An Assessment of the Risk Associated with the Movement of Broiler Day-old Chicks Into, Within, and Out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak

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Table of Contents

..... 1
1. Abbreviations and Definitions 5
2. Executive Summary 8
3. Introduction 11
4. Scope 13
4.1 Facilities Covered Under This Risk Assessment 13
4.2 Types of Movements Addressed Under This Risk Assessment 13
5. Overview of Data Analysis Approaches 14
6. Significant Assumptions Used in the Risk Assessment 16
7. Background Information on Broiler Day-Old Chick Production 17
7.1 Definition of Day-old Chicks and Hatcheries 17
7.2 Overview of Day-Old Chick Production in the United States 17
7.2.1 Hatchery Operations 17
7.2.2 Day-Old Chick Distribution and Logistics 17
7.3 Major Steps in the Production and Processing of Day-Old Chicks During Routine Operations 18
7.4 Sanitary Efforts and Current Disease Prevention in the Production of Day-Old Chicks 18
8. Hazard Identification - HPAI Overview 20
8.1 Agent and Host Range 20
8.1.1 Definition of Highly Pathogenic Notifiable Avian Influenza 20
8.1.2 Host Range 21
8.2 Geographic Distribution of H5N1 HPAI 21
8.3 Virus Shedding 22
8.4 Chemical and Physical Inactivation 22
8.5 Persistence of HPAI in Manure and Other Media 22
8.6 Transmission 27
8.7 Dose Response 28
8.7.1 Dose Response in Chickens 28
8.7.2 Route of Entry and 50 Percent Infectious Dose Estimate Used in this Assessment 29
8.8 Latently Infected and Infectious Periods 30
8.9 Clinical Signs 31
8.10 Diagnosis 31

8.11 Differential Diagnosis 31
9. Risk Evaluation 32
9.1 Introduction 32
9.2 Risk of Day-old Chicks Becoming Infected via Exposure to Contaminated Visitors, and Essential and Non-essential Personnel 34
9.2.1 Introduction 34
9.2.2 Preventative Measures 34
9.2.3 Evaluation of Risk 35
9.2.4 Conclusion 41
9.3 Risk of Day-old Chicks at a Hatchery Within the Control Area Becoming Infected with HPAI Virus from an Infected Poultry Flock via Fly Transmission 42
9.3.1 Introduction 42
9.3.2 Current Preventive Measures 42
9.3.3 Evaluation of Risk 42
9.3.4 Conclusion 46
9.4 Risk of Day-old Chicks in a Hatchery in the Control Area Becoming Infected with HPAI Virus from an Infected Flock in the Control Area via Aerosols 47
9.4.1 Introduction 47
9.4.2 Evaluation of Risk 47
9.4.3 Conclusion 57
9.5 Risk of Day-old Chicks in a Hatchery in the Control Area Becoming Infected with HPAI Virus from Essential Vehicles 58
9.5.1 Introduction 58
9.5.2 Preventive Measures 58
9.5.3 Evaluation of Risk 59
9.5.4 Conclusion 60
10. Overall Conclusion 61
Appendix 1: Relevant Current Preventive Measures from the NPIP Program (9CFR 145 and 147) 62
Appendix 2: Supporting Data and Analysis for Evaluation of the Risk of Aerosol Transmission of HPAI Virus to Day-old Chicks 63
Appendix 3: AI Virus Survival on Various Substrates 69
Appendix 4: The Definition of Non negligible Risk Levels Used in this Assessment 71
Appendix 5: The Use of "Negligible Risk" in this Assessment 73
Appendix 6: Qualitative Scales of Likelihood 75
11. References 77

1. Abbreviations and Definitions

APHIS	Animal and Plant Health Inspection Service (USDA)
AI	Avian influenza
AMS	Agricultural Marketing Service
BSL-2	Biosafety Level-2
BWG	Broiler Working Group
CEAH	Centers for Epidemiology and Animal Health
CFM	Cubic feet per minute
CFR	Code of Federal Regulations
C&D	Cleaning and Disinfection
EPA	Environmental Protection Agency
GMP	Good Manufacturing Practice
HA	Hemagglutination
HPAI	Highly pathogenic avian influenza
LPAI	Low pathogenic avian influenza
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
PM	Particulate Matter
PPE	Personnel protective equipment
RH	Relative Humidity
RRT-PCR	Real-time reverse transcription polymerase chain reaction
SPF	Specific Pathogen Free
U.S.	United States of America
USDA	United States Department of Agriculture
VS	Veterinary Services (USDA:APHIS:VS)

AERMOD

Aerosol dispersion model developed by the EPA and recommended to be used for regulatory decisions associated with air quality.

Breeder farm

Farms with multiplier broiler breeder flocks that produce broiler hatching eggs. The hatching eggs from a breeder farm are transported to a hatchery.

Broiler sector working group

A collaborative working group to address business continuity issues with representatives from the Association of Veterinarians in Broiler Production, the University of Minnesota Center for Animal Health and Food Safety, and USDA APHIS.

Buffer surveillance zone

The zone immediately surrounding the infected zone; the buffer surveillance zone and the infected zone comprise the Control Area.

CID_{50}

50 percent chicken infectious dose. One CID_{50} unit is the amount of virus that will infect 50 percent of inoculated chickens.

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 are 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.

Downtime for Visitors

For the purpose of this assessment, downtime refers to the time interval between when a visitor enters the hatchery and the time of last contact with other domestic poultry, other avian species, and/or related organic material from the Control Area.

EID_{50}

50 percent chicken embryo infectious dose. One EID_{50} unit is the amount of virus that will infect 50 percent of inoculated embryos.

ELD_{50}

50 percent chicken embryo lethal dose. One ELD_{50} unit is the amount of virus that will be lethal to 50 percent of inoculated embryos. Since most HPAI virus are embryo lethal the ELD_{50} estimates would be similar to EID_{50}.

Free Premises

Poultry premises that are not in a HPAI Control Area.

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.

Local area spread

Refers to risk pathways which have an increased likelihood for disease transmission with proximity to infected flocks.

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 poultry industry products from a premises or a geographic area described in a quarantine order.

Secure Broiler Supply Plan (SBS Plan)

A science-based plan that is comprised of outbreak measures and protocols proposed by the broiler sector working group to mitigate the risk of HPAI spread associated with the movement of hatching eggs and day-old chicks into, within, and outside of a Control Area. The SBS Plan includes various categories of measures such as active surveillance, holding time, biosecurity, cleaning, and disinfection.

$TCID_{50}$

50% tissue culture infectious dose. One $TCID_{50}$ unit is the amount of virus that will cause cytopathic effects in 50 percent of exposed host cells. The Madin-Darby Canine Kidney cell line is often used to estimate $TCID_{50}$ for HPAI viruses.

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 is acceptable. Performing the risk assessments prior to an HPAI outbreak can enhance emergency response and facilitate timely movement permitting decisions during an outbreak. This document assesses the risk that the movement of broiler day-old chicks, during an HPAI outbreak, from a hatchery, located within the Control Area, will result in HPAI virus spread to a virus free poultry premises (e.g., broiler farm).

This risk assessment is a joint effort between the broiler industry working group, 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 broiler day-old chicks and associated materials during an HPAI outbreak. This assessment is applicable to commercial broiler hatcheries that participate in the USDA-APHIS National Poultry Improvement Plan (NPIP) Appendix 1 and follow the Secure Broiler Supply Plan (SBS Plan) in the event of an HPAI outbreak. The SBS Plan contains science based outbreak measures developed by the broiler sector working group to mitigate the risk of HPAI spread associated with the movement of day-old chicks and hatching eggs. This risk assessment considers applicable current industry practices and biosecurity measures (NPIP) as well as outbreak specific measures from the SBS Plan. The main categories of outbreak measures from the SBS Plan considered here include:

- Biosecurity measures for essential visitors (e.g., protective clothing, a shower and change of clothes)
- Cleaning and disinfection of vehicles associated with essential visitors
- Biosecurity measures for hatchery personnel (e.g., footwear protocol)
- Measures pertaining to the movement of broiler hatching eggs from breeder premises in the Control Area

The emphasis in this assessment is on the risk of entry of HPAI virus onto a broiler premises associated with the movement of day-old chicks from a hatchery located within a Control Area. Given that day-old chicks are moved from the hatchery within a short duration after hatching, it is unlikely that HPAI is detected by the time of movement if they had become infected at the hatchery. Therefore, pathways for HPAI infection of day-old chicks in the hatchery during chick processing or holding, prior to movement were considered in order to evaluate the risk of spread (i.e. entry) associated with movement of day-old chicks.

An assessment of the risk that movement of broiler hatching eggs and associated handling materials results in HPAI infection of day-old chicks at the hatchery was completed beforehand, and the risk was estimated to be *negligible* to *low*. In this risk assessment, we focus on the pathways for the infection of day-old chicks via potential components of local area spread when the hatchery is located within a Control Area. By local area spread, we refer to risk pathways which have an increased likelihood for disease transmission with proximity to infected poultry flocks. The components of local area spread considered in this analysis include bio-aerosols generated from neighboring infected flocks; transmission of HPAI virus through insects;

transmission via essential visitors or hatchery employees who may have had contact with poultry, poultry waste or wild bird droppings; and through vehicles associated with essential visitors.

Risk pathways for HPAI transmission to the hatchery via movement of chick-handling materials from broiler farms in a Control Area were not included in the assessment scope. Hence this risk assessment only applies to movement of day-old chicks to premises outside the Control Area (Free Premises) and to premises in the Control Area for which the risk of transmitting HPAI virus back to the hatchery is *negligible to low*. In general, it is unlikely for day-old chicks to be placed onto premises with a high likelihood of HPAI virus being present. In the following, we summarize the main risk pathways and the corresponding risk ratings based on our evaluation:

- **The risk of day-old chicks at a hatchery in the Control Area becoming infected via exposure to essential visitors, and essential and non-essential personnel, contaminated with HPAI virus**. The current hatchery employee requirements (e.g., restrictions from having backyard poultry), as well as outbreak specific measures such as protocols requiring employees to wear protective footwear; and a minimum downtime with a shower and change of clothes for essential visitors, were considered in the risk evaluation. The risk through this pathway was rated to be *negligible* to *low* provided that the SBS Plan measures are followed.
- **The risk of day-old chicks at a hatchery in the Control Area becoming infected with HPAI Virus from an infected poultry flock via insect transmission.** We reviewed previous outbreak studies implicating flies in transmission of HPAI; survivability of AI viruses in flies; dispersion rates of specific types of flies implicated in HPAI spread; and a summary of expert opinion on the likelihood of transmission of HPAI to day-old chicks in a hatchery. The risk of day-old chicks becoming infected via flies transmitting HPAI virus from a nearby poultry facility at a distance of 1.5 km or more was rated to be *negligible*.
- **The risk of day-old chicks in a hatchery in the Control Area becoming infected with HPAI virus from an infected flock in the Control Area via aerosols.** The evaluation was based on a review of the scientific literature, aerosol dispersion modeling scenarios, and expert opinion. Alternate scenarios were modeled using the EPA aerosol dispersion model AERMOD, where the source flock is either known to be infected, or is infected, and undetected. The risk of exposure of day-old chicks from bio-aerosols was estimated to be *negligible* to *low* if the broiler hatchery is located at 1.5 km or farther from an infected but undetected poultry farm, and low if the broiler hatchery is located at 1.5 km or farther from a known infected poultry farm.
- **Risk of day-old chicks in a hatchery in the Control Area becoming infected with HPAI virus from essential vehicles.** The focus here is on essential visitors such as those transporting hatchery waste, delivering vaccines, equipment service personnel, and company veterinarians. The risk through this pathway was rated to be *negligible* to *low* provided that the SBS Plan measures are followed.

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 an 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 day-old chicks.

Overall Finding and Conclusion

The risk that movement of day-old chicks into, within, and out of a Control Area during an HPAI outbreak results in spread of HPAI to another operation (broiler farm) is *negligible* to *low*, provided that applicable preventive measures from NPIP regulations 9CFR145 and 9CFR147 and the Secure Broiler Supply Plan are strictly followed.

3. Introduction

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. 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 birds 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. Broiler hatcheries have limited holding capacity for day-old chicks and hatching eggs. Extended movement restrictions may result in the need to dispose of day-old chicks and loss of the value of hatching eggs. Proactive risk analysis identifies areas of risk and incorporates mitigation steps in order to minimize the spread of infection. Evaluating risk before an outbreak occurs facilitates timely emergency response and movement permitting decisions and minimizes unintended disruptions to business continuity.

The purpose of this assessment is to: (1) identify plausible risk pathways for the spread of HPAI infection associated with the movements of day-old chicks and chick handling materials; and (2) to assess the corresponding likelihoods of spread of HPAI onto another poultry premises (e.g. a broiler grow-out operation) despite all current and future preventive measures that will be in place during an outbreak.

There are two types of movements between hatcheries producing day-old chicks and broiler farms receiving them:

- Movement of broiler day-old chicks from a commercial broiler hatchery to a broiler farm
- Movement of chick handling materials from a broiler farm to the hatchery

The emphasis in this evaluation is on the risk of HPAI virus entry on to a broiler farm, with respect to the movement of day-old chicks potentially exposed at the hatchery. As day-old chicks are moved from the hatchery within three days of hatching, it is unlikely that HPAI is detected by the time of movement if they had become infected at the hatchery. This is because the spread of HPAI virus within a batch of day-old chicks, and its associated disease mortality, in the short duration between hatching and movement is expected to be minimal. Therefore, in order to evaluate the risk of entry of HPAI virus onto a broiler farm, we focused on pathways for HPAI infection of day-old chicks in the hatchery prior to movement.

One potential pathway for HPAI infection of day-old chicks at the hatchery is via horizontal transmission of HPAI virus associated with incoming hatching eggs and egg-handling materials, from breeder premises in the Control Area. The risk through this pathway has been evaluated previously (see the *Broiler Hatching Egg* risk assessment) and found to be *negligible* to *low* when the outbreak measures specified in the SBS Plan are implemented. In this risk assessment, we focused on pathways for the infection of day-old chicks via components of local area spread, assuming that the hatchery could be located within a Control Area. By local area spread, we refer to risk pathways which have an increased likelihood for disease transmission due to proximity to

an infected premises. The components of local area spread considered in this analysis include bio-aerosols generated from neighboring infected flocks; transmission of HPAI virus through insects; transmission via essential visitors or hatchery employees who may have had contact with infected poultry or poultry waste; and through vehicles associated with essential visitors.

In scenarios where day-old chicks are delivered to broiler farms in a HPAI Control Area, there may be risk pathways for virus to be transmitted back to the hatchery via the movement of chick handling materials. These pathways were not included in the risk assessment scope. The assessment only applies to the movement of day-old chicks to premises outside the Control Area (Free Premises) and to premises in the Control Area for which the risk of transmitting HPAI virus back to the hatchery is *negligible* to *low*. It is assumed that it is unlikely for day-old chicks to be placed onto premises where there is a high likelihood of HPAI virus being present.

