, gross lesions on necropsy may consist of subcutaneous edema of the head and neck; fluid in the nares, oral cavity, and trachea; congested conjunctivae and kidneys; and petechial hemorrhages which cover the abdominal fat, serosal surfaces, peritoneum, and surface under the keel. In layers, the ovary may be hemorrhagic or degenerated and necrotic. The peritoneal cavity is frequently filled with yolk from ruptured ova, causing severe airsacculitis and peritonitis in birds that survive longer than 7 days.

7.10 Diagnosis

HPAI is a differential diagnosis to be considered in any flock in which marked depression, inappetence, and/or a drastic decline in egg production are followed by sudden deaths; however, a conclusive diagnosis is dependent on the isolation and identification of the virus.

The reference standard for diagnosis of AI virus is virus isolation. In the laboratory, 9- to 11-day-old embryonated chicken eggs are inoculated with swab or tissue specimens. Additional tests on fluids from the egg are required to confirm the presence of AI virus and determine its serologic identity (HA and NA type) [3].

The application of molecular methods for detection of viral nucleic acid has become an important tool in the recent years. The real time reverse transcription polymerase chain reaction (RRT-PCR) has advantages for outbreak surveillance such as speed, scalability for high through put, high sensitivity and specificity (Swayne, 2008 #1529).

Antigen detection immunoassays kits have also been used in prior outbreaks and have advantages of speed (15-20 minutes) and good specificity. While the high analytical sensitivity (greater than 10^4 EID_{50}) is a limiting factor, birds presenting from clinical disease or that died due to AI infection generally shed adequate virus titers for detection with these kits. In contrast the assays are not recommended for screening of apparently healthy poultry due to the lower level of shedding before the disease is clinical[3].

7.11 Differential Diagnosis

HPAI can resemble several other avian diseases including velogenic viscerotropic Newcastle disease, infectious bronchitis, infectious laryngotracheitis, mycoplasmosis, infectious coryza, fowl cholera, aspergillosis, and *Escherichia coli* infection. It also must be differentiated from heat exhaustion and severe water deprivation.

8. Background Information on the Production of Eggshells and Inedible Egg Product from Egg-processing Operations

In this section, we provide an overview of the production and distribution process for eggshells and INEP.

8.1 Eggshells

Eggshells are a byproduct of normal egg-grading and egg-processing activities. The bulk of eggshells produced are from egg-breaking facilities. Breaking facilities can be categorized into inline (i.e. live poultry on the premises) or standalone facilities (i.e. no live poultry on the premises). Standalone egg-breaking facilities receive nest-run eggs from offline farms that have no egg-processing facility on the premises.

A smaller quantity of eggshells is also produced from processing restricted eggs from grading and packing facilities. In routine operations, restricted eggs from grading facilities may be transported to approved processing facilities in barrels. Some of the restricted eggs, such as checks and dirties, may be broken to recover edible liquid egg followed by centrifuging or screening of eggshells to separate liquid INEP and wet eggshells. Other restricted eggs such as inedible or loss eggs may be directly processed to recover INEP and wet eggshells. Restricted eggs produced on smaller facilities may be disposed of in a landfill or used as feed for furbearing animals or starter pigs.

For the purpose of this risk assessment, eggshells are divided into two categories depending on their processing stage: “wet eggshells” and “dry eggshells.” Eggshell waste from the breaking process contains approximately 29 percent moisture as it includes adhering egg whites. Eggshells from egg breaking are continuously conveyed to a centrifuge or a screen where the adhering egg whites are recovered as liquid INEP and the moisture content of the eggshells is reduced to about 16%.[60] This process produces wet eggshells. The major disposal or reuse options for wet eggshells include disposal in a municipal landfill, land application as a soil amendment, or drying to produce dry eggshells (**Figure 2**). Eggshells are usually dried to 4 percent or less moisture content. Dry eggshells are an excellent source of calcium and phosphorous in proportions required by laying hens and are frequently included in laying hen rations.

8.1.1 Major Processes in Eggshell Production at an Egg-breaking Plant

Shell-eggs for breaking are received either on transfer belts from a layer flock (inline facility) (**Figure 1**) or from an offline flock packed on flats (standalone facility).

Figure 1. Incoming eggs on a transfer belt from an inline operation.

8.1.1.1 Shell Egg Washing

The initial phase of pre-wetting and washing shell-eggs occurs as inline eggs are received via conveyor belts and accumulated on egg-washer conveyors for entry into the washing machines, or when the offline shell eggs are removed from their bulk packaging (pallets, racks, and flats) and introduced via vacuum lifts to the egg-washer conveyor. The pre-wetting of shell-eggs is optional, and not all egg-washing operations use this procedure. Eggs are then moved into the egg-washing machine where they are washed with a detergent. Agriculture Marketing Service (AMS) regulations 7CFR56.76 (f) require the temperature of the wash water to be 32.2 °C (90 °F) or higher, and at least 11°C (20 °F) warmer than the egg temperature. The AMS regulations also list requirements for maintaining the condition of the wash water.[2]

8.1.1.2 Sanitizing Shell-Egg Surfaces

Immediately prior to breaking, shell-eggs are sanitized with a potable water rinse with a chlorine concentration of 100 (min) to 200 (max) ppm (9CFR590.516). As an optional process after sanitizing and drying, the surface of shell-eggs may be coated with a food grade mineral oil to maximize conservation of quality.

8.1.1.3 Breaking

Eggs are broken in specialized machines located in a room separate from where the transfer and washing processes are performed. Breaking machines are mechanical devices designed to open individual shell-eggs in a series of operations that allow control and inspection of each individual egg (**Figure 3**). The machines may be set up to produce liquid whole egg (natural proportions of yolk and white), egg yolk, and egg whites. Wet eggshells from the breaking machine are conveyed through an enclosed auger system to a centrifuge or a screen (**Figure 4** and **Figure 5**).

8.1.1.4 Centrifuging or Screening

Eggshells exiting the breaker are transferred to the eggshell centrifuge via an auger or conveyor line. An alternative to the centrifuge is a perforated screen system in which a screw of progressively reducing pitch rotates gradually, increasing pressure and forcing the liquid INEP to extrude through the screen. Wet eggshells leaving the centrifuge, or the screen system, have approximately 14 to18 percent moisture content. The centrifuge process also helps break

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[2] An Assessment of the *Risk Associated with the Movement of Washed and Sanitized Shell Eggs Into, Within, and Outside of a Control Area during a Highly Pathogenic Avian Influenza Outbreak*, October 10, 2009 (pp. 19-20)

eggshells into smaller pieces. The liquid collected during the centrifuge process is transferred to the liquid INEP storage tank.

8.1.1.5 Transporting Wet Eggshells

At the end of each processing day, wet eggshells not dried on site are transported either to a breaking facility with a dryer, to a landfill, or are spread on land as a soil amendment. Typically, dump trucks, or live bottom trailers, are used to move wet eggshells (**Figure 7** and **Figure 6**). A tarp may be used to cover the wet eggshells to prevent spillage during transport.

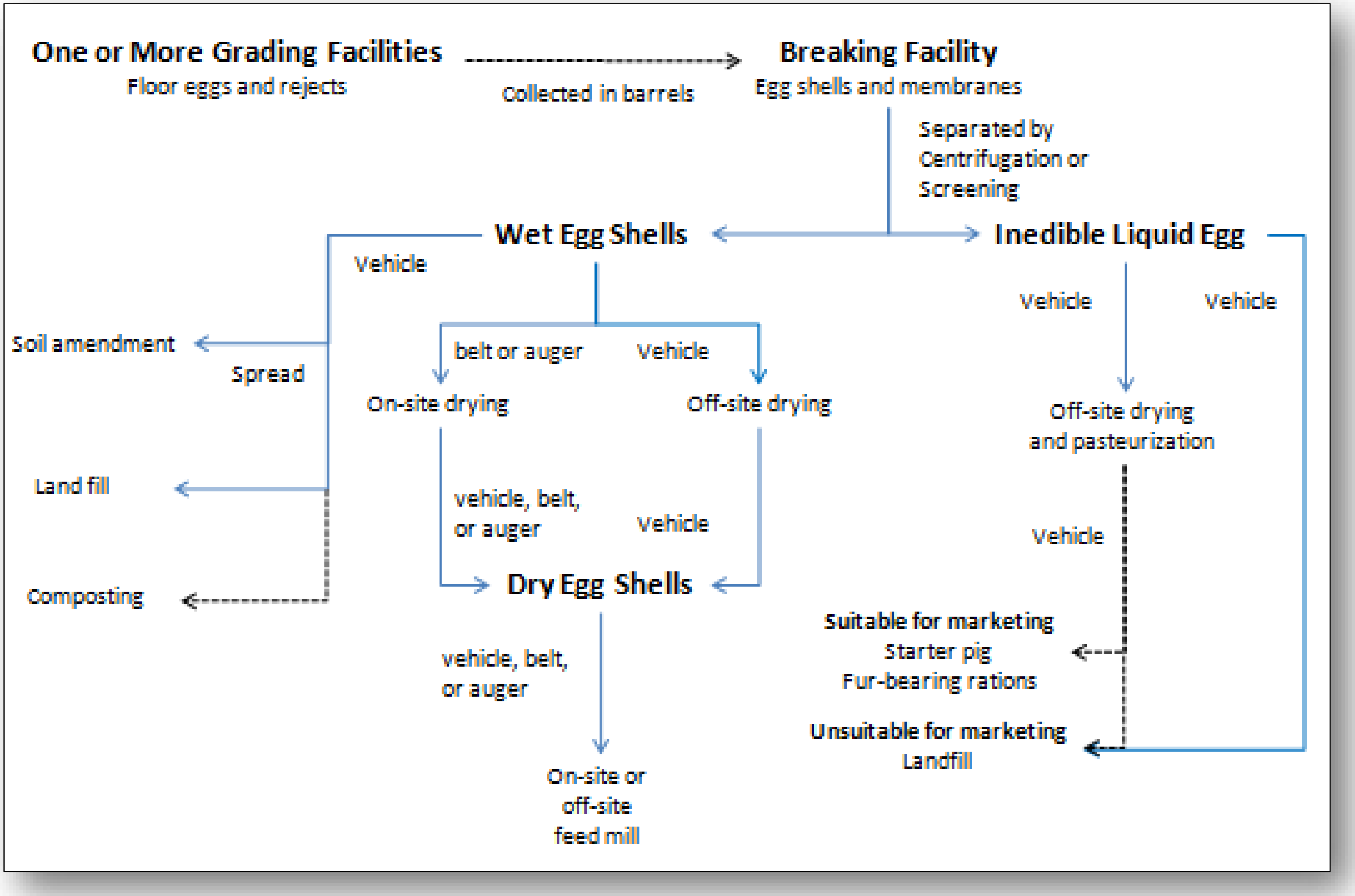

Figure 2. Source, transport, and fate of wet eggshells, dry eggshells, and inedible egg product in current practice. The solid lines represents movements addressed in this risk assessment.

Figure 3. Photo of inspectors with a high-speed egg breaker.

Figure 4. Eggshells transferred from the breaking room to the centrifuge. Note that the auger is fully enclosed.

Figure 5. Eggshells entering a centrifuge.

8.1.1.6 Drying Wet Eggshells

Both drum and belt dryers are used to dry eggshells. **Figure 8** shows a rotary drum dryer. The centrifuged shells are conveyed into the

Figure 7. Receiving wet eggshells from another breaking plant.

horizontal drum dryer where they are mixed with air heated to temperatures in the range of 537.8 to 815.6 °C (1,000 to 1,500 °F). A typical rotary dryer consists of a cylindrical shell slightly inclined to an angle to the horizontal (0 to 5 degrees) that rotates around its longitudinal axis. The dryer functions both as a conveyor and as a heating device. Rotary dryers are normally equipped with lifting flights that continuously enhance the transport of the material and increase the contact surface area between the material and the hot medium.

Wet eggshells, that need to be dried, are introduced in a continuous way through one end of the dryer. Hot air is introduced either concurrently or counter-currently, where it contacts the eggshells. Inside the dryer, there are three main transport phenomena occurring simultaneously: transport of wet materials, heat exchange between hot air and the material, and water vapor transfer from the solids to the hot air stream. Wet eggshells cascade off the flights and pass through the hot moving gas stream. Each time an eggshell particle cascades it is moved along the length of the drum—a result of gas particle interaction. Convective heat and mass transfers are the major modes of drying. Moisture and exit temperature are monitored in the drying process. Water is evaporated such that the moisture content of the dry eggshells, with adhering membranes, is less than 4 percent.[61] The exit temperature of the exhaust air is in the range of 98.9 to 104.4 °C (210 to 220 °F).[35] The exit temperature of the eggshells may range from 70 to 93.3 °C (158 to 200 °F).[61] Although not standard practice, some facilities may treat the dry eggshell with formaldehyde as a sanitizer (*pers. comm.*, Rich Dutton).

Belt dryers use a fluidized bed of hot air (up to 315.6 °C (600 °F)) to remove moisture from eggshells. Air in a belt dryer flows from below the eggshells upwards through the belt, which carries them through the dryer. Heated air passes through the product at high velocities, "fluidizing" the product to reduced backpressure and provide thorough mixing of the product for even processing.[62] Typical operating temperatures for eggshells reported by the manufacturer are 315.6 °C (600 °F) (air temperature) and 148.9 °C (300 °F) (eggshell temperature). Dry eggshells are moved from the drier in an enclosed auger and are stored in a hopper (**Figure 9**).

Figure 8. Rotary drum driers used to dry eggshells.

Figure 9. Hopper used to store dried eggshells.

8.1.2 Distribution of Eggshells

Figure 11. Mill used to grind eggshells for feed.

Figure 10. Transporting dry eggshells to an offsite feed mill in a covered hopper trailer.

Dry eggshells are either transported directly to the on-site feed mill via an enclosed pipe system or collected and transported off-site (**Figure 11** and **Figure 10)**. Dry eggshells are predominantly used as an animal feed additive (calcium source). Less frequently, dry eggshells are included as litter material in horse barns and riding arenas. Disposal options for wet eggshells include disposal in a landfill; land application on farms as liming source; drying; and less frequently for composting. **Table 4** provides examples of the variable types of eggshell products, their origin, quantity, and end use or disposal option.

Table 4. Examples of the type of eggshell products by origin, quantity, and fate. Farms indicate inline plants with live poultry, whereas plants are standalone facilities. Storage bins may be not be located on a plant or farm.

Origin	Type of product	Quantity	Fate

Plant A[a]	Hard cooked shells	5,023 tons annually	Land applied
Plant A[a]	Wet eggshells	4,033 tons annually	• 50% dried for feed • 50% land applied
Farm A[b]	Wet eggshells	10–12 tons/day	• 50% dried for feed; transported to feed mills (contract farms or company farms) • 50% raw shells land applied within 30 miles of origin
Farm B[b]	Wet eggshells	15 tons/day	Nearly all dried and used for feed on the farm
Farm C[b]	Wet eggshells	6–8 tons/day	Nearly all dried and used for feed
Wet bin [c]	Wet eggshells	40–50 tons/day	Transported to an inline plant with a drier to be dried and transported to a feed mill

[a] Offline breaking plant with further processing
[b] Inline breaking plant with poultry on the premises
[c] Common storage bin that receives wet eggshells from several inline plants without driers

8.2 Inedible Egg Product

8.2.1 Major Processes in INEP Production

INEP may be generated at several different stages of the egg-processing supply chain.

From Egg-Grading Operations

Restricted eggs from the shell-egg grading process include eggs classified as inedible, loss, checks, dirties, and leakers. Some of the restricted egg types from larger operations may be transported to an approved egg-processing facility for specialized processing while smaller operations may dispose of them in a landfill, or ship them to a furbearing animal operation as a feed ingredient. The inedible, loss, and other types of eggs not fit for human consumption, as specified in 9CFR590.510, are clearly identified with inventory control and mixed with a denaturant. In routine operations, the inedible and loss eggs from an egg-packing operation are collected in barrels and processed onsite or held under refrigeration and transported offsite for further processing. At the receiving egg-processing facility, the inedible or loss eggs are pumped through a centrifuge where the eggshells are separated and the liquid is processed as INEP.

From Egg-breaking and other Processing Operations

Large quantities of INEP are produced by recovery of liquid from the eggshell centrifuge or screen coming from the breaking machines (**Figure 12**). INEP is also produced from various egg-processing operations at the interface of production runs where water is used to displace the egg from the processing lines and equipment. It may also be generated as a result of a mishap in production or distribution of egg that renders it unfit for human consumption while retaining its value as animal food. Breaking plants may receive liquid INEP from other breaking plants, which is held and blended for further processing.

Figure 12. Liquid inedible egg recovered from the centrifugation of wet eggshells.

The fat content of INEP is measured prior to final processing, and INEP is then blended to get the correct fat level for customers (e.g. pet food manufacturer). The difference in fat level is related to breaking machine function and shell quality. This is mostly due to the efficiency of the breaking operation.

Figure 13. Inedible egg holding tank.

There is no forced mixing of products when added to the holding tank (**Figure 13**) but some mixing occurs due to differences in temperature and fat content. INEP from shell-egg grading, or from breaking, pasteurization, and packaging plants, is generally chilled and held at temperatures below 7.2 °C (45 °F) until transported to drying facilities or processed. There may be variable levels of bacteria by tanker, age, plant, and time. INEP is blended with hydrogen peroxide at a specified concentration to minimize spoilage.

Pasteurization of INEP generally exceeds the World Organization for Animal Health (OIE) standards for inactivation of AI virus in liquid egg products with a minimum hold time of 188 seconds at 60 °C (140 °F).[63] Pasteurized INEP then is sprayed into a dryer (200 to 300 °F) and the moisture removed by heat and air movement producing dried INEP. Dried INEP is then packaged for shipping (**Figure 14**).

8.2.2 Distribution of INEP

Figure 14. Dried and packaged INEP.

Unpasteurized liquid INEP is pumped from the inedible storage tank directly into tankers for transport. The tankers are loaded in the same loading bays as edible liquid egg, but have separate piping. These tankers may then move between premises to collect a full tanker batch. INEP is usually transported off the premises several times per week. Most facilities will have a 3 to 4 day storage capacity. Smaller volumes of inedible egg from small egg-breaking facilities may be stored in reusable plastic drums, or carboys, and then transferred to trucks via pallets.

Liquid INEP is commonly pasteurized, dried, and used in pet feeds. Dried INEP may also be used as a protein source for fur-bearing animals and starter pigs. A large quantity of dried INEP is exported. Rarely, liquid INEP is incorporated into manure wastes for compost or landfills.

8.3 Rate of Eggshell and Liquid INEP Production

We asked industry experts to estimate the total weight of wet eggshells and liquid INEP for both breaking and grading operations, and the weight of wet eggshells and liquid INEP produced after centrifugation assuming an average egg weight of 60 grams (**Table 5**).[61] We estimated the weight of wet eggshells and inedible liquid produced from a table-egg layer flock, assuming that a 100,000-hen table-egg layer house would produce 70,000 eggs per day (194 cases/day-house) (**Table 6**). Because of the high volume of wet eggshells produced, wet eggshells typically need to move off the farm daily.

Table 5. Daily liquid INEP and wet eggshell yield on a per case basis in pounds from breaking and grading operations, assuming a 47.62-pound case weight (360 eggs in a case).

		After centrifugation		
Operation	**Total**	**Wet eggshells**	**Inedible liquid**	**Handling liquid**
Breaking	7.14 [a]	5.71 [b]	1.43 [c]	1.21 [d]
Grading and packing	1.43 [e]	0.17	1.26	

[a] Estimate of wet shell and adhering liquid from breaking assuming 15% of starting weight of a case of eggs as an upper bound.

[b] Estimate of wet shells assuming 12 % of weight of an intact case of eggs as an upper bound.

[c] Estimate of inedible liquid assuming 3% of weight of an intact case of eggs.

[d] Inedible liquid from line and equipment rinses after product transfers within the plant including tankers, chilling, pasteurization, and mixing. 1,000 pounds of liquid egg (may include non-egg ingredients) yields 30 pounds waste liquid. Yield per case assumes 40.5 pounds liquid per case using 3% waste liquid weight and includes loss eggs (eggs dropped on the floor, rejected from the process because they are either leakers or inedible eggs).

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[e] This estimate is for those operations which separate the inedible eggs obtained during grading or packing via centrifuging. Assumes 3% loss of eggs during grading that goes into inedible waste.

Table 6. Pounds of wet eggshells and liquid INEP produced each day by 100,000 table-egg layers (360 eggs per case, 194.4 cases per day).

		After centrifugation		
Operation	**Total**	**Wet eggshells**	**Inedible liquid**	**Handling liquid**
Breaking	1388.9	1111.13	277.8	236.1
Grading and packing	277.8	33.33	244.4	

9. Entry Assessment

An entry assessment determines the likelihood of a commodity (eggshells and INEP) being contaminated with a hazard (e.g. HPAI virus) and describes the biological pathways necessary for that hazard to be introduced into a particular environment with susceptible poultry. It includes an estimation of the likelihood (i.e., qualitative or quantitative) of each of the pathways.[2]

The entry assessment of this risk assessment evaluates the likelihood of transmitting HPAI virus onto the destination premises via movement of contaminated eggshells, INEP, or the vehicle or driver, from a breaking facility that receives eggs from flocks in the control area.

The entry assessment considers outbreak specific preventive measures (future measures) proposed by the egg-sector working group and the SES Plan. The active surveillance protocol described in the SES Plan is an important measure for reducing the likelihood of moving HPAI contaminated eggshells and INEP. The movement of wet and dry eggshells is considered separately as some breaking plants may not have a dryer to process wet eggshells onsite. The vehicle and driver biosecurity measures from the SES plan are also considered in the evaluation.

9.1 Likelihood and Degree of HPAI Contamination of Wet Eggshells and Liquid INEP Moved from an Egg-breaking Facility

Estimated HPAI Viral Titer in Wet Eggshells or Liquid INEP Moved from an Egg-breaking Facility

Risk Factors: HPAI virus contamination of eggs; late detection of HPAI infection in a flock

Current Preventive Measures: Requirements for washing and sanitization of shell-eggs presented for breaking according to Federal regulations 9CFR590

Future Preventive Measures: Targeted active surveillance protocol described in the SES Plan; 2 day (48 hour) on-farm holding of eggs from off-line operations

Conclusions: The fraction of HPAI contaminated wet eggshells out of all wet eggshells produced from a 100,000-hen flock per day is 9×10^{-6} (90 percent P.I. 0-5.7×10^{-5}) for offline operations, and 7.6×10^{-5} (90 percent P.I. 0-2.4×10^{-4}) for inline operations. Assuming weekly movement of INEP and homogeneous mixing, the average viral titer in liquid INEP moved from the facility is < 1.5 EID_{50}/ml for both types of operations. There is a 99 percent chance that the titer is less than 10^1 EID_{50}/ml.

9.1.1 Background Information

Natural outbreak and experimental studies have found HPAI H5N2 virus in internal contents and on shell surfaces of eggs laid by infected chickens.[13-15] Therefore, wet eggshells and INEP produced by breaking plants may be contaminated if eggs are sourced from a HPAI infected but undetected, flock in the control area.

The likelihood of moving contaminated wet eggshells or liquid INEP from an egg-breaking facility depends on the following factors:

- The dynamics of HPAI infection spread within the flock given the HPAI strain characteristics.
- The time it takes to detect HPAI infection in the flock, given the surveillance protocol followed during an outbreak.
- The frequency of movement and holding time for eggs from the farm to the breaking plant (for offline facilities), and the frequency of movement of eggshells, or INEP, from the breaking plant.

We previously developed discrete event simulation models of HPAI disease transmission and the active surveillance protocols for use in proactive assessments for evaluating the risk of HPAI spread via movement of egg-industry products.[22] These models were modified according to the movement protocols for eggshells and INEP. Scenarios where eggs are sourced from offline or inline layer farms were considered separately.

9.1.2 Preventive Measures

9.1.2.1 Current Preventive Measures

An important current preventive measure is washing and sanitization of shell-eggs presented for breaking according to Federal regulations 9CFR590. The washing operation uses a combination of heat, pH, detergent action, contact time, and mechanical agitation to accomplish the removal of soil from the shell-egg surface. Detergents used in the washing process must be USDA approved and labeled for such use by the EPA. In addition, the wash temperatures are assumed to reach a minimum of 90°F. Unclean eggs are disposed of or rewashed. A spray rinse with a sanitizer containing 100 to 200 ppm chlorine, or its equivalent, is applied to all surfaces of the egg. A previous risk assessment on the movement of washed and sanitized shell-eggs estimated the viral load on the eggshell to be reduced by a factor of at least 1,000 (a 3-log reduction) given a 1 to 8 second exposure time with 100 to 200 ppm chlorine rinse. [64]

9.1.2.2 Future Preventive Measures

Relevant future measures evaluated in this assessment include:

- The targeted active surveillance protocol described in the SES Plan reduces the risk of moving contaminated egg products before infection is detected in the flock. This protocol includes monitoring of flocks for clinical signs such as changes in feed and water intake, a drop in egg production, an increase in daily mortality above an established threshold, and submission of a pooled sample of swabs for RRT-PCR testing each day from 5 randomly selected birds for every 50 dead birds among the daily mortality pool.
- The SES Plan requires a two-day hold after production prior to the movement of nest-run eggs from offline farms to an egg-breaking plant. This holding period further reduces the likelihood of moving contaminated eggs from an undetected flock—in addition to active surveillance. A similar holding time was not considered for movement of eggshells or INEP from an inline facility, as in this case, the contiguous processing lines and other equipment are not conducive to holding eggs, liquid INEP, or wet eggshells.

9.1.3 Evaluation

9.1.3.1 Number of Internally Contaminated Eggs from a HPAI Infected but Undetected Flock Contributing to the Batch of Wet Eggshells or Liquid INEP Moved from the Breaking Facility

We used a stochastic disease transmission model to simulate HPAI spread within a table-egg layer flock to estimate HPAI infection prevalence, disease mortality, and the number of contaminated eggs produced over time. We then used an active surveillance-protocol-simulation model, that interfaces with the transmission model, to estimate the number of contaminated eggs in a batch of wet eggshells or inedible egg that is moved after different hold times. The active surveillance protocol model was modified from that used in previous risk assessments to incorporate the movement logistics of eggshells and liquid INEP. Specifically, we considered the lower frequency of movement of liquid INEP and considered the risk reduction due to holding time before movement of nest-run eggs to a standalone breaking plant. Details on the implementation of these modifications are provided in Appendix 1.

For the purposes of this risk assessment, we defined a batch of wet eggshells to be 1144.5 pounds of wet eggshells. This is the average estimated quantity of wet eggshells generated with breaking and processing of eggs laid by a 100,000 hen layer flock in a day. We note that multiple batches of wet eggshells or INEP originating from different layer flocks may be combined together in one truckload moved from a breaking plant. For the purposes of this risk assessment, we have assumed that each of the batches would have originated from an infected but undetected flock.

The estimated number of internally contaminated eggs from a HPAI infected but undetected, flock contributing to a batch of wet eggshells or liquid INEP moved from the breaking facility depends on whether the breaking plant is a standalone breaking facility (i.e. without poultry onsite) or an inline facility (i.e. produces eggs from a layer flock on the premises). The difference between the risk estimates between a standalone and inline breaking facilities is due to different holding times—after production of eggs—before they are processed and when the resulting wet eggshells, or inedible egg, can be moved. The SES plan requires a two-day holding time before eggs from an offline farm can be moved to a standalone breaking facility. For an inline facility, there may not be any holding time before eggs are moved into the breaking facility (e.g. each morning eggs are collected by conveyor, washed, and broken). We evaluated these two scenarios separately. Liquid INEP is typically moved from the plant on a weekly basis. We also consider scenarios where liquid INEP is moved twice weekly.

The main assumptions of the simulation models are summarized here. Further discussion of these assumptions is provided in the *Washed and Sanitized Shell Eggs Risk Assessment*.[22]

- The effective contact rate for disease transmission estimated from HPAI outbreak data from the Netherlands (2 birds/6 hours) is applicable to table-egg layer flocks (i.e. in the U.S.).
- The mean infectious period and latent period for the HPAI strain in table-egg layer flocks is similar to those for Asian H5N1 strains.
- Apart from active surveillance of mortality via RRT-PCR, other clinical indicators of HPAI infection—such as a drop in egg production and decreased feed intake—are not considered towards detecting infection in the model.

- Daily mortality sampling is random. Swabs from dead birds with and without clinical signs are equally likely to be included in the pooled sample for RRT-PCR testing.

9.1.3.1.1 Key Disease Transmission Model Parameter Estimates

The parameter estimates for the disease transmission model are summarized in **Table 7**. Most of the parameter estimates are similar to those used in the *Washed and Sanitized Shell Eggs Risk Assessment.*

Table 7. Key parameter estimates for the within flock HPAI transmission and surveillance models used in this risk assessment.

Parameter Name	Parameter Description	Value
Effective contact rate (transmission parameter)	The number of birds an infectious hen comes into contact with that is sufficient to transmit infection per-unit-time	2 hens/6-hours [25, 26 23]
Expected latent period	----	13.8 hours[22]
Expected infectious period	----	29.6 hours[22]
Expected time to death	Sum of the expected latent and infectious periods	43.4 hours[22]
Basic reproductive number	The expected number of new infections in other chickens caused by an infected chicken in its lifetime	9.86[a]
Normal egg production rate	Average egg production rate in layer hens that are not infected with HPAI	0.7 eggs/hen-day[22]
$V_{T(i)}$	HPAI viral titer in albumin from a contaminated egg *i*	Empirical distribution mean: $10^{4.81}$ $EID_{50/}$ml, 95th percentile $10^{5.5}$ $EID_{50/}$ml
Infected HPAI egg production rate	Estimated egg production rate during the period in which HPAI infected hens may lay contaminated eggs	0.49 eggs/hen-day[22]
Flock size	Current industry estimate of the number of birds per henhouse	100,000 birds
Normal flock daily mortality (percent of flock)	Estimated normal mortality independent of HPAI disease	Empirical distribution: mean= 0.028; SD= 0.033 percent of flock.
Sensitivity of the RRT-PCR test	The probability of RRT-PCR detection of HPAI virus when a pooled sample containing HPAI virus is tested	86.5 percent[27]
Mortality threshold (percent of flock)	HPAI infection would automatically be detected regardless of RRT-PCR testing if the percent daily mortality exceeds this threshold.	0.5 percent [22]

[a] The reproductive number equals the product of the expected infectious period (29.6 hrs) and effective contact rate (0.333/hr).

9.1.3.1.2 Disease Transmission and Surveillance Model Results

The disease transmission and surveillance models were implemented in @RISK and Excel and simulated for 6,000 iterations. **Figure 15** shows the simulation results on the number of contaminated eggs produced and the cumulative probability of detecting HPAI on various days post infection of the flock. **Table 8** summarizes the main transmission model results.

Table 8. A summary of key results from the disease transmission and active surveillance simulation models. The proportion of HPAI infectious birds prior to detection is used to estimate the degree of HPAI contamination of handling materials after eggs are held for two-days.

Output Parameter	Value
Number of days the flock is infected before HPAI infection is detected	3.19 (95 percent P.I. 2-4) days
Proportion of HPAI infectious birds in the flock two days before the day on which HPAI infection is detected	0.009 (90 percent P.I. 0-0.06) percent
Proportion of HPAI infectious birds in the flock one day before the day on which HPAI infection is detected	0.08 (90 percent P.I. 0.002-0.53) percent

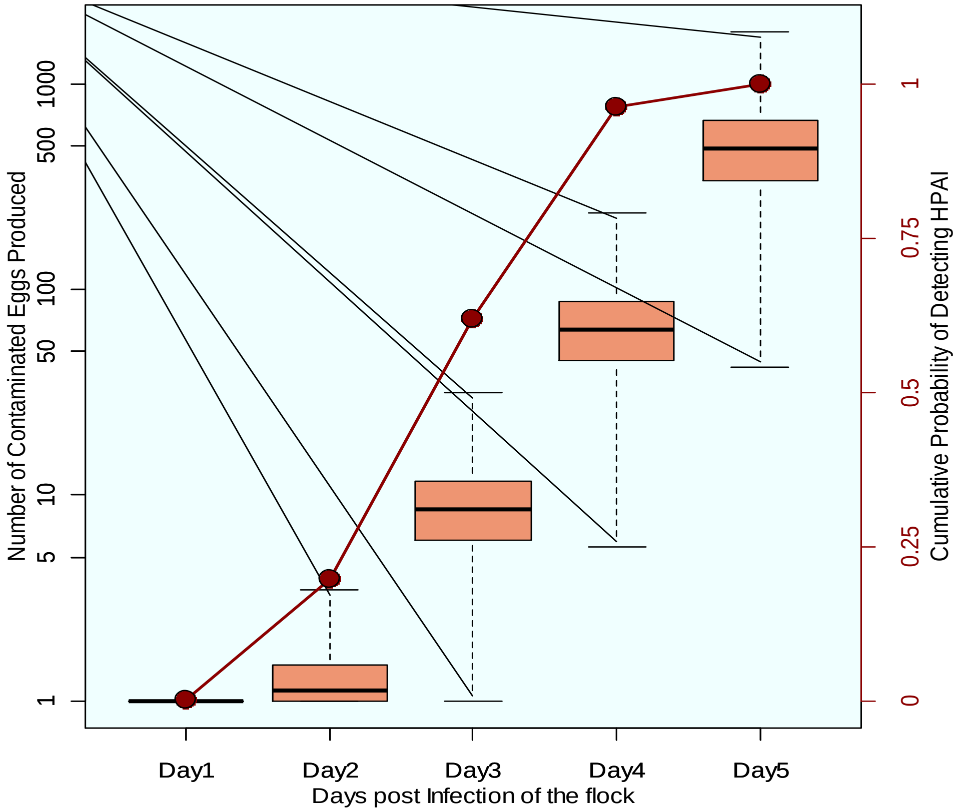

Figure 15. Boxplots of number of contaminated eggs produced (left axis) and the cumulative probability of detecting HPAI infection (right axis) at various days post infection of the flock. Although the number of contaminated eggs produced increases on progressive days post

infection of the flock, the likelihood of detection also increases reducing the chances of moving a higher number of contaminated eggs on days 4 and 5 post infection of the flock.

Table 9 shows the estimated maximum number of internally contaminated eggs (E_{max}) from a HPAI infected but undetected flock contributing to a batch of wet eggshells moved from the breaking facility. This variable's value is different for inline and standalone egg-breaking plants due to the different holding times after the production of eggs and before they are processed.

Table 9. Simulation results for the estimated maximum number (E_{max}) of internally contaminated eggs from a HPAI infected but undetected flock contributing to a 1144.5 pound batch of wet eggshells moved from a breaking facility (mean with 90 percent P.I.).

0 Days Holding (Inline Breaking Facilities)	**2 Days Holding (Standalone Breaking Facilities)**
5.3 (90 percent P.I. 0-17) eggs/day [a]	0.64 (90 percent P.I. 0-4) eggs/day [a]

a: Two-sided 90 percent probability interval from simulation results. A batch of wet eggshells includes 1144.5 lb of eggshells generated through processing of eggs from a single layer hen flock and included in a single movement.

Table 10 shows the results for the estimated number of internally contaminated (E_{sum}) eggs from a HPAI infected but undetected flock contributing to a batch of liquid INEP moved from inline and standalone breaking facilities. For inedible egg product that is moved twice per week, the batch size is 2654 lb and on average includes inedible egg product generated from 245, 000 eggs from a single layer flock. For inedible egg moved once per week, the corresponding batch size is 5308 lb and includes inedible egg product generated from 490, 000 eggs.

Table 10. The total number of contaminated eggs (E_{sum}) contributing to a batch of liquid INEP moved at a frequency of once or twice per week (mean with 90 percent P.I.).

0 Days holding (Inline Breaking Facilities)	**2 Days holding (Standalone Breaking Facilities)**
1.60 (90 percent P.I. 0-17) eggs/day [a]	0.016 (90 percent P.I. 0-1) eggs/day [a]

a: Two-sided 90 percent probability interval from simulation results. With weekly twice movement, a batch included 2654 lb of inedible egg product generated through processing of eggs from a single layer hen flock and included in a single movement. With once weekly movement, a batch is 5308 lb of inedible egg product.

9.1.3.2 Estimated Quantity of HPAI Virus in Wet Eggshells and Liquid INEP Originating from Infected but Undetected Flocks

9.1.3.2.1 Estimated Quantity of HPAI Virus in Wet eggshells from Infected but Undetected Flocks

Standalone Egg-breaking Facilities Receiving Eggs from Offline Farms

A standalone egg-breaking facility may source eggs from multiple–table egg-layer flocks. We conservatively assumed that all of the flocks supplying eggs to the breaking facility are in an infected but undetected state. Based on the estimated number of contaminated eggs from Section 9.1.3.1, and assuming minimal mixing of contaminated and virus free eggshells, we estimated a mean HPAI contaminated fraction of 9×10^{-6} (90 percent P.I. $0\text{-}5.7\times10^{-5}$) in a batch of wet eggshells.

To calculate the quantity of HPAI virus in this fraction, we assumed that HPAI virus is present in the liquid egg adhering to the mineral portion of the shell and in protein matrices of the eggshell. The variability in the virus titer of the contents of individual eggs was modeled using an empirical distribution with a mean of $10^{4.81}$ EID_{50} estimated from data presented in Swayne and Eggert (2010). The mean of the total HPAI virus infectivity in a batch of wet eggshells was $10^{4.84}$ EID_{50} for movements from a standalone breaking plant (**Table 11**).

Table 11. Estimated quantity of HPAI virus (EID_{50}) in wet eggshells from inline and standalone breaking plants and the fraction of the total amount of wet eggshells contaminated with HPAI virus (mean with 90 percent P.I.).

	Inline	**Offline**
Fraction of total wet eggshells contaminated assuming no mixing	7.6×10^{-5} ($0\text{-}2.4\times10^{-4}$)	9×10^{-6} ($0\text{-}5.7\times10^{-5}$)
Total quantity of HPAI virus in 1,144.5 pound batch of wet eggshells	$10^{5.74}$ ($0\text{-}10^{6.44}$) EID_{50}	$10^{4.84}$ ($0\text{-}10^{5.70}$) EID_{50}
Virus concentration in a 2-pound portion of wet eggshells mixed with eggshells generated from HPAI contaminated eggs	$10^{2.78}$ ($0\text{-}10^{3.48}$) EID_{50}/g	$10^{1.88}$ ($0\text{-}10^{2.74}$) EID_{50}/g
Virus concentration in a 5-pound portion of wet eggshells mixed with eggshells generated from HPAI contaminated eggs	$10^{2.38}$ ($0\text{-}10^{3.08}$) EID_{50}/g	$10^{1.48}$ ($0\text{-}10^{2.34}$) EID_{50}/g

Although there is no specific mixing step in the processing of wet eggshells, some limited mixing may occur during transport via augers, or while centrifuging to recover liquid egg from eggshells. Given limited mixing, the contamination is expected to be relatively concentrated in a few pounds of eggshells. The HPAI virus concentrations were estimated for example scenarios in which the contamination is contained within 2 or 5 pounds of eggshells to approximate the range of virus concentrations, when limited mixing occurs. Note that other scenarios of mixing apart from the examples considered here are possible. However, it is not possible to enumerate all the potential scenarios given the uncertainty and variability associated with the extent of mixing.

Overall, the simulation models predicted a 37.4% chance that eggshells from at-least one contaminated egg are present in the batch of eggshells produced from an infected but undetected layer flock and moved from a standalone egg-breaking facility. Finally, as most breaking facilities are likely to process eggs from multiple layer flocks, a truckload of wet eggshells would likely contain eggshells from multiple egg production batches from different flocks. For this assessment, we assumed that each batch of wet eggshells from the control area is from an infected but undetected flock.

Inline Breaking Facilities Receiving Eggs from Flocks on the Premises

For an inline facility, there may be no holding time before eggs are broken and wet eggshells are moved off the premises (e.g. eggs are collected by conveyor each morning, washed, and broken). In this case—assuming there is no mixing of contaminated and virus free eggshells—we estimated a contaminated fraction of 7.6 x 10^{-5} (90 percent P.I. 0 to 2.4×10^{-4}) in a batch of wet eggshells moved from the facility. Given the previously stated assumptions, we estimated approximately $10^{5.74}$ EID_{50} (0 to $10^{6.44}$) EID_{50} of HPAI virus in 1,144.5 pounds of wet eggshells produced each day from the flock (Appendix 2). Overall, the simulation models predicted a 81.4% chance that wet eggshells from at least one contaminated egg are present in a batch of eggshells moved from an inline egg breaking facility.

Table 12. Estimated average quantity of HPAI virus infectivity and concentration in liquid INEP from inline and standalone egg-breaking plants.

Movement	**Batch Estimate**	**Inline (without a two-day hold)**	**Standalone (eggs received after two-day hold)**
Once weekly	Total virus quantity in a batch (5308 lb)	$10^{6.18}$ EID_{50} (0-$10^{6.7}$)	$10^{4.32}$ EID_{50} (0-0)
	Average concentration in the batch	0.65 (0-2.15) EID_{50} /ml	0.009 (0-0) EID_{50} /ml
Twice weekly	Total virus quantity in a batch (2654 lb)	$10^{6.17}$ EID_{50}(0-$10^{6.7}$)	$10^{4.32}$ EID_{50}(0-0)
	Average concentration in the batch	1.27(0-4.3) EID_{50} /ml	0.018 (0-0) EID_{50} /ml

9.1.3.2.2 Estimated Concentration of HPAI Virus in Liquid INEP from Infected but Undetected Flocks

INEP may be moved once or twice per week from the egg-breaking plant. Similar to the wet eggshell scenarios, we conservatively assumed that all of the flocks supplying eggs to the breaking plant are in an infected but undetected state. The degree of mixing of inedible egg during storage or transfer is variable as tanks are designed with different mixing capabilities. Even if the storage tanks used do not have agitators, there would be some mixing during the addition of liquid to the tank by force of movement of the liquid by pumps. In the scenario where there is no mixing, HPAI virus contamination would be localized to a very small portion of INEP, given the small proportion of contaminated eggs. Conversely, in the scenario of homogeneous mixing, the virus concentration would become considerably diluted.

Overall, the simulation models predicted a 1.5% chance that inedible egg from at-least one contaminated egg is present in a batch of INEP produced from an infected but undetected layer flock and moved from a standalone egg breaking facility. The models predicted a 28.6% chance that inedible egg from at-least one contaminated egg is present in a batch of INEP moved from an inline egg breaking facility.

Assuming once or twice weekly movement of liquid INEP, and sufficient mixing for the inedible egg to be homogeneous, we estimated the average viral titer in liquid INEP moved from an inline facility to be less than 1.5 EID_{50}/ml (**Table 12**). There is a 99 percent chance that the titer is less than 10^1 EID_{50}/ml.[64]

9.1.4 Conclusion

In this chapter, we used a simulation model of HPAI disease transmission (representative of Asian H5N1 strains) to estimate the number of internally contaminated eggs produced by an infected but undetected layer flock, following the active surveillance protocol, according to the USDA-APHIS-VS Draft Secure Egg Supply Plan. We then estimated the potential amount of HPAI virus in contaminated wet eggshells and liquid INEP separately for inline breaking plants and for plants that source eggs from offline operations. Here, we summarize the results while also considering the extent of mixing that might occur in storage and during handling.

Wet Eggshells

- The stochastic simulation models predicted a 37.4% and 81.4% chance that some HPAI virus is present in a batch of wet eggshells produced using eggs from an infected but undetected flock and moved from a standalone or inline egg-breaking facility respectively.
- The total quantity of HPAI virus in a 1144.5 pound batch of wet eggshells moved from a standalone or inline egg-breaking facility receiving eggs from infected but undetected flocks was predicted to range from 0 and $10^{6.44}$ EID_{50} for movement from inline breaking facilities, and from 0 and $10^{5.70}$ EID_{50} for movement from standalone egg-breaking facilities. This contamination is expected to be localized to a small fraction of wet eggshells as there is no specific mixing step in their processing.
- The estimated fraction of wet eggshells that is contaminated with HPAI virus, moved from a breaking facility, was 9×10^{-6} (90 percent P.I, 0 to 3×10^{-5}) for offline plants, and 7.6×10^{-5} (0 to 2.4×10^{-4}) for inline plants, when mixing is not considered. In an explorative scenario where HPAI virus becomes diluted into a 2- pound portion of the batch due to mixing during handling, the mean virus concentration within this portion is predicted to be less than $10^{3.48}$ EID_{50}/g using simulation results.

Inedible Egg Product

- The stochastic simulation models predicted a 1.5% and 28.6% chance that some HPAI virus is present in a 2654 lb batch of INEP produced from an infected but undetected layer flock and moved from a standalone or inline egg-breaking facility respectively.
- The total quantity of HPAI virus in a 2,654 lb pound batch of INEP produced from a standalone or inline egg-breaking facility receiving eggs from infected but undetected flocks was predicted to be $10^{4.32}$ (90% P.I., 0-0) EID_{50} and $10^{6.17}$ EID_{50}(90% P.I., 0-$10^{6.7}$) EID_{50} for movements from standalone and inline egg-breaking facilities respectively.

- Data on the extent of mixing of INEP during handling and storage are unavailable. In an explorative scenario with homogenous mixing, the estimated average concentration of HPAI virus in a 2654 lb batch of INEP moved from a standalone or inline egg-breaking facility was 0.018 (90% P.I., 0-0) EID_{50}/ml and 1.27(90% P.I., 0-4.3) EID_{50} /ml respectively.

9.2 Likelihood of Dry Eggshells Moved from an Inline Egg-breaking Facility Being Contaminated with HPAI Virus

Likelihood of Dry Eggshells Moved from an Inline Egg-breaking Facility Being Contaminated with HPAI Virus

Risk Factors: HPAI contamination of wet eggshells; potential HPAI contamination of egg-breaking plant surfaces via personnel with duties in the henhouse, or those who have contacted eggs, liquid egg, or wet eggshells; personnel contacting dry eggshells

Current Preventive Measures: Segregation of duties between employees working in the henhouse and those working in egg-processing areas; egg-breaking plant design and operation guidelines from 9CFR590

Future Preventive Measures: Targeted active surveillance protocol described in the SES Plan; drying process that reduces moisture content of incoming wet eggshells to 4 percent, or lower, with an exhaust air temperature greater than 200°F

Conclusions: The likelihood of HPAI contaminated dry eggshell being moved from an inline breaking facility is *negligible*.

9.2.1 Background Information

A considerable proportion of wet eggshells produced at egg-breaking plants are dried and incorporated into poultry feed as a calcium source. In this section, we evaluate the likelihood of moving HPAI contaminated dry eggshells from an inline egg-breaking facility with an infected but undetected flock. As described in Chapter 7, eggshells are dried either through rotary drum, or belt type driers, to reduce the moisture content to 1 to 4 percent. Given that dry eggshells are incorporated into poultry rations, the drying process is a critical event, or critical control point, for the risk of HPAI spread. We evaluated the likelihood of HPAI virus not being thermally inactivated given the operational parameters of the drying process. In addition, we also evaluated the risk that dry eggshells, or finished poultry feed, becomes cross-contaminated with HPAI virus from incoming wet eggshells or from employees who may have had direct contact with poultry.

9.2.2 Preventive Measures

9.2.2.1 Current Preventive Measures

Several of the current industry practices in inline egg-breaking plants and egg-processing facilities are preventive factors for this risk event. The key factors considered in the risk evaluation include:

- Enclosed conveyer lines, augurs, and hoppers for handling wet and dry eggshells within the breaking facility

- Segregation of duties between employees working in the henhouse and those working in egg-processing and dry eggshell loading areas
- Breaking plant design and operation guidelines from FSIS regulations 9CFR590

9.2.2.2 Future Preventive Measures

- As described in Section 9.1.2.1 , the active surveillance protocol in the SES Plan reduces the time to detect HPAI infection in the flock.
- The SES Plan requires eggshells to be treated with a drying process that reduces moisture content to 4 percent or less with an exhaust air temperature greater than 200 °F.

9.2.3 Evaluation of Risk

We evaluated this risk pathway in two parts:

a) Likelihood of HPAI virus on wet eggshells not being inactivated with drying.
b) Likelihood that dry eggshells become cross-contaminated with HPAI virus from contaminated wet eggshells before they are moved from an inline egg-breaking facility.

9.2.3.1 Likelihood of HPAI Virus on Wet Eggshells not Being Inactivated by Drying

9.2.3.1.1 Process Conditions for Drying Wet Eggshells and HPAI Virus Inactivation

The objective of this section is to estimate exposure time and temperature associated with the drying process for HPAI virus inactivation in dry eggshells, based on operational characteristics of dryers. **Table 13** summarizes the approximate ranges for the operational conditions of typical drum driers from a dryer manufacturer and egg-processing industry experts. We note that the operational conditions may vary within a certain range among dryers in different processing plants, based upon their design.

Table 13. A range of values for operation of a rotary drier used to dry wet eggshells from a dryer manufacturer and egg-processing industry experts.

Variable	Range from dryer manufacturer	Estimates from egg-processing industry
Average resident time of wet eggshell particles in the dryer	15-20 minutes	___
Feed-flow rate	3,000 - 15,000 lbs/ HR @ 17% moisture	
Length of the dryer	15 - 25 ft. Long	___
Hot gas flow rate	2,000 - 9,000 average cubic feet per minute	___
Inlet air temperature	800 –1200 ° F (427–649 °C)	1000 –1500 ° F (539–815 °C)
Outlet air temperature	200 – 230 °F (93–110°C)	210-220 ºF (98.9–104 °C)
Outlet dry eggshell temperature	175 –180 °F (79.4–82.2 °C)	158–200 °F (70–93.3 °C)
Outlet moisture content		Average 1 to 4%

The key operational parameters impacting the inactivation of HPAI virus by the drying process include the average resident time and temperature of wet eggshells in the dryer. The average resident time refers to the mean duration that eggshells are present in the dryer. The resident time is difficult to measure directly and depends on dryer design and operational parameters, such as the length of the drier, speed of rotation, angle of inclination, hot gas flow rate, etc. However, in general, for a specific outlet temperature, the outlet moisture content would provide an indirect measure of the resident time. **Figure 16** shows the temperature and moisture content profiles for rotary drying of wood chips. The plots from this figure indicate that achieving a lower final moisture content, would require a greater dryer length, which is proportional to the resident time.[1] For this evaluation—based on discussions with eggshell dryer manufacturers—we considered a resident time of 15 to 20 minutes for wet eggshells. In addition, process conditions expected to produce outlet moisture content of 4 percent or less would also ensure adequate resident time.