This assessment considers current industry practices and biosecurity measures as well as outbreak measures applicable for the movement of day-old chicks in the risk evaluation. The current biosecurity measures considered include guidelines followed by participants in the NPIP (9CFR145 and 9CFR147). Categories of outbreak specific measures from the SBS Plan considered here include:

- Restriction of non-essential visitors to the hatchery
- Biosecurity measures for essential visitors (e.g., protective clothing, a shower and a change of clothes)
- C&D of vehicles associated with essential visitors
- Biosecurity measures for hatchery personnel (e.g., footwear protocol).

This assessment 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 an 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 day-old chicks.

4. Scope

This section describes the scope of the assessment with respect to the type of movements addressed and the facilities covered.

4.1 Facilities Covered Under This Risk Assessment

This risk assessment is applicable to commercial hatcheries producing broiler day-old chicks that meet all of the criteria listed below:

- Are in a HPAI Control Area
- Participate in the USDA-APHIS National Poultry Improvement Plan (NPIP) as stated in 9CFR145 and 9CFR147 (Appendix 1)
- Implement the SBS Plan in the event of an HPAI outbreak
- Do not have other poultry on the premises except for day-old chicks hatched onsite and held for one or two days before shipping to broiler farms.

4.2 Types of Movements Addressed Under This Risk Assessment

This risk assessment will only address the movement of day-old chicks to broiler farms outside the Control Area or to broiler farms in the Control Area for which the risk of transmitting HPAI virus back to the hatchery is *negligible* to *low*.

5. Overview of Data Analysis Approaches

The assessment follows the general qualitative risk assessment principles recommended by the OIE import risk analysis guidelines.[1] However, the risk assessment organization has been modified from that proposed in the OIE import risk analysis handbook as appropriate for the movement of day-old chicks to broiler farms. As mentioned in the introduction, the focus of the risk assessment is on entry assessment (i.e., entry of HPAI virus onto broiler farms through movement of the day-old chicks). An exposure assessment step was not included as the spread of HPAI onto a virus free broiler farm was assumed to be the final risk event with considerable adverse consequences.

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 5.1**). 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. Quantitative simulation model results from previously completed proactive risk assessments were used to estimate the prevalence of infectious birds in potentially infected and undetected poultry flocks located near the hatchery. Steady state aerosol dispersion models recommended by the EPA were used to partly inform the risk of aerosol spread from infected and undetected farms, along with other approaches. The likelihood for main steps in each pathway was assessed and categorized using the descriptive scale in **Table 5.1**.

Table 5.1 Descriptive scale 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 entry 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 lowest likelihood in the chain. The risk rating scale used in this assessment is provided below.

Negligible Risk: The likelihood of HPAI spread to day-old chicks and their movement off the hatchery premises through the risk pathway is insignificant or not worth considering.

Low Risk: HPAI spread to day-old chicks and their movement off the hatchery premises through the risk pathway is very unlikely.

Moderate Risk: HPAI spread to day-old chicks and their movement off the hatchery premises through the risk pathway is unlikely to but does occur.

High Risk: There is more than an even chance that the HPAI spread to day-old chicks and their movement off the hatchery premises through the risk pathway will occur.

Extremely High Risk: The spread of HPAI infection to day-old chicks and their movement off the hatchery premises through the risk pathway is almost certain to occur.

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 5.1**. 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. For example, a *negligible* to *low* rating was used with regards to aerosol transmission where there is considerable uncertainty in the aerosol dose response relationship in chickens and the particle size distribution of aerosols generated in poultry houses depends on the ventilation design, production type, and age of the birds. Other areas of uncertainty were handled similarly during the analysis.

6. Significant 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 must make some assumptions to establish context and applicability. These assumptions are:

- 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.
- The movement of incoming hatching eggs and associated materials from broiler breeder farms in the Control Area is in accordance with the SBS Plan.
- This assessment conservatively assumes that there is at least one HPAI infected poultry flock located near the hatchery within the Control Area.
- Based on the results of a previously completed risk assessment for the movement of broiler hatching eggs from a HPAI Control Area, it is assumed that the risk of day-old chicks at the hatchery becoming infected with HPAI virus associated with contaminated hatching eggs or hatching egg handling materials is *negligible* to *low*.
- The hatchery is a standalone facility without other poultry on the premises except for day-old chicks produced at the hatchery, which may be held for up to two days before shipping to broiler farms.
- That all relevant preventive measures from the SBS Plan are strictly followed. The assessment does not evaluate the risk that the preventive measures are incorrectly implemented either intentionally or unintentionally.
- The adverse consequences of movement of infected day-old chicks to broiler farms are assumed to be very high. Hence, the risk rating was determined on the basis of the likelihood of HPAI spread to a broiler farm and the consequences of the event were not evaluated.
- In situations where preventing cohabitation of broiler hatchery employees with household members having direct contact with poultry or poultry waste from flocks located within the Control Area is not feasible during a HPAI outbreak, it is assumed that effective measures to mitigate the risk pathway are taken by the employees and household members as approved by the Incident Command.

7. Background Information on Broiler Day-Old Chick Production

7.1 Definition of Day-old Chicks and Hatcheries

A day-old chick is a newly hatched chick produced at the hatchery and typically moved to a broiler grow out facility within three days of age. Day-old chicks may be used for commercial broiler production or to supply birds for breeding farms.

A hatchery is an establishment dedicated to the hatching of eggs for the production of chicks. Commercial hatcheries receive hatching eggs from breeder farms, incubate and hatch the eggs, then ship the day-old chicks to broiler farms.

This risk assessment focuses specifically on the production and movement of day-old chicks intended to supply the broiler industry with commercial broiler chickens. Day-old chicks used as replacements for breeder flocks are outside the scope of this assessment.

7.2 Overview of Day-Old Chick Production in the United States

7.2.1 Hatchery Operations

Broiler chicks hatched in the United States during 2011 totaled 9.06 billion. The placement of broiler chicks on feed for meat production was approximately 8.56 billion. As of 2012, there were 302 broiler hatcheries in the U.S. with an incubator capacity of 894 million eggs.[2]

The number of day-old chicks per hatch day varies between hatcheries. On average 144,000 eggs would be set per day in a hatchery with 4 hatch days per week. Broiler hatchery operations producing and distributing broiler chicks in the United States can be categorized into two types:

1. Integrators: Integrators own hatcheries, broiler flocks, processing plants and feed mills and contract with broiler farms to raise the birds to market weight. The broiler industry is vertically integrated and most of the hatcheries supply day-old chicks to growing facilities where the birds or the facilities themselves are owned by the integrators. In a few instances, excess day-old chicks may be supplied to a different company's broiler farms.

2. Mail order and small order suppliers: These hatcheries generally supply backyard flocks or other niche operations that are not supplied by integrator-owned hatcheries.

7.2.2 Day-Old Chick Distribution and Logistics

The supply chain members involved in the day-old chick process include breeder farms, hatcheries, and broiler farms. During normal operations, day-old chicks processed within the hatchery are transported to a broiler grow-out facility where they are raised for 7.2 weeks on average before harvest. [3]

Typically, day-old chicks are placed in chick boxes which are stacked onto buggies (or dollies) for transport to broiler farms. A single truck and driver may perform hatching egg pick-up and day-old chick delivery; however, this is usually not on the same day and the truck is generally C&D between pick-up and delivery. A 2010 USDA NAHMS survey[3] found that most broiler

companies ship birds from hatcheries to broiler farms within state. However 28 percent of company owned hatcheries shipped birds out of state in a one year time frame. Typically, the driver off-loads day-old chicks at only one broiler grow-out facility and the truck is C&D before the driver delivers to another facility. Chick boxes are returned to the hatchery and are C&D prior to reuse. Hatching trays and chick boxes may be C&D within the same area of the hatchery (*pers. comm.* Deidre Johnson 2012).

7.3 Major Steps in the Production and Processing of Day-Old Chicks During Routine Operations

The major steps in the production, processing, and distribution of hatching eggs during routine operations are detailed in proactive risk assessment for movement of broiler hatching eggs.[4] We provide a brief summary below:

- *Day-old chick processing at the hatchery:* After incubating in the hatcher, the day-old chicks present in hatcher trays and buggies are moved to a conveyor belt system for processing. During this process, chicks will be separated from the hatch debris, loaded into chick boxes (with or without paper), spray vaccinated for respiratory diseases, and then held in chick boxes until loading for delivery.
- *Transportation to broiler growing facility*: In general, trucks and drivers used for day-old chick placement are either company-owned or hired through contractual agreements. The same truck and truck driver may perform hatching egg pick-up and chick delivery; however, not on the same day and the truck is generally C&D between pick-ups and deliveries. Delivery vehicles usually have mechanisms to hold chick boxes and dollies in place during transport. These delivery trucks are equipped with adequate ventilation to maintain appropriate temperature for day-old chick delivery.
- *Day-old chick placement at the grow-out facility:* Upon delivery, grow-out facility personnel or the delivery driver will roll the dollies with the chick boxes directly to the barn floor on the broiler farm. Chick boxes and dollies are returned to the hatchery and C&D prior to reuse.

7.4 Sanitary Efforts and Current Disease Prevention in the Production of Day-Old Chicks

The key sanitation goals in the production of day-old chicks are 1) to produce and process healthy day-old chicks; and 2) to structure hatchery operations such that day-old chick contamination is minimized. Some of the preventive biosecurity measures practiced in the hatching egg industry currently include: 1) cleaning and disinfection of reusable materials and the delivery vehicle; and 2) segregation of setting, hatching, and chick processing operations in the hatchery.

- Cleaning and disinfection of reusable materials: Chick handling materials are cleaned and disinfected in each work cycle. The effectiveness of the C&D process is monitored by testing swabs from trays, racks, and other environmental surfaces on a regular basis for microbial contamination.[5]
- Segregation of setting, hatching, and chick processing operations: The incubation and hatching, chick pull and chick processing operations are performed in separate rooms,

reducing the potential for cross contamination.[5] Strict sanitary measures are in place for personnel working in the hatching and chick processing areas. For example, personnel typically do not handle stored hatching eggs and chicks on the same day.

The NPIP is a cooperative Industry-State-Federal program focused on disease prevention in poultry and safety of poultry products throughout the country. Participation in NPIP provides breeders and hatcheries with standardized guidelines for poultry and egg management, as well as biosecurity practices. A USDA 2010 survey of the top broiler producing companies (accounting for 81 percent of broiler production in the U.S.) found that most broiler breeder farms participated in the NPIP program.[6] Hatcheries often implement additional biosecurity measures beyond the NPIP requirements that are considered to be minimum standards. NPIP provisions 9CFR145 and 9CFR147 are pertinent to hatchery and breeder facilities and contain various C&D and biosecurity measures for the production and transportation of day-old chicks (Appendix 1).

In the event of an outbreak, additional outbreak specific measures from the SBS Plan will be implemented at the breeder farm in order to further reduce the risk of exposure of day-old chicks at the hatchery. Further details and hatchery workflow and sanitary practices are provided in the *Proactive Risk Assessment for the Movement of Broiler Hatching Eggs, Into, Within, and Out of an HPAI Control Area.* [4]

8. 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.[1] For movement of broiler hatching eggs and day-old chicks, the pathogenic agent of concern is HPAI virus. Properties of HPAI viruses, including environmental persistence, transmission characteristics, and physical and chemical inactivation, have been extensively reviewed in comprehensive texts.[7] 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.

8.1 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 humans and occasionally pigs.[7,8]

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.[7] 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.

8.1.1 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.[9] 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).

8.1.2 Host Range

Wild waterfowl 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.[10]

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.[8] 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.[10]

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.[11] 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.[10]

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.[12] 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.[13] 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.[14] 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.[15] 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.[10]

Experimental studies have shown that various LPAI viruses can replicate in pigs, ferrets, and cats to levels comparable to human influenza viruses.[16] 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.[17] A survey of wild raccoons in the United States found a prevalence of 2.4 percent for AI antibodies.[18] Asian HPAI H5N1 strains have a wider host range and have been isolated from up to 176 species including wild birds and mammals.[19]

8.2 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.

8.3 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.[20-22]

H5N2 HPAI viruses have been isolated from the eggshell surface, yolk and albumen of eggs laid by experimentally inoculated hens.[23] 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.[24] Italian HPAI H7N1 viruses have also been isolated from eggs laid by infected hens.[25]

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 albumen and from 0.93 to $10^{4.8}$ EID_{50}/ml in yolk of eggs laid by infected hens.[23]

8.4 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.[26-28] 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.

8.5 Persistence of HPAI in Manure and Other Media

Persistence of AI viruses in the environment in different media is summarized in **Table 8.1**. 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 and 35 days at 4º C.[21,29] H5N1 HPAI were viable for four days at 25 to 32 ºC when kept out of direct sunlight[30].