The temperature of eggshells is an important operational factor impacting the inactivation of AI viruses in the dryer. In general, the temperature of solid particles increases very rapidly in the initial segments of the drier and is reasonably close to the outlet eggshell temperature. The general profile of particle and gas temperatures and particle moisture content at various points in a dryer from Kamke and Fredrick (1984) are shown in **Figure 16**[1]. From this figure, we observe that the particle temperature approaches the outlet temperature (also called “drying temperature”) well before the first 1/3rd of the length of the dryer. Other simulation and observational studies of concurrent[a] rotary driers have found a similar shape for the temperature profiles of the drying substrate.[65, 66] . The concurrent dryers are those where the hot gas and the solid particles flow in the same direction through the drier. The concurrent dryers are most frequently used for drying

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wet eggshells. Considering this general particle temperature profile in a dryer, and based on discussions with dryer manufacturers, we assumed that the eggshell temperature is relatively similar to the outlet temperature of 70 to 93 ºC (158 to 200º F) for at least 10 minutes in our evaluation.

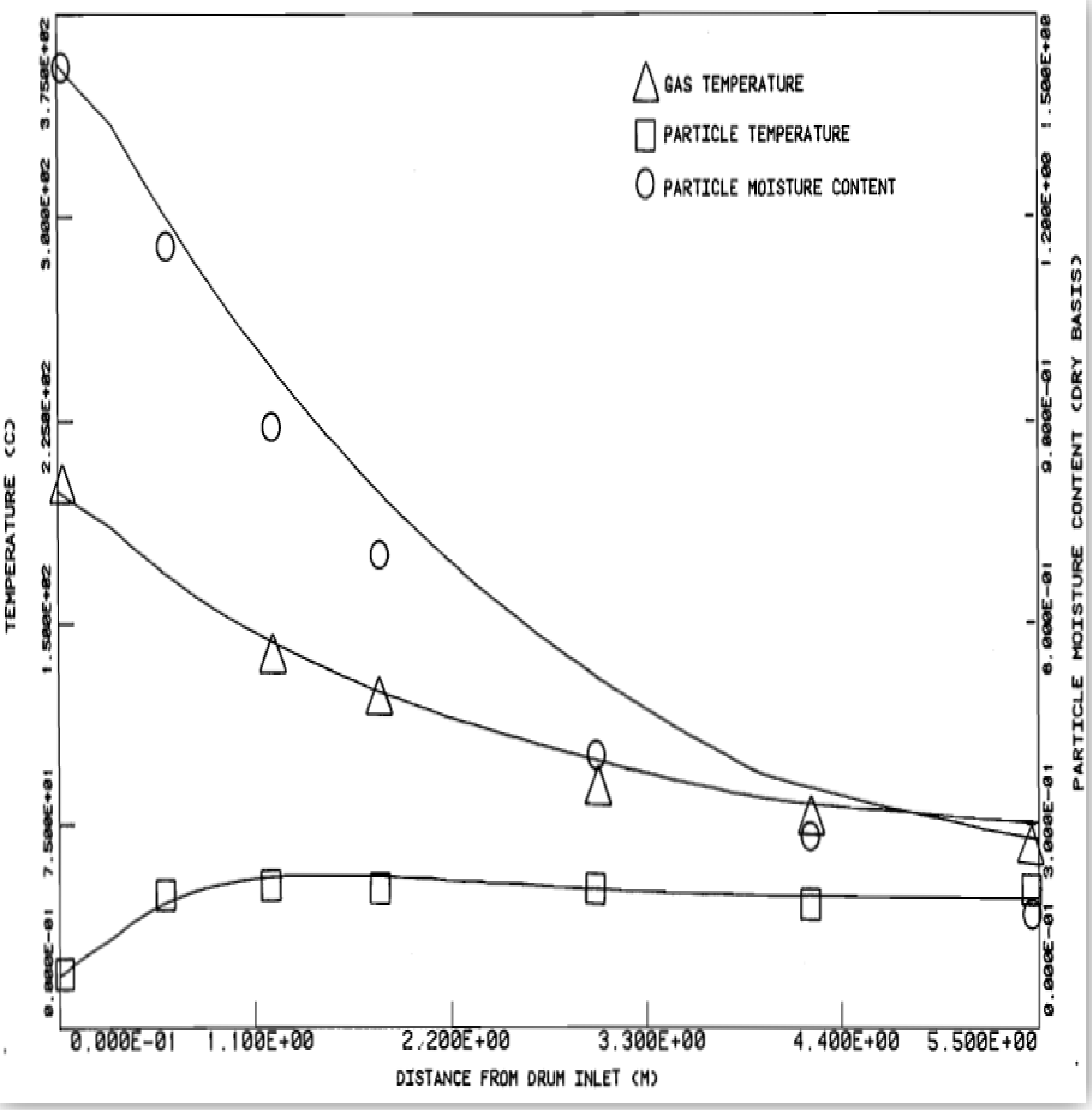

Figure 16. General profile of particle and gas temperatures and particle moisture content at various points in a concurrent rotary dryer from Kamke and Fredrick 1984[1]

We also considered that eggshells would have adequate exposure to hot gas as they are lifted and released by the dryer baffles and would be at a relatively uniform temperature.[61] The thickness of the eggshells, excluding cuticles, is approximately 300 to 340 µm.[67] Given this thickness, we assume that the entire eggshell particle is at a relatively similar temperature.

9.2.3.1.2 Survival of AI Viruses in Poultry Products at High Temperatures

A review of the scientific literature suggests that the survival of AI viruses in poultry products at higher temperatures (55 to 70 ˚C) appears to depend on the type of product (Appendix 4). The most recent studies suggest various types of liquid egg (whole egg, yolk, albumen, and whole-egg blend) have been completely inactivated with more than a 4.9 log inactivation in 19 seconds to 4 minutes at a temperature of 57 to 61 ºC.[68] Earlier studies, using different methodologies, have found inactivation of approximately 10^5 EID_{50}/ml of virus in liquid egg, within 10 minutes, at 56 to 60 ºC.[69] HPAI viruses are least stable in meat and are inactivated within a few seconds at 70 ºC (11). HPAI virus has been most stable in spray-dried egg whites, where 3 hours was required to reduce titers to 10^0 EID_{50} at 67 °C.[68] Avian influenza experts have suggested that this could be due to the lower thermal conductivity of spray-dried egg whites.

We considered inactivation data for AI viruses in liquid egg whites as the closest approximation to inactivation times in wet eggshells with adhering liquid egg (Appendix 7). Given this assumption, HPAI virus in wet eggshells would be expected to be inactivated in less than 4 minutes at 57 to 60 ºC. Avian influenza experts indicated that HPAI virus in eggshells is expected to be inactivated in a few seconds when the core temperature of eggshells reaches 70 ºC.[38] In addition, studies on the thermal inactivation of HPAI virus have also found the Z-value[b] to be between 2 to 5 ºC for various types of liquid egg.[68, 70]. The Z- value is the temperature increase necessary to reduce D_t value one log cycle. D_t value is the time required to decrease the concentration of the virus by 1-log unit, or by a factor of 10. The Z-value suggests that the D_t value at a drying temperature of 70 °C would be at least 2-logs (1/100) lower compared to the value at 60 °C. Consistent with expert opinion and observations in chicken meat, the extrapolation based on Z values indicates that HPAI virus in liquid egg at 10^5 EID_{50}/ml would be inactivated in less than 10 seconds at 70 °C.

As discussed in the previous section, we expect eggshells to be at a relatively uniform temperature during the drying process given their thickness. Considering a resident time of at least 10 minutes at 70 ºC, we conclude that the likelihood that HPAI virus is not inactivated in wet eggshells during drying is *negligible* when the moisture content of wet eggshells is reduced to 4 percent or lower and the exhaust air temperature of the dryer is greater than 200 °F.

9.2.3.2 Likelihood of Dry Eggshells Becoming Cross-contaminated with HPAI Virus before Movement from an Inline Egg-breaking Facility

We considered the following potential pathways for cross-contamination of dry eggshells at an inline egg-breaking facility.

- Employees contact HPAI contaminated wet eggshells, liquid INEP, or non-pasteurized liquid egg and subsequently contact dry eggshells.

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- A sequence of events where: 1) employees working in the henhouse contact breaking plant surfaces or equipment; and 2) HPAI virus from contaminated surfaces is subsequently transferred to dry eggshells via other employees or equipment.

We considered the following factors in our evaluation:

a) Most processes in egg-breaking plants are automated, reducing employee contact with wet eggshells or non-pasteurized liquid egg, and minimizing the chance for cross-contamination. Wet eggshells and liquid egg are usually transferred through closed lines and augurs minimizing the opportunity for contact with employees and spillage. Wet eggshells are moved from the centrifuge by a screw auger into the intake hopper of a drum or rotary dryer where the auger mechanism is fully enclosed by a pipe casing.

b) Dry eggshells are transferred from the dryer to a storage hopper and then to the truck through closed augers and pipes (**Figure 17** and **Figure 18**). Given limited contact, there is limited opportunity for cross-contamination of dry eggshells via employees.

c) Egg-breaking plants are under continuous inspection by FSIS. Egg-breaking room regulations require the floor to be kept clean and reasonably dry during breaking operations and free of egg-meat and eggshells. Most egg-breaking room equipment is cleaned at least once per day. (9CFR Sec. 590.522 Breaking Room Operations)[71].

d) The duties of those working in the henhouse and egg-processing operations are segregated in inline egg-processing facilities and there is limited movement of personnel from the henhouse to egg processing areas and to the dry eggshell loading area.[64]

e) For wet eggshells originating from flocks under active surveillance—as specified in the SES Plan—and because there is no mixing step in their handling, the contamination is expected to be concentrated to a few pounds out of a 1144.5 lb batch. Even in the unlikely scenario where some employees contact wet eggshells (e.g. spillage), the contacted wet eggshells are unlikely to be contaminated.

Figure 17. Auger that moves dry eggshells from a storage silo to the truck.

Figure 18. Covered truck used to transport dry eggshells.

Based on the above qualitative factors, we rated the likelihood of dry eggshells becoming cross-contaminated with HPAI virus before movement from the inline egg-breaking facility as *negligible.*

9.2.4 Conclusion

In this section, we evaluated the likelihood of HPAI contaminated dry eggshells being moved from an inline breaking facility. The main preventive measures considered in the evaluation are:

a) The drying process that reduces moisture content of incoming wet eggshells to 4 percent or lower with an exhaust air temperature greater than 200°F.

b) RRT-PCR testing based on the active surveillance protocol specified in the SES Plan.

Provided the above measures are strictly followed, we conclude that the likelihood of HPAI contaminated dry eggshells being moved from an inline breaking facility is *negligible*.

9.3 Likelihood of the Vehicle or Driver Moving Eggshells or Liquid INEP Being Contaminated with HPAI Virus

Risk Factors: Failure of biosecurity practices for plant personnel and the driver; cross-contamination of other vehicles or drivers at the destination premises; and susceptible poultry is present at the destination premises.

Current Preventive Measures: Good industry manufacturing practices.

Future Preventive Measures (to be implemented by industry during an outbreak)**:** C&D procedures for the vehicle and biosecurity measures and protective clothing for the driver prior to entering premises.

Overall Risk: Provided the above future preventive measures are strictly followed, the overall risk is *low*.

9.3.1 Background Information

This chapter addresses the likelihood of the driver and vehicles used to transport wet eggshells and liquid INEP from a breaking facility being contaminated with HPAI virus. Shipments of wet eggshells may originate from inline egg-breaking facilities with live poultry on-site or from offline egg-breaking plants (see Sections 8.1.1 and 8.1.2, **Table 4**; and **Figure 2**). Wet eggshells may be transported to different types of facilities, depending on the end use. Destinations include egg-breaking plants for drying and processing into feed; agricultural land application as a soil amendment; composting; or disposal in a municipal landfill.

Vehicles moving wet eggshells, or liquid inedible egg, have not been implicated in HPAI spread in previous outbreaks, although in general, movements of vehicles are considered a risk factor. The SES Plan includes general C&D requirements for vehicles and provides specific C&D guidelines for different product movements (e.g., wet eggshells truck or liquid INEP tanker). Risk mitigation measures are applied along each risk pathway (**Figure 2**). We consider these guidelines in our risk evaluation.

9.3.2 Preventive Measures

Future preventive measures from the SES Plan would be implemented during a HPAI outbreak. The relevant preventive measures for this risk event are summarized below.

9.3.2.1 Eggshell Trucks

- The tires and wheel wells must be cleaned and disinfected before leaving the premises of origin within the Control Area.
- The driver is not allowed outside the cab, or else the cab interior must also be cleaned and disinfected (at the breaking plant and at the destination).
- Dump trucks are securely covered with a tarpaulin or equivalent cover.
- The tires and wheel wells must be C&D before leaving the destination premises after delivering wet eggshells. The interior and exterior of the vehicle is C&D after delivering wet eggshells if traveling to a different poultry premises.
- The driver wears protective clothing such as disposable boots, and gloves, and removes

them before getting back into the cab (at the breaking plant and at the destination).

9.3.2.2 Trucks Transporting Liquid INEP

- The tires and wheel wells must be cleaned and disinfected before leaving the premises of origin within the Control Area.
- The driver is not allowed outside the cab, or else the cab interior must also be cleaned and disinfected(at the breaking plant and at the destination).
- The driver wears protective clothing such as disposable boots, and gloves, and removes them before getting back into the cab (at the breaking plant and at the destination).
- The exterior of the vehicle moving liquid INEP is C&D before entering the destination premises.
- The vehicle is C&D after delivering liquid INEP and before returning to a poultry premises.

9.3.2.3 All Vehicle Drivers

- Do not leave the cab or the cab interior must be cleaned and disinfected (at the breaking plant and at the destination).
- If leaving the cab, wear protective coveralls, boots, and head cover while outside the cab and remove them immediately before reentering the cab (at the breaking plant and at the destination).

Figure 19. Dump truck loaded with wet eggshells covered with a tarp.

9.3.3 Evaluation of Likelihood

9.3.3.1 Wet Eggshells

Wet eggshells are moved over the road in dump trucks (**Figure 19**). Typically, these trucks are directly loaded by auger, or from a high-rise storage bin (see Section 8). We considered the following potential pathways for the contamination of the vehicle exterior:

- Spillage of any wet eggshells onto the vehicle exterior or onto ground which may come in contact with vehicle tires.
- Aerosolized dust from infected birds in an adjacent henhouse on an inline operation.

- Through mechanical transport of flies via the wet eggshell truck.
- Through contamination of the loading areas via movement of personnel from processing areas (**Figure 20**).

Figure 20. Grounds around the entrance of the eggshell loading bay.

We consider the following factors when estimating the likelihood of the wet eggshell truck exterior being contaminated:

a) From Section, 9.1.3 HPAI virus is expected to be localized to small portions in a batch of eggshells. Therefore, even if the vehicle exterior or tires contact wet eggshells, it is unlikely that they become contaminated.

b) There is limited potential for the wet eggshell loading area to become contaminated via movement of personnel from other egg-processing areas or through spillage. Eggshells and liquid egg are usually transferred through closed lines (conveyer systems), minimizing the opportunity for spillage. In addition, most processes in egg-breaking plants are automated reducing employee contact with eggshells, or liquid egg. Egg breaking room regulations require the floor to be kept clean and reasonably dry during breaking operations and free of egg meat and eggshells. (9CFR Sec. 590.522 Breaking Room Operations)[71] For inline plants, the duties of those working in the henhouse and egg-processing operations are segregated; there is limited movement of personnel from the henhouse to egg-processing areas and to the wet eggshell loading area.[64] The lower likelihood of contamination of the wet eggshell loading area would reduce the likelihood of vehicle tires or other exterior areas from becoming contaminated.

c) HPAI transmission through flies on wet eggshells, or in the cab interior, is a possibility where wet eggshells are moved from one inline (poultry on-site) facility to another inline facility. Although, there is no evidence for transmission via flies present in vehicles, it has been hypothesized as a possible pathway.[72] There is limited opportunity for flies to access wet eggshells during storage, before movement, as they are held in a closed room within the egg-processing facility, or in an enclosed storage hopper. FSIS regulations 9CFR590.500 also require various sanitary measures to minimize conditions, which engender flies or insects in egg-processing areas and on premises.[73] However, in case of inline facilities, flies may become contaminated via manure from an infected but undetected flock.

   Only a small proportion of flies around a HPAI infected but undetected flock, under active surveillance, is expected to be contaminated (approximately 5 percent of flies in heavily infected flocks). The prevalence of infectious birds a day before HPAI is detected in the flock, when movement of wet eggshells may occur, was predicted to be *low* (0.081, 90 percent P.I., 0 to 0.053). The proportion of contaminated flies is expected to be correspondingly lower given the low prevalence of infectious birds. We qualitatively rated the likelihood of HPAI spread to a susceptible poultry flock, via flies present in vehicles moving wet eggshells to be *low*.

d) A low degree of aerosol contamination of air, in the vicinity of a HPAI infected flock, has been observed in previous outbreaks.[74, 75] Given this observation, there is a possibility of contamination of vehicles via deposition of bio-aerosols, either directly onto the vehicle or onto the ground, which may come in contact with the truck tires. The degree of contamination of the vehicle exterior through this pathway would likely be *low*. In a natural outbreak study, there was a 100 factor (2 log $TCID_{50}$) reduction in HPAI viral titer from the inside of an infected barn (with a high proportion of infectious birds) to the outside of the barn (at a titer of 12 $TCID_{50}/m^3$).[74] The HPAI viral concentration in air, from an undetected farm under active surveillance, would be lower compared to the estimates from the above study due to the lower prevalence of infectious birds (0.081, 90 percent P.I 0-0.53). Based on the above qualitative review, we conclude that the degree of HPAI virus contamination of the vehicle exterior through aerosols from an infected but undetected flock is *low.*

e) The C&D of tires and wheel wells with an EPA registered disinfectant would inactivate potential HPAI virus transferred to them via pathways discussed above, given the susceptibility of AI viruses to detergents and disinfectants[22-24, 76, 77], and documentation of effectiveness by EPA.[78] The likelihood of spillage during transportation is reduced due to covering of wet eggshells with a tarp or cover.

Based on the above evaluation, we qualitatively rate the likelihood of the exterior of the vehicle moving wet eggshells from an egg-breaking plant, receiving eggs from an infected but undetected flock, as being *low*. Note that since the most of the interior surface of the wet eggshell vehicle bed (**Figure 19**) is in direct contact with wet eggshells, we rate the likelihood of contamination of the wet eggshell cargo interior to be *low to moderate.*

The potential pathways we considered for contamination of the vehicle driver are listed below:

- Contact with contaminated wet eggshells.
- The loading floor area or other surfaces become contaminated via spillage of wet eggshells, or via movement of processing plant personnel. The driver subsequently becomes contaminated via direct contact with these surfaces.
- The driver passes across potentially contaminated areas of the egg-breaking plant and becomes contaminated via direct contact.

We considered the following factors in our qualitative evaluation of these pathways.

a) Only a small proportion of eggshells in each movement are estimated to be contaminated given active surveillance. Even if the driver contacts some wet eggshells, they are unlikely to be contaminated.

b) There is limited potential for the wet eggshell loading area to become contaminated via movement of personnel from other egg-processing areas.

c) The requirement to don protective clothing such as disposable boots, gloves, and coveralls and their removal before getting back into the cab would reduce the likelihood of the driver becoming contaminated. Expert opinion and studies of transmission of other viruses (porcine reproductive and respiratory syndrome) indicated that PPE measures are mostly, but not 100 percent, effective in preventing viral transfer to hands and subsequently to environmental surfaces.[79] Potential cross-contamination while removing PPE was suggested as a possible explanation for infectiveness of PPE in a few rare instances.

Based on the above evaluation, we qualitatively rate the likelihood of the vehicle driver moving wet eggshells from an egg-breaking plant receiving eggs from an infected but undetected flock as being *low*. The main pathway for contamination of the cab interior is by contact with the contaminated driver. Similar to the case of the driver, we rate the likelihood of the cab interior of the vehicle moving wet eggshells from an egg-breaking plant receiving eggs from an infected but undetected flock as being *low*. However, we note that the SES Plan includes an additional preventive measure to C&D the cab interior if the driver steps outside the cab.

9.3.3.2 Dry Eggshells

Dry eggshells are loaded by auger into an enclosed trailer as described in Section 8.1.1.6. The pathways for the contamination of the vehicle or driver transporting dry eggshells are similar to those for wet eggshells with the following additional considerations:

a) In Section 9.2.3, we concluded that the likelihood that HPAI virus is not inactivated in wet eggshells during drying is *negligible*. Even in the unlikely case that there is spillage of dry eggshell (loaded by an auger), the likelihood of the portion of dry eggshell that is spilt being contaminated is *negligible*. We conclude that likelihood of vehicle exterior or driver becoming contaminated via spillage of dry eggshells is *negligible*.

b) The exterior of the dry eggshell vehicle is required to be C&D according to the SES Plan. In this case, the entire exterior of the trailer can be C&D since the eggshells are transported in an enclosed trailer. Given the susceptibility of AI viruses to disinfectants, any HPAI virus on the exterior would be inactivated.

We qualitatively rate the likelihood of the contamination of the vehicle or driver moving dry eggshells from an egg-breaking plant sourcing eggs from an infected but undetected flock being contaminated with HPAI virus as being *negligible.*

9.3.3.3 Liquid INEP in Tankers

Unpasteurized INEP is pumped from the holding tank through enclosed lines into tankers for over-the-road transport. Spillage from a system of enclosed lines is unlikely, but some spills may occur from hoses or couplings when the lines are being connected or disconnected from the tanker. The tanker driver does not have to enter the egg-breaking facility to handle INEP apart from the loading docks, which reduces the chances of cross-contamination. From Section 9.1.3.2.2, the expected quantity of virus from a randomly selected gram of liquid INEP from a batch ranged from zero to 4.28 EID_{50}/ml under alternate scenarios. These results suggest that even if the driver’s shoes, gloves, or protective clothing contact some liquid INEP, the degree of contamination is expected to be low. The exterior of the INEP tanker is required to be C&D according to the SES Plan. Given the susceptibility of AI viruses to disinfectants, any HPAI virus on the exterior would be inactivated. We conclude that the likelihood of the exterior of the tanker moving liquid INEP from an egg-breaking facility sourcing eggs from flocks in a HPAI control area being contaminated is *negligible.*

9.3.3.4 Liquid INEP in Carboys

INEP is also transported in large plastic carboys. The carboys are not prone to leakage once filled. Therefore, we assume that contamination of the exterior of the carboy and surrounding floor areas would occur through splashes and spills related to filling. After filling, carboys are then loaded into a trailer by a pallet jack or forklift. During normal operations, the truck driver may participate in loading the trailer. The key pathway for the interior of the trailer being contaminated is the potential for contamination of shoes from spilled INEP on the floor, or on the wheels of the forklift/pallet jack. There is also a potential pathway where the interior of the cab could be contaminated by hands and clothing through contact with surfaces contaminated with liquid INEP on the outside of the carboy. We considered the following factors when estimating the likelihood of the truck driver, truck interior and exterior, and cab interior being contaminated:

a) Given the smaller size of carboys compared to an INEP tanker, there would be less dilution of HPAI virus due to mixing with virus-free inedible egg. Therefore, HPAI virus is expected to be limited to a few carboys, which could potentially be contaminated to a higher virus concentration compared to INEP in tankers.

b) Carboys filled with INEP are moved from the egg-breaking room into a separate storage area where they are held until they are moved off-site. Aerosol contamination of the egg-breaking room, or storage room floors, by dust from the henhouse on an inline operation is unlikely. The henhouse is physically separated from the egg-breaking room and storage area and there is no direct airflow from the henhouse to the egg-breaking room. Therefore, there is no direct pathway of contamination of the wheels of pallet jacks, forklifts, or the shoes of the driver loading the trailer via infectious aerosols from the henhouse.

The likelihood of the driver and the vehicle exterior and interior being contaminated with HPAI virus—at the point of origin of movement of wet eggshells and liquid INEP transported in tankers or carboys—is shown in **Table 14**. When making these likelihood estimates, we assumed that—at a minimum—C&D of tires and wheel wells would occur before leaving premises, as well as C&D of exterior of the vehicle (except for wet eggshells in dump truck beds where only tires and wheel wells are C&D) before entering the destination premises. We also assumed that requirements for the driver to wear PPE were strictly followed if they exit the cab.