Table 8.1 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 ºC; 20 days 0 ºC	Webster *et al.* (1978) [31]
	Duck feces	Relatively high humidity (sealed in a vial)	30 days 4 ºC; 7 days 20 ºC	
Pennsylvania HPAI H5N2	Wet manure	In a barn (winter under freezing conditions)	105 days after depopulation	Fichtner et al. (2003)[29]
Pennsylvania HPAI H5N2	Wet feces	Closed vial	35 days > 4 ºC Between 2 to 3 days at 25 °C	Beard et al. (1984)[21]
	Wet feces	Open vial	Between 9 to 14 days 4 ºC Between 1 to 2 days at 25 °C	Beard et al. (1984)[21]
2 Clades of HPAI H5N1	Duck feathers	Relatively high humidity (sealed in a vial)	160 days 4 ºC; 15 days 20 ºC; Titers of $10^{4.3}$ EID_{50}/ml for 120 days at 4 °C	Yamamoto et al. (2010) [32]
	Drinking water (commercial mineral water)	Collected at 3 days post infection and stored at 4 C or 20 ºC	Inconsistently isolated from water stored at 4 ºC over a 30 day period; no virus isolated from drinking water at 20 ºC 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 ºC; 2 days at 28 to 30 ºC	Lu et al. (2003) [33]

Virus strain	Media	Conditions	Survival duration and temperature	Reference
8 wild type LPAI viruses, H5 and H7 subtypes; and 2 HPAI H5N1 subtypes	Water 0, 15, and 30 parts per thousand salinity;	Simulated winter and summer coastal marshland temperatures in LA, 17 and 28 °C	H5N1 had shorter environmental survival times compared to wild-type LPAI viruses; 2 clades persisted for 94 to 158 days at 17 ºC	Brown et al. (2007) [34]
HPAI H5N1 from chickens in central Thailand	0.2ml $10^{6.3}$ EID_{50}/ml in allantoic fluid, feces, water, one cubic inch of meat or eggs	Virus added to allantoic fluid or feces	In *shade* (25-32 ºC): 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 ºC): 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 ºC	
3 isolates from hunter killed ducks from various 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) [35]
5 HA subtypes from hunter killed ducks in Louisiana (H3N8, H4N6, H6N2, H12N5,	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 ºC depending on subtype	Stallknecht et al. (1990) [36]

Virus strain	Media	Conditions	Survival duration and temperature	Reference
H10N7)				
H5N1 HPAI	Virus added to normal chicken manure; 2.38 x $10^{5.25}$ ELD_{50}	pH 9.23; 13.7% moisture (dry manure)*	No virus recovered after 24 hrs. at 25 ^{o}C; or 15 minutes at 40 ^{o}C. Virus recovered after 4 hrs of UV exposure at room temperature (25 ºC)	Chumpolbanchorn et al.(2006) [37]
3 LPAI viruses (H4N6, H5N1, H6N8)	3 different water types: Starting titers ranged from $10^{4.14}$/ml – $10^{5.14}$/l	DW distilled water (pH 7.8); NS normal saline 0.9% (pH 7.2); SW surface water from Lake Constance, Europe. Incubated at -10, 0, 10, 20, and 30 ºC.	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) [38]
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, and 0 ºC	Persistence highest in lake sediment (5 to 394 days), feces (1 to 75 days), meat (1 to 81 days)	Nazir *et al.*(2011) [39]
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 ºC	Infectivity in meat at 4 ºC: 135 days in chicken meat; 90 days in turkey meat; 75 days in duck meat	Beato *et al.* (2009) [40]

Virus strain	Media	Conditions	Survival duration and temperature	Reference
	HPAI and LPAI: virus inoculated into allantoic fluid	Infectivity assayed after holding allantoic fluid samples at 4 ºC and 20 ºC	Infectivity in allantoic fluid: HPAI up to 210 days at 4 ºC; LPAI up to 270 days at 4 ºC; HPAI not detectable at 60 days at 20 ºC, LPAI 2.9 log EID_{50} at 60 days at 20 ºC.	
		Infectivity assayed after holding allantoic fluid samples at 4 ºC and 20 ºC with pH adjusted to 5 and 7	Persistence time higher for viruses at pH 7 than for pH 5; HPAI more persistent at pH 7; LPAI more persistent at pH 5	
H13N7 LPAI	Steel, wood, tile, tire, gumboot, feather, egg shell, egg tray (cardboard), plastic, latex, cotton fabrics, polyester fabric; 10 µl of 6.3 X 10^6 $TCID_{50}$/ml	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) [41]
H6N2	Treatments: Virus in allantoic fluid mixed with chicken manure, used litter, and feed; homogenized embryonated chicken egg in corn silage. 3.4 x 10^8 EID_{50}	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 ºC)	Treatments: Virus in all mesh bag specimens inactivated at 40-50 ºC by day 3 except for one manure sample at 40 ºC; Viable virus from allantoic fluid in vials on day 3 (46- 43 ºC); day 7 (55.5 ºC); and day 10 (62.2 ºC) Controls: Viable virus at 21 days (22.7 – 25.7 ºC)	Guan *et al.* (2009)[42]

Virus strain	Media	Conditions	Survival duration and temperature	Reference

*Chicken fecal moisture may be as high as 60%

Table 8.2 summarizes the literature on inactivation of Avian Influenza viruses at 35 to 37 °C which is comparable to the range of incubation temperatures (37 to 39 º C). Most of these data suggest more than a 6.8 log EID_{50} inactivation of AI virus within 15 days at incubation temperature. Muhmmad *et al.,* (2001) reported that HPAI H7N3 virus retained infectivity in allantoic fluid for a period of 35 days at 37 ºC although the hemagglutination titers were reduced to undetectable levels, suggesting very low virus concentrations.[43]

Table 8.2. Summary of literature on thermal inactivation of Avian Influenza virus in wet media when incubated at 35 to 37 ºC.

Study	Virus and Media	Temperature and Time	Inactivation Log (EID_{50})
Terregino (2009) [44]	HPAI H7N1 A/turkey/Italy/1387/00 (Allantoic fluid)	37 ºC for 15 days	>6.5
Terregino (2009) [44]	HPAI H7N1 A/turkey/Italy/4580/99 (Allantoic fluid)	37 ºC for 15 days	>6.6
Terregino (2009) [44]	LPAI H7N1 A/turkey/Italy/3675/99 (Allantoic fluid)	37 ºC for 15 days	5.5
Terregino (2009) [44]	LPAI H7N1 A/turkey/Italy/4608/03 (Allantoic fluid)	37 ºC for 15 days	6.1
Shortridge *et al*., (1998) [22]	HPAI H5N1 (Wet feces)	35 ºC for 2 days	~4
Davidson *et al.,* (2009) [45]	LPAI H9N2 (Allantoic fluid)	37 ºC for 4 days	>6.8
Davidson *et al*., (2009) [45]	LPAI H9N2 (Allantoic fluid)	37 ºC for 2 days	~5
Negovetich and Webster, (2010) [46]	LPAI H2N3 (Allantoic fluid mixed with various liquid media)	37 ºC for 10 days	>6.8
Homme and Easterday, (1970) [47]	LPAI A/Turkey/ Wisconsin/1966 (Allantoic fluid)	37 ºC for 4 days	8

8.6 Transmission

Contact with migratory waterfowl, sea birds, or shore birds is a risk factor for introduction of AI virus into domestic poultry populations.[48] 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[7]. 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.[49,50]

Evidence of vertical transmission of avian influenza from infected hens to day-old chicks or turkey poults has been lacking thus far, as most strains are lethal to chicken embryos.[40,51-53] Chicks hatched from eggs produced by two HPAI H7N3 infected broiler breeder flocks tested negative for AI during an outbreak in British Columbia in 2004. The outbreak report of the Canadian Food Inspection Agency states, "Because AI does not survive long at incubator temperatures, day-old chicks are not a likely source of infection for broiler growers." [54] In the 1983 Pennsylvania HPAI H5N2 outbreak, eggs from four severely infected layer breeder flocks were incubated and assayed for AI virus. None of the dead embryos yielded HPAI virus in this study.[21] Also the 214 chicks hatched from these eggs showed no sign of AI disease and had not developed AI antibodies.[21]

Transmission of HPAI or LPAI viruses from infected breeder flocks to day-old poults via hatchery dissemination has not been observed in previous outbreaks. Turkey industry veterinarians and avian influenza experts have stated that although there have been several LPAI outbreaks in the United States, vertical transmission or hatchery transmission has not been observed to-date. In a small scale survey conducted by the University of Minnesota, turkey industry representatives provided reports of 26 flocks which had undergone Avian and other Influenza A virus infections (swine origin) and where eggs from the flocks were set and not removed. There was no evidence of horizontal or vertical transmission of AI within the hatchery to day-old poults in any of these instances.

8.7 Dose Response

8.7.1 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}.[55] 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.[56]

Single hit dose response models (e.g., exponential) have been used for HPAI virus in chickens and mammals.[57,58] 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. Kwon and Swayne (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}.)[59] 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[60]. 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.[61] However, in the Purchase et al. 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.[62] The authors suggested contamination of the nasal mucosal membranes from the oral cavity via the 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 after 24 hours of exposure to more than 10^3 EID_{50}/m^3 of H5N1 HPAI virus concentration in air coming from a room housing infectious chickens.[63] 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}.[63] 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.[64]

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.[62] 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).[63] 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.[63]

8.7.2 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 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.

8.8 Latently Infected and Infectious Periods

Table 8.3 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. In individual birds, 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 level of clinical signs and the ability of the grower to detect them.[65] For trade purposes, the OIE defines the flock incubation period as 21 days.[66]

Table 8.3. 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) [67]
HPAI H5N1	36-48 hours			Pfeiffer et al. (2009)[68]
HPAI H7N7	-	Between 1 and 2 days	6.3 days (3.9-8.7)[#]	Van Der Goot et al. (2005) [69]; Bos et al. (2007) [70]
LPAI H5N2	-		Inoculated chickens, 4.8 days ([±] 9%); contact animals, 4.25 days (2.57-5.93[#])	Van Der Goot et al. (2003) [71]
HPAI H5N2			Contact chickens, 6.8 days (4.91-8.69[#])	

*95% credible interval; [#] 95% confidence interval; [±] Coefficient of variation; - was not determined

8.9 Clinical Signs

The presence and severity of clinical signs of HPAI infection depends on the type of bird species affected.[12] Infected wild and domestic ducks may be asymptomatic, whereas clinical signs in gallinaceous poultry are usually severe, resulting in high mortality. In 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.[66]

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.

8.10 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).[7]

The application of molecular methods for detection of viral nucleic acid has become an important tool in 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.[7]

Antigen detection immunoassays kits have also been used in prior outbreaks and have advantages of speed (15-20 minutes) and good specificity. While the low analytical sensitivity (detection limit greater than 10^4 EID_{50}) is a limiting factor, birds presenting for clinical disease or that died due to AI infection generally shed adequate virus antigen 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.[7]

8.11 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.

9. Risk Evaluation

9.1 Introduction

The emphasis of this assessment is on the risk of entry of HPAI virus to a broiler farm via movement of HPAI infected day-old chicks from a hatchery located within a Control Area. In particular, we consider pathways for HPAI infection of day-old chicks in a broiler hatchery via components of local area spread.

Table 9.1 Previous HPAI outbreak investigations results on proximity as a risk factor for exposure.

HPAI Strain	**Study Approach**	**Key Findings**	**Source**
H7N1	Cox regression, people and equipment flow not controlled for in model.	Population attributable fraction 11-31% for proximity i.e., flocks ≤ 1.5 km from infected premises (IP).	Mannelli (2006) [72]
H7N1	Multivariate analysis, people and equipment flow not controlled for in model.	Flocks ≥ 4.5 km from infected premises had lower risk. Flocks ≤1.5 km from infected premises had highest risk.	Busani *et al*., (2009) [73]
H7N7	Spatial transmission model with distance as the only risk factor	Farms ≤1km from IP about at 3-4 times higher risk compared to farms ≥5 km	Boender *et al*., (2007) [74]
H5N1	Outbreak observation	No transmission to 78 other farms within 3 km. Author concludes no evidence of local transmission above 1 km	Sharkey (2007) [75]

Previous HPAI outbreak investigations have identified proximity to infected premises as a risk factor for HPAI infection of poultry flocks (Table 9.1). However, the relative impact of different pathways on local area spread is ambiguous. In addition, there is considerable uncertainty as to the distance from infected premises within which local spread may present a considerable risk for HPAI spread. Given these uncertainties, we chose to evaluate the potential components of local area spread that may be relevant for risk of HPAI transmission to day-old chicks in the hatchery individually. Note that we have considered the pathways associated with essential visitors and the hatchery vehicle as components of local area spread. The reason for including these pathways in the evaluation is because the hatchery is in a Control Area, and it is perceived that the hatchery is at a relatively higher risk compared to Free Premises, due to potential proximity to infected flocks.

The presence of wild mammals on poultry premises has been identified as a risk factor for AI spread in previous outbreaks. We did not evaluate the pathways associated with wild mammal transmission in this assessment as they do not represent a considerable risk for hatcheries. Hatchery waste is handled through enclosed systems and storage bins prior to transport and would be inaccessible to wild mammals and rodents. Most hatcheries also implement rodent control as part of their biosecurity program. Wild mammal or rodent movements onto the hatchery premises would therefore be unlikely. In addition, [76,77]. However, we note some of the Asian HPAI H5N1 strains can replicate efficiently in mice[78].

The key risk pathways evaluated in this assessment are as follows.

- **The risk of day-old chicks in a hatchery in the Control Area becoming infected via exposure to essential visitors, and/or essential and non-essential personnel contaminated with HPAI virus.** The current hatchery employee requirements as well as outbreak specific measures, such as the requirement for employees to wear protective footwear and a minimum downtime with a shower and change of clothes for essential visitors, were considered in the risk evaluation.
- **The risk of day-old chicks at a hatchery in the Control Area becoming infected with HPAI virus from an infected poultry flock via insect transmission.** We reviewed previous outbreak studies implicating flies in transmission of HPAI; survivability of AI viruses in flies; dispersion rates of specific types of flies implicated in HPAI spread; and a summary of expert opinion on the likelihood of transmission of HPAI to day-old chicks in a hatchery.
- **The risk of day-old chicks in a hatchery in the Control Area becoming infected with HPAI virus from an infected flock in the Control Area via aerosols**. The evaluation is based on a scientific literature review, aerosol dispersion modeling scenarios, and expert opinion. Alternate scenarios were modeled using the EPA aerosol dispersion model AERMOD where the source flock is either known to be infected, or is infected and undetected.
- **Risk of day-old chicks in a hatchery in the Control Area becoming infected with HPAI virus from essential vehicles.** The focus here is on vehicles associated with essential visitors, such as those transporting hatchery waste, delivering vaccines, equipment service personnel, and company veterinarians.

9.2 Risk of Day-old Chicks Becoming Infected via Exposure to Contaminated Visitors, and Essential and Non-essential Personnel

Risk Factors: Hatchery personnel or essential visitors having direct or indirect contact with live poultry.

Current Preventive Measures: Limitations on contact with live poultry; limitations on cohabitation; downtime and PPE for visitors.

Outbreak Specific Measures: Additional requirements to cohabitation protocols required by industry and approved by the Incident Command; downtime and PPE for essential visitors; guidelines on removal of solid waste from the hatchery.

Overall Risk: We conclude that the risk of day-old chicks becoming infected with HPAI Virus released into the hatchery via hatchery personnel or essential visitors who have had prior direct or indirect contact with infected but undetected poultry to be *negligible* to *low*. This risk rating assumes that the measures in the SBS Plan are strictly followed.

9.2.1 Introduction

In this section, we consider potential indirect pathways of exposure of day-old chicks in the hatchery, by hatchery personnel and essential visitors. Generally, hatchery managers restrict unnecessary human traffic—especially non-essential visitors—and these restrictions would likely be more stringent during an outbreak. However, visitors who may be required to visit the hatchery during the outbreak include company veterinarians, vaccine suppliers, pest control professionals, and waste removal and equipment repair personnel.

Farm employees keeping poultry or visiting other poultry farms have been considered to be a high priority biosecurity risk by poultry health specialists.[79] Current industry practice contains several preventive measures to mitigate the risk of disease introduction by personnel and visitors. Protective caps/hairnets, clothing, and footwear are generally provided, and some hatcheries require a shower and change of clothes. The OIE also provides recommendations for hygiene and biosecurity applicable to commercial hatcheries.[80] The applicable current and outbreak specific risk mitigation measures for U.S. commercial broiler hatcheries as well as outbreak specific measures from the SBS Plan were considered in the risk evaluation.

9.2.2 Preventative Measures

9.2.2.1 Current Preventative Measures

Industry standard current preventative measures, as noted by the BWG, are summarized below:

Limitations on poultry contact: Most commercial hatcheries have protocols that limit outside contact with live poultry upon employment. These restrictions include restrictions on employment with another company that may involve direct contact with live poultry or owning personal flocks or pet birds.

*Downtime for visitors***:** Most commercial hatcheries have protocols in place outlining the downtime and additional measures undertaken by visitors who have had contact with poultry outside the hatchery.

PPE for visitors: Visitors are typically required to don a different set of PPE such as disposable or cleaned and disinfected footwear before entering the hatchery.

9.2.2.2 Outbreak Specific Preventative Measures

9.2.2.2.1 Hatchery personnel

Footwear: Personnel must use dedicated hatchery footwear or should clean and disinfect their footwear upon entering the hatchery.

Cohabitation protocol: Hatchery employees should not cohabit with household members having direct contact with other poultry, avian species or associated organic waste from operations in the Control Area. In scenarios where such cohabitation is unavoidable, it is assumed that the household members and employees follow measures applicable for poultry operations in the Control Area to effectively mitigate the associated risk pathway, as approved by the Incident Command.

9.2.2.2.2 Essential Visitors

At a minimum, essential visitors must meet the same biosecurity requirements as hatchery personnel and require company permission for entry. In the event of an HPAI outbreak, non-essential visitors would not be permitted to enter the hatchery according to the SBS Plan.

Downtime: Essential visitors who have had contact with domestic poultry, other avian species, and/or related organic material are required to have at least 12 hours downtime before visiting the hatchery. A shower and change of clothing is also required before visiting the hatchery.

Visitors collecting hatchery waste: Visitors collecting hatchery waste should not enter the hatchery building. Hatchery personnel should keep waste bins outside for collection to facilitate this protocol. Waste bins should be stored in a way to prevent access to wild birds or wildlife.

PPE: All vehicle drivers must put on disposable plastic boots or clean rubber boots before getting out of the truck cab and follow guidelines for using PPE as described in NPIP (9CFR147.24). These protocols also specify the use of a hand sanitizer. Other essential visitors should follow equivalent procedures for wearing PPE as required by the broiler company.

9.2.3 Evaluation of Risk

We evaluated the risk of day-old chicks becoming infected with HPAI virus transferred into the hatchery via hatchery personnel or essential visitors who had direct or indirect contact with infected but undetected poultry in two component steps.

- The likelihood of hatchery personnel or essential visitors being contaminated with HPAI virus from prior direct or indirect contact with infected but undetected poultry at the time of entering the hatchery premises.
- The likelihood of HPAI virus contamination from prior direct or indirect contact of hatchery personnel or essential visitors with infected but undetected poultry being released into the hatchery building.

9.2.3.1 Likelihood of Hatchery Personnel or Essential Visitors Being Contaminated with HPAI Virus from Prior Direct or Indirect Contact with Infected but Undetected Poultry at the Time of Entering the Hatchery Premises

9.2.3.1.1 Hatchery Personnel

Direct contact with live poultry: Most commercial hatcheries have protocols in place for hatchery personnel that restrict any outside contact with live poultry upon employment. This would include refraining from working with other poultry or keeping backyard flocks. Industry experts stated that these requirements would be strictly enforced in the event of a HPAI outbreak, considering the heightened awareness.

Therefore, we rated the likelihood of contamination of hatchery personnel via direct contact with other poultry to be *negligible* when broiler company protocols are strictly followed.

Indirect contact through organic materials: We defined indirect contact to be contact of personnel with HPAI virus originating from infected poultry either via contaminated organic materials or through multiple virus transfer events between contact surfaces. We considered the following factors in the evaluation:

- Hatchery personnel are typically restricted from ongoing living arrangements that include cohabitation with people that are in regular contact with domestic poultry, other avian species, and/or related organic material. In situations where preventing such cohabitation is not feasible during a HPAI outbreak, effective measures to mitigate the risk pathway are assumed to be taken by the employees and household members having contact with poultry in the Control Area, as approved by the Incident Command. The mitigation steps required by Incident command would be strictly implemented during an HPAI outbreak, considering the heightened awareness.
- Social contact between personnel working on different poultry premises has been implicated as a transmission mechanism for a few of the affected premises in previous AI outbreaks.[81] Similarly, contact between neighbors, friends, and relatives has been implicated as a transmission mechanism between poultry premises in a few cases in previous HPAI outbreaks.[82] Movement of people and equipment associated with poultry services and operations was considered to account for most of the spread in the 1983 Pennsylvania H5N2 HPAI outbreak. Based on a small scale survey, a study of the contact structures in the broiler industry in the United States found an average frequency of social contacts between growers and employees of other poultry premises of once in 30 days (90 % confidence interval: 10 days to no visits).[83] However, a study of broiler farms in Georgia found a higher frequency of social contacts between broiler growers working on different premises. The frequency of contacts in this study was estimated to be 1.24 (range for mean 0.92-1.6) per week (approximated based on the presented data) with around 40% of contacts being between growers of different companies.[84] However, there was a substantial variation between different producers in the number of social contacts, and 20 percent of producers had social contact with other growers 5 or more times per week. In addition, we should note that this is a small scale survey limited to Georgia and is not representative of all broiler growers in the United States. Broiler industry representatives have also commented that the number of

contacts can be substantially higher in some operations, depending on poultry density and location. Overall, there is a high degree of uncertainty and variability regarding the frequency of social contacts between hatchery employees and other poultry growers, with the mean frequency being 0.25 to 1.24 visits per week in different small scale studies, as extrapolated from the contact rate between broiler farm employees. Transmission of HPAI virus via social contact between growers would involve virus transfer events between contact surfaces. The final surface concentration of HPAI virus transferred through such contact steps would be lowered by the multiple steps. This is because only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface in each direct contact step (personal communication Drs. Sayed Sattar and Susan Springthorpe).[20,85,86 a]

- Another possible means of indirect contamination is via shoes of hatchery personnel if they were to walk across surrounding ground areas of an infected but undetected poultry house. In this case, the surrounding ground areas could have become contaminated via bio-aerosols originating from the poultry house that have settled onto environmental surfaces. Exploratory aerosol modeling scenario analysis suggests that there would be a very low degree of contamination (1-10 EID_{50}/shoe sole) if a poultry employee were to walk at a distance of 500 m from an infected but undetected flock[b]. The likelihood of this event occurring may depend on the poultry density in the geographical area where the hatchery is located. However, we note that it is highly unlikely that hatchery employees walk across surrounding ground areas of other poultry operations, due to contact restrictions placed on them by their employer. In addition, only hatcheries that do not have other poultry on the premises are included in the scope of this assessment.
- As mentioned above, the likelihood of transmission of any HPAI virus contamination due to direct or indirect contact on a previous day would be reduced due to a shower and change of clothes by personnel at home.
- A considerable portion of any virus potentially transferred onto clothing, shoes or hands via indirect contact is likely inactivated within one day. Literature studies summarized in Appendix 3 show that AI and other Influenza A viruses are likely inactivated within one day at room temperature (25° C) and humidity conditions on dry surfaces. Relatively, there is a greater degree of uncertainty regarding inactivation rates of AI viruses on porous surfaces compared to non-porous surfaces.
- We rate the likelihood of hatchery personnel being contaminated with HPAI virus due to prior direct or indirect contact with infected poultry to be *negligible* to *low* when broiler company protocols are strictly followed. This rating considers the impact of preventive measures associated with cohabitation as well as other preventive factors as detailed above.

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[a] As a hypothetical example, suppose a risk pathway involved 4 virus transfer steps (poultry premises floor → shoes person A → hatchery floor → shoes person B → hands) with each step having a transfer efficiency of 27%, the virus concentration on the finial recipient surface (hands) would be 2.27 log lower than that of the original surface. Such a reduction in the virus surface concentration can result in a considerable decrease in the probability of infection, given the dose response relationship.

[b] Using the EPA AERMOD model scenarios described in chapter 9.4 and assuming an effective sole surface area of 200 cm^2. It was assumed that the virus concentration of the sole surface would be similar to the ground area on which the person had walked.

9.2.3.1.2 Essential Visitors

- As mentioned previously, the SBS Plan recommends non-essential visitors be restricted during an HPAI outbreak. In regards to essential visitors, the transmission risks associated with the hatching egg delivery driver was previously evaluated in the *Broiler Hatching Egg Risk Assessment* and that pathway is not revaluated here.[4] As mentioned in the scope, day-old chicks are assumed to be delivered to premises in an HPAI free area or to premises in a Control Area which the Incident Commander has determined to have an acceptable level of risk of HPAI virus being present on the premises. Hence, we do not consider risk of HPAI virus being transmitted to the hatchery via the day-old chick delivery driver returning from a broiler farm in this evaluation.
- Apart from the day-old chick and hatching egg delivery drivers, other essential visitors include those transporting hatchery waste, delivering vaccines, equipment service personnel, company veterinarians, etc. It is possible that essential visitors may have had prior indirect or direct contact with HPAI virus contaminated organic material originating from an infected but undetected flock, if they had previously visited poultry premises in the Control Area.

The down-time protocol mentioned in Section 9.2.2.2.2 is a key preventive measure that reduces the likelihood of essential visitors being contaminated with viable HPAI virus at the time of entering hatchery premises. From Section 9.2.3.1, data from experimental disease transmission studies show that a shower and change of clothes is very effective in preventing pathogen transmission. This protocol is expected to be particularly effective for the enveloped AI viruses that are susceptible to detergents and soaps.[28,87] In addition, a considerable (but not complete inactivation, which requires 24 hours) inactivation of AI viruses on dry surfaces is expected at room temperature and humidity within 12 hours (Appendix 3).

We rate the likelihood of essential visitors being contaminated with HPAI virus at the time of entering hatchery due to direct or indirect contact with infected but undetected poultry to be *negligible* to *low* when the downtime protocol and shower and change of clothes as specified in the SBS Plan are implemented.

9.2.3.2 Likelihood of Hatchery Personnel or Essential Visitors Being Contaminated with HPAI Virus from Indirect Contact with Infected wild birds at the Time of Entering the Hatchery Premises

There is a possibility of the shoes of essential visitors or personnel becoming contaminated due to contact with wild bird droppings before entering the hatchery building. We consider the following factors for evaluating the likelihood of this event:

1) Wild birds have not been considered among the main factors for the secondary spread of AI viruses between domestic poultry in an outbreak although they have been mentioned as a possibility [7]. Analysis of previous AI outbreaks indicates that local spread is mostly limited to a distance of 3km or less[72,75,88]. If wild birds contributed significantly to secondary AI spread between poultry premises, then local spread to greater distances than observed in most previous outbreaks would be expected.
2) As discussed in the hazard identification, except for Asian lineage HPAI H5N1 strains, very few HPAI viruses have been isolated from wild birds. Once adapted to gallinaceous poultry, HPAI viruses are unlikely to return back to circulate among

wild birds, because they are adapted to poultry.[12,76,89]. Asian HPAI H5N1 strains that have been isolated from several species of wild birds and mammals may be an exception. Similarly, LPAI viruses adapted to domestic poultry have rarely been transmitted back into aquatic wild bird host, and wild birds have played very limited or no role in secondary [7,89]

3) Wild birds have never been found to be a source of introduction of LPAI or HPAI viruses into a hatchery.

4) In general, hatchery waste is handled and stored in enclosed bins prior to transport, and there would not be any easily accessible food source for wild birds within and around the hatchery premises.

We rated the likelihood of hatchery personnel or essential visitors being contaminated with HPAI virus from indirect contact with infected wild birds at the time of entering the hatchery to be *low to negligible.*

9.2.3.3 The Likelihood of HPAI Virus Contamination from Prior Direct or Indirect Contact of Hatchery Personnel or Essential Visitors with Infected but Undetected Poultry Being Released Into the Hatchery Building

9.2.3.3.1 Hatchery personnel

In Section 9.2.3.1.1, the likelihood of personnel being contaminated with HPAI virus at the time of entering the hatchery due direct or indirect contact with infected but undetected poultry was qualitatively rated to be *negligible* to *low.* The use of dedicated footwear upon entry into the hatchery or footbaths will further reduce the risk of transferring HPAI contamination onto hatchery floors.

If shoes of hatchery personnel are contaminated, there is a possibility of their hands becoming contaminated with virus due to contact while removing footwear (*personal communication* Dr Lisa Cassanova). However, there would likely be a considerable reduction in the virus concentration transferred to the hands through contact. This is because 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 Drs. Sayed Sattar and Susan Springthorpe).[20,85,86]

We qualitatively rate the likelihood of HPAI virus contamination from prior direct or indirect contact of hatchery personnel with infected but undetected poultry being released into the hatchery building to be *negligible* to *low* when following the SBS Plan.

9.2.3.3.2 Essential Visitors

In Section 9.2.3.1.2, the likelihood of essential visitors being contaminated with HPAI virus at the time of entering the hatchery due to direct or indirect contact with infected but undetected poultry was qualitatively rated to be *negligible* to *low.* We considered the following factors in the evaluation of the likelihood of release of contamination into the hatchery.

- Restrictions *on entering the hatchery building*: In the event of an HPAI outbreak, the SBS Plan recommends that hatchery waste bins be rolled outside of the hatchery building so that

waste collection staff do not have to the enter the hatchery building. This measure reduces the likelihood of HPAI virus being transferred onto the hatchery room environmental surfaces.

- *Protective clothing*: In the event of an HPAI outbreak, the SBS Plan requires all vehicle drivers to put on disposable plastic boots or clean rubber boots before getting out of the truck cab, and to follow guidelines for using PPE as described in NPIP (9CFR147.24). These protocols also specify the use a hand sanitizer and smock or coveralls. Essential visitors would also be expected to follow similar PPE protocols before entering the hatchery building during an HPAI outbreak. A change of PPE, such as disposable boots and coveralls, is expected to be effective in preventing disease transmission in most cases when the recommended sequence of removing and segregating PPE is followed.[4] However, PPE was not found effective in some scenarios, potentially due to cross-contamination while removing them. In general, hand sanitizers are expected to be effective against Influenza A viruses when the hands are not visibly dirty or the organic load is not high.[87]

We qualitatively rate the likelihood of HPAI virus contamination from prior direct or indirect contact of essential visitors with infected but undetected poultry being released into the hatchery building to be *negligible* to *low* when following the SBS Plan.

9.2.3.4 The Risk of Day-old Chicks Becoming Infected with HPAI Virus Transferred Into the Hatchery via Hatchery Personnel or Essential Visitors who had Direct or Indirect Contact with Infected but Undetected Poultry

- *Hatchery* personnel *coming into contact with day-old chicks*: Some of the hatchery employees would directly contact day-old chicks as part of their duties (e.g., manual separation of chicks from eggshell debris). In this scenario, day-old chicks would likely become infected if their hands are contaminated. We qualitatively rate the risk of day-old chicks becoming infected with HPAI virus transferred into the hatchery via personnel working directly with day-old chicks to be *negligible* to *low* considering the likelihood of the steps in the entire risk pathway as evaluated in previous sections.
- *Essential visitors and hatchery* personnel *not having direct contact with day-old chicks:* In this case, the risk pathway would involve dissemination of HPAI virus within the hatchery rooms via multiple virus transfer steps associated with movements of people and equipment within the hatchery. We considered the following factors in evaluating this risk pathway.
    - The temperature in most rooms (except for the egg-storage room) is typically 77°F (25°C) or higher.[5] Experimental studies show that HPAI H5N1virus is inactivated at 77°F (25°C) within a day on hard dry surfaces (Appendix 3). Considerable inactivation of HPAI virus on hard surfaces is likely before day-old chicks are potentially exposed via dissemination of virus in the hatchery.
    - Risk pathways for transmission of HPAI virus via hatchery environmental surfaces or equipment would involve multiple transfer steps through direct contact between surfaces. The final surface concentration of HPAI virus transferred through such contact steps would be lowered by the multiple steps. This is because only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface

in each direct contact step (personal communication Drs. Sayed Sattar and Susan Springthorpe).[20,85,86]

- Hatcher and chick processing rooms are C&D at routine intervals between processing different batches of day-old chicks. These routine C&D and sanitation protocols further reduce the likelihood of HPAI virus dissemination throughout the hatchery.