Table 14. Likelihood of the driver and vehicle being contaminated at the point-of-origin for each product, assuming that preventive measures are followed.

Source of Contamination	Likelihood		
	Driver	**Vehicle Exterior**	**Vehicle Interior**
Wet eggshells	Low	Low	Low to Moderate (dump truck bed)
Inedible egg (tankers)	Negligible	Negligible	N/A
Inedible egg (carboys)	Low	Negligible	Low

9.3.4 Conclusion

In this chapter, we evaluated the likelihood of the vehicle or driver moving wet eggshells or liquid INEP from an egg-breaking facility being contaminated with HPAI virus while considering the vehicle C&D, driver biosecurity, and other risk mitigation measures from the

SES Plan. Provided the risk mitigation measures from the SES Plan are strictly followed, we have the following conclusions:

- The likelihood of the driver moving wet eggshells from an egg-breaking facility being contaminated with HPAI virus is *low*. The likelihood of the exterior of the vehicle moving wet eggshells (other than the dump truck bed) being contaminated with HPAI virus is *low.*
- The likelihood of the vehicle or the driver moving dry eggshells from an inline egg-breaking facility being contaminated with HPAI virus is *negligible*.
- The likelihood of the driver moving liquid INEP in a tanker from an egg-breaking facility being contaminated with HPAI virus is *negligible*. The likelihood of the exterior of the INEP tanker being contaminated with HPAI virus is *negligible.*
- The likelihood of the driver moving liquid INEP in carboys from an egg-breaking facility being contaminated with HPAI virus is *low.* The likelihood of the exterior of the vehicle moving INEP in carboys being contaminated with HPAI virus is *negligible.* The likelihood of the cargo interior surfaces of the vehicle being contaminated with HPAI virus is *low.*

10. Exposure Assessment

An exposure assessment is the process of describing the biological pathways necessary for exposure of animals to a pathogen hazard, released from a given risk source, and an estimate of the probability of the exposure occurring. [2] The exposure assessment of this risk assessment describes pathways for susceptible poultry to become infected with HPAI virus associated with movement of wet eggshells or INEP from an egg-breaking facility sourcing eggs from table-egg layer flocks in the Control Area. The exposure assessment begins after the movement of wet eggshells and INEP for disposal (e.g., land fill) or other end uses (incorporation into poultry feed, pasteurization, land application etc.). We organized the exposure assessment into four main pathways as follows.

1. Risk that spreading wet eggshells for agricultural land application results in HPAI spread to poultry.
2. Risk that the movement of wet eggshells to a standalone egg-breaking plant for drying results in HPAI spread to a susceptible poultry flock.
3. Risk that disposal of wet eggshells in landfills exposes susceptible poultry.
4. Risk that movement of liquid INEP to a pasteurization facility results in HPAI spread to poultry.

10.1 Risk that the Movement of Wet Eggshells to an Agricultural Land Application Site Results in HPAI Spread to Susceptible Poultry

Risk that the Movement of Wet Eggshells to an Agricultural Land Application Site Results in HPAI Spread to Susceptible Poultry

Risk Factors: Application or storage of wet eggshells in close proximity to susceptible poultry

Current Preventive Measures: Industry good manufacturing practices

Future Preventive Measures (to be implemented by industry during an outbreak)**:** Active surveillance protocol, in conjunction with the two-day holding time before the movement of eggs for offline facilities. For inline operations, wet eggshells are held for 48 hours at the point-of-delivery before land application. The land application site for wet eggshells is at least a distance of 3 km away from premises with commercial poultry. The vehicle driver wears PPE.

Overall Risk: Provided the above additional preventive measures are strictly followed, the overall risk is *negligible.*

10.1.1 Background Information

In this chapter, we evaluate the risk of HPAI spread to other poultry premises via movement of wet eggshells for agricultural land application. Wet eggshells are useful as a liming source to reduce the pH of soil and can be applied directly, or mixed with manure, and then subsequently applied. Spreading contaminated manure or chicken litter on nearby fields has been implicated as a risk pathway in previous HPAI outbreaks.[75] Potential component pathways for HPAI spread to susceptible poultry associated with the land application of wet eggshells including aerosols, flies, wild mammals or cross-contamination of other vehicles and equipment destined for other poultry premises. In our evaluation, we consider the land application site to be some minimum distance away from poultry flocks as a future (outbreak) risk mitigation measure.

10.1.2 Future Preventive Measures

- The active surveillance, holding time, vehicle C&D, and driver biosecurity measures—as evaluated in the entry assessment—are relevant mitigation measures for this risk event.
- The land application site for wet eggshells is at least a distance of 3 km away from poultry production facilities. This measure acts as a mitigation measure for transmission pathways associated with aerosols and flies.
- Wet eggshells from an inline egg-breaking facility are required to be held at the destination premises for 2 days before land application.

10.1.3 Evaluation of Risk

10.1.3.1 Risk of Susceptible Poultry Becoming Infected due to Transmission of HPAI Virus from Wet Eggshells that are Land Applied via Flies

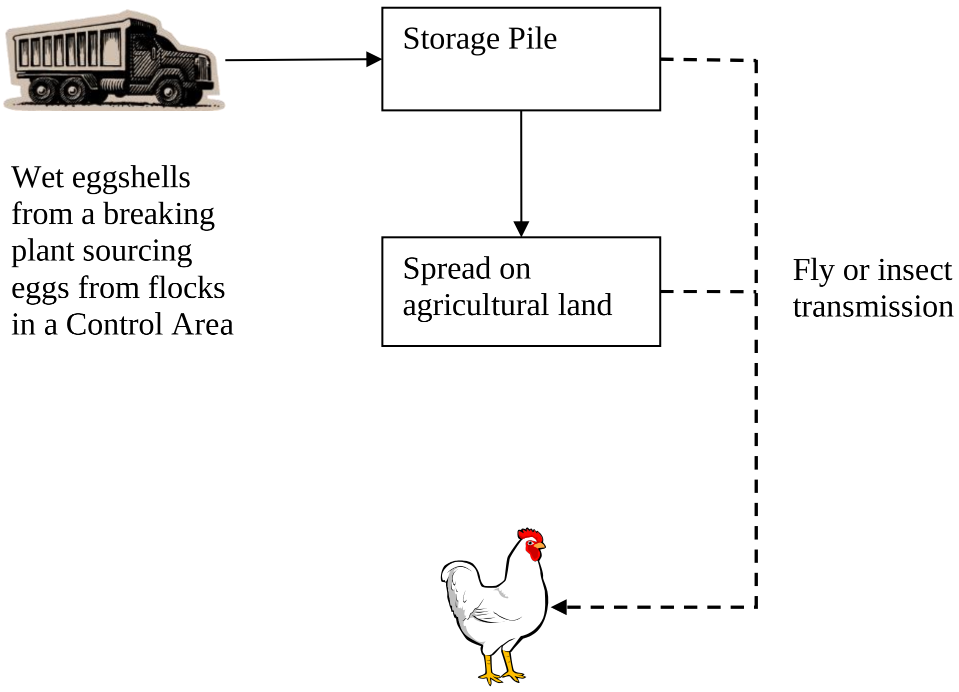

Figure 21. Exposure pathway for HPAI spread to susceptible poultry via fly or insect transmission from wet eggshells.

Land application on agricultural land to increase soil PH as a replacement for lime is a key end use option for wet eggshells. Typically, wet eggshells are held in storage piles or stacks at farm headlands (area near the farm border) before land application in spring or fall. The storage duration before land application is variable depending on the status of the farm and the time of the year with the longest holding time occurring in winter. The land application rates may vary between 2 to 7 tons per acre depending on the soil PH.

The risk pathway evaluated here involves potential transmission of HPAI virus from eggshells at the land application site to susceptible poultry on nearby farms via flies (**Figure 21**).

10.1.3.1.1 Implication in Previous Outbreaks

Blow flies (Calliphoridae) and house flies (Muscidae) are reservoirs and vectors of a wide variety of pathogenic organisms affecting poultry.[80] Flies have been implicated, although not conclusively demonstrated, as a vehicle for HPAI virus transmission between poultry flocks. Transmission through flies should be considered a possibility; however, as HPAI virus was isolated from flies near infected henhouses during the 1983-84 HPAI H5N2 outbreaks in Pennsylvania.[75] A study of 300 pools of insects found HPAI virus could be isolated from 7.7 percent of pools of house flies, 2.8 percent of pools of black garbage flies, and 2.5 percent of pools of small dung flies. Flies were implicated as a possible source of transmission for several flocks during the outbreak.

Blow flies were also considered a potential transmission route in the 2004 HPAI H5N1 outbreak in Japan.[81, 82] In this outbreak, HPAI virus genes could be detected via PCR (matrix gene and H5) in up to 24 percent of flies surrounding the infected premises (2.3 km radius). The prevalence of H5 virus genes was highest in blow flies collected 600 to 700 m from the infected farm (20 to 30 percent), and HPAI virus gene positive flies (10 percent) could be detected up to two kilometers from the infected premises. Viable virus was isolated from 2 of 10 gene-positive flies that were tested. We estimated that prevalence of viable virus (as opposed to PCR + ve results) was 5 percent in flies around the epidemic area.[82]

10.1.3.1.2 Survivability of HPAI Virus in Flies

Sawabe *et al.* also evaluated the survivability of H5N1 virus in blow flies after experimental exposure at 10 and 20°C over a period of 14 days.[83] Viable virus was recovered in the crop and intestine up to 24 hours post exposure, and at 48 hours in feces and vomit matter. However, there was a steady decrease in viral titers from the gut contents with time. Most of the flies had viral titers below the level of detection for the assay (0.50 log $TCID_{50}$/0.05 ml of fly homogenate using MDCK kidney cells) at 24 hours. All of the flies had viral titers below the level of detection at 48 hours post exposure.

Wanaratana *et al.* evaluated the potential of the house fly to serve as a mechanical vector of the H5N1 virus.[84] Virus isolation revealed that H5N1 virus could survive within the body of the house fly and remain infective for up to 72 hours post-exposure. The viral titers in a house fly homogenate varied between $10^{5.43}$ EID_{50}/ml at 6 hours post exposure and to 10^{2} EID_{50}/ml at 72 hours post exposure.

Infective LPAI virus (H7N1 and H5N7) was isolated from the alimentary tract of house flies for at least 24 hours post feeding, but the level isolated depended on temperature, time period post feeding, and quantity of virus ingested. Only 3 percent of flies (one group out of 36 groups tested) was found virus positive after 24 hours at 25 and 35 °C.[85] In summary, the experimental studies show that house flies and blow flies can ingest infective quantities of AI viruses with the viability of the virus being relatively low after 24 hours. However, the virus may remain viable for up to 48 to 72 hours in a small number of cases.

10.1.3.1.3 Dispersion Rate of Flies

Table 15 summarizes data on the dispersion rate of blow flies and house flies. The experimental data indicate that house flies, as well as blowflies, may travel between 1-3 km/day in most cases. House flies tend to remain close to the breeding site (an approximate radius of 328 to 1,640 feet) as long as they find suitable food, breeding sites, and shelter.

Table 15. Reported dispersal rates for flies implicated in the mechanical transmission of H5N1 HPAI.

Common Name	Reported Dispersal Rates	Reference
House fly	1–3 km / day	Herms *et al.*[86]
House fly	Generally range less than 2 miles (3.2 km); range in a radius of 328-1,640 feet from breeding site if suitable food available; only 8-30% disperse beyond a poultry facility	Stafford [87]
Blow fly	Estimated 1250 –1789 m/day on average	Tsuda *et al.* [88]
Blow fly	2–3 km in 24 hours	Sawabe *et al.* [82]

10.1.3.1.4 Qualitative Factors Considered in Determining the Risk Rating

- For wet eggshells originating from flocks under active surveillance—as specified in the SES Plan—the contamination is expected to be concentrated to a few pounds out of a 1144.5 lb batch because there is no mixing step in their handling. Field studies by Brugh and Johnson [75], and Sawabe *et al.*[81] indicate around 5 percent of flies from a severely infected poultry farm could be infectious. The percentage of flies foraging and breeding around a pile of wet eggshells that are contaminated would be lower, compared to that in field studies around infected flocks. As an example scenario, if we consider around 5,000 infectious birds in the infected flocks from these studies, and then assume around 80 grams of contaminated manure per bird, the amount of infectious material in 1,144.5 pounds of wet eggshells would be a very small fraction (less than 1/12,000) of the amount of infectious material exposed to flies in field studies[c]. The percentage of contaminated flies around the wet eggshells is also expected to be considerably lower than the 5 percent as observed around heavily infected flocks.
- The SES Plan requires wet eggshells from an inline egg-breaking facility to be held onsite for a minimum of 2 days before land application. The actual holding time might be higher in practice as wet eggshells are only applied once or twice a year at each farm site. For

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[c] The total EID_{50} in contaminated manure from field studies was estimated as 5,000 (birds) * 80 (gm manure/bird)*(10^5 EID_{50}/gm manure) = $4*10^{10}$ EID_{50}. Based on confidence interval from simulation results (entry assessment, $10^{6.5}$ EID_{50} was considered a conservative estimate of the quantity of HPAI virus present in a 1,144.5-pound load of wet eggshells.

eggshells held in a pile, the exposed surface area that flies can access would be lower than for eggshells that are spread. The holding time increases the likelihood that HPAI infection is detected before eggshells are spread.

- There is a lack of direct evidence of transmission of AI virus via live flies. Although experiments have shown that transmission of AI virus via flies is a potential pathway (flies can ingest and carry viable virus for 24 to 48 hours and can fly 1 to 3 km in that time period), there has been no evidence of chickens becoming infected by directly feeding on live contaminated flies. An experimental attempt to demonstrate fly transmission to chickens was unsuccessful partly owing to technical difficulties. Feeding dead flies (*C. nigribarbis*) contaminated with H5N1 virus did not result in transmission (unpublished data) [61]. However, the frozen dead flies were not attractive to chickens and only small numbers of flies were consumed in this experiment [61].
- The intra-gastric infectious dose for HPAI virus in chickens is relatively high. Wanaratana *et al.*, have found a considerable decrease in the external HPAI virus concentration on an exposed fly within 24 hours. While HPAI virus is deactivated at a slower rate in fly gut content, the likelihood of infection due to the virus encapsulated in the fly gut would be reduced due to the higher infectious dose for the intra-gastric route. Kwon and Swayne 2010 found a CID_{50} of $10^{6.2}$ EID_{50} for the intra-gastric route in chicken.[45] Sergeev *et al.*, 2012 found an infectious dose above 10^5 EID_{50} for the intra-gastric route[48].
- The land application site, or a temporary holding location for the wet eggshells, is required to be at least 3 km away from commercial poultry establishments. Even in the unlikely event that a few flies have ingested HPAI virus from wet eggshells, there is a low likelihood of the flies travelling to the poultry facility within a day (3 km is a higher value for the effective or straight-line distance traveled by a fly in a day).
- Wet eggshells from a standalone egg-breaking facility have a lower likelihood of being moved before HPAI infection is detected given the holding time before eggs from offline farms are brought into the facility. The estimated quantity of HPAI virus present in wet eggshells moved from standalone egg-breaking facilities was $10^{4.52}$ EID_{50} (0 to $10^{5.32}$ EID_{50}). This estimate is lower than the corresponding value from eggshells moved from inline facilities.

We rated the risk of susceptible poultry becoming infected via contaminated flies mechanically transmitting HPAI virus from the wet eggshell land application site that receives eggshells from an inline egg-breaking facility—to be *negligible.* We rated the risk of susceptible poultry becoming infected via contaminated flies mechanically transmitting HPAI virus from the wet eggshell land application site that receives shells from a standalone egg-breaking facility—to be *negligible.*

10.1.3.2 Risk of Susceptible Poultry Becoming Infected due to Transmission of HPAI Virus from Wet Eggshells that are Land Applied via Aerosols

Wet eggshells may be land applied either directly or mixed with manure. Typically, manure spreading equipment, such as box spreaders or slingers, is used in their application (**Figure 22**). The slinging of the material during spreading causes the potential to generate aerosols. Aerosols generated during the spreading of manure and other biosolids have been hypothesized as potential means of transmission for various microorganisms, including viruses. Hence, land application of wet eggshells is a potential risk pathway for HPAI spread to nearby poultry flocks. We evaluated this risk by using conservative aerosol-dispersion model scenarios and qualitative review of factors.

Figure 22. Spreader equipment used for land application of wet eggshells.

10.1.3.2.1 Exploratory Dispersion Modeling Scenarios for Aerosol Transmission of Avian Influenza Virus Due to Land Application of Wet Eggshells

Aerosol dispersion models have been extensively used to predict aerosol particle concentrations at different distances from a generating sou of the concentration of particles from a gen meteorological conditions. Recently, aeroso pathogen transport through bioaerosols generated during land application of biosolids. The concentration of bioaerosols at a specific distance from a source depends on factors such as:

a) The source emission rate, which is the relative amount of particles/species emitted by the source per-unit-time depending on the aerosolization process.

b) Dispersion, or dilution, of the particles given the wind speed, meteorological conditions, and local terrain topography.

c) The depletion of particles due to settling, or due to precipitation given the particle size distribution.

d) The decay of microorganisms in bioaerosols with time, depending on their survival ability in aerosols and environmental factors.

The dispersion models most frequently used can be categorized into two main groups: 1) steady-state plume models[93], and 2) non-steady-state particle dispersion trajectory models.[94] The steady-state model is adequate for scenarios where a source is emitting at a constant rate and there are no time or space varying wind fields. Direct data on aerosol generation rate and characteristics are not available for land application of wet eggshells. In this section, we develop conservative estimates of the potential aerosol concentration at different distances from a land application site based on simplifying assumptions and using data on the land application of other biosolids as a proxy. The conservative approach enables the results to be broadly applicable for a wide range of meteorological conditions and reduces the data collection effort. The scenarios developed here support the qualitative review of this pathway presented in the next section.

The key conservative assumptions used in this section are summarized below:

- The use of a steady-state plume model to estimate aerosol concentration, given that only a small portion of wet eggshells (1 to 5 pounds) are expected to be contaminated and may result in a transient release of HPAI contaminated bioaerosols.
- The AERSCREEN model utilizes cautious meteorological parameters to produce conservative estimates of bioaerosol concentrations at various distances from the source.
- Aerosol depletion due to dry deposition (gravitational settling) or inactivation of HPAI virus is not considered. Data on the particle size information for aerosols generated during spreading of wet eggshells is unavailable. The particle size is a critical factor that influences the risk of aerosol transmission. Here, we conservatively assume that all generated particle sizes during spreading are very small resulting in minimal gravitational settling.
- The wind direction is along a straight line to the poultry premises from the land application premises.

The EPA recommends the use of the AERMOD steady state plume dispersion modeling system for regulatory applications to determine pollution exposure.[95] The AERMOD system takes into account various meteorological factors and terrain attributes such as wind-speed, temperature, and terrain profile in predicting the concentration of pollutants.[96] The AERSCREEN model is a conservative screening version of AERMOD, which utilizes conservative meteorological data and terrain assumptions and is recommended by EPA for use in risk assessments.

We considered 3 AERSCREEN scenarios for estimating the HPAI virus concentration at a distance of 3 or 8 km from the land application site for wet eggshells as shown in **Table 16**. Scenario 1 and Scenario 2 consider a two-day hold before land application of wet eggshells while eggshells are land applied after one day of holding in Scenario 3. We consider that there is limited mixing resulting in dilution of HPAI virus into 1 to 5 pounds of wet eggshells. Details of the parameter estimation for the scenario analysis are provided in Appendix 8.

Table 16. Input parameters used in conservative AERSCREEN scenarios to predict HPAI virus concentration at different distances from the wet eggshell land-application site from infected but undetected table-egg layer flocks.

Parameter description	Source/Formula	Scenario 1	Scenario 2	Scenario 3
Total quantity of HPAI virus in 1,144.5 pound batch of wet eggshells Q (EID_{50})	Estimated from disease transmission and surveillance models	$10^{4.84}$	$10^{5.74}$	$10^{5.95}$

Pounds of contaminated eggshells among 1,145.5 pounds of wet eggshells P	Assumption related to mixing of contaminated and virus free eggshells	3	5	1
Slinger equipment spreading rate of wet eggshells S (pounds/second)	Paez-Rubio *et al.*, 2007[97]	4	4	4
Aerosolization efficiency (E)	Paez-Rubio *et al.*, 2007, EPA Spray Irrigation experiments.[97]	0.0000076	0.000123	0.0033
Aerosol source rate (EID_{50}/s)	$\frac{S*Q*E}{P}$	0.7010	54.074	11764
Source duration (seconds)	P/S	0.75	1.25	0.25
Minimum wind speed (m/s)	Conservative Assumption	0.5 m/s	1m/s	1m/s
Terrain type	Assumption	cultivated	cultivated	cultivated

The results shown in **Table 17** and **Figure 23** indicate very low concentrations of HPAI virus at both 3km and 8 km distances in all the scenarios due to land application of wet eggshells from infected but undetected premises. The mean 50 percent chicken infectious dose (CID_{50}) with intranasal inoculation for various HPAI strains (reviewed in Swayne and Slemons, 2008) mostly ranged between 100 to 7,943 EID_{50} for 3 to 4 week old chickens.[42] Also, note that the emission and exposure duration is less than a couple of seconds in these scenarios, given the small quantities of contaminated wet eggshells estimated in Chapter 7. **Table 17** shows the overall estimated dose for a susceptible chicken assuming 4 CFM air requirement and exposure duration calculated previously.[98] The estimated overall dose is relatively small and suggests that it is unlikely for a susceptible chicken to become infected through this pathway.

Table 17. Estimated dose of HPAI virus to a susceptible laying hen (EID_{50}) from 3 AERSCREEN scenarios—at different distances from the land application site of wet eggshells—assuming a ventilation rate of 4 CFM per chicken.

	Estimated HPAI exposure EID_{50}		
Distance from Source	**Scenario 1**	**Scenario 2**	**Scenario 3**
3 km	1.4×10^{-6}	7.08×10^{-5}	3.7×10^{-3}
8 km	2.82×10^{-7}	1.25×10^{-5}	6.1×10^{-4}

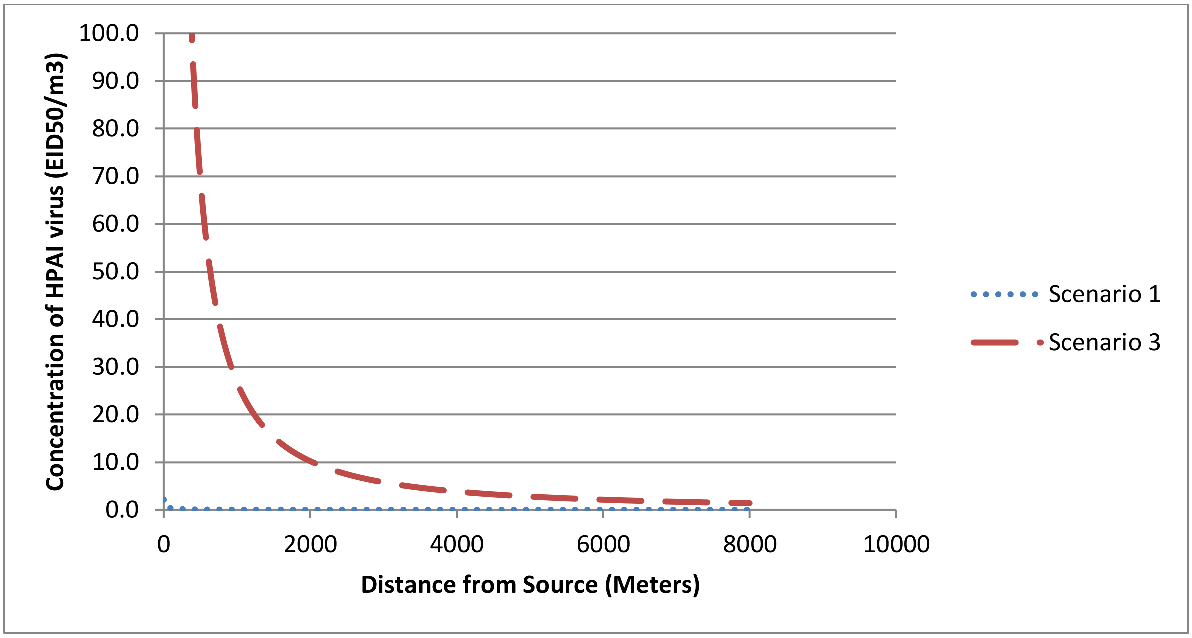


Figure 23. Conservative estimates of HPAI concentration at different distances from the wet eggshell land application site.

10.1.3.2.2 Qualitative Factors Considered in Determining the Risk Rating

- Several experimental studies indicate that airborne transmission of HPAI infection between chickens on the same premises is possible but inefficient (by inefficient, we mean a slow rate of spread). Specifically, experimental studies showed aerosol transmission was not efficient from a cage with only one or two infectious chickens to chickens in adjacent cages. Transmission was efficient from a cage housing four or more infectious chickens to those in adjacent cages.[18,99] Given this inefficient transmission, some experts have suggested that long-range aerosol transmission of HPAI virus between different premises is unlikely.
- Based on disease transmission and surveillance model results and due to low extent of mixing, only a small fraction of wet eggshells in a 1,144.5-pound batch is likely to be contaminated. The two-day holding time would further reduce the likelihood of contaminated wet eggshells being land applied before HPAI infection is detected in the flock.
- Experimental studies indicate only a very small proportion of the biosolids and the microorganisms present in them are aerosolized during their land application (**Table 13**).