We qualitatively rate the risk of day-old chicks becoming infected with HPAI virus transferred into the hatchery via essential visitors and hatchery personnel not having direct contact with day-old chicks to be *negligible* to *low.*

9.2.3.5 Previous Outbreak Experiences and Expert Opinion

Given the uniformly negative test results in hatching eggs and chicks from infected broiler breeder flocks during the 2004 HPAI outbreak in British Columbia—and considering the high temperature inside the setters—the Canadian Food Inspection Agency concluded that the movement of chicks does not pose a risk for HPAI transmission.[54] In the 2002 HPAI outbreak in Queretaro, Mexico, eggs already present in the hatchery at the time of infection in broiler breeder flocks were allowed to hatch following normal procedures. The broilers hatched from these chicks were not infected until 3 weeks old, suggesting that dissemination of virus in the hatchery did not occur. Broiler industry veterinarians and avian influenza experts have stated that, although there have been several LPAI outbreaks in the United States, vertical transmission or hatchery transmission has not been observed to-date (*personal communication, Dave Halvorson).*

A potential explanation for the lower likelihood of hatchery transmission based on previous outbreaks is that most hatcheries have a high degree of biosecurity, even under routine operations, to ensure chick quality. In addition, several key risk factors for AI spread between poultry premises identified in previous outbreaks such as movement of rendering trucks, manure equipment, contact with live bird markets, external crews working in contact with live birds, and mammalian wildlife on farm premises are not applicable for hatchery operations.[48,79,90,91]

9.2.4 Conclusion

We conclude that the risk of day-old chicks becoming infected with HPAI virus released into the hatchery via hatchery personnel or essential visitors who have had prior direct or indirect contact with infected but undetected poultry to be *negligible* to *low*.

9.3 Risk of Day-old Chicks at a Hatchery Within the Control Area Becoming Infected with HPAI Virus from an Infected Poultry Flock via Fly Transmission

Risk Factors: Neighboring known infected or infected but undetected poultry flocks.

Current Preventive Measures: Hatchery waste management.

Outbreak Specific Measures: None.

Overall Risk: We rated the risk of newly hatched chicks becoming infected via contaminated flies, mechanically transmitting HPAI virus from a nearby poultry facility (at distances up to 1.5 km) to be *negligible.*

9.3.1 Introduction

Flies have been implicated, although not proven, as a vehicle for HPAI virus transmission between poultry flocks. House flies (Muscidae) and Blow Flies (Calliphoridae) are reservoirs and vectors of a wide variety of pathogenic organisms affecting poultry.[92] The house fly is usually the most abundant and pestiferous fly species in poultry houses.[92] Most blowflies result from improper disposal of dead birds in a poultry operation, with very little production from manure.[92]

In scenarios where the hatchery is located within the control zone, transmission of HPAI to day-old chicks through flies should be considereda possibility. In this section, we review previous studies implicating flies in transmission of HPAI; survivability of AI viruses in flies; dispersion rate of specific types of flies implicated in HPAI spread; and a summary of expert opinion on the likelihood of transmission of HPAI to day-old chicks in a hatchery. We consider current hatchery management practices in evaluation of this risk.

9.3.2 Current Preventive Measures

Most of the hatchery waste is stored in dump bins before disposal to land fill or to composting sites. Most hatcheries use a vacuum extraction system or auger that places solid waste into a bin or a system of conveyor belts to separate live chicks from the solid waste stream. Hatchery waste is removed from the site daily, to control odors and reduce the potential for rodent and fly infestation within the hatchery. Most solid hatchery waste is sent to a land fill or is composted, while some waste is rendered.

9.3.3 Evaluation of Risk

9.3.3.1 Previous Outbreak Studies

HPAI virus was isolated from flies near infected poultry houses during the 1983-84 HPAI H5N2 outbreaks in Pennsylvania. [93,94] 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. Brugh and Johnson (1986) state, "While flies were not considered to be a factor in the spread of H5N2 virus in Virginia, they were implicated as probable sources of infection for several flocks in Pennsylvania". However, specific epidemiological data supporting fly transmission were not provided in this article.

Blow flies were also considered a potential transmission route in the 2004 HPAI H5N1 outbreak in Japan.[95,96] 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. The authors estimated that prevalence of viable virus was 5 percent in flies around the epidemic area.[96]

9.3.3.2 Survivability of AI virus in and on Flies

Sawabe *et al.* [97] evaluated the survivability of H5N1 virus in blow flies after experimental exposure at 10 and 20°C over a period of 14 days. Viable virus was recovered in the crop and intestine up to 24 hours post-exposure.. 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 In the same study, agar gels and environmental surfaces of a container in which flies were reared were sampled at 6,9,24 and 48 hours (3 replicates per time point). Virus could only be isolated from 1 of the 12 samples tested (positive sample was taken at 48 hours) and at a concentration less than 0.5 TCID50/.05 ml.

Wanaratana *et al.* [98] evaluated the potential of the house fly to serve as a mechanical vector of the H5N1 virus. Virus isolation revealed that H5N1 virus could survive within the body of the house fly and remain infective for up to 72 h post-exposure. The viral titers in housefly homogenate varied between $10^{5.43}$ EID_{50}/ml at 6 hours post exposure to 10^{2} EID_{50}/ml at 72 hours post-exposure. In this study, the potential for virus transmission via virus on the fly body was also investigated. Specifically, the fluid used to wash the flies body was sampled at various times post-exposure. At 24 hours post-exposure, the virus concentration was 1.90 log ELD_{50}/ml (the concentration at time 0 was 4.70 log ELD_{50}/ml) whereas virus could not be recovered by 48 hours post-exposure.

Nielsen *et al.* [99] isolated infective low pathogenic avian influenza viruses (H7N1 and H5N7) from the alimentary tract of houseflies for at least 24 hours post-feeding, but the level isolated depended on temperature, time period post-feeding, and load of virus ingested. Only 3% of flies (one group out of 36 groups tested) was found virus positive after 24 hours at 25 and 35°C. In summary, the experimental studies show that houseflies and blow flies can ingest some quantities of AI viruses, with the viability of the virus being relatively low after 24 hours. However, virus may remain viable for up to 48-72 hours in a few cases. Nielsen *et al.* concluded that house flies represent potential carriers of AI virus among chickens placed in the same building or farm, or between poultry located on premises in proximity to infected farms.

Tsuda *et al* [100] proposed a new mechanism of transmission where wild birds and poultry directly feed on HPAI infected blow flies. It has been shown that a chicken can eat 31 blow flies placed

in its cage in just 7 minutes.[97] However, feeding dead flies (*C. nigribarbis*) contaminated with H5N1 virus did not result in transmission (unpublished data) (***pers. comm.*** Tsuda, 2012). The frozen dead flies were not attractive to chickens, and only small numbers of flies were consumed by the chickens in this experiment (pers. comm. Tsuda 2012). We were unable to identify published experimental studies where whole (i.e. not fly homogenate) flies contaminated with HPAI virus were consumed by chickens, resulting in HPAI transmission. However, transmission of H5N1 HPAI virus to chickens by feeding a homogenate from contaminated flies has recently been reported [101].

9.3.3.3 Dispersion Rate of Flies

Table 9.2 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 328-1,640 feet) as long as they find suitable food, breeding sites and shelter.

Table 9.2 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.*[102]
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 [103]
Blow fly	Estimated 1250 –1789 m/day on average	Tsuda *et al.* [100]
Blow fly	2–3 km in 24 hours	Sawabe *et al.* [96]

9.3.3.4 Summary of Expert Opinion on the Risk of Exposure of Newly Hatched Chicks by Flies

A summary of expert opinion on the likelihood that newly hatched chicks are exposed to HPAI virus from flies associated with poultry production is included in **Table 9.3**. Experts were asked to estimate the risk of exposure, assuming that a hatchery is located near (up to 1.5 km) an infected undetected broiler farm (50,000 to 100,000 birds) within a HPAI control zone[c]. Qualitative likelihood descriptors used by experts to estimate exposure risk are provided in Appendix 6.

Table 9.3 Summary of expert opinion on the likelihood that newly hatched chicks are exposed to HPAI virus in the hatchery from flies from nearby infected flocks.

	Risk Estimate		
Distance (km)	**Expert 1**	**Expert 2**	**Expert 3**

[c] Participating experts were Dr Amos Ssemtimba, Dr David Halvorson and Dr David Swayne in no particular order.

0.5	Low	Low	Negligible
1.0	Low	Negligible	Negligible
1.5	Low	Negligible	Negligible

9.3.3.5 Qualitative Factors Considered in the Determining the Risk Rating

- Hatchery waste is handled in an enclosed system and disposed of daily, reducing the potential for waste to serve as an attractant for flies within or near the hatchery. Handling waste in an enclosed system and frequently removing waste material decreases the likelihood that flies would enter the hatchery. Hatcheries have several sanitary measures as part of routine biosecurity programs. In particular, chick processing rooms in a hatchery are C&D frequently to reduce the accumulation of organic debris on surfaces and are unlikely to attract flies.
- 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 with AI virus by directly feeding on whole contaminated flies.
- Winpisinger et al. found the number of house flies was significantly higher near (within 3.2 km) large (> 2 million) caged layer operations, compared with background fly levels in rural areas, suggesting that the majority of flies within this distance dispersed from the layer facility.[104] However, the number of flies caught at a distance of 0.8 km (3-22% of the mean value at layer farm) and 1.6 km (2-8% of the mean value at layer farm) was much lower compared to the number of flies trapped at the layer facilities.
- 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 inactivated 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. Sergeev et al., 2012 found an infectious dose above 10^5 EID_{50} for the intra-gastric route.
- Contamination of the surfaces that the fly lands on with virus from the fly body, vomit or feces is a possibility. However, available experimental studies indicated that there would be a considerable reduction in the virus concentration in fly body, vomit or feces by 6 to 24 hours post exposure of the fly to virus (9.3.3.2). The relatively rapid inactivation of virus present externally on flies would result in reduced likelihood of transmission to greater distances.
- Field studies by Brugh and Johnson [93], Wilson [94], and Sawabe *et al*.[95] indicate around 5% of house flies and blow flies from severely infected poultry farms could contain viable virus.
- Expert opinion on local area spread: 2 among three experts rated the risk of fly transmission to day-old chicks in a hatchery that is at 1.5km from an infected farm to be negligible.

9.3.4 Conclusion

We rated the risk of newly hatched chicks becoming infected via contaminated flies mechanically transmitting HPAI virus from a nearby poultry facility (at a distance of 1.5 km or more) to be *negligible.*

9.4 Risk of Day-old Chicks in a Hatchery in the Control Area Becoming Infected with HPAI Virus from an Infected Flock in the Control Area via Aerosols

Risk Factors: Known infected, or infected but undetected, poultry flocks near the hatchery.

Current Preventive Measures: None.

Outbreak Specific Measures: None.

Overall Risk: The risk of exposure of day-old chicks in the hatchery from bioaerosols ranges from *negligible* to *high*, depending on distance from, and prevalence in, the source flock. We estimated the risks of exposure of day-old chicks to be *negligible* to *low* if the broiler hatchery is located at 1.5 km from an infected but undetected poultry farm, and *low* if the broiler hatchery is located at 1.5 km from a known infected poultry farm.

9.4.1 Introduction

This chapter considers the scenario where a broiler hatchery is located within the Control Area, giving rise to the possibility of aerosol transmission to day-old chicks from infected poultry flocks located near the hatchery. Previous HPAI outbreak investigations have identified proximity to infected premises as a risk factor for HPAI infection of poultry flocks.[49,72] Although fecal-oral rather than aerosol transmission has been considered to be the primary transmission mechanism for HPAI H5N1 virus within a flock, [22] aerosol transmission is a potential component of local area spread of HPAI virus between infected and susceptible poultry flocks. Recent evaluation of genetic and epidemiological data for the HPAI H7N7 outbreak in the Netherlands provided statistical evidence that the direction of spread of avian influenza A (H7N7) is correlated with the direction of the wind at the date of infection. These data suggest that aerosol transmission is a possibility, depending on the HPAI strain characteristics. However, within-flock transmission by aerosol has been shown to occur at a slower rate compared to transmission between chickens in direct contact. Here we focus on risk pathways for infection of day-old chicks due to aerosolization of HPAI virus from infected poultry flocks at specific downwind distances from the hatchery.

9.4.2 Evaluation of Risk

The risk evaluation is based on a scientific literature review, expert opinion and aerosol dispersion modeling scenarios. Alternate scenarios are modeled using the EPA aerosol dispersion model AERMOD, where the source flock is either known to be infected, or is infected and undetected.

9.4.2.1 Literature review

9.4.2.1.1 Role of Aerosol Transmission in Previous AI Outbreaks

Aerosol transmission was not identified as a primary mode of transmission in most of the previous AI outbreaks. However, it has been proposed as a transmission mechanism in some outbreaks in the Netherlands, Australia and Canada.[49,105] In general, the outbreak experiences and air sampling studies suggest that some aerosol spread is possible over short distances.