Figure 24. Close-up of wet eggshells intended for land application.

Similarly, we expect a very small proportion of HPAI virus present in wet eggshells to be aerosolized during land application.

- Scenarios of aerosol dispersion models using conservative assumptions and metrological parameters (AERSCREEN) suggest the concentration of any HPAI virus aerosolized during land application of wet eggshells would be considerably diluted before a distance of 3km from the land application site and has a *low* likelihood of being infectious to susceptible chickens.
- The reduction in concentration due to gravitational settling of aerosolized particles was not considered in AERSCREEN model scenarios. However, we note that wet eggshell pieces are coarse particles and would likely settle before being dispersed over long distances (**Figure 24**).[100] However, it is ambiguous whether any egg protein from liquid egg would be aerosolized during land application.

Overall, we conclude that the risk of susceptible poultry becoming infected via aerosols generated during land application of wet eggshells is *negligible* provided that there is a two-day hold after production of eggs before wet eggshells are land applied and a 3 km minimum distance between the land application site and other susceptible poultry premises.

10.1.3.3 Risk of Susceptible Poultry Becoming Infected due to Transmission of HPAI Virus from Wet Eggshells Moved to Land Application via Wild Mammals

Thus far, wild mammals have not specifically been implicated as a biological vector for AI viruses, although mechanical transmission has been considered a possibility. The spotting of raccoons, possums or foxes near poultry houses has been identified as a risk factor (odds ratio 1.9 in multivariate analysis) during the 2002 LPAI H7N2 outbreak in Virginia[101]. Mechanical transmission was proposed as a possible explanation for the risk factor in that study. In this section, we evaluate the risk pathways for HPAI virus spread from wet eggshells moved for land application through mechanical or biological transmission associated with mammalian wildlife.

We considered the following factors in evaluating the risk through mechanical transmission.

- The transmission pathway would likely involve successive virus transfer steps between contact surfaces. For example, a fox or raccoon may act as a mechanical vector and transfer small quantities of eggshell onto the poultry premises or its surroundings. Subsequently, the virus may be transferred into the poultry barn through movement of equipment or farm personnel. The final surface concentration of HPAI virus transferred through such contact steps would be diluted through multiple steps. Only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface in each direct contact event (*personal communication*, Dr. Sayed Sattar; Dr. Susan Springthorpe).[16, 102-105]. The final quantity of virus transferred through such pathways is also likely small. We note that the number of virus transfer steps and the transmission risk could be higher for free range or backyard poultry compared to poultry raised in commercial barns.
- Only a small proportion of wet eggshells moved from an egg-breaking facility sourcing eggs from flocks in the control area are expected to be contaminated. Given this, even if we consider that the extent of mixing and dispersion of HPAI virus into a batch of eggshells is unknown, and that a susceptible chicken is exposed to a gram of wet eggshell

randomly taken from this batch, the upper bound on the probability of infection is 1.1 percent based on the exponential dose response model (Appendix 12). The likelihood of infection of the exposed chicken decreases with reduced mixing where the virus is localized to smaller volumes (under exponential dose response model). For example, in the scenario where HPAI virus is present in a 5 pounds segment within the batch and a chicken is exposed to 1 gram of wet eggshells from this batch, the predicted probability of infection is 0.18 percent (95 % P.I., 0- 0.4 percent).

We qualitatively rated the risk of HPAI virus spread from wet eggshells moved for land application through mechanical transmission associated with mammalian wildlife to be *negligible* to *low*.

We considered the following factors in evaluating the risk through biological transmission.

- This risk pathway involves the following steps: 1) The wild mammal eats a sufficient quantity of contaminated wet eggshells to become infected; 2) the wild mammal sheds virus in quantities that are infectious to poultry at the poultry premises; and 3) the virus is transferred into the poultry barn through movement of equipment or farm personnel and infects poultry.
- Bird eggs are a part of the diet of foxes and raccoons and they are opportunistic animals that feed on whatever is available. However, wet eggshells that have been centrifuged to recover inedible egg have low protein content (5-6 %) and calorific value (approximately 90 Kcal/pound)[60]. The majority of wet eggshell weight is due to calcium carbonate. In an experimental study, a fox meal intake averaged around 320 gm per day with a maximum of 540 gms when prey was available [106]. Given the low protein and caloric content, it is ambiguous whether wild mammals would consume considerable quantities of wet eggshells.
- As discussed in the entry assessment, given that the HPAI virus contamination is likely to be localized to a small fraction of the 1144.5 lb batch of wet eggshells (2-5 pounds), the likelihood of wild mammals accessing the contaminated portions of the eggshells would be *low*, even if they eat some wet eggshells from the storage pile at farm headlands.
- While foxes and other mammals have shown to become infected via oral consumption of high doses of HPAI contaminated material (e.g., uncooked infected chicken meat), there are limited dose response data for the oral route at low doses. As discussed in Section 7.7, available data in chickens and mice suggests a higher infectious dose for the oral and intra-gastric routes compared to the intranasal route.

We qualitatively rated the risk of HPAI virus spread from wet eggshells moved for land application through biological transmission associated with mammalian wildlife to be *low*.

10.1.4 Conclusion

We considered the following key measures from the SES Plan in evaluating this pathway:

- The land application site for wet eggshells is at least a distance of 3 km away from poultry production facilities.
- Wet eggshells from an inline egg-breaking facility are required to be held at the destination premises for two days before land application.

Provided that the preventive biosecurity measures from the SES Plan are strictly followed, we conclude that:

- The risk of susceptible poultry becoming infected via contaminated flies transmitting HPAI virus from the land application site of wet eggshells moved from an inline breaking facility to be *negligible.*
- The risk of susceptible poultry becoming infected via aerosols generated during land application of wet eggshells is *negligible.*
- The overall risk of movement of wet eggshells to an agricultural land application site resulting in HPAI spread to susceptible poultry is *negligible.*
- The risk of susceptible poultry becoming infected due to transmission of HPAI virus from wet eggshells moved to a land application site via wild mammals is *low*.

10.2 Risk that the Movement of Wet Eggshells to Another Egg-breaking Plant for Drying Results in HPAI Spread to a Susceptible Poultry

Risk Factors: HPAI contamination of employees or equipment used to handle eggshells.

Current Preventive Measures: Industry good manufacturing practices described in 21CFR110; dry eggshell storage and handling areas are relatively separated from wet eggshell handling areas; enclosed handling system for dry eggshells.

Future Preventive Measures (to be implemented by industry during an outbreak)**:** Active surveillance as specified in SES Plan; the vehicle driver wears PPE; C&D of vehicle.

Overall Risk: *low*, provided the above additional preventive measures are strictly followed.

10.2.1 Background Information

In some cases, wet eggshells may be transported to a different egg-breaking plant or processing facility for drying. Based on the evaluation in Chapter 9.2, drying wet eggshells under process conditions specified in the SES Plan would be effective for inactivating HPAI virus. When the receiving egg-processing facility is standalone, there may be a possibility of cross-contamination of dry eggshells used as a poultry-feed ingredient from HPAI virus associated with incoming wet eggshells, the vehicle, or the driver. Potential pathways for this event include personnel or equipment used to handle wet eggshells, which then contact other egg-breaking plant surfaces or equipment used for handling dry eggshells.

We considered preventive factors from current egg-processing industry practices and FSIS egg-processing plant regulations in evaluating this risk. The risk pathways considered here are shown in **Figure 25.**

10.2.2 Preventive Measures

10.2.2.1 Current Preventive Measures

The current preventive measures include existing regulations as well as current egg-industry practices that are preventive factors for this risk.

- FSIS regulations 9CFR590 provide guidelines for maintenance and operation of egg-processing plants. FDA regulations 21CFR110 provide good manufacturing practices for food-processing facilities in general. These measures include sanitary measures for personal and C&D of equipment and egg-processing rooms.
- Most of the handling systems (augers and storage hoppers) used to move eggshells are mechanized and enclosed.

10.2.2.2 Future Preventive Measures

- The vehicle is C&D after delivering wet eggshells and before returning to a poultry premises.

- The cab interior must be cleaned and disinfected if the vehicle driver steps out of the cab.
- If leaving the cab, the driver should wear protective coveralls, boots, and head cover while outside the cab and remove them immediately before reentering the cab

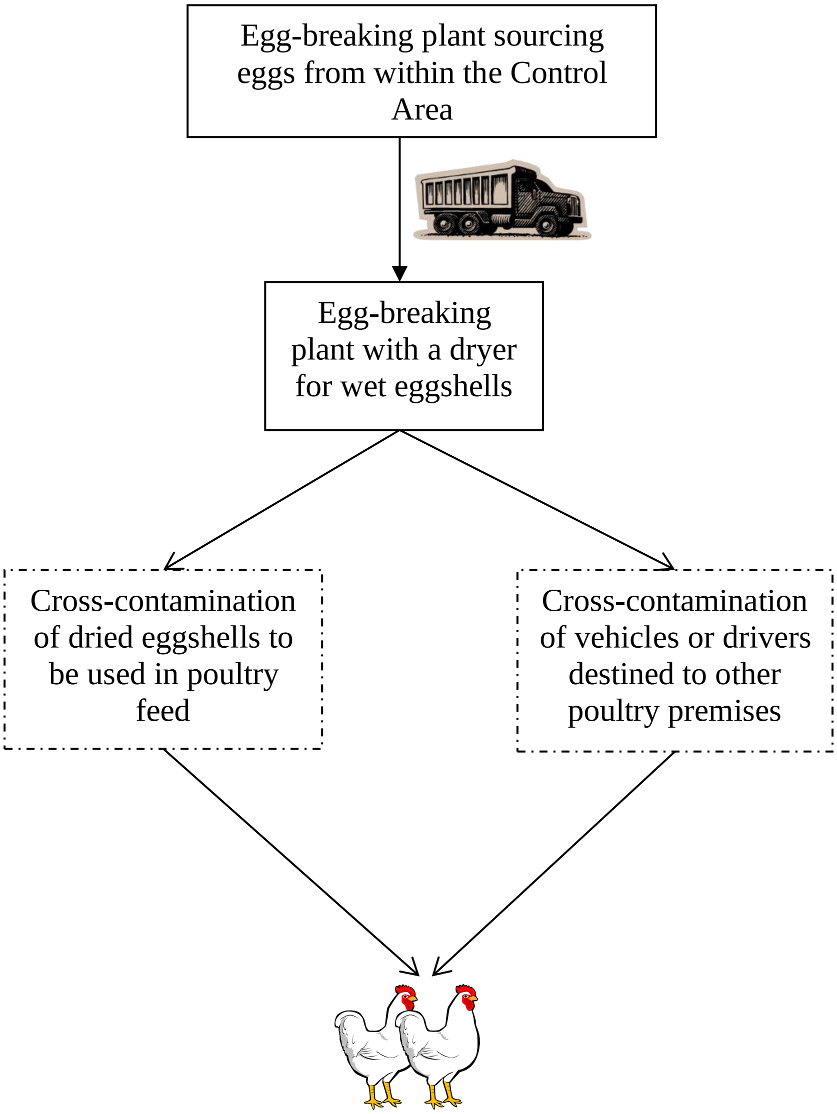


Figure 25. The risk pathways for HPAI spread to susceptible poultry associated with movement of wet eggshells to a standalone egg-breaking plant for drying.

10.2.3 Risk of the Movement of Wet Eggshells to a Standalone Egg-breaking Plant for Drying Results in HPAI Spread to a Susceptible Poultry Flock

10.2.3.1 Risk of Cross-contamination of Dry Eggshells with HPAI Virus via Personnel or Equipment Handling Wet eggshells

Wet eggshells are delivered by road and unloaded into a drive-over hopper (road-pit)[107] or dumped into a hopper from a dump truck (**Figure 26**). A conveying system removes the wet eggshell material from the intake hopper as it is being delivered and transports it to a bulk storage bin. We considered the following factors in evaluating this risk:

Figure 26. Dumping wet eggshells into the auger intake.

a) For wet eggshells originating from flocks under active surveillance—as specified in the SES Plan—and because there is no mixing step in their handling, the contamination is expected to be concentrated to a few pounds out of a 1144.5 lb batch. Even in the unlikely scenario where some employees contact wet eggshells (e.g. spillage), the contacted wet eggshells are unlikely to be contaminated.

b) The factors considered in section 9.2.3.2 such as the separation of wet eggshell and dry eggshell storage and handling areas, segregation of duties between different plant areas, and the use of enclosed handling and storage system for dry eggshells are also applicable here. Consequently, there is limited opportunity for employees to come into contact with, and cross contaminate, dry eggshells to be used in poultry feed.

We conclude that the risk that an employee handling contaminated wet eggshells resulting in infection of susceptible poultry by cross-contaminating dry eggshells to be used in poultry feed with HPAI virus is *negligible*.

10.2.3.2 Risk of HPAI Spread due to Cross-contamination of the Vehicle or Driver Destined to other Poultry Premises with HPAI Virus from Wet eggshells Moved to a Standalone Egg-breaking Facility

In situations where an egg-processing facility receives wet eggshells from multiple poultry premises for drying, there is a possibility that a vehicle destined to a poultry premises is cross contaminated with HPAI virus associated with wet eggshells from a different poultry premises. The pathway for this risk involves a sequence of events where 1) there is spillage of contaminated wet eggshells around the intake hopper; and 2) the tires of another vehicle or the driver's shoes become contaminated due to contact with spilled wet eggshells around the intake hopper. We considered the following factors in evaluating the likelihood of this event:

a) Only a small proportion of wet eggshells moved from an egg-breaking facility sourcing eggs from flocks in the control area are expected to be contaminated (10.2.3.1). Given this, even if the vehicle's tires or driver's shoes contact some spilled wet eggshells, the specific portion contacted is unlikely to be contaminated.

b) The SES Plan requires C&D of the vehicle after delivering wet eggshells before going to a different poultry premises. As evaluated in previously completed risk assessments and considering the susceptibility of AI virus to most detergents and disinfectants, the C&D process is expected to effectively inactivate any HPAI virus transferred to the vehicle via cross contamination[108].

c) The SES Plan requires the vehicle driver to don protective clothing such as gloves and disposable boots if they step out of the cab. PPE is effective in preventing transmission of pathogens in most cases.[109]

We conclude that the risk of HPAI spread due to cross-contamination of the vehicle or driver destined to poultry premises with HPAI virus from wet eggshells moved to a standalone egg-breaking facility is *low*.

10.2.4 Conclusion

We conclude the following, provided that the preventive biosecurity measures from the SES Plan are strictly followed:

- The risk of susceptible poultry becoming infected due to cross-contamination of dry eggshells to be used in poultry feed with HPAI virus from incoming wet eggshells is *negligible*.
- The risk of HPAI spread due to cross-contamination of the vehicle or driver destined to poultry premises with HPAI virus from wet eggshells moved to a standalone egg-breaking facility is *low.*
- The overall risk of the movement of wet eggshells to another egg-breaking plant for drying resulting in HPAI spread to a susceptible poultry flock is *low.*

10.3 Risk That the Movement of Wet Eggshells or Liquid INEP from an Infected but Undetected Flock to Landfills Results in HPAI Spread to Susceptible Poultry

Risk Factors: Cross-contamination of other vehicles or drivers at the landfill. Wildlife.

Current Preventive Measures: EPA Regulations 40 CFR Parts 239 through 259 which provide minimum standards for design and operation of municipal landfills; state and local regulations.

Future Preventive Measures (to be implemented by industry during an outbreak)**:** Vehicle C&D and driver biosecurity measures from the C&D guidelines; operational measures taken at the landfill in state response plans.

Overall Risk: *negligible*, provided the above additional preventive measures are strictly followed.

10.3.1 Background Information

Municipal solid waste landfills (also termed Sanitary landfills , or “Subtitle D” landfills) have been used as a disposal option during avian influenza outbreaks in the United States.[46] In the 2002 LPAI outbreak in Virginia, carcass disposal was carried out in sanitary landfills within 24 hours of detection of a diseased flock.[110] Municipal landfills, which are actively receiving new wastes, are required to follow the design and operational requirements specified in EPA regulations 40CFR258 under the Resource Conservation and Recovery Act subtitle D.

Landfills have been a preferred disposal option for carcasses from infected birds in previous AI outbreaks and they have not been implicated in secondary spread of AI.[111, 112] However, poultry waste from the control area was not accepted in several of the smaller municipal landfills in previous AI outbreaks. A considerable portion of wet eggshells are disposed of in landfills in routine operations while liquid INEP is less frequently disposed of in landfills given its higher value as a feed ingredient.

We evaluate risk pathways associated with cross-contamination of other vehicles or drivers destined to poultry premises with HPAI virus from wet eggshells and the vehicle or driver during disposal at a municipal solid waste landfill. For completeness, we also evaluate pathways involving mechanical transport of the virus by vectors, such as flies, or through environmental contamination through landfill leachate. Exposure of HPAI virus to wildlife (birds) which might feed on wet eggshells at a landfill site is a possibility but is outside the scope of this assessment.[113] The requirement to immediately cover wet eggshells with 6 inches of earthen materials as recommended by the SES Plan would reduce the risk associated with this pathway.

10.3.2 Preventive Measures

10.3.2.1 Current Measures

Current regulatory measures that apply to municipal solid waste landfills are design, operation and management guidelines specified by the EPA (minimum standards for operation and monitoring in 40 CFR Parts 239 through 259) as well as State and local regulations.[114-116]

- Use of appropriate cover material at the end of each operating day, or as frequently as necessary, to control disease vectors, fires, odors, blowing litter, and scavenging.
- Pollution control requirements for run-off, air, and litter.
- Design requirements to prevent release of leachate from the landfill cell.
- On-site control of disease vector populations.
- Appropriate disposal of obnoxious loads to avoid contamination of other landfill users.

10.3.2.2 Future Preventive Measures

- The vehicle C&D and driver biosecurity measures specified in the SES Plan (see the Entry Assessment section for details).
- Covering wet eggshells or inedible egg by 6 inches of earthen material (or equivalent) immediately after disposal to restrict access to flies, insects, and other vermin.

10.3.3 Evaluation of Risk

Municipal sanitary landfills are engineered sites designed to provide safe containment of municipal solid waste and protect the environment.[106, 115] In Subtitle D municipal landfills, the leachate is contained to minimize seepage and groundwater contamination.[117] Containment landfills are lined with clay and a membrane liner and have a leachate collection system installed, preventing release into groundwater.

At the landfill, truck drivers are directed to place the load at a particular location by a spotter.[118] The spotter considers various factors to properly identify the load. Obnoxious loads (foul smelling or offensive material) are placed as near to the working face of the cell as possible and far enough away from other landfill users so as not to cause problems.[118] The driver may exit the cab to remove the tarpaulin and perform duties associated with dumping the load of wet eggshells. Once the load is dumped, the waste is pushed to the working face of the cell and compacted. Obnoxious loads are pushed and covered immediately to minimize scavenging and reduce vector populations. State and federal regulations require placement of a minimum of six inches of cover material on all exposed refuse at the end of each operational day, or as frequently as necessary after the waste is compacted.[118]

In our qualitative analysis, we considered factors such as environmental conditions within the landfill environment, scientific studies on avian influenza in buried waste, and current landfill management practices relative to waste handling and vehicle traffic flow at the disposal site as we evaluated the likelihood of each potential transmission pathway.

10.3.3.1 Likelihood of Cross-contamination of Other Trucks or Drivers Destined to a Poultry Premises at the Landfill

At the landfill, other vehicle’s tires or wheel wells could become contaminated with HPAI virus from wet eggshells spilled onto common areas before they are pushed into the landfill cell. The chain of events that would result in cross-contamination of the vehicle or driver through this pathway requires 2 viral transfer steps: 1) spillage of contaminated wet eggshells or liquid INEP onto environmental surfaces at the landfill; and 2) contamination of the truck’s wheels or the driver's shoes by contacting the spilled material. We considered the following factors in our evaluation:

- The C&D guidelines require the driver to wear PPE and C&D of the wet eggshell vehicle's tires, wheel-wells, and under-carriage. For the liquid INEP tanker, the entire exterior is required to be C&D.
- Wet eggshells are transported to the landfill in dump trucks covered with a tarpaulin. Covering the batch reduces spillage of wet eggshells along access roads at the landfill site. There is no opportunity for inedible egg to spill from the tanker during transport.
- Drivers hauling obnoxious loads are directed by the landfill spotter to dump the loads near the face of the active (open) landfill cell. Landfill management practices and C&D guidelines recommend immediate coverage of obnoxious loads with an appropriate material.
- For wet eggshells originating from flocks under active surveillance as specified in the SES Plan, only a small proportion of eggshells in each movement are expected to be contaminated before detection of HPAI infection in the flock. Similarly, the average virus titer of inedible egg moved from the egg-breaking facility was estimated to be low.

Given the small proportion of contaminated eggshells in an incoming batch, it is unlikely that the specific portion of wet eggshell spilled from the truck onto environmental surfaces is contaminated with HPAI virus. Moreover, the wet eggshell batch is immediately pushed to the landfill cell and covered after being dumped. Therefore, a truck or driver hauling waste to the site would have limited opportunity to directly contact wet eggshells dumped from the previous batch. Liquid INEP is likely dumped directly into the working face of the landfill reducing the likelihood of cross-contamination via common areas. Trucks and drivers utilizing the landfill from outside the control zone may not be required to C&D the vehicle or use PPE before returning to the premises of origin. Based on the above evaluation, we qualitatively estimate the likelihood that a cross-contaminated truck or driver leaves the landfill site to be *negligible*.

10.3.3.2 Likelihood that HPAI Virus is Released from the Landfill Site via Leachate

A theoretical pathway for this event includes the possibility of a breach in the landfill containment system before HPAI virus, from the wet eggshells or liquid INEP, is inactivated. We considered the following factors:

- Subtitle D municipal solid waste landfills have a leachate containment system, which includes a composite liner consisting of clay covered by a polymer sheet. In addition, leachate collection systems are employed to remove and treat leachate to minimize the stress on the composite liner. The design lifetime of the containment system exceeds 30 years. In addition, the groundwater in the vicinity of the landfill is monitored according to 40CFR258 to detect the presence of any leachate chemicals.
- The survivability of a low pathogenic H6N2 avian influenza strain in methanogenic landfill leachate and reverse osmosis water as a function of temperature, conductivity, and pH was evaluated by Graiver *et al.*.[8] Inoculated samples were incubated (21, 37, 60 °C) for 0, 0.5, 1, 2, 4, 7, 14, 28, and 56 days. Waste temperatures between 30 to 60 °C are common during the methanogenic degradation phase although the temperatures and the rate of degradation would be lower in cold weather. The authors concluded that

inactivation times calculated in the study (< 2 years) were within design lifetimes of the composite barrier and leachate collection systems at typical Subtitle D landfills.

- Landfills have been used as a disposal option for infected birds in several previous avian influenza outbreaks and release of virus via leachate has never been reported. The quantity of HPAI virus in wet eggshells or liquid INEP moved from infected but undetected flocks in the control area (estimated in the entry assessment section) is expected to be substantially lower than that in dead carcasses from known infected flocks.

We conclude that the risk of HPAI spread due to release of leachate from a landfill, where wet eggshells or liquid INEP from an infected but undetected flock are disposed, is *negligible.*

10.3.3.3 Likelihood of Mechanical Transport of Avian Influenza Virus from the Landfill by Flies

In this part of the analysis, we evaluate the risk of transmission of HPAI virus to poultry from flies associated with disposal of wet eggshells in a landfill. EPA regulations 40 CFR Parts 239-259) establishes national standards for landfill design and operation and require that the landfill cell is covered at the end of the operational day or more frequently if necessary with 6 inches of earthen material at the end of the day. Moreover, obnoxious loads are typically covered immediately after being dumped in current landfill operations.[118] In the event of a HPAI outbreak, the SES plan requires covering of wet eggshells or inedible egg by 6 inches of earthen material or equivalent immediately after disposal. Considering landfill management practices, we conclude that the risk of susceptible poultry becoming infected due to transmission of HPAI virus from the wet eggshells, or inedible egg via flies, is *negligible.*

10.3.4 Conclusion

We conclude the following, provided the preventive biosecurity measures from the SES Plan are strictly followed:

- The likelihood of cross-contamination of other vehicles or drivers leaving from the landfill and destined to a poultry premises with HPAI, from wet eggshells or liquid INEP, is *negligible.*
- The risk of HPAI spread due to release of leachate from a landfill, where wet eggshells or liquid INEP from an infected but undetected flock were disposed, is *negligible.*
- The risk of susceptible poultry becoming infected due to transmission of HPAI virus from wet eggshells, or liquid INEP disposed of in a landfill, via flies is *negligible.*
- The overall risk of the movement of wet eggshells or liquid INEP from an infected but undetected flock to landfills, resulting in HPAI spread to susceptible poultry, is *negligible*

10.4 Risk of the Movement of Liquid INEP to a Pasteurization Facility Resulting in HPAI Spread to Susceptible Poultry

10.4.1 Background Information

In current industry practice, liquid INEP may be pasteurized before use in pet food and animal feeds. The pasteurization may be performed at egg processing facilities that also have inedible egg product processing equipment or it may be performed at a dedicated animal food processing facilities. In most cases, there is no direct pathway for susceptible poultry to become exposed to pasteurized INEP as it is not used as an ingredient in poultry feed. However, other animals for which INEP may be used as a feed ingredient may also be susceptible to some strains of HPAI virus. We evaluate the pasteurization process as a “kill step” for completeness and so that evaluation of potential downstream exposure pathways, after pasteurization, is not necessary.