- In several AI outbreaks such as the LPAI H7N2 outbreak in Virginia, the geographic distribution of affected farms was relatively random suggesting that aerosols were not a primary mode of transmission.[106]In a HPAI H5N1 outbreak in United Kingdom, there was no transmission to 78 other farms within 3 km of an infected turkey premises. The author concludes that there was no evidence of local area spread above 1 km.[75]
- Generation of dusts or aerosols associated with trucking actively infected birds with AI virus within 200 yards (182m) of a susceptible flock; this represents a risk for aerosol transmission (personal communication, *Dave Halvorson*).[82]
- Depopulation activities up to 400 yards (366 m) up-wind from a susceptible flock can represent a risk for aerosol transmission (personal communication, *Dave Halvorson*).
- Spreading of non-composted contaminated litter on adjacent fields was suspected as a transmission mechanism during the 1983 HPAI H5N2 AI outbreak (personal communication, *Dave Halvorson*.[82] Ypma *et al*., (2012) estimated the contribution of a possible wind-mediated mechanism to the total amount of spread during the 2003 HPAI H7N7 outbreak to be around 18%.[50] This estimate was based on the observed correlation between the wind direction and the direction of spread of disease estimated through genetic and epidemiological data. The possibility of the direction of spread coinciding with the wind direction by chance was also accounted for in their statistical analysis. We note that this outbreak had occurred in a region of very high poultry density (around 4 farms per km^2), increasing the possibility of spread over short distances.[74]
- Only a couple of studies thus far report air sampling results from or around HPAI infected houses in field outbreaks. High volume air sampling was conducted in and near an infected layer flock where flocks experienced high mortality during the HPAI H7N7 outbreak in Canada.[107] Inside the barn, a viral titer of 292 $TCID_{50}/m^3$ was detected in air samples. Air sampling at a command post outside the barn showed a much lower viral load of 12.5 $TCID/m^3$. In the 1983 H5N2 HPAI Pennsylvania outbreak, 5 of 6 samples taken 3-6 m downwind of affected flocks on 6 premises were virus-positive, whereas only 1 of 12 samples taken 45-85 m downwind of affected flocks on 8 premises was virus positive; the positive sample was taken 45 m downwind.[82] These studies demonstrate the effect of dilution on aerosol concentration with increasing distance from the generating source.

9.4.2.1.2 Experimental Studies on Aerosol Transmission

Several experimental studies indicate that airborne transmission of HPAI infection between chickens in adjacent pens or cages is possible but inefficient. These studies also suggest that aerosols may not be a primary route of transmission within a flock.

- HPAI H7N7 virus was not transmitted from a group of 14 inoculated birds to 10 birds housed 3m away with direct airflow from the inoculated pen.[108]
- HPAI H5N1 virus was not transmitted between chickens in adjacent cages, suggesting that the fecal-oral route was the primary mode of transmission.[22]
- HPAI H5N2 virus from infected chickens was not transmitted to sentinel chickens in another corner of the room.[109]
- LPAI H7N6 viruses were not transmitted to chickens placed in a cage with direct airflow below a cage with infected birds.[8]
- Two out of six strains of LPAI H9N2 were transmitted via aerosol from a cage with four infected chickens to chickens in an adjacent cage 100 cm apart. [110]
- Airborne transmission of HPAI H5N1 occurred inefficiently when 1 to 2 chickens were infected, but efficiently when 4 to 8 chickens were infected. [111]
- For H5N1, Spekreijse *et al.,* (2011) [63,112] estimated an aerosol transmission rate parameter of 0.10 birds per day for chickens housed 1 m away. For LPAI H6N2, Yee *et al*., (2009) and Yee *et al*., (in review) found a transmission rate parameter of 0.008 birds per infectious chicken per day for chickens in adjacent stacks of cages with the aerosol route. [64] These results indicate a slower spread via aerosol route compared to direct contact, for which the transmission rate estimates in the literature ranged from 0.5 to 32 birds per day.

Several studies have found that influenza A viruses experience decreased survivability in aerosols at higher temperature and relative humidity.[113,114] Given the chick holding room conditions of 77 to 84.2°F (25° to 29°C) and 50 to 70 percent relative humidity, the estimated first order rate constant for reduction of virus titer is approximately 14 per day (equivalent to a 6-log reduction in viral titer per day[d]).[5, 7]

9.4.2.2 Exploratory Aerosol Modeling Scenarios

Aerosol dispersion models have been extensively used to predict aerosol particle concentrations at different distances from a generating source.[115] Essentially, these models estimate the dilution of aerosol concentration with distance from a generating source due to dispersion in air or gravitational settling, considering the meteorological conditions. The concentration of bioaerosols at a specific distance from a source depends on factors such as:

1) The source emission rate, which is the relative amount of particles/species emitted by the source per-unit-time, depending on the aerosolization process.
2) Dispersion or dilution of the particles, given the local meteorological conditions and topography.
3) Depletion of particles due to settling or precipitation, given the particle size distribution.

[d] The log reduction in viral titer in t days given a first order rate constant of 14 per day is $\log e^{(-14t)}$. For t =0.5 days, the log reduction is $\log e^{(-7)} = -3.04$.

4) The decay of aerosolized microorganisms with time due to environmental factors acting upon them.

The EPA recommends the use of the AERMOD steady state plume dispersion modeling system for regulatory applications for determining pollution exposure.[116] 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.[117]

We evaluated separate aerosol model scenarios for transmission of HPAI virus from a known severely infected poultry flock, and from an infected but undetected flock. Performing the analysis separately enables us to consider the differences for within flock prevalence and aerosol emission rates in these cases.

9.4.2.2.1 Aerosol Dispersion Modeling Assumptions and Key Uncertainties

Key factors contributing to uncertainty:

Particle size distribution: There is considerable uncertainty and variability regarding the particle size distribution of aerosols generated from birds or their excreta in poultry houses. While some studies have estimated the particle size distribution of poultry house aerosols, only a few have adjusted for the particle size distribution of background incoming air in their analysis. The particle size distribution across operations is highly variable as well, depending on their management practices and age of the flock. The uncertainty and variability in these factors impacts the model estimates of aerosol depletion due to gravitational settling.

Dose response: Ssematimba *et al.* estimated a 50% chicken infectious dose of around 2.5 log EID_{50}, based on oronasal inoculation data from Spekreijse et al., (2011) as a proxy for dose response via the aerosol route.[49,112] However, direct aerosol data from Spekreijse et al. (2012) suggests very low transmission rates— even at 24 hours of exposure— to more than 10^3 EID_{50}/m^3 of H5N1 HPAI virus concentration in air coming from a room housing infectious chickens.[63] As a possible explanation for the higher infectious dose through the aerosol route observed in Spekreijse et al. (2012), the authors suggested the possibility that the immune system could clear a small virus load inhaled over a prolonged period of time better than when it is overwhelmed with a large amount of virus all at one time. We fit exponential and logistic dose response models to data from Spekreijse et al. (2012). Data and maximum likelihood estimation suggested a 50 percent chicken infectious dose for the aerosol route between 5 to 6 log EID_{50}. 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. [64]

Sergeev et al., (2012) found considerably lower 50% chicken infectious dose 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. [62] The results from this paper are not consistent with other studies, which indicate lower aerosol transmission rates between infected and susceptible chickens housed in adjacent cages, and is also inconsistent with data published in Spekreijse et al. (2012). A possible explanation for the differences between this study and Spekreijse et al. (2012) is that the 0.5 to 2 μm diameter contaminated aerosols generated by nebulizing embryonating egg contents are different from naturally contaminated aerosols emanating from a chamber with infectious chickens.

We used the maximum likelihood estimates from our analysis based on data presented in Spekreijse et al. (2012) to estimate the exponential dose response parameter (Figure 1). These data from air flowing through infectious chickens may be more representative of the dose response relationship for aerosol spread between farms. In addition, these data are most consistent with the several studies that showed inefficient aerosol spread.[62]

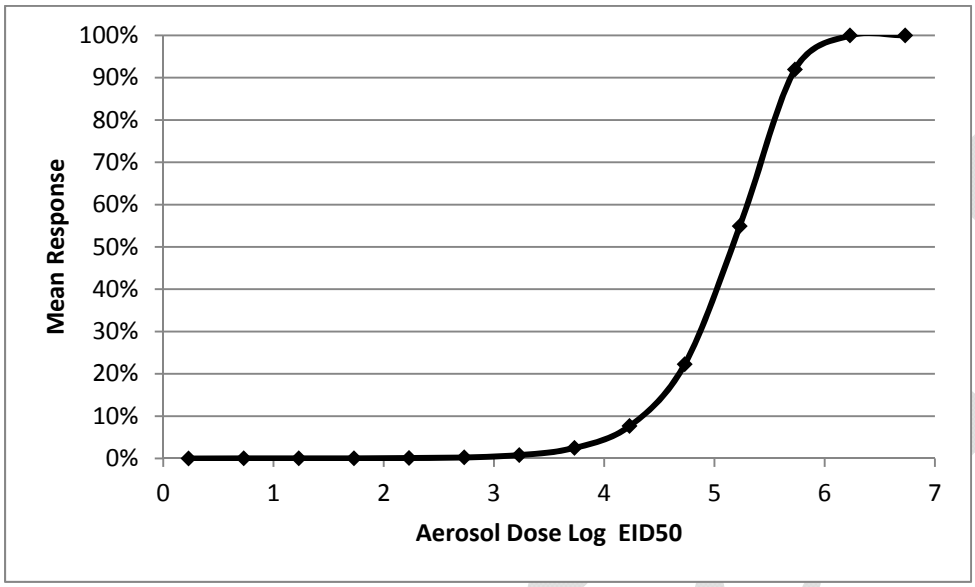


Figure 1 Exponential dose response model for aerosol transmission in chickens

Key assumptions for aerosol dispersion scenarios:

1) The decay of HPAI virus titer with time was not considered in the model.
2) HPAI virus would be uniformly distributed across aerosol particles of varying dimensions. In practice there would be an increased likelihood of aerosol transmission if HPAI virus was preferentially distributed on smaller size particles and vice versa.
3) Meteorological and surface parameters averaged over a year from Lovett, Georgia USA were used for the main results. To consider the variability in the weather conditions, estimates using meteorological parameters that resulted in the highest 3-hour average concentration of aerosolized virus in a year were also provided. In addition, we performed sensitivity analysis with meteorological parameters representative of other locations in Minnesota and South Carolina, which showed a lower risk of aerosol spread (Appendix 2).
4) The hatchery is located downwind from an infected poultry house.
5) A single-hit or independent action dose response model where each virus particle has an independent likelihood of causing infection was used. However, it is not known whether there is a threshold dose below which there is no likelihood of infection.

9.4.2.2.2 Dispersion Modeling Scenarios for Known Infected Flocks

We evaluated three modeling scenarios (A, B and C) for aerosol transmission of HPAI virus from known infected flocks at different distances to day-old chicks at the hatchery (Table 9.4). The HPAI virus emission rates were chosen to be relatively higher in these scenarios due to the greater within flock infection prevalence compared to infected but undetected flocks. The emission rates were calculated by assuming that a specific proportion of aerosolized particles are contaminated with HPAI virus and by using direct HPAI outbreak studies, as detailed in Appendix 2. The HPAI infected flock was modeled to be a broiler meat flock in scenarios A and B, and a table-egg layer flock in scenario C.

The scenario results show a decrease in aerosol concentrations with distance, due to dilution as well as gravitational settling (Figure 2). The results also show a considerable difference in the predicted aerosol concentration with average meteorological conditions and extreme meteorological conditions (in parenthesis in Table 9.4). At 0.5 km from a known infected flock, the predicted probability of infection in the three scenarios ranged from *low* to *high*, depending on the meteorological parameters. The predicted probability of infection ranged from *low* to *moderate* at 1.5 km and was *low* at 3 to 5 km distance from infected flocks.

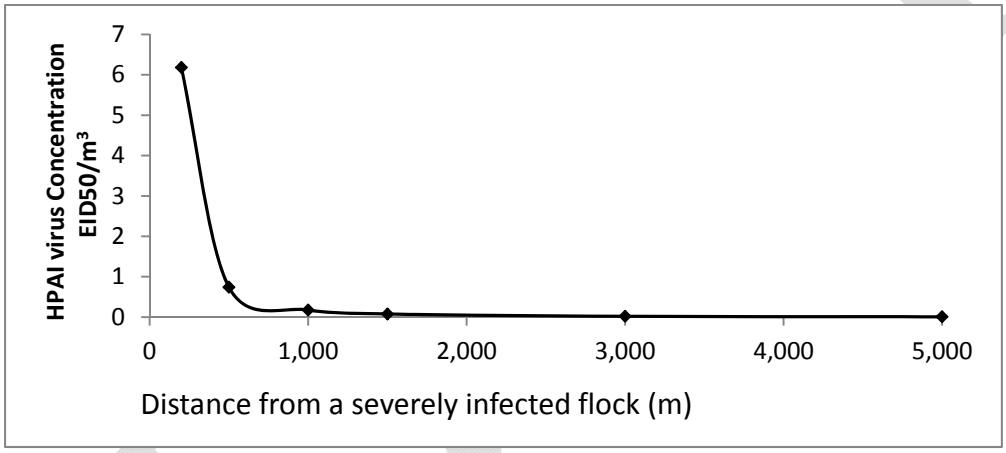


Figure 2 AERMOD Model predicted HPAI aerosol concentration with distance from an infected poultry house (Scenario A).

Table 9.4 AERMOD Modeled scenarios to predict HPAI virus concentration at different distances from a known severely infected poultry house.

Parameter description	Source/Formula	Scenario A (Broiler)	Scenario B (Broiler)	Scenario C (Layer)
(C) HPAI virus concentration in the air of a commercial poultry facility (EID_{50}/m^3)	Experimental studies, Calculation Appendix 2	$10^{2.6}$	NA	$10^{3.46}$

Table 9.4 AERMOD Modeled scenarios to predict HPAI virus concentration at different distances from a known severely infected poultry house.

Parameter description	Source/Formula	Scenario A (Broiler)	Scenario B (Broiler)	Scenario C (Layer)
Mean ventilation rate (cfm/bird)	Appendix 2	7	NA	3
(N_s) Number of birds	Scenario parameter	20,000	25,000	100,000
Aerosol emission rate (EID_{50})/s	$E*N_s*C$	$10^{4.42}$	$10^{3.42}$	$10^{5.6}$
Estimated concentration at 0.5 km (EID_{50}/m^3)	AERMOD	0.74 (92)	0.19 (10)	23 (647)
Estimated concentration at 1km (EID_{50}/m^3)	AERMOD	0.17 (16)	0.04 (2.6)	4.7 (261)
Estimated concentration at 1.5 km (EID_{50}/m^3)	AERMOD	0.07 (6.3)	0.02 (1.2)	2.0 (87)
Estimated concentration at 3 km (EID_{50}/m^3)	AERMOD	0.01 (1.2)	0.005 (0.29)	0.5 (14)
Estimated concentration at 5 km (EID_{50}/m^3)	AERMOD	0.007 (0.39)	0.002 (0.11)	0.18 (5.3)
Probability (%) of infection with one day of exposure and for 100,000 chicks at 0.5km	Exponential dose response model fitted to data from Speckrisje et al.	0.53 (48)	0.14 (7.1)	15 (98)
Probability (%) of infection with one day exposure and for 100,000 chicks at 1 km	Exponential dose response model	0.12(11.4)	0.035 (1.9)	3.3 (84)

Table 9.4 AERMOD Modeled scenarios to predict HPAI virus concentration at different distances from a known severely infected poultry house.

Parameter description	Source/Formula	Scenario A (Broiler)	Scenario B (Broiler)	Scenario C (Layer)
Probability (%) of infection with one day exposure and for 100,000 chicks (1.5 km)	Exponential dose response model	0.06 (4.5)	0.016 (0.84)	1.4 (46)
Probability (%) of infection with one day exposure and for 100,000 chicks (3 km)	Exponential dose response model	0.014 (.88)	0.004(0.21)	0.37 (10)
Probability (%) of infection with one day exposure and for 100,000 chicks (5 km)	Exponential dose response model	0.0053(.28)	0.0015(0.082)	0.13(3.8)

The main results are average values based on meteorological parameters in a year for Lovett, Georgia. The results in parentheses are for meteorological parameters which resulted in the highest 3-hour average concentration of aerosolized virus in a year.

9.4.2.2.3 Dispersion Modeling Scenarios for Infected, Undetected Flocks

We evaluated three modeling scenarios (D, E, and F) for aerosol transmission of HPAI virus from an infected but undetected flock to day-old chicks at the hatchery (Table 9.5). In these scenarios, the HPAI virus emission rates were based on relatively lower values for the within flock infection prevalence compared to scenarios for infected flocks (see Appendix 2). The HPAI infected flock was modeled to be a broiler meat flock in scenarios D and E and was modeled as a table-egg layer flock in scenario F. For scenario C (layer flock), the impact of active surveillance with daily RRT-PCR testing in reducing the time to detection and resulting in a lower prevalence in the time period before detection was considered in estimating the aerosol emission rate. Further details on these scenarios are provided in Appendix 2.

The scenarios for infected but undetected flocks show *low* risks at 1.0 km and *negligible* to *low* risks at 1.5 km distance. These results suggest that aerosol transmission may not represent a considerable risk for HPAI virus in the time period before a flock is detected when the HPAI strain characteristics are similar to Asian HPAI H5N1 strains.

Table 9.5 AERMOD Model scenarios to predict HPAI virus concentration at different distances from an infected, undetected poultry house.

Parameter description	Source/Formula	Scenario D (Broiler)	Scenario E (Broiler)	Scenario F (Layer)
(C) HPAI virus concentration in the air of a commercial poultry facility (EID_{50}/m^3)	Estimated from disease transmission and surveillance models. Experimental studies	$10^{1.7}$	_	$10^{1.46}$
Emission ventilation rate from a poultry house cfm/bird	Appendix 2	7	-	3
(N_s) Number of birds	Scenario parameter	20,000	25,000	100,000
Aerosol Emission rate (EID_{50})/s	$E*N_s*C$	$10^{3.52}$	$10^{2.2}$	$10^{3.6}$
Estimated concentration at .5 km (EID_{50}/m^3)	AERMOD	0.09 (11)	0.012 (0.60)	0.23 (6.47)
Estimated concentration at 1km (EID_{50}/m^3)	AERMOD	0.02 (2)	0.003 (0.15)	0.04 (2.61)
Estimated concentration at 1.5 km (EID_{50}/m^3)	AERMOD	0.009 (0.76)	0.0013 (0.07)	0.02 (0.87)
Probability (%) of infection with one day exposure and for 100,000 chicks (0.5km)	Exponential dose response model fitted to data from Spekisje et al.	0.06 (7.7)	0.009 (0.44)	0.31 (4.6)
Probability (%) of infection with one day exposure and for 100,000 chicks (1 km)	Exponential dose response model	0.015 (1.4)	0.0021 (0.12)	0.06 (1.87)

Table 9.5 AERMOD Model scenarios to predict HPAI virus concentration at different distances from an infected, undetected poultry house.

Parameter description	Source/Formula	Scenario D (Broiler)	Scenario E (Broiler)	Scenario F (Layer)
Probability (%) of infection with one day exposure and for 100,000 chicks (1.5 km)	Exponential dose response model	0.0067 (0.55)	0.0009 (0.051)	0.03 (0.63)

9.4.2.3 Expert Opinion

We elicited opinion from three experts on the risk of aerosol transmission for day-old chicks in a hatchery that is located at specific distances from an infected flock (Drs. David Swayne, David Halvorson and Amos Ssematimba). The experts were requested to provide separate risk ratings for aerosol transmission from known severely infected flocks and infected but undetected flocks.

Table 9.6 Qualitative ranking for the likelihood of exposure of day-old chicks in a hatchery by aerosol transmission from a known severely infected flock based on expert opinion.

Hatchery distance from severely infected poultry farm	**Expert 1**	**Expert 2**
0.5 km	Moderate	Extremely high
1 km	Moderate	Extremely high to high
1.5 km	Low	Moderate

Table 9.7 Qualitative ranking for the likelihood of exposure of day-old chicks in a hatchery by aerosol transmission from an infected but undetected flock based on expert opinion.

Hatchery distance from infected, undetected poultry farm	**Expert 1**	**Expert 2**	**Expert 3**
0.5 km	Low	Low	High
1 km	Low	Low	High
1.5 km	Negligible	Negligible	Moderate

In general, experts have rated the risk of aerosol transmission from a known infected flock to be higher compared to the scenarios for an infected but undetected flock, which is likely to have lower infection prevalence. Two out of 3 experts have rated the risk of day-old chicks at the

hatchery becoming infected with aerosolized HPAI virus from an infected undetected poultry flock at a distance of 1.5 km or more to be *negligible,* while the third expert rated this risk to be *moderate.* In addition, several industry experts and field emergency response personnel have stated that, based on previous AI outbreak experiences and spread patterns, aerosol transmission does not play a substantial role in transmission of AI infection.

9.4.2.4 Risk Rating

9.4.2.4.1 Risk of HPAI Spread to Day-old Chicks at a Hatchery in a Control Area via Aerosol Transmission from a Known HPAI Infected Flock

Given the higher predicted prevalence of infectious birds in known infected flocks, the expert opinion ratings as well as exploratory dispersion modeling results indicated higher potential risk for this category. Literature review and most previous outbreak reports indicate that local area spread and aerosol transmission were not an important factor at distances more than 1.5 km from an infected flock, while there is some evidence of aerosol transmission over shorter distances. We provided the following risk ratings, considering the above factors.

- *Moderate* to *high* if the broiler hatchery is located at 0.5 km from a known infected poultry farm.
- *Moderate* if the broiler hatchery is located at 1 km from a known infected poultry farm.
- *Low* if the broiler hatchery is located at 1.5 km from a known infected poultry farm.

9.4.2.4.2 Risk of HPAI Spread to Day-old Chicks at a Hatchery in a Control Area via Aerosol Transmission from an Infected but Undetected Flock

In this case, the expert opinion ratings as well as dispersion modeling results indicated lower risks. We rated the risk of day-old chicks at a hatchery becoming infected with HPAI via aerosols from an infected but undetected poultry flock at a specific distance from the hatchery as follows.

- *Low* to *moderate* if the broiler hatchery is located at 0.5 km from an infected but undetected poultry farm.
- *Low* if the broiler hatchery is located at 1 km from an infected but undetected poultry farm.
- *Negligible* to *low* if the broiler hatchery is located at 1.5 km from an infected but undetected poultry farm.

9.4.3 Conclusion

The risk of exposure of day-old chicks in the hatchery from bioaerosols ranges from *negligible* to *high*, depending on the distance from, and prevalence in, the source flock. We estimated the risks of exposure of day-old chicks to be *negligible* to *low* if the broiler hatchery is located at 1.5 km from an infected but undetected poultry farm, and *low* if the broiler hatchery is located at 1.5 km from a known infected poultry farm.

9.5 Risk of Day-old Chicks in a Hatchery in the Control Area Becoming Infected with HPAI Virus from Essential Vehicles

Risk Factors: Contamination of essential vehicles after visiting a farm with poultry within the Control Area.

Current Preventive Measures: None.

Outbreak Specific Measures (to be implemented by industry during an outbreak)**:** C&D requirements of essential vehicles; use of PPE by the vehicle driver.

Conclusions: We conclude that the risk of day-old chicks in a hatchery in the Control Area becoming infected with HPAI virus from essential vehicles is *negligible* to *low* provided the measures in SBS Plan are strictly followed.

9.5.1 Introduction

This chapter evaluates the risk of essential vehicles (e.g. waste removal, commercial delivery, privately owned conveyances) being contaminated with HPAI virus and resulting in infection of day-old chicks at the hatchery. The transmission risks associated with the hatching egg delivery vehicle were evaluated in the broiler hatching egg RA and were determined to be low. As mentioned in the scope, RA addresses the scenarios where day-old chicks are delivered to premises in an HPAI Free Area or to premises in the Control Area with a *negligible* to *low* risk of HPAI virus being present on the premises. Hence, we do not consider risk of HPAI virus being transmitted to the hatchery via a day-old chick delivery vehicle returning from a broiler farm after placing chicks, in this evaluation.

Other essential vehicles, apart from the day-old chick and hatching-egg trucks, include those vehicles transporting hatchery waste, delivering vaccines, equipment service personnel, company veterinarians, etc. It is possible that these visitors and vehicles previously visited poultry premises in the Control Area. Movement of vehicles (rendering or manure hauling) in general has been implicated in the spread of HPAI in previous outbreaks, although spread to day-old chicks at hatcheries via vehicles has not been documented.[48,91,118] At the hatchery, there are theoretically risk pathways for disseminating HPAI virus from the vehicle through the hatchery environment, via movements of personnel and equipment, resulting in infection of day-old chicks. We considered the preventive measures from the SBS Plan for evaluating this risk.

9.5.2 Preventive Measures

9.5.2.1 Outbreak Specific Measures

- The vehicle exterior should be C&D after visiting a poultry premises in a HPAI Control Area and before entering the hatchery premises.
- A route should be selected so as to avoid other poultry premises by a reasonable distance once the C&D of vehicle is completed.

- All vehicle drivers will be required to put on disposable plastic boots or clean rubber boots before getting out of the cab and follow guidelines for using PPE as described in NPIP (9CFR147.24). These protocols also specify the use of a hand sanitizer.
- Essential visitors who have had contact with domestic poultry, other avian species, and/or related organic material are required to have at least 12 hours downtime before visiting the hatchery. A shower and change of clothing is also required before visiting the hatchery.
- Other essential visitors should follow equivalent procedures for wearing PPE as required by the broiler company.

9.5.3 Evaluation of Risk

In this chapter, we evaluate the risk of exposure of day-old chicks to HPAI viruses associated with essential conveyances to the hatchery, other than the day-old chick and hatching egg vehicles. Specifically, the focus here is on essential visitors, such as those transporting hatchery waste, delivering vaccines, equipment service personnel, and company veterinarians.

The likelihood and degree of HPAI virus contamination of the exterior surfaces of essential vehicles is difficult to estimate, given the different types of vehicles that could visit a hatchery. The SBS Plan contains provisions for C&D of vehicles during an outbreak. The SBS Plan requires C&D of the exterior, tires and wheel-wells of vehicles. Similarly, other relevant guidelines, such as the NAHEMS guidelines, address the C&D of vehicles in detail.[119] These C&D procedures would effectively inactivate HPAI virus on the vehicle exterior, given the sensitivity of HPAI virus to most detergents and disinfectants. [26,27] We conclude that the likelihood of HPAI virus remaining on the exterior of a vehicle that has been cleaned and disinfected as specified in the SBS Plan is *negligible* to *low*.

We considered the following factors in evaluating the risk associated with the cab or trailer interior:

- *Use of PPE and hand sanitizer*: The use of PPE by vehicle drivers and essential visitors when getting out of the cab would reduce the likelihood of transmitting HPAI virus from vehicle interior to environmental surfaces in the hatchery. Similarly, the use of commercial hand sanitizer formulations is also expected to be effective in preventing transmission from the vehicle interior.[87,120]
- *Impact of shower and change of clothes*: A shower and change of clothes would likely mitigate any recontamination of the vehicle driver from the cab interior and would also likely increase the number of virus transfer steps in the risk pathway. For example, given this intervention, the risk pathway associated with the cab interior would involve contamination of interior by the driver and recontamination of the driver from the cab interior after a shower and change of clothes.
- *Multiple virus transfer steps*: Transmission of HPAI virus from the vehicle cab interior would involve multiple virus transfer events between contact surfaces. Most essential visitors would not directly contact day-old chicks, and the risk pathway thus involves hatchery dissemination via movements of people and equipment. In addition, some of the essential visitors (e.g., waste collection personnel) may not enter the hatchery premises. In this case, the risk pathway would involve transmission of virus from hatchery premises ground areas into the building via hatchery personnel. However, hatchery personnel have specific footwear

protocols (Section 9.2.2.2.1) to reduce such transmission. The final surface concentration of HPAI virus transferred through such contact steps would be lowered by the multiple steps involved in the pathway and would reduce the likelihood of transmission. This is because only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface in each direct contact step (personal communication Drs. Sayed Sattar and Susan Springthorpe). [20,85,86]

We qualitatively rate the risk of day-old chicks becoming infected with HPAI virus associated with the cab interior surfaces of essential vehicles to be *negligible* to *low,* given that the preventive measures from the SBS Plan are strictly followed.

9.5.4 Conclusion

We conclude that the risk of day-old chicks in a hatchery in the Control Area becoming infected with HPAI virus from essential vehicles is *negligible* to *low* provided the measures in SBS Plan are strictly followed.

10. Overall Conclusion

The objective of this assessment was to estimate the risk that the movement of broiler day-old chicks into, within, and out of a Control Area during a HPAI outbreak in the poultry industry in the United States would result in the introduction of HPAI infection onto another poultry premises (broiler farm). The assessment focused on the risk pathways for HPAI infection of day-old chicks in a broiler hatchery located within a HPAI control zone and near a HPAI infected flock, via components of local area spread.

The pathways for transmission of HPAI virus from broiler farms located in a Control Area back to the hatchery were not included in the scope of this assessment. Therefore, this assessment applies to the movement of day-old chicks to premises outside of Control Area or to premises in the Control Area for which the risk of transmitting HPAI virus back to the hatchery is *negligible* to *low*. The risk of HPAI infection of day-old chicks at the hatchery associated with the movement of hatching eggs from broiler breeder flocks in a Control Area has been evaluated previously and found to be *negligible* to *low* when the outbreak measures specified in the SBS Plan are implemented.

With respect to the major component risks that were analyzed, this assessment concludes the following:

- The risk of day-old chicks becoming infected with HPAI virus released into the hatchery via hatchery personnel or essential visitors is estimated to be *negligible* to *low*.
- The risk of newly hatched chicks becoming infected via contaminated flies mechanically transmitting HPAI virus from a nearby poultry facility (at distances up to 1.5 km) is estimated to be *negligible.*
- The risks of exposure of day-old chicks from bio-aerosols is estimated to be *negligible* to *low* if the broiler hatchery is located at 1.5 km from an infected but undetected poultry farm, and *low* if the broiler hatchery is located at 1.5 km from a known infected poultry farm.
- The risk of day-old chicks in a hatchery in the Control Area becoming infected with HPAI virus from essential vehicles is estimated to be *negligible* to *low.*

The overall risk of the movement of day-old chicks from a hatchery in a Control Area resulting in HPAI spread to a broiler farm is *negligible* to *low* provided the hatchery is 1.5 km or more from known infected premises and the preventive measures from the NPIP program (9CFR145-147) and the SBS Plan measures, including those pertaining to movement of hatching eggs, are strictly implemented.