There is a possibility that a vehicle or driver returning from the pasteurization facility (standalone plant), can become cross-contaminated with HPAI virus associated with incoming liquid INEP, or the vehicle or driver hauling liquid INEP to the plant. We evaluate this risk, while considering the entry assessment results and the vehicle and driver biosecurity guidelines from the SES Plan.

Finally, in the scenario where liquid INEP is moved to an inline egg-processing facility, we evaluate the risk of susceptible poultry on the premises becoming infected with HPAI virus associated with the incoming vehicle or driver.

10.4.2 Preventive Measures

10.4.2.1 Current Preventive Measures

The current preventive measures include existing regulations and industry practices that are preventive factors for this risk.

- Most of the handling system used to move liquid INEP is enclosed, minimizing opportunity for spillage and employee contact with INEP.
- The duties of those working in the henhouse and INEP processing operations are segregated in inline egg-processing facilities. There is limited personnel movement from the INEP processing room to the henhouse.
- Pasteurization of INEP occurs prior to use in pet food and animal feed rations.

10.4.2.2 Future Preventive Measures

- Pasteurization of INEP according the USDA FSIS standards for inactivating *Salmonella* in edible whole egg, or whole-egg blends, depending on the percent of non-egg ingredients as described in 9CFR590.570. [119]
- The vehicle is C&D after delivering liquid INEP and before returning to a poultry premises.
- If leaving the cab at the destination egg-processing premises, the driver should wear protective coveralls, boots, and head cover while outside the cab and remove them immediately before reentering the cab.

10.4.3 Risk of the Movement of Liquid INEP to a Standalone Pasteurization Facility Resulting in HPAI Spread to Susceptible Poultry

10.4.3.1 Likelihood of Viable HPAI Virus Being Present in Liquid INEP after Pasteurization

Liquid INEP from breaking and grading/packaging facilities is pasteurized at an offsite processing facility to remove *Salmonella* before drying and incorporation into pet or other animal feed (mink rations, starter pig rations, etc.). We evaluate the effectiveness of the pasteurization process in reduction of HPAI virus titers in liquid INEP.

Background Information

Relatively few studies have evaluated the inactivation of HPAI virus in egg products. The available studies indicate that the USDA FSIS pasteurization requirements to inactivate *Salmonella* in various egg products are adequate to inactivate HPAI viruses as well. Further details are provided in *Pasteurized Liquid Egg Risk Assessment* and a comparison of D-values for *Salmonella* and AI virus is presented in Appendix 7.[120]

King (1991) evaluated the effect of pasteurizing yolk, albumen, and allantoic fluid inoculated with HPAI virus (A/chicken/Pennsylvania/83) at 57 and 62°C (143.6°F). In this study, HPAI virus at 5.2 log EID_{50}/ml in albumen held at 57 °C (134.6 °F) was not completely inactivated by 5 minutes and was inactivated within 10 minutes.[17, 69]

Swayne and Beck (2004) studied the effect of pasteurizing homogenized whole egg, liquid egg-white, 10% salted yolk, and dried egg-white artificially infected with HPAI/PA/H5N2 virus at standard pasteurization temperatures. The study found that the standard pasteurization temperatures and holding times described in 9CFR590.570 were sufficient to inactivate HPAI virus in all tested liquid-egg products.[68]

Differences in results of the two studies may be due to use of different experimental methods. In a side-by-side comparison, Swayne and Beck explain the differences in results between the two studies by suggesting that their heat inactivation studies may have been more precise than previous studies due to the use of thin-walled, small volume, plastic tubes and precision thermocycler plates rather than thick-walled, large volume glass vials and a water bath method of heating. Previous inactivation studies using *Salmonella* have demonstrated that thin-walled capillary tubes provide more accurate results than large glass tubes (such as those used in the study performed by King), because capillary tubes have instant come-up and cool down times.[121, 122] Use of the 12 ml flat-bottomed glass-vials employed in the King (1991) study may have prevented uniform heating of the sample and accurate assessment of inactivation of the AI virus at specific times and temperatures.[69]

Likelihood of HPAI Virus not Being Inactivated after Pasteurization According to FSIS Standards for Inactivating Salmonella in Whole Egg

In the event of a HPAI outbreak, the C&D guidelines require liquid INEP to be pasteurized according to the FSIS guidelines for pasteurization of whole egg blends as described in 9CFR590.570.[119] These protocols are designed to achieve 5-$\log_{10}$ inactivation of most *Salmonella* strains.

Although liquid INEP contains mostly egg whites by weight from the breaking process, the addition of whole egg from the grading/packaging process introduces yolk into the mixture. In some cases, liquid INEP may also include non-egg ingredients such as salt, sugar, gums, starch, non-fat dried milk, etc., that are normal ingredients in egg-product formulations. In this case, FSIS pasteurization requirements for whole egg blends (less than 2 percent added non-egg ingredients) should be followed for pasteurization of liquid INEP during a HPAI outbreak. Pasteurization requirements for these products from FSIS and OIE guidelines are listed in **Table 18**.

Swayne and Beck (2004) estimated times to complete inactivation of 4.9-$\log_{10}EID_{50}$/ml of HPAI virus in various egg-products at the standard pasteurization times and temperatures listed in 9CFR590.570 (**Table 19**).[68] The authors estimated D_t, the time required to inactivate HPAI H5N2 virus by one-log, to be 27.2 seconds for whole egg and whole-egg blends at 60 °C. The D_t for whole-egg blends at 61.1 °C was estimated to be 13.6 seconds. In this article, the D_t for whole-egg blends, which may have up to 2 added ingredients, was assumed to be similar to that for whole egg.

Using the D_t values calculated by the authors of this study, pasteurization of whole-egg blends at 60 °C for 6.2 minutes should reduce virus titer by 13.7-$\log_{10}$, and pasteurization at 61.1 °C for 3.5 minutes should reduce virus titer by 15.4-$\log_{10}$ (**Table 19**).[68] Note that with homogeneous mixing, the viral titer of liquid INEP from an infected but undetected flock was estimated to be less than 1-log EID_{50}/ml in the entry assessment section. There is a possibility that there would be less dilution of the HPAI viral titer due to incomplete mixing. The estimates of HPAI concentration in the albumen of individual contaminated eggs mostly ranged from $10^{3.2}$ to $10^{4.9}$ EID_{50}/ml, with an observed maximum value of $10^{6.1}$ EID_{50}/ml. However, some mixing would likely occur resulting in the virus concentration being lower than that found in individual eggs. Overall, considering the estimated HPAI virus inactivation given the pasteurization temperatures and holding times, we conclude that the likelihood of HPAI virus not being inactivated after pasteurization, according to FSIS standards for inactivating *Salmonella* in whole egg or whole-egg blends, is *negligible*.

Effect of pH on AI Virus Survival During Pasteurization

We were unable to find information describing the effect of pH on HPAI virus survival in egg products during pasteurization. However, stability of *Salmonella* in egg products during pasteurization depends on pH. Several studies and industry experts report that lower pH among the ranges found in liquid whole egg increases the heat resistance of *Salmonella*.[121, 123, 124] Though the pH of liquid whole egg can vary depending on freshness (pH 7 to 7.6 on the same day of breaking; 8.0 to 8.5 as it becomes more alkaline due to evolution of CO_2), the pH of liquid INEP is generally lower, ranging from 6 to 6.5 due to bacterial growth or mixing with phosphate.[60] To counter the effect of increased thermal resistance of *Salmonella* at this lower pH, inedible egg is commonly pasteurized for longer periods of time.

Table 18. USDA FSIS (9CFR590.570) and OIE pasteurization standards for liquid whole egg and whole-egg blend products.

USDA Requirements (9CFR590.570)			OIE Guidelines		
Product	Temperature ºF (ºC)	Time Minutes (Seconds)	Product	Temperature ºF (ºC)	Time Minutes (Seconds)
Whole egg	140 (60)	3.5 (210)	Whole egg	140 (60)	3.1 (188)
Whole egg blends [a]	142 (61)	3.5 (210)	Whole egg blends [b]	140 (60) 142 (61)	3.1 (188) 1.6 (94)

[a] less than two percent added non-egg ingredients
[b] all blends including whole egg blends

Table 19. USDA FSIS required pasteurization holding times and temperatures and estimated times to inactivate 4.9-log10EID$_{50}$ HPAI virus in various egg products. Adapted from Swayne and Beck (2004) [68].

Egg product	Pasteurization Temperature (°C)	Pasteurization Time (min)	Time to Inactivate 4.9-log$_{10}$EID$_{50}$/ml HPAI virus (min)	Reduction of HPAI viral titer with FSIS standard (log$_{10}$)
Whole egg	60	3.5	2.2	7.7
Whole egg blends	60	6.2	2.2	13.7
Whole egg blends	61.1	3.5	1.1	15.4

Some studies evaluated the survival of AI viruses in water at varying pH values. Brown *et al.* (2009) tested the survival of low pathogenic AI viruses in water with eight different pH values ranging from 5.8 to 8.6 and at temperatures of 17 °C and 28 °C. Brown *et al.* found that at both temperatures, AI viruses persisted longest in slightly basic water with a pH value of 7.4 to 8.2. AI viruses were least stable in acidic water with a pH less than 6.6.[125] Stallknecht *et al.* (1990) used similar methods, finding a peak infectivity in water samples with pH ranging from 7.4 to 7.8.[31] Studies evaluating AI viruses in egg products were less conclusive. Shahid *et al.* (2009) found that HPAI virus in amnioallantoic fluid was inactivated at pH 5 after 24 hours but remained infective after 24 hours at pH values of 7 and 9 at a temperature of 28 °C.[77] Another study found that all samples of AI virus in chorioallantoic fluid were 100 percent infective after a 15 minute sampling period at pH 5, 7, 10, and 12.[28] In a study by Wanaratana *et al.* (2010) incubation of H5N1 HPAI viruses in allantoic fluid for 10 minutes at room temperature was not sufficient to inactivate any of the strains tested at pH values of 5, 7, and 9.[126]

Based on these studies, there is no evidence that AI viruses are more heat stable at lower pH values. We conclude that liquid INEP does not need to be pasteurized for longer periods of time considering potential variations in pH to eliminate AI virus. We conclude the following:

- The likelihood of HPAI virus in liquid INEP from an infected but undetected flock not being inactivated by pasteurization, according the USDA FSIS standards for inactivating *Salmonella* in whole egg described in 9CFR590.570, is *negligible*.[119]
- If the liquid INEP produced at a facility has a considerable portion of non-egg ingredients, the use of USDA FSIS pasteurization standards for inactivating whole egg blends may be considered.

10.4.3.2 Risk of HPAI Spread Due to Cross-contamination of the Vehicle or Driver Destined to Other Poultry Premises with HPAI Virus Associated with Incoming Liquid INEP, the Vehicle, or Driver

In situations where an egg-processing facility receives liquid INEP from multiple poultry premises for pasteurization, there is a possibility that a vehicle destined to a poultry premises from the facility becomes cross-contaminated with HPAI virus associated with liquid INEP from a different poultry premises. We performed the evaluation separately for liquid INEP transported in tankers and in carboys.

Liquid INEP in Tankers

Liquid INEP is pumped from the tanker through an enclosed system into a holding tank at the pasteurization facility. Spillage from enclosed lines is unlikely, however in a few cases, a small quantity may spill from hoses and couplings when the lines are being connected to, or disconnected from, the tanker. From the entry assessment, the average HPAI virus concentration of liquid INEP in a tanker was estimated to be less than 10^1 EID_{50}/ml. In comparison, the mean 50 percent chicken infectious dose (CID_{50}) with intranasal inoculation for various HPAI strains (reviewed in Swayne and Slemons, 2008) mostly ranged between 100 to 7,943 EID_{50} for 3- to 4-week-old chickens.[42]

In a scenario where liquid INEP is mixed and would have a homogeneous virus concentration, even if the driver's shoes, gloves, or protective clothing were to contact some liquid INEP, the degree of contamination would be very low. Conversely, with less mixing, the contamination would be limited to a few isolated portions of liquid INEP and it would be unlikely that the specific portion spilled is also contaminated. Suppose a susceptible chicken is exposed to one gram of INEP from a batch where the degree of mixing is unknown, the upper bound on the probability of infection is 0.9 percent based on the exponential dose response model and using a 50% chicken infectious dose of 2.5 log EID_{50} (Appendix 12). Note that the infectious dose of 2.5 log EID_{50} was conservatively based on intranasal inoculation and the infectious dose for oral route may be higher. We had concluded a *negligible* risk of the vehicle exterior or driver moving liquid INEP in tankers being contaminated in the entry assessment section. In summary, we conclude that the risk of cross-contamination of other vehicles or drivers at the egg-pasteurization facility, due to the movement of liquid INEP sourced from an infected but undetected flock in tankers, is *negligible.*

Liquid Inedible Egg in Carboys

The evaluation of the likelihood of other vehicles, or driver, being cross-contaminated with HPAI virus from liquid INEP transported in carboys is similar to that for liquid INEP in tankers, with the following additional considerations.

a) Spillage of liquid INEP from carboys while unloading is unlikely as they are closed containers.

b) A low likelihood of the vehicle interior, or the driver transporting liquid INEP, in carboys being contaminated was estimated in the entry assessment section. The driver is required to wear a different set of PPE at the destination egg-processing facility.

c) Cross-contamination of other vehicles or the driver would involve multiple virus transfer steps via direct contact between surfaces. For instance, the pathway “trailer interior→ driver shoes→ loading dock→ other vehicle driver shoes→ environmental surfaces at destination poultry premises” involves more than four virus transfer steps. The degree of contamination transferred decreases with each virus transfer step via direct contact as only a fraction of virus on the donor surface is transferred to the recipient surface in each step.[108, 127]

We conclude that the risk of cross-contamination of other vehicles or drivers at the egg-pasteurization premises, due to the movement of liquid INEP sourced from an infected but undetected flock in carboys, is *low.*

10.4.4 Risk of the Movement of Liquid INEP to an Inline Pasteurization Plant Results in HPAI Spread to a Susceptible Poultry Flock

The risk pathways for HPAI spread via cross-contamination of vehicles or drivers destined to other poultry premises with HPAI virus from incoming liquid INEP, the vehicle, or driver are similar for inline and standalone facilities. Based on the evaluation in Section 10.4.3.2 , we rate the risk for this pathway to be *negligible* for liquid INEP transported in tankers and *low* for liquid INEP transported in carboys.

For inline pasteurization facilities, there is a possibility of susceptible poultry on the premises becoming infected with HPAI virus associated with incoming liquid INEP, the vehicle, or driver via movements of personnel or equipment. The likelihood of spillage and egg-processing plant personal contacting liquid INEP before it is pasteurized is *low* for INEP transported in tankers. This is because liquid INEP is pumped from the tanker through enclosed lines into a holding tank at the pasteurization facility. In addition, considering the *low* estimated HPAI concentration of liquid INEP, we rate the risk of the movement of INEP in a tank truck, to an inline pasteurization plant resulting in HPAI spread to a susceptible poultry flock, as being *negligible.*

We considered the following factors in evaluating this risk for movement of liquid INEP to a pasteurization premises in carboys:

a) Spillage of liquid INEP from carboys while unloading is unlikely as they are closed containers. A small quantity may spill as INEP is being transferred into a holding tank. INEP is subsequently transferred from the holding tank to the pasteurization equipment through enclosed lines minimizing opportunity for spillage and contact with employees.

b) The duties of those working in the henhouse and egg-processing operations are segregated in inline facilities. There is limited movement of personnel or equipment from

INEP processing areas to the henhouse.[64] Hence, it is unlikely for personnel to transmit HPAI virus from INEP to the henhouse in an inline facility.

c) The expected concentration of HPAI virus in INEP moved from an egg-breaking plant sourcing eggs from an infected but undetected flock is considerably lower compared to the 50-percent chicken infectious dose.

We conclude that the risk of susceptible poultry on an inline premises becoming infected with HPAI virus associated with incoming INEP in carboys, the vehicle, or driver is *low*.

10.4.5 Conclusion

We conclude the following regarding specific risk pathways associated with the movement of INEP to a pasteurization facility, provided that the applicable preventive measures from the SES Plan are strictly followed:

- The likelihood of HPAI virus in INEP from an infected but undetected flock not being inactivated by pasteurization, according the USDA FSIS standards for inactivating *Salmonella* in whole egg or whole-egg blends specified in 9CFR590.570, is *negligible.*[119]
- The risk of cross-contamination of other vehicles or drivers at the egg pasteurization premises due to the movement of INEP sourced from an infected but undetected flock in tankers is *negligible.*
- The risk of cross-contamination of other vehicles or drivers at the egg pasteurization premises due to the movement of INEP sourced from an infected but undetected flock in carboys is *low.*
- The risk of susceptible poultry on an inline premises becoming infected with HPAI virus associated with incoming INEP in tankers, the vehicle, or driver is *negligible*.
- The risk of susceptible poultry on an inline premises becoming infected with HPAI virus associated with incoming INEP in carboys, the vehicle, or driver is *low*.

The overall risk of the movement of liquid INEP in tankers to a pasteurization facility resulting in HPAI spread to susceptible poultry is *negligible.* The overall risk of the movement of INEP in carboys to a pasteurization facility resulting in HPAI spread to susceptible poultry is *low.*

11. Overall Conclusion

This risk assessment evaluates the risk of HPAI spread via movement of eggshells and INEP from egg-breaking and processing plants that receive eggs from table-egg layer flocks located within a Control Area. The risk evaluation considers applicable regulations, relevant egg-industry practices, as well as future preventive measures proposed to be included in the SES Plan. The key quantitative results from this evaluation are:

- Only a small fraction of wet eggshells moved from an egg-breaking plant that receives eggs from an infected but undetected flock is expected to be contaminated with HPAI virus (7.6×10^{-5}, 90% P.I., 0- 2.4×10^{-4}) when dilution of HPAI virus concentration, due to mixing of contaminated and virus free eggshells, is *minimal* .
- The mean HPAI virus concentration of liquid INEP moved from an inline egg-breaking facility that receives eggs from an infected but undetected flock was estimated to be less than 1.5 EID_{50}/ml. The results also indicate a 99 percent chance of the average virus titer being less than 10^1 EID_{50}/ml.
- The mean HPAI virus concentration of liquid INEP moved from a standalone egg-breaking facility that receives nest-run eggs from an infected but undetected offline flock, after a two-day holding period, was estimated to be less than 0.018 EID_{50}/ml.

We conclude the following with respect to major risk pathways evaluated in this document:

- The risk of movement of wet eggshells to an agricultural land application site resulting in HPAI spread to susceptible poultry is *negligible.*
- The risk of the movement of wet eggshells or liquid INEP for disposal in a landfill resulting in HPAI spread to a susceptible poultry flock is *negligible.*
- The risk of movement of dry eggshells to a poultry feed mill resulting in HPAI spread to susceptible poultry is *negligible.*
- The risk of the movement of liquid INEP in tankers to a pasteurization facility resulting in HPAI spread to susceptible poultry is *negligible.*
- The risk of the movement of liquid INEP in carboys to a pasteurization facility resulting in HPAI spread to susceptible poultry is *low.*
- The risk of the movement of wet eggshells to a standalone egg-breaking plant for drying resulting in HPAI spread to a susceptible poultry flock is *low.*

It is concluded that the overall risk of moving wet eggshells and INEP into, within, and outside of a control area during a HPAI outbreak is *low*, provided that the future preventive measures proposed by the egg-sector working group, as listed below, are strictly followed:

- The active surveillance protocol described in the SES Plan is implemented for all flocks supplying eggs to the egg-breaking facility.
- The exterior of the vehicle moving dry eggshells and INEP is cleaned and disinfected before entering the destination premises.
- The tires and wheel wells of all vehicles should be cleaned and disinfected before leaving the premises within the Control Area.

- The driver is not allowed outside the cab, or else the cab interior must also be cleaned and disinfected.
- Vehicle drivers should wear protective clothing such as disposable boots, gloves, and coveralls and remove them before getting back into the cab.
- The drying process used to produce dry eggshells that are intended to be used in poultry feed must reduce the moisture content of incoming wet eggshells to 4 percent or lower with an exhaust air temperature greater than 200 °F.
- Wet eggshells from an inline egg-breaking facility are required to be held at the destination premises for two days before land application.
- The land application site for wet eggshells is at a distance of 3 km away from poultry production facilities.
- The wet eggshells or INEP disposed of at a landfill should be immediately covered by 6 inches of earthen material or equivalent approved material (40 CFR 258.21).
- INEP intended to be used in animal food and is pasteurized according to USDA FSIS pasteurization requirements for whole egg or whole-egg blends as described in 9CFR590.570 depending on the proportion amount of non-egg ingredients. [119]

Appendix 1. Modifications to the HPAI Disease Transmission Model Presented in the Washed and Sanitized Shell-egg Risk Assessment

Modeling Associated with the Movement of Eggshells

Movement of Eggshells from a Standalone Egg-breaking Facility Receiving Eggs from an Offline Farm

According to the SES Plan, nest-run eggs from offline flocks within the Control Area will be allowed to move to a standalone processing (including egg-breaking) facility two days after production so that negative RRT-PCR test results from two days of testing are available. This protocol implies that if infection from a flock is detected on a particular day by RRT-PCR testing, then the eggs from the flock produced in the past two days would not have been moved to an egg-breaking facility. Therefore, for evaluating the likelihood of moving contaminated eggshells from a standalone egg-breaking plant, we assumed that eggs produced at the offline farm in the two days prior to detecting HPAI infection would not have been moved to the egg-breaking plant and hence, would not be included in eggshells generated in the plant. Given this assumption, we defined the maximum daily number of contaminated eggs E_{max}, as the highest daily number of contaminated eggs among all the days starting from the day the flock is infected to two days before the infection is detected in the flock.

A stand-alone processing plant may receive eggs from multiple offline farms. We make a simplifying and conservative assumption that all offline farms supplying eggs to the plant are in an infected but undetected state. To calculate the proportion of contaminated wet eggshells, we estimate the maximum number of contaminated eggs contributing eggshells in a batch of eggshells generated by breaking one day's production from a flock of 100,000 layers. Given an egg production rate of 0.7 eggs per-hen per-day, we consider that at most, eggshells from E_{max} contaminated eggs may be present in a 1,144.5 pound batch of wet eggshells generated by breaking 70,000 eggs.

In the targeted active surveillance protocol described in the SES Plan, swabs from 5 randomly selected birds per each 50 birds among the daily mortality sample are pooled together and tested via RRT-PCR each day. For this analysis, we define a pooled sample as oropharyngeal swabs from 5 dead or euthanized sick chickens in a single tube which is tested via RRT-PCR. If less than 5 dead or sick chickens are present on a day, swabs from the available dead or sick chickens are included in the pooled sample. The number of days to detect infection under this protocol depends on the variability in the normal mortality independent of HPAI and the variability in the mortality due to HPAI disease. We used outputs from the transmission model, in conjunction with the simulation model of the active surveillance protocol, to estimate the number of days to detect infection in a flock. Specifically, the HPAI disease mortality on various days post HPAI infection of the flock was obtained from the transmission model described in Chapter 7. The normal daily mortality in table-egg layer flocks was estimated from the daily percentage mortality data for table-egg layer flocks (described in the Washed and Sanitized Shell Egg Risk Assessment[64]). The following pages describe the simulation model for surveillance.

Notation

t = index of days, $t = 1,\ldots,t_d$; where t=1 is the first day on which the flock is infected and $t = t_d$ is the day on which infection is detected in the flock via the active surveillance protocol.

$M^d(t)$ = mortality due to HPAI on day t (birds/flock of 100,000 layers) estimated from the HPAI disease transmission model (*Washed and Sanitized Shell-egg Risk Assessment*).[64]

$M^n(t)$= normal mortality independent of HPAI on day t (birds/flock of 100,000 layers) estimated from daily mortality percentage data for table-egg-layer flocks.[64]

$M^t(t)$= total mortality on day t (birds/flock of 100,000 layers). $M^t(t) = M^d(t) + M^n(t)$

M_{lim} = threshold for total mortality above which HPAI infection in the flock would be detected due to increased mortality regardless of diagnostic testing. M_{lim} was set at 500 birds based on a 0.5 percent percentage mortality threshold.

$N(t)$= number of pooled samples tested via RRT-PCR on day t.

n = number of swabs in the pooled sample submitted for RRT-PCR testing each day (swabs), n = min (5, $M^t(t)$).

k = index of a pooled sample submitted for RRT-PCR testing.

$X^{pool}(t,k)$ = number of swabs from HPAI infected birds present in pooled sample k on day t. $0 \le X^{pool}(t,k) \le n$.

$$X^{pcr}(t,k) = \begin{cases} 1 \text{ if RRT-PCR test result for the } k^{\text{th}} \text{ pooled sample submitted on day } t \text{ is positive} \\ 0 \text{ otherwise} \end{cases}$$

$$X(t) = \begin{cases} 1 \text{ if infection in the flock is detected on day } t \text{ by either RRT PCR or increased mortality} \\ 0 \text{ otherwise} \end{cases}$$

Se = sensitivity of the RRT-PCR testing procedure.

$E(t)$ = calculated number of HPAI contaminated eggs produced on day t based on the disease transmission model (contaminated eggs/flock of 100,000 layers).

E_{max} = maximum number of contaminated eggs per day that may be moved among all days $t = 1,\ldots, t_d - 2$ prior to detection of infection in the flock.

Assumptions

- Apart from active surveillance of mortality via RRT-PCR, other clinical indicators of HPAI infection, such as a drop in egg production and decreased feed intake, are not considered towards detecting infection.

- The sampling of daily mortality is random (i.e., swabs from dead birds with clinical signs are not more likely to be included in pooled samples tested via RRT-PCR).
- The active surveillance protocol with daily RRT-PCR testing of a pooled sample from the flock was implemented before the flock became infected with HPAI. The assumption is well justified after the initial few days of RRT-PCR testing in the HPAI outbreak. However, the differences in the model results without this assumption are not substantial as discussed in Appendix 11 of *Washed and Sanitized Shell-egg Risk Assessment*.

Simulation Model

The number of pooled samples that are tested on day t, $N(t)$ according to the SES Plan is given by

$$N(t) = \left\lceil \frac{M^t(t)}{50} \right\rceil$$

The number of infected birds included in a pooled sample of n birds was modeled via the hyper geometric distribution as shown below.

$$X^{pool}(t,1) \sim HyperGeometric(M^t(t), n, M^d(t))$$

$$X^{pool}(t,k) \sim HyperGeometric(M^t(t) - n(k-1), n, M^d(t) - \sum_{l=1}^{l=k-1} X^{pool}(t,l)) \; \forall \; k \in 2, \dots, N(t)$$

Depending on the sensitivity of the test, there is a chance that infection may not be detected even if a pooled sample contains a contaminated swab. We modeled the outcomes of RRT-PCR testing as a simple Bernoulli trial with probability P equal to the sensitivity of the test Se.

$$X^{pcr}(t,k) \sim Bernoulli(Se) Min(1, X^{pool}(t,k))$$

Finally, disease is detected on the day t either by RRT-PCR testing or due to the daily mortality being higher than the threshold, $M_{lim.}$

$$X(t) = \left\{ \begin{matrix} 1 \; if \; \sum_1^{N_k} X^{pcr}(t,k) \geq 1 or \, M^t(t) > M_{\lim} \\ 0 \; otherwise \end{matrix} \right\}$$

The probability that infection is detected on a particular day $P(t = t_d)$, is given by

$$P(t = t_d) = P\{X(t) = 1\} \; if \; t = 1$$

$$P(t = t_d) = \left(\prod_{h=1}^{h=t-1} P\{X(h) = 0\}\right) P\{X(t) = 1\}\ \forall\ t > 1)$$

The maximum number of contaminated eggs contributing wet eggshells in a batch of wet eggshells generated by breaking the daily egg production from a 100,000 bird infected but undetected offline flock (E_{max}) was calculated as shown below.

$$E_{\max} = Max(E(t)\,|\,1 \le t \le t_d - 2)$$

Movement of Eggshells from an Inline Egg-breaking Facility Receiving Eggs from an Inline Farm

In an inline facility, eggs are directly transferred from the henhouse to the egg-breaking machine through conveyer lines and processing (washing and sanitizing) machines. This system is not suitable for implementation of a two-day holding time. In this case, we considered that an RRT-PCR result from samples taken on the day the eggs are collected is available before eggshells from those eggs are moved from the facility. In this case, E_{max} the maximum number of contaminated eggs contributing wet eggshells in a batch of wet eggshells generated by breaking the daily egg production from a 100,000 bird infected but undetected inline flock (E_{max}) was calculated as shown below.

$$E_{max} = \max(E(t)|1 \le t \le t_d - 1) = E(t_{d-1})$$

Movement of Liquid INEP from a Standalone Egg-breaking Facility Receiving Eggs from an Offline Farm

INEP from an egg-breaking plant is moved once or twice per week. The lower frequency of movement reduces the likelihood that HPAI contaminated inedible egg is moved from the processing facility before HPAI infection is detected in a flock supplying eggs. We have the following additional notation for the simulation model for the movement of INEP:

t^* -Days post infection of the flock that INEP is moved from the standalone processing facility.

E_{sum}= The total number of contaminated eggs contributing inedible egg in a batch of INEP per movement from one flock. For example, a 100,000 hen flock would produce approximately 700,000 eggs per week, from which inedible egg would be generated. In this case, E_{sum} is the total number of contaminated eggs among the 700,000 eggs from which INEP would be generated and moved once per week.

We considered conservative (cautious) scenarios for the day of movement t^* relative to the day disease is introduced into the flock. Based on the HPAI disease transmission model results with a contact rate of 2-birds/6 hours, HPAI infection would be detected due to observation of increased mortality by day 6 regardless of RRT-PCR testing. In this case, we conservatively considered scenarios where the day of movement (testing needs to be done within a day of movement) is days 4 or 5 post infection of the flock (i.e., $t^* = 4$ or 5). If the day of movement is 6 or more days post infection in the flock, it would lead to the trivial case of disease being detected due to increased mortality above the threshold and little chance that contaminated INEP would be released. Conversely, if the day of testing were day 1, 2, or 3 post infection, then contaminated eggs would not be moved from the flock given a two-day holding time. Appendix 1 Table 1 provides a hypothetical example for the scenarios associated with the day that HPAI disease is introduced into the flock.

Appendix 1 Table 1. A hypothetical example where the offline layer flock is assumed to be infected between 4 to 5 days on the day of movement of liquid INEP from the facility (Wednesday).

Weekday	**Sun**	**Mon**	**Tue**	**Wed (Movement day)**
HPAI disease mortality scenarios	D_2	D_3	D_4	D_5
		D_2	D_3	D_4

D_n refers to the number of days post HPAI infection of the flock.

In this example, testing is conducted on Wednesday to move eggs by Wednesday evening or later. We considered that the flock may have been infected 2, 3, or 4 days on the day of RRT-PCR testing (Wednesday in the example) for simulations with a contact rate of 2-birds/6 hours. Scenarios where the flock is infected for 5 or more days on the day of testing (Wednesday in th example) were not considered as they would lead to the trivial case of HPAI infection being automatically detected on the following day (due to very high mortality) and little chance that infected eggs would be released. We also considered scenarios where the flock may have been infected between 4-5 days on the day of movement of inedible egg (Wednesday in the example) for simulations with a contact rate of 8-birds/6 hours i.e., $t^* = 5$ or 4. For the scenario where eggs are moved once per week, E_{sum} was calculated according to equation below.

$$E_{sum} = \left\{ \begin{matrix} 0 \; if \sum_{t=1}^{t^*} X(t) \geq 1 \\ \sum_{t=1}^{t^*-2} E(t) \; if \sum_{t=1}^{t^*} X(t) = 0 \end{matrix} \right\}$$

Movement of INEP from a Standalone Egg-breaking Facility Receiving Eggs from an Inline Egg-breaking Facility

Inline egg-processing facilities use contiguous conveyors and egg-processing systems with limited egg-holding capacity. In this scenario, we assume RRT-PCR test results, from pooled samples submitted on the day of movement of liquid INEP, are available before movement of INEP from the premises. We conservatively considered scenarios where the day of movement (testing needs to be done within a day of movement) is days 2, 3, 4, or 5 post infection of the flock i.e., $t^* = 2, 3, 4$, or 5. In this case, E_{sum} was calculated according to the equation below.

$$E_{sum} = \left\{ \begin{matrix} 0 \ if \sum_{t=1}^{t^*} X(t) \geq \ 1 \\ \sum_{t=1}^{t^*} E(t) \ if \ \sum_{t=1}^{t^*} X(t) = 0 \end{matrix} \right\}$$

Simulation Results

The simulation models of disease spread and active surveillance were implemented using Excel, Visual Basic, and @ Risk. The estimates of E_{sum} are provided in Appendix 1 Table 2 below.

Appendix 1 Table 2. The estimated total number of HPAI contaminated eggs contributing inedible egg in a batch of INEP from one flock's egg production, moved at a frequency of once or twice per week (mean with 90 percent P.I.).

	Inline	**Offline**
E_{sum} Total number of HPAI contaminated eggs from which inedible egg is generated	1.60 (0-17) eggs	0.016 (0-1) eggs

Appendix 2. Estimation of HPAI Viral Batch in Wet Eggshells and Liquid INEP

Amount of HPAI Virus in a 1144.5 pound batch of Wet Eggshells

In Appendix 1, we estimated that eggshells from 0.64 (90 percent P.I. 0 to 4) contaminated eggs may be included in a 1,144.5-pound batch of wet eggshells. To calculate the quantity of HPAI virus in the batch, we assumed that HPAI virus is present in the liquid egg adhering to the mineral portion of the shell. We also conservatively assumed that HPAI virus may be found in the protein matrices of eggshell. We have the following notations and input values for this section.

Appendix 2 Table 1. Inputs used to estimate the HPAI viral batch in liquid inedible egg.

Variable	Description	Value
$W_{eggshell}$	Grams of wet eggshell per broken egg	7.200 grams (industry estimate)
M_C	Percentage moisture content in centrifuged wet eggshells	18% conservative estimate from industry experts
P_C	Protein content on dry weight bases	6%
ρ	Average density of egg white adhering to eggshell	1.033 g/cm^3 (Paganelli et al., 1974)[128]
$V_{T(i)}$	HPAI viral titer in albumin from a contaminated egg *i*	Empirical distribution from data presented in Swayne and Eggert (2012) from 43 contaminated eggs[19] mean: $10^{4.81}$ EID_{50}/ml, 95th percentile $10^{5.5}$ EID_{50}/ml

The total amount of HPAI virus in a 1,144.5-pound batch of wet eggshells $A_{weteggshell}$ was calculated according to Appendix 2 equation 1.

$$A_{wetshell} = \sum_{i=\min(1,E\_\max)}^{i=E_{max}} \frac{(W_{eggsshell})(M_C + (1 - M_C) * (P_C))V_{T(i)}}{\rho}$$

(Appendix 2 Eq. 1)

Given the input parameter estimates summarized in Appendix 2 Table 1, we estimated approximately $10^{4.84}$ (0 to $10^{5.70}$) EID_{50} of HPAI virus among 1,144.5 pounds of wet eggshells produced daily by breaking eggs from a 100,000 bird layer offline flock. The $A_{wetshell}$ for movement of wet eggshells from an inline facility can be calculated similarly using Appendix 2 Equation 1, except that E_{max} estimates for an inline facility needs to be used in this case. We estimated approximately $10^{5.74}$ EID_{50} (0-$10^{6.44}$) EID_{50} of HPAI virus among 1,144.5 pounds of wet eggshells produced daily by breaking eggs from a 100,000 bird infected but undetected inline layer flock.

Appendix 2 Table 2. Estimated quantity of HPAI virus in wet eggshells from inline and standalone breaking plants and the fraction of the total amount of wet eggshells contaminated with HPAI virus (mean with 90 percent P.I.).

	Inline	**Offline**
Fraction of total wet eggshells contaminated assuming no mixing	7.6×10^{-5} ($0\text{-}2.4\times10^{-4}$)	9×10^{-6} ($0\text{-}5.7\times10^{-5}$)
Total quantity of HPAI virus in 1,110 pound batch of wet eggshells	$10^{5.74}$ EID_{50} ($0\text{-}10^{6.44}$) EID_{50}	$10^{4.84}$ EID_{50} ($0\text{-}10^{5.70}$) EID_{50}
Virus concentration in a 2-pound portion of wet eggshells mixed with eggshells generated from HPAI contaminated eggs	$10^{2.78}$ EID_{50} ($0\text{-}10^{3.48}$) EID_{50}/g	$10^{1.87}$$EID_{50}$ ($0\text{-}10^{2.75}$) EID_{50}/g
Virus concentration in a 5-pound portion of wet eggshells mixed with eggshells generated from HPAI contaminated eggs	$10^{2.39}$ EID_{50} ($0\text{-}10^{3.08}$) EID_{50}/g	$10^{1.47}$$EID_{50}$ ($0\text{-}10^{2.35}$) EID_{50}/g

Concentration of HPAI Virus in Liquid INEP in the Holding Tank

The HPAI virus concentration of liquid INEP was estimated using the disease transmission model and estimates of the quantity of inedible egg during grading or breaking from industry experts (Appendix 2 Table 3). We analyzed the movement of liquid INEP from inline egg-breaking plants (no holding time) and standalone egg-breaking plants (two-day hold time) separately.

Appendix 2 Table 3. Inputs used to estimate the HPAI viral batch in liquid INEP.

Variable	Description	Input
W_E	Average weight of a hen's egg	60 grams
$V_{T(i)}$	HPAI viral titer in albumin from a contaminated egg *i*	Empirical distribution from data presented in Swayne and Eggert 2012 from 43 contaminated eggs mean : $10^{4.81}$ EID_{50}/ml, 95th percentile $10^{5.5}$ EID_{50}/ml,
I_B	Inedible liquid from centrifuging eggshells post breaking	1.44 pounds/case of eggs
I_G	Inedible liquid from grading	1.27 pounds/case of eggs
H_L	Handling liquid from breaking (includes floor eggs)	1.22 pounds/case of eggs
T_D	Number of eggs produced each day (100,000 hens)	70,000 eggs/day
E_{sum}	Number of HPAI contaminated eggs from breaking (inline and offline sources) per movement	Number of contaminated eggs from the disease spread model
E_G	HPAI contaminated eggs with defects rejected at grading that go into the inedible egg barrel	Number of contaminated eggs from the disease spread model
E_T	Total number of HPAI contaminated eggs released	Number of contaminated eggs from the disease spread model
p	Days of inedible liquid egg production from breaking	3.5 (emptied twice a week) or 7 (emptied once a week)
N_C	Number of eggs in a case	360
ρ	Density of egg white	1.033 gms/ml

Estimation of the HPAI Virus Titer of Liquid INEP Moved from an Inline Egg-breaking Facility Sourcing Eggs from an Infected but Undetected Flock on the Premises

We considered the quantity of liquid INEP moved per shipment (A_{in} ml/shipment) given weekly once or weekly twice movement and facility sources eggs from a 100,000-hen inline-layer flock. We first calculated the total inedible production in a week according to Appendix 2 equation 2. Here, the number 454 is the conversion factor from pounds to grams as (I_B), (H_L) and I_G are in units of pounds-per-case of eggs.

$$A_{in} = (453.592)\,((p\,(\mathrm{I_B} + \mathrm{I_g} + \mathrm{H_L})\,\mathrm{T_d}))/(\mathrm{Nc} * \rho) \qquad \text{(Appendix 2 Eq. 2)}$$

We then calculated the amount of HPAI virus in liquid INEP from breaking and handling, per shipment of inedible egg C_{break} (EID$_{50}$/shipment), as shown in Appendix 2 Eq.3.

$$C_{break} = \sum_{\text{i=min }(1,E_{sum}}^{E_{sum}} (453.592)\,((V_{T(i)} * (I_B + H_L)))/(N_c*\rho) \qquad \text{(Appendix 2 Eq. 3)}$$

In the next step, we calculated the amount of HPAI virus from HPAI contaminated whole eggs that are rejected at grading (due to being defective) and diverted to the inedible (barrel) tank. We considered that 20 percent of HPAI contaminated eggs may have defects, such as being soft shelled or thin shelled, and would be diverted into the inedible egg tank during the egg-grading process. We used the binomial distribution as shown in Appendix 2 Eq.4 to estimate

$$E_G \sim \text{Binomial}(E_{sum}, 0.2) \qquad \text{(Appendix 2 Eq. 4)}$$

$$C_{grade} = \sum_{\text{i=min }(1,E_G)}^{E_G} V_{T(i)} * W_E / \rho \qquad \text{(Appendix 2 Eq. 5)}$$

In the next step, the final concentration C_{final} of HPAI virus in the holding tank for shipment was calculated as total amount of HPAI virus (C_{break} + C_{grade}) divided by the estimated amount of inedible in the tanker A_{in}

$$C_{final} = (C_{break} + C_{grade})/A_{in} \qquad \text{(Appendix 2 Eq. 6)}$$

Appendix 2 Table 4. Estimated average quantity of HPAI virus infectivity and concentration in liquid INEP from inline and standalone egg-breaking plants.

Movement	Batch Estimate	Inline (without a two-day hold)	Standalone (eggs received after two-day hold)
Once weekly	Total virus quantity in a batch (5308 lb)	$10^{6.18}$ EID$_{50}$ (0-$10^{6.7}$)	$10^{4.32}$ EID$_{50}$ (0-0)
	Average concentration in the batch	0.65 (0-2.15) EID$_{50}$ /ml	0.009 (0-0) EID$_{50}$ /ml
Twice weekly	Total virus quantity in a batch (2654 lb)	$10^{6.17}$ EID$_{50}$(0-$10^{6.7}$)	$10^{4.32}$ EID$_{50}$(0-0)
	Average concentration in the batch	1.27(0-4.3) EID$_{50}$ /ml	0.018 (0-0) EID$_{50}$ /ml

Appendix 3. Regulations that Pertain to Poultry Feed or Poultry Feed Ingredients

TITLE 9 Animals and Animal Products

PART 145—NATIONAL POULTRY IMPROVEMENT PLAN FOR BREEDING POULTRY

Subpart B—Special Provisions for Multiplier Egg-Type Chicken Breeding Flocks and Products

§ 145.23 Terminology and classification; flocks, and products.

(ii) All feed fed to the flock shall meet the following requirements:

(A) Pelletized feed shall contain either no animal protein or only animal protein products produced under the Animal Protein Products Industry (APPI) *Salmonella* Education/Reduction Program. The protein products must have a minimum moisture content of 14.5 percent and must have been heated throughout to a minimum temperature of 190 °F., or above, or to a minimum temperature of 165 °F for at least 20 minutes, or to a minimum temperature of 184 °F under 70 lbs. pressure during the manufacturing process.

(B) Mash feed may contain no animal protein other than an APPI animal protein product supplement manufactured in pellet form and crumbled: *Provided*, that mash feed may contain nonpelleted APPI animal protein product supplements if the finished feed is treated with a salmonella control product approved by the Food and Drug Administration.

(iii) Feed shall be stored and transported in such a manner as to prevent possible contamination

Appendix 4. Survival of Avian influenza Virus in Various Poultry Products at Higher Temperatures

Appendix 4 Table 1. Survival of AI viruses in chicken meat.

Temp	Time to Inactivation	Amount Left/Description	Source
61 °C	28-34 s (D value)	Time to reduce titer by 90% (from 7.5–8 log/ml)	Thomas and Swayne, 2007[117]
70 °C	<40 seconds to lose infectivity (ramped up from ambient temperature, 25 °C – 70 °C)	$10^{\wedge 2.3}$ EID 50 (detection limit) reduction from $10^{\wedge 6.8}$ to $10^{\wedge 2.3}$ [thermocycler took 40 seconds to reach 70 °C, some virus was detected at 70 °C but after a 5 second treatment, no virus was detected]	Swayne, 2006[118]
70 °C	5.5 s	Predicted for 11 log reduction	Thomas and Swayne, 2007[117]
73.9 °C	0.8 s		
70 °C	0.2 s (D value)	Time to reduce titer by 90%	Thomas, King, and Swayne 2008[129]
74 °C	0.03 s (D value)		

Appendix 4 Table 2. Survival of AI viruses in liquid egg yolk, albumen, and whole egg.

Material	Temp	Time to inactivation	Amount left/ description	Source
Egg yolk	57 °C	30 min	No virus isolated (from 4.8 log/ml)	King, 1991[69]
Albumen	57 °C	10 min	No virus isolated (from 5.2- 8.3 log/ml)	
Salted yolk (10%)	57–59 °C	<19 s (D value)	Time to reduce titer by 90% (from 4.9 log/ml)	Swayne and Beck, 2004[68]
Liquid egg white	59 °C	<19 s (D value)	Time to reduce titer by 90%	
Whole egg	57 °C	4 min	No virus isolated from 4.9 log EID 50	
	59, 61 °C	2 min		
	63 °C	1 min		
Homogenized whole egg	63 °C	<19 s (D value)	Time to reduce titer by 90%	

Appendix 4 Table 3. Survival of AI virus in dried and freeze-dried egg white.

Material	Temp	Time to inactivation (D value)	Source
Dried egg white	63 °C	0.2 days (4.8 h)	Swayne and Beck, 2004[68]
	67 °C	0.12 days (2.88 h)	
Freeze-dried egg white (moisture: 7.4-7.6%)	65.5 °C	400.6 min	Thomas and Swayne, 2009[129]
	71.5 °C	43.7 min	

Appendix 4 Table 4. Survival of AI virus in chorioallantoic and allantoic fluid.

Material	Temp	Time to inactivation	Amount left/ description	Source
Chorioallantoic fluid	56 °C	40 min, 60 min	80-100% infectivity lost, (at 90 min 100% infectivity lost)	Lu et al. 2003[28]
Chorioallantoic fluid	60 °C	10 min	100% infectivity lost	
Allantoic fluid	56 °C	30 min	No virus isolated (from 4.0 log/ml)	Shahid et al. 2009[77]
	60 °C	30 min		
Allantoic fluid	60 °C	5 min	No virus isolated (from 5.0 log10/ml)	King, 1991[69]
Allantoic fluid, diluted	70 °C	60 min to lose infectivity	Assay performed by inoculating incubated heat treated AF into embryonated eggs	Wanaratana et al 2010[126]

Appendix 5. Inactivation Times for Industry Standard Temperatures for Inactivation of AI Virus in Eggs, Egg- products, and Poultry Meat

The times for industry standard temperatures that are suitable for the inactivation of AI virus present in eggs and egg products and meat from the OIE are shown in Tables 1 and 2.[130] The listed temperatures are indicative of a range that achieves a 7-log kill. Where scientifically documented, variances from these times and temperatures may also be suitable when they achieve inactivation of the virus.

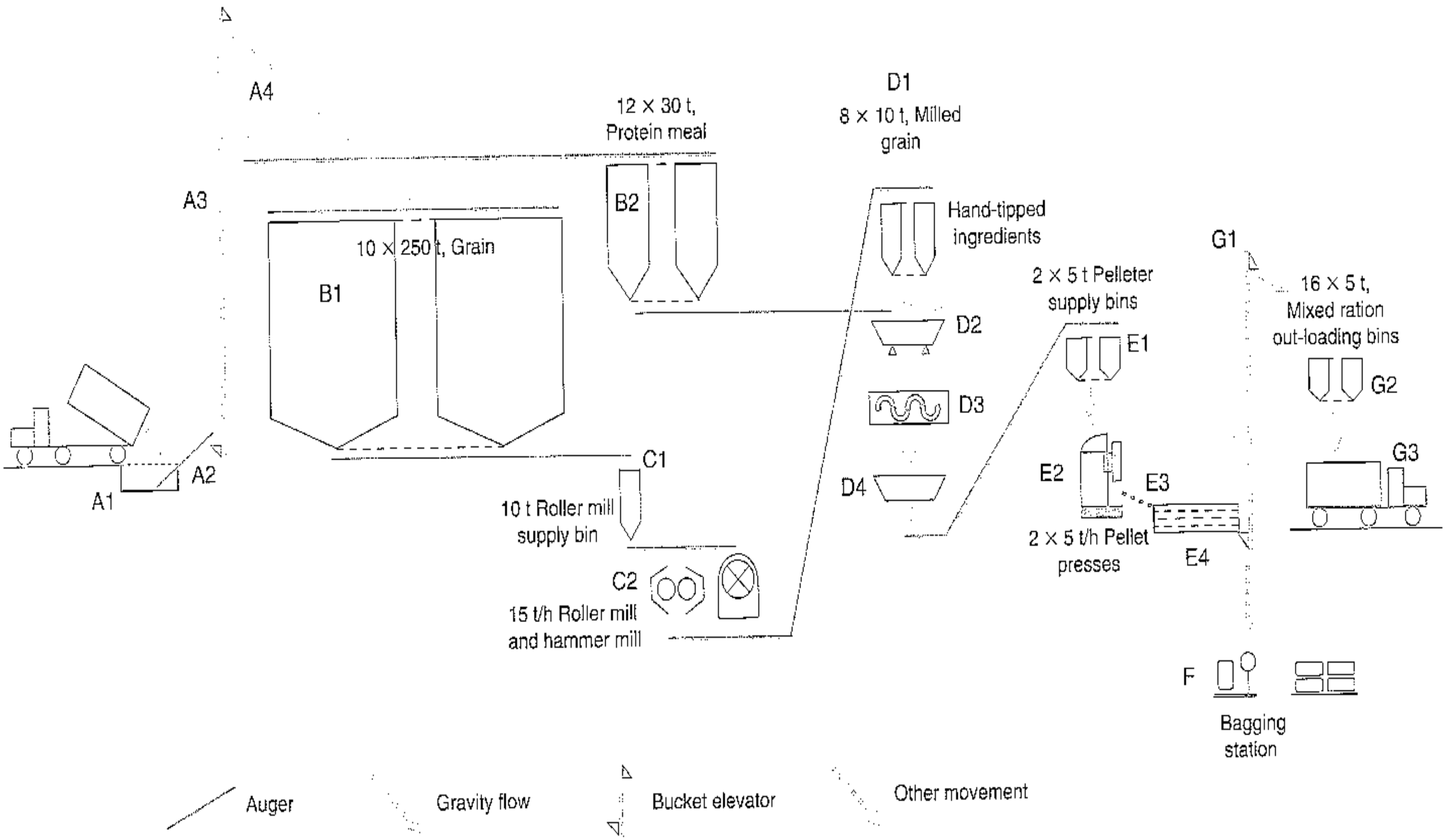
Appendix 5 Table 1. Procedures for the inactivation of the AI virus in eggs and egg products.