It should be remembered that:

- This assessment is based on current (October 2013) information and will need to be reviewed and revised as circumstances warrant.
- The assessment does not replace the judgment of on-scene officials with first-hand knowledge of the outbreak situation and the premises in question.

Appendix 1: Relevant Current Preventive Measures from the NPIP Program (9CFR 145 and 147)

Recommended Measures at Hatchery

9CFR 147.23 (b): The hatchery building should be arranged so that separate rooms are provided for each of the four operations: Egg receiving, incubation and hatching, chick processing, and egg tray and hatching basket washing. Traffic and airflow patterns in the hatchery should be from clean areas to dirty areas (i.e., from egg room to chick processing rooms) and should avoid tracking from dirty areas back into clean areas.

9CFR 147.23 (e): Only clean eggs should be used for hatching purposes.

9CFR 147.23 (f): Only new or cleaned and disinfected egg cases should be used for transportation of hatching eggs. Soiled egg case fillers should be destroyed.

9CFR 145.6 (2): Incubator room walls, ceilings, floors, doors, fan grills, vents, and ducts should be cleaned and disinfected after each set or transfer. Incubator rooms should not be used for storage. Egg trays and buggies should be cleaned and disinfected after each transfer. Cleaning and disinfection procedures should be as outlined in 9CFR 147.24.

9CFR 147.23 (c) The hatching compartments of incubators, including the hatching trays, should be thoroughly cleaned and fumigated or otherwise sanitized after each hatch.

9CFR 147.23 (g) Day-old chicks, poults, or other newly hatched poultry should be distributed in clean, new boxes and new chick papers. All crates and vehicles used for transporting birds should be cleaned and disinfected after each use.

Recommended Egg Truck and Driver Biosecurity 9CFR 147.24 (c)

The chick/poult delivery truck drivers and helpers should use the following good biosecurity practices while delivering chicks/poults:

1) Spray truck tires thoroughly with disinfectant before leaving the main road and entering the farm driveway.
2) Put on sturdy, disposable plastic boots or clean rubber boots before getting out of the truck cab. Put on a clean smock or coveralls and a hairnet before entering the poultry house.
3) After loading eggs or unloading chicks/poults, remove the dirty smock/coveralls and place into a plastic garbage bag before loading in the truck. Be sure to keep clean coveralls separate from dirty ones.
4) Reenter the cab of the truck and remove boots before placing feet onto floorboards. Remove hairnet and leave with disposable boots on farm.
5) Sanitize hands using appropriate hand sanitizer.
6) Return to the hatchery or go to the next farm and repeat the process.

Appendix 2: Supporting Data and Analysis for Evaluation of the Risk of Aerosol Transmission of HPAI Virus to Day-old Chicks

In this appendix, we provide supporting data and analysis for the AERMOD dispersion modeling scenarios for estimating the aerosolized HPAI virus concentration in air surrounding the hatchery when an HPAI infected poultry flock is located at specific distances from the hatchery. We considered three modeling scenarios in our evaluation.

Particle size distribution of particles generated in a poultry house

The deposition of particulate matter due to gravitational settling is a key factor impacting the risk of aerosol transmission of HPAI viruses. Larger size particles tend to settle faster and are unlikely to be suspended and transported for long distances via aerosol. There is considerable variability in the particle size distribution of aerosols in poultry houses depending on the ventilation design, production type, and age of the birds.

For risk evaluation, the particle size distribution (PSD) for the particles generated within the poultry house is more pertinent compared to the PSD for all particles emitted from a poultry house which may also include background aerosols from incoming air. A greater importance was placed on studies which estimated the PSD by controlling for particle source concentrations. This is particularly relevant for estimating the proportions and concentrations of smaller size particles such as particle sizes measured at 2.5 μm or less ($PM_{2.5}$) that can be suspended for a longer time and hence may be present in greater quantities in ambient air. Recent industry data indicate that the background concentration did not have a considerable impact on $PM_{2.5}$ at distances more than 30 m from a broiler house.

Appendix Table 1 summarizes the studies on size fractions for PM_{10} (particles with a diameter less than or equal to 10μm) and $PM_{2.5}$ (particles with a diameter less than or equal to 2.5 μm).

PSD used in aerosol modeling scenarios:

Scenario A: We considered that 25.5 percent of particles are PM_{10} and 13.3 percent of PM_{10} particles are $PM_{2.5}$. These particle fractions represent averages for data presented in **Appendix Table 1** for studies that have controlled for background concentration. For particles greater than 10μm in size, a diameter of 25 μm was used based on the mean mass diameter estimate from Redwine (2002).

Scenario B: We considered that 41 percent of particles are PM_{10} and 27 percent of PM_{10} particles are $PM_{2.5}$. The particle size fraction in this scenario was chosen cautiously from the data points in **Appendix Table 1** to have a greater proportion of small size particles. For particles greater than 10μm in size, a diameter of 25 μm was used based on the mean mass diameter estimate from Redwine (2002).

Scenario C: We considered that 17 percent of particles are PM_{10} and 19 percent of PM_{10} particles are $PM_{2.5}$. These are average values from the literature review for layer houses presented in **Appendix Table 1** of Li *et al*., (2010). [121] For particles greater than 10μm in size a diameter of 15 μm was used.

Appendix Table 1. Fraction of particles from poultry operations with size less than or equal to 10µm (PM10) and 2.5µm (PM2.5).

Study	PM_{10} fraction of total suspended particles (%)	$PM_{2.5}$ fraction of PM_{10} (%)
Burns *et al.*, (2008) [122] (background PM controlled)	41	
Roumeliotosis (2010) , [123] Canada (background PM controlled)		15.6
Wathes et al. (1997) [124]	10	
Li (2008) , [125] turkey toms		11
Redwine (2002) (background PM not controlled)	5.9 (2.7-8.4)	
Takai (1998) , [126] inhalable vs. respirable dust (background PM not controlled)	13	
Li et al. (2010), layers (background PM not controlled)		6
Roumeliotosis (2007) [127] (background PM not controlled)	77	27
Unpublished Industry data	50-70	50

HPAI virus aerosol concentration in an infected poultry house (C EID_{50}/m^3)

Scenario A: One approach to estimate the HPAI virus concentration in poultry house air is based on the concentration of aerosolized particulate matter in different poultry barn types. **Appendix Table 2** summarizes studies on the ventilation rates and concentrations of particle matter in different poultry house types. A literature review by Li et al. (2010) indicates that the total particle concentration was mostly in the range of 1-11 mg/m^3 for broilers depending on age of the birds.

Poultry house dust may be composed of dried particles of skin (squames), feathers, litter, fecal matter, urine crystals and feed. Some studies report that the majority of particle matter in a broiler house air originates from skin and feathers, with the rest originating from the feed and litter. [128] Conversely, other studies reported bedding, feces and feed to contribute most of the inhalable and respirable particles from a broiler house. [129] Bioaerosol dust components originating from infected birds such as feathers and manure can be contaminated at high virus titers (10^4 to 10^7 EID_{50}/g) . [21,130]

Using a total particulate matter concentration of 5 mg/m^3 and assuming 25 percent of the particles are contaminated at a titer of $10^{5.5}$ EID_{50}/g, the resulting total virus concentration would

be $10^{2.6}$ EID_{50}/m^3. This virus titer is comparable to the observed aerosol concentrations in field and experimental studies. We used a HPAI virus titer of $10^{2.6}$ EID_{50}/m^3 in scenario A.

Scenario B: This parameter was not calculated for scenario B as a different approach was used for predicting the aerosol emission rate in this case.

Scenario C: Thus far, only one study has estimated AI virus concentration in the air from infected barns. High volume air sampling was conducted in and near an infected layer flock where birds experienced a high mortality during the HPAI H7N7 outbreak in Canada.[85] Inside the barn, a viral titer of 292 $TCID_{50}/m^{\cdot}$ was detected in air samples. For HPAI H7N7 virus, 1 $TCID_{50}$ is approximately equal to 10 EID_{50} (personal communication *David Swayne*). We used an estimate of $10^{3.46}$ EID_{50}/m^3 in Scenario C for the aerosol concentration in a severely infected layer house. Spekreijse et al., found HPAI virus titers between $10^{2.8}$ to 10^4 EID_{50}/m^3 in air from an experimental cage with 8 inoculated and 6 contact birds. The ventilation rate used in this experiment was within the range used in commercial poultry farms of 4.7 cfm/bird. Note that aerosol concentrations used above pertain to scenarios where the flock is severely infected (i.e. majority of birds are in an infectious state).

The aerosol source emission rate (E EID_{50}/s)

We modeled the exhaust coming from a poultry house as an aerosol point source. The source emission *E* was calculated as the product of the ventilation rate per bird (CFM), the number of birds in the house and the aerosol virus concentration C. We note the ventilation rate would vary depending on the age of the bird. Data from Li et al. (2008) indicates that as the ventilation rate increases, the particle concentration in the poultry house air decreases. However, the net emission rate was observed to increase with the ventilation rate. [131]

Scenario A: A conservative ventilation rate of 7 CFM/bird was used. The resulting emission rate was calculated to be $10^{4.42}$ EID_{50}/s = 7 CFM/bird* 20,000 birds*0.0000472 $(m^3/s/cfm)*10^{2.6}$ EID_{50}/m^3.

Scenario B: The emission rate was directly estimated from the total suspended particle emission rate for a broiler house in the literature. Burn et al. (2008) estimated a mean particle emission rate of 2.78 ± 1.87 kg/day-house for a broiler house (Kentucky, Tyson Foods) with average placement of 25, 000 chickens. Assuming that 50 percent of the suspended particles were contaminated at a HPAI virus titer of 10^5 EID_{50}/g and a particle emission rate of 4.65 (2.78+1.87) kg/day-house, the aerosol source emission rate would be $10^{3.42}$ EID_{50}/s = 50%*4.65*1000 g/day/(24*3600 s/day)*$10^5$$EID_{50}/g$.

Scenario C: A ventilation rate of 3 CFM/bird Wathes et al., (1997) was used to estimate the source emission rate (**Appendix Table 2**). The resulting emission rate was calculated to be $10^{5.61}$ EID_{50}/s = 3CFM/bird* 100,000 birds*0.0000472 $(m^3/s/cfm)*10^{3.46}$ EID_{50}/m^3.

Emission rates in scenarios D, E and F, are for infected but undetected source flocks in Control Area.

Scenario D: In a previously completed *Risk Assessment for the Movement of Broiler Hatching Eggs*, it was estimated that up to 4.5 percent of a flock could be infectious before detection of HPAI virus.[4] Assuming a value of 37.5 percent (possible value based on disease transmission models) for the prevalence of infectious birds in a known infected flock, the aerosol emission rate would be 12% of the emission rate in scenario A for known infected flocks.

Scenario E: In this scenario, it was considered that the aerosol emission rate would be 6% of the emission rate in scenario B for known infected flocks. Note that the estimated mean percent of infectious birds in undetected flocks in a previously completed *Risk Assessment for the Movement of Broiler Hatching Eggs* was 0.57%.

Scenario F: In this scenario, it was considered that the aerosol emission rate would be 1% of the emission rate in scenario C for known infected flocks. Note that the predicted 95th percentile of infectious birds in undetected flocks in a previously completed *Risk Assessment for the Movement of Washed and Sanitized Shell-eggs* was 0.53%.

Appendix Table 2. Concentration of particulate matter and ventilation rates in poultry houses.

Study	Housing type	House Size (birds)	Ventilation rates (cfm/bird)	Findings
Mahirang **et al**. (1998) [132]	Caged Layers	112, 000	5.5(1-5)	99% of particles < 5 µm in size. Indicate majority generated in henhouse > 1 µm. PM concentration 0.8 mg/m^3
Redwine (2002) [128]	Tunnel broiler	27, 500	1- 6.85	5.9(2.7-8.4) % of total suspended particles were PM_{10}. Respirable particle concentration 0.5 to 5 mg/m^3
Roumeliotis (2007) [127]	Cross ventilated broilers	34, 000	0.5 -3	Total PM concentration 1-5 mg/m^3. About 20 percent of particles < 1 µm
Wathes et al. (1997) [124]	Caged layers, broilers	Layers: 26, 000 Broilers 13,000	1.5 to 3	Respirable concentration between 1-2 mg in broiler and layer houses. Broiler houses had a higher inhalable dust concentration of 8 to 12 mg/m^3 compared to 2 to 4 mg/m^3 for caged layers.

Meteorological Conditions

The meteorological data used were for Lovett, Georgia for year 1988. We also repeated Scenario A with metrological parameters for Florence, South Carolina, and Morris, Minnesota. The baseline scenario (Lovett Georgia) is relatively more conservative than scenarios with above meteorological stations, as they resulted in concentration estimates that were lower by 50 percent or more. The baseline model results are for meteorological conditions which result in the 6th highest 1-month average concentrations. In parentheses, the model results for the 1st highest 3-hour concentration in a year are provided to show the impact of meteorological conditions favorable for aerosol transmission.

Dose Response

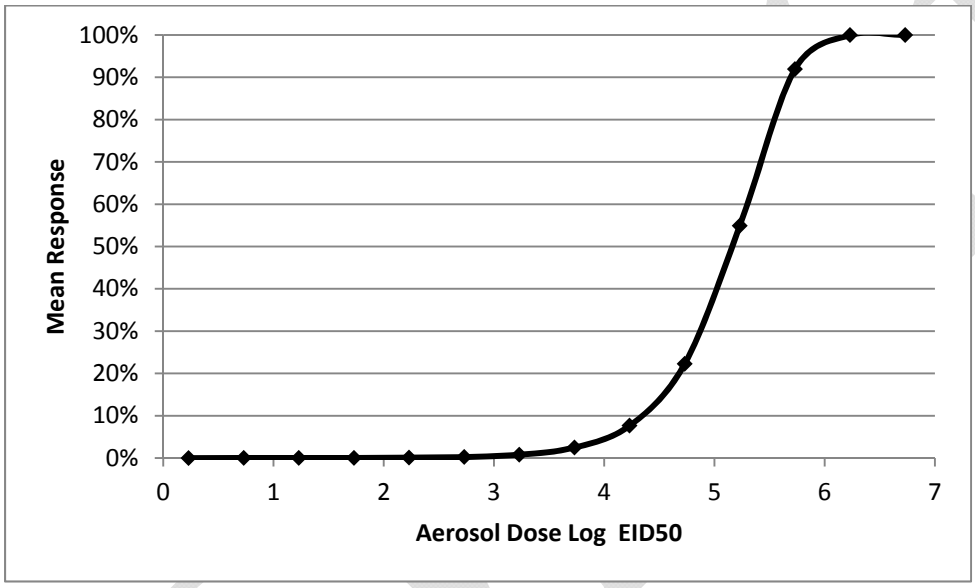
As mentioned in the literature review, we used an exponential dose response model parameterized from data presented in Spekreijse et al. (2012). The specific data points from Spekresijse et al. (2012) that we included in our analysis are shown in **Appendix Table 3**. We excluded data points where the concentration of HPAI virus was zero