Product	Core temperature (°C)	Time
Whole egg	60	188 seconds
Whole egg blends	60	188 seconds
Whole egg blends	61.1	94 seconds
Liquid egg white	55.6	870 seconds
Liquid egg white	56.7	232 seconds
10% salted yolk	62.2	138 seconds
Dried egg white	67	20 hours
Dried egg white	54.4	513 hours

Appendix 5 Table 2. Procedures for the inactivation of the AI virus in meat.

Core temperature (°C)	Time
60.0	507 seconds
65.0	42 seconds
70.0	3.5 seconds
73.9	0.51 seconds

Appendix 6. Specific Process Flow Charts for a Feed Mill



Appendix 7. A Comparison of D-values for *Salmonella* sp. and Avian Influenza Virus

Thermal inactivation time for Salmonella in egg products depends on the type of product, temperature, and strain of Salmonella. For liquid egg white, D-values ranged from 0.25 minutes at 56.7 °C (Garibaldi, Straka et al., 1969) to 7.99 at 55.1 °C (Schuman and Sheldon, 1997).[121, 131] In egg yolk D-values ranged from 0.087 at 62.2 °C (Schuman and Sheldon, 1997) to 0.75 at 60 C (Palumbo, Beers et al. 1995).[121, 132] D-values for homogenized whole egg ranged from 0.2 at 60 °C to 34.3 at 55 °C (Humphrey, Chapman et al., 1990).[133]

D-values for AI virus in liquid egg white ranged from <0.32 minutes 59 °C to 4.28 minutes at 55 °C (Appendix 7 Table 6. There was greater variability for homogenized whole eggs as D-values ranged from <0.32 at 61 degrees to 10.74 at 55 °C (Swayne and Beck, 2004).[68]

We excluded D-values for Salmonella (Seftenberg). This strain is particularly heat resistant with the high D-values and is not as important in terms of foodborne illness, but can be used in the lab as a benchmark to evaluate effectiveness of inactivation procedures for Salmonellas in general. Since none of the required FSIS pasteurization temperatures were under 55.6 °C, we also excluded D-values calculated for temperatures of 55 °C or less. One of the AI data points (from King, 1991) was a rough calculation of a D-value using virus titer at the start of the experiment and time to inactivation.[69] Since the actual time to inactivation could have varied from 5 to 10 minutes (samples were taken to assess inactivation at 5 minute intervals) we excluded this value from our evaluation.

Because D-values are within similar ranges for Salmonella and AI virus at standard pasteurization temperatures, protocols designed to inactivate most strains of Salmonella should be sufficient to inactivate AI virus.

FSIS standards call for specific pasteurization protocols designed to inactivate 7 $\log_{10}$ for Salmonella. For whole egg, egg must be heated at 60 °C for 3.5 min. Liquid egg white must be heated either at 55.6 °C for 6.2 min or 56.7 °C for 3.5 min (Chmielewski and Swayne, 2011).[70]Although inedible egg contains mostly egg white by weight, the addition of whole egg from the grading/packaging process introduces yolk to the mixture. Therefore, FSIS pasteurization requirements for whole egg/whole egg blends should be followed for the pasteurization of inedible eggs.

Appendix 7 Table 1. D-values for the inactivation of various strains of Salmonella in egg yolk from two studies.

Strain	Temp °C	D-value (min)	Source
	58.9	0.7	Garibaldi, Straka et al. 1969[131]
	60	0.4	
SE Y8-P2	60	0.55	Palumbo, Beers et al. 1995[132]
SE 2000	60	0.62	
SE 5-19	60	0.65	
ST	60	0.67	
SS	60	0.73	
SE 92-008	60	0.75	
ST	61.1	0.2	
SE 2000	61.1	0.27	
SE Y8-P2	61.1	0.27	
SS	61.1	0.28	
SE 5-19	61.1	0.29	
SE 92-008	61.1	0.35	
ST	62.2	0.14	
SE Y8-P2	62.2	0.21	
SS	62.2	0.21	
SE 2000	62.2	0.23	
SE 92-008	62.2	0.25	
SE 5-19	62.2	0.3	

Appendix 7 Table 2. D-values for the inactivation of Salmonella. in homogenized whole egg (source: Humphrey, Chapman et al. 1990).[133]

Strain	Temp °C	D-value (min)
Typhimurium PT141	55	2.3
Enteritidis PT13a	55	3.9
Typhimurium PT110	55	4.7
Enteritidis PT8	55	5.9
Seftenberg	55	34.3
Enteritidis PT4	55	6.4
Typhimurium PT141	60	0.2
Enteritidis PT13a	60	0.22
Typhimurium PT110	60	0.26
Enteritidis PT8	60	0.3
Seftenberg	60	5.6
Enteritidis PT4	60	0.44
Seftenberg	64	2.8
Enteritidis PT4	64	0.22

Appendix 7 Table 3. D-values for the inactivation of Salmonella in whole egg (source: Garibaldi, Straka et al. 1969)[131]

Temp °C	D-value (min)
55	4
59	0.5
60	0.28

Appendix 7 Table 4. D-values for the inactivation of Salmonella in egg white and raw liquid egg white.

Material	pH	Temp °C	D-value (minutes)	Source
Egg white		56.6	1.44	Palumbo, Beers et al. 1996[124]
		57.7	0.78	
	9.2	54.8	0.64	Garibaldi, Straka et al. 1969[131]
Raw liquid egg white	9.1	56.7	1.58	Schuman and Sheldon, 1997[121]
	8.2	56.7	2.96	
	9.1	58.3	0.52	
	8.2	58.3	1	

Appendix 7 Table 5. D-values for the inactivation of Salmonella in raw liquid egg yolk (source: Schuman and Sheldon, 1997).[121]

pH	Temp °C	D-value (min)
6.3	60	0.28
	61.1	0.16
	62.2	0.087

Appendix 7 Table 6. D-values for the inactivation of Avian Influenza HPAI strain H5N2 in two egg products (source: Swayne and Beck, 2004).[68]

Material	Temp °C	D-value (min)
Homogenized whole egg	57	4.48
	59	0.37
	61	0.32
	63	0.32
Liquid egg white	57	0.38
	59	0.32
	61	0.32
	63	0.32

Appendix 8. Scenarios for Transmission of HPAI Virus via Bioaerosols Produced During Land Application of Wet Eggshells

In this appendix, we provide details of the AERSCREEN modeling scenarios for conservative estimation of the HPAI virus concentration at specific distance from the land application site. The key input parameters of the AERSCREEN model are summarized below.

- *Total quantity of HPAI virus in 1,144.5 pounds of wet eggshells:* This parameter represents the total quantity of HPAI virus in wet eggshells generated by breaking eggs from a 100,000 bird layer flock. The values for this parameter under different scenarios were based on the simulation results presented in Chapter 9.1.
- *Pounds of contaminated wet eggshells among 1,144.5 pounds of wet eggshells*: For this analysis, we assumed 1 to 5 pounds of wet eggshells would become contaminated through mixing of contaminated and virus free eggshells. The amount of virus that may be present in a 1,144.5-pound batch of wet eggshells originating from an infected but undetected flock that is moved after a two day hold was estimated to be $10^{4.84}$ EID_{50} (0-$10^{5.74}$) EID_{50} from simulation models presented in the Entry Assessment section. We considered scenarios where the quantity of HPAI virus in a batch of wet eggshells could be $10^{4.84}$ and $10^{5.74}$ EID_{50} in different scenarios. This quantity of HPAI virus was assumed to be diluted into 1, 3, or 5 pounds of eggshells in alternate scenarios.
- *Slinger equipment spreading rate of wet eggshells (pounds/second)*: The rate at which the equipment spreads wet eggshells would impact the source emission rate and duration for which contaminated eggshells are land applied. Paez-Rubio *et al*, 2007[97] reported a side discharge slinger (Kuhn Knight Inc.) operated at 4.04 dry kg per second.[83] Egg-industry experts mentioned that similar slinger equipment is used in land application of wet eggshells as well.
- *Aerosolization efficiency*: We define aerosolization efficiency as the ratio of the amount of microorganisms aerosolized to the amount of microorganisms present in the solids that are spread. One approach to estimating the aerosolization efficiency is to assume that the fraction of specific microorganisms aerosolized is the same as the fraction of biosolid mass that is aerosolized. For example, an EPA study found a mean fraction of irrigated wastewater that is aerosolized of 0.0033 (approximate range 0.0009 to 0.018).[134] Paez-Rubio *et al.*, 2007 estimated emission rates of aerosols produced while spreading dewatered class B biosolids.[97] We estimated aerosolization efficiency of 0.000123 based on HPC (Heterotrophic Plate Count of Bacteria) concentration in solids, solids spreading rate, and the HPC emission rate estimates from Paez-Rubio *et al.*, 2007 as shown in Appendix 8 Equation 1. [97]An alternate estimate of aerosol emission rate 0.0000076 was based on the ratio of the amount of biosolids aerosolized to the total amount of biosolids spread on a dry weight basis from the same study.

$$Aerosolization\ Efficieny\ E = \frac{Aerosol\ Emission\ rate}{(Biosolid\ concentration) * (spreading\ rate)}$$

(Appendix 8 Eq. 1)

Appendix 8 Table 1. Input parameters used in conservative AERSCREEN scenarios to predict HPAI virus concentration at different distances from the land application site of wet eggshells from infected but undetected table-egg layer flocks.

Parameter Description	Source/Formula	Scenario 1	Scenario 2	Scenario 3
Total quantity of HPAI virus in 1,144.5 pounds of wet eggshells (Q) (EID_{50})	Estimated from disease transmission and surveillance models	$10^{4.84}$	$10^{5.74}$	$10^{5.95}$
Pounds of contaminated eggshells among 1,144.5 pounds of wet eggshells P	Assumption related to mixing of contaminated and virus free eggshells	3	5	1
Slinger equipment spreading rate of wet eggshells S (pounds/second)	*Paez-Rubio et al., 2007*[97]	4	4	4
Aerosolization efficiency (E)	*Paez Rubio et al., 2007, EPA Spray Irrigation experiments*.[97]	0.0000076	0.000123	0.0033
Aerosol source rate(EID_{50}/s)	$\frac{S * Q * E}{P}$	0.7010	54.074	11764
Source duration (seconds)	P/S	0.75	1.25	0.25
Minimum wind speed (m/s)	Conservative Assumption	0.5 m/s	1m/s	1m/s
Terrain type	Assumption	cultivated	cultivated	cultivated
Estimated concentration at 3 km EID_{50}/m^3)	From Aerscreen model	0.001	0.03	5.75
Estimated concentration at 8 km (EID_{50}/m^3)	From Aerscreen Model	0.0002	0.0053	1.3

Appendix 8 Table 2. Estimated HPAI virus concentrations (EID_{50}/m^3) from 3 AERSCREEN scenarios at different distances from the land application site of wet eggshells.

	Estimated HPAI virus concentration (EID_{50}/m^3)		
Distance from Source	**Scenario 1**	**Scenario 2**	**Scenario 3**
3 km	0.001	0.03	5.75
8 km	0.0002	0.0053	1.3

Appendix 9. The Definition of Non Negligible Risk Levels Used in this Assessment

Low Risk

For this risk analysis, the term "low risk" means it is very unlikely that moving eggshells and inedible egg product will cause infection in susceptible poultry. The determination of "low risk" suggests that, although not a requirement, additional resources to further evaluate or mitigate this risk may be considered (depending on circumstances).

Use in Risk Analysis

The term "low risk" has been frequently used in risk-rating systems for qualitative risk analysis. These risk-rating systems are often customized according to the specific objectives of the risk assessments. Consequently, there is considerable variation in the interpretation of the terms used to describe risk among various risk assessments. For example, in the USDA-APHIS Guidelines on Pathway-Initiated Pest Risk Assessments, the rating of *low* is interpreted as "the pest will typically not require specific mitigation measures".[135] The FDA Guidance Document 152 states that "for a drug to be ranked as low risk overall, two of three major components (release, exposure and consequence) of the risk assessment should be ranked as low and the third component ranked as moderate".[136] In a risk-rating system used in USDA APHIS for qualitative risk assessment for potential Federal noxious weeds, the overall pest risk potential is *low* as long as the likelihood of introduction of the weed is *low*, regardless of the consequences of introduction.[137] Overall, various definitions of "low risk" have been used as appropriate in different situations.

Negligible to Low Risk

When there is a considerable uncertainty in the risk estimate, we may not be able to ascertain whether the risk is *negligible* or *low*. This uncertainty can be expressed as a probability distribution for the risk in a fully quantitative risk assessment. For a qualitative risk assessment, there are no universally followed guidelines for expressing this uncertainty. Therefore, when there is uncertainty about whether the risk is *negligible* or *low*, we rate it as *negligible* to *low* risk. With *negligible* to *low* risk, depending on the circumstances, further evaluation to determine whether the risk is *negligible* or *low* may be conducted.

Definitions of Moderate, High and Extremely High Risk Levels Considered in the Risk Evaluation Process

These risk levels were defined on the basis of the likelihood of the spread of HPAI infection to susceptible poultry. The specific levels are defined as follows.

Moderate Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is unlikely to but does occur.

High Risk: There is more than an even chance that the spread of HPAI infection to susceptible poultry through the risk pathway will occur.

Extremely High Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is almost certain to occur

Appendix 10. The Use of "Negligible Risk" in this Assessment

Negligible Risk Defined for this Analysis

For this risk analysis, the term "negligible risk" means that the spread of HPAI infection to susceptible poultry through the risk pathway is insignificant or not worth considering. In quantitative terms, this is defined as a likelihood of less than 1/1,000,000 that the risk pathway will result in infection in other premises. This particular likelihood is used to be consistent with other common meanings for the term, as discussed below. The determination of "negligible risk" suggests that allocating additional resources to mitigate this risk pathway may not be a cost-effective use of resources (depending on circumstances).

Negligible Risk as Less Than 1/1,000,000

Origins

Use of the term "negligible risk" originated in efforts to regulate chemical exposures. While there is no formal definition, the term evolved in the human exposure risk assessment literature as a lifetime cancer risk of less than 1/1,000,000. This particular level was selected as it was thought to be a level of "essentially zero" risk.[138-141] While this level has not been formally defined in legislation, The House Committee on Commerce evaluated the use of this term by the Environmental Protection Agency, and agreed that the agency's interpretation of the term "negligible risk" to be approximately a one-in-a-million lifetime risk, as appropriate.[142]

Use in Agricultural Risk Analysis

The use of risk analysis for imports of agricultural products became mandatory with the adoption of the SPS Agreement[a] in 1995.[b] Specific recommendations and standards were to be established by the appropriate technical body. For animals and animal products, this is the Office International des Epizooties (OIE, or World Organization for Animal Health).[144] The OIE has published standards and guidance for conducting risk analysis, but has not formally defined "negligible" in a quantitative sense.[145, 146] However, in a World Trade Organization trade dispute case,[147, 148] negligible risk was considered to be a risk whose probability is very low, or, as an expert consultant to the WTO Dispute Panel put it, "the standard scientific definition of "negligible" was a likelihood of between zero and one-in-one million." [149,150]

Policy Implications of a Quantitative Definition for Negligible Risk

While the 1/1,000,000 definition for negligible risk has substantial precedence (as shown above), there are difficulties with this approach. The 1/1,000,000 likelihood has been described as "folklore," vague, and inconsistent, and has been "used and (abused) in various policy

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[a] Formally known as the "Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) and Agreement on Technical Barriers to Trade (TBT)."

[b] Risk analysis is also required for moving animals and animal products during a HPAI outbreak (Kwon et al., 2005)143. Kwon, Y. K., S. J. Joh, M. C. Kim, H. W. Sung, Y. J. Lee, J. G. Choi, . . . J. H. Kim. Highly Pathogenic Avian Influenza (H5N1) in the Commercial Domestic Ducks of South Korea. Avian Pathology 34:367-370. 2005.

contexts."[151,141,152] However, use of this figure is meant to be a very rough approximation and should not be given the same degree of certainty that may be applied when quantitative risk assessments can be used.

Negligible Risk as a Qualitative Measure for Agricultural Risk Analysis

The OIE has issued guidance that recommends using "negligible" to mean "not worth considering; insignificant."[145] The use of qualitative risk analysis methods by APHIS and the implied non-requirement for attaching a specific number to a level of risk has been challenged in the U.S. Court system and has been upheld as appropriate, if the analysis presents adequate scientific information.[153] When used in this manner, the courts have held that the determination of risk may be based on "the cumulative effects of the multiple, overlapping, safeguards." Furthermore, the courts have held that an "imposition of such a bright-line prohibition on qualitative standards was incorrect," and that the Animal Health Protection Act does not require a quantified permissible level of risk.[147] These opinions by the court system are also consistent with U.S. views expressed in WTO trade disputes.

Appendix 11. Qualitative Scales of Likelihood

This appendix defines the qualitative likelihood scale used to describe the probability of events in this risk assessment. Qualitative scales attach a specific narrative phrase which conveys a meaning to terms used to describe the likelihood of an event occurring. Generally, it is best to choose an expression where there is some evidence for a high degree of consensus for its interpreted meaning.[154] For example, use of the narrative phrase *"there is a high likelihood that the event will occur"* has been interpreted as a probability that ranges from 0.60 to 0.97 (60 to 97 percent chance of occurrence); and the expression *likely* has been interpreted to range from 0.63 to 0.77.[155, 156] To date, there is no one universally accepted or utilized likelihood scale, and the scales are customized as appropriate for specific assessments. The OIE handbook on qualitative risk analysis does not prescribe a specific likelihood scale although it provides examples for terms which might be used in likelihood scales such as *low, negligible, high* etc.[157] **Appendix 11 Table 1** provides examples of qualitative scales used in risk assessments elsewhere and **Appendix 11 Table 2** lists adjectives to describe likelihoods considered appropriate by the OIE. The likelihood scale used in this assessment is defined by **Appendix 11 Table 3**.

Appendix 11 Table 1: An example likelihood scale adapted from Standards Australia for qualitative risk assessment in fisheries management.[158]

Category	Probability Range
Likely	It is expected to occur
Occasional	May occur sometimes
Possible	Some evidence to suggest this is possible here
Unlikely	Uncommon, but has been known to occur elsewhere
Rare	May occur in exceptional circumstances
Remote	Never heard of, but not impossible

Appendix 11 Table 2: Terms used as adjectives to qualify likelihood estimates considered appropriate by the OIE.[2]

Category	Descriptor
Extremely	Outermost, furthest from the center; situated at either end; utmost; the highest or most extreme degree of anything
High	Extending above the normal or average level
Highly	In a high degree
Significant	Noteworthy; important; consequential
Average	The usual amount, extent, rate
Low	Less than average; coming below the normal level
Remote	Slight, faint
Insignificant	Unimportant; trifling
Negligible	Not worth considering; insignificant

Appendix 11 Table 3: Qualitative likelihood scale used in this assessment.

Category	Descriptor
Extremely High	The event is almost certain to occur
High	There is more than an even chance that the event will occur
Moderate	The event is unlikely but does occur
Low	It is very unlikely that the event will occur
Negligible	The likelihood that the event will occur is insignificant: not worth considering

Appendix 12 Upper Bound Estimation for Probability of Infection for Chickens Exposed to a known Quantity of Wet Eggshells or Inedible Egg Product from a Batch

The extent of dispersion of HPAI virus in a contaminated batch of wet eggshells and inedible egg is challenging to predict due to the uncertainty and variability regarding the degree of mixing during their processing. In this appendix, we derive theoretical upper bounds for the probability of infection of a chicken intranasally exposed to a specific quantity of wet eggshells or INEP from a batch where the extent of dispersion of the virus in the batch is not known. The upper bound estimates are beneficial in informing the exposure assessment for HPAI virus present in wet eggshells and INEP.

Notation and Assumptions

We have the following notation in the theoretical model:

T-Total amount of wet eggshells or inedible egg in a batch (g)

A- Contaminated quantity (or segment) of wet eggshells or inedible egg (g) $A \leq T$

d – The exposure quantity of wet eggshells or inedible egg (g)

Q –Total quantity of virus dispersed within wet eggshells and inedible eggs (EID_{50})

P –Probability of infection when a quantity d is randomly selected from either the contaminated or virus free portions of the media and is transmitted to a susceptible animal.

r- Exponential dose response parameter

We make the following key assumptions in our analysis:

- Virus is homogeneously distributed within contaminated segment of the total quantity of wet eggshells or inedible egg.
- The exposure quantity is randomly selected from either the contaminated or the virus free wet eggshells and inedible egg.

Derivation

Probability of infection if a quantity *d* from either the contaminated or the virus free segments of a batch of wet eggshells or inedible egg is transmitted to a susceptible animal according to exponential dose response function is given by,

$$P = \frac{A\left(1 - e^{-\frac{rdQ}{A}}\right)}{T}$$

(Appendix 12 Equation 1)

$$\frac{dP}{dA} = \frac{1 - e^{-\frac{rdQ}{A}}\left(1 + \frac{rdQ}{A}\right)}{T}$$

(Appendix 12 Equation 2)

$$\frac{dP}{dA} = \frac{1 - ee^{-(\frac{rdQ}{A}+1)}(1 + \frac{rdQ}{A})}{T}$$

(Appendix 12 Equation 3)

Let z be equal to the term $e^{-(\frac{rdQ}{A}+1)}(1 + \frac{rdQ}{A})$. Now, $\lim_{A\to\infty} \text{z} = 1/\text{e}$ and $\frac{dz}{dA} = \frac{d^2r^2\, q^2\, (e^{-(\frac{rdQ}{A}+1)}(1+\frac{rdQ}{A}))}{A^3}$. So $\frac{dz}{dA}>0$ in the interval (0,∞). Therefore, the function z approaches a maximum of 1/e as A→ ∞ in the interval (0, ∞). Therefore, $\frac{dP}{dA}>0$ when A is in the interval (0,∞). This result $\frac{dP}{dA}>0$ indicates that the probability of infection if a susceptible chicken is exposed to a quantity of wet eggshells from a batch increases as the amount of wet eggshells the virus is dispersed into increases under the exponential dose response model. The result also implies that the risk of infection with homogeneous dispersion of virus in the batch of wet eggshell (i.e., A = T), would be an upper bound on the probability of infection when virus is present only in a segment of the wet eggshells.

Numerical Scenarios

In this section, we provide numerical examples for the risk of infection for a chicken exposed to specific quantities of wet eggshells or inedible egg from a batch. We calculated the parameter *r* for the exponential dose response model based on a 50 percent chicken infectious dose of 2.5 log EID_{50} for the intranasal route. Using this approach, *r* = 0.0021919. Estimates of the maximum (among wet eggshell batches with different degrees of dispersion) likelihood of infection when a chicken is exposed to 1 or 2 grams of wet eggshells from a batch were calculated using Appendix 12 equation 4. Here the total amount of HPAI virus in batch Q was taken from **Table 11** and **Table 12**. The results are shown in Appendix 12 table 1.

$$P_{max} = \left(1 - e^{-\frac{rdQ}{T}}\right)$$

(Appendix 12 Equation 4)

Appendix 12 Table 1. Upper bound on the probability of infection when a chicken is exposed to 1 or 2 grams of wet eggshells or inedible egg from a contaminated batch when the degree of dispersion is not known.

Exposure Quantity	**Estimated upper bound on the probability of infection**	
	Inline	**Offline**
1 g from a 1144.5 lb batch of wet eggshells	0.23 (0-1.1) percent	0.02 (0-0.2) percent
2 g from a 1144.5 lb batch of wet eggshells	0.46 (0-2.2) percent	0.05 (0-0.4) percent
1 g from a 2654 lb batch of INEP	0.26 (0-0.9) percent	$3.8*10^{-3}$ percent
2 g from a 2654 lb batch of INEP	0.53 (0-1.8) percent	$7.65*10^{-3}$ percent

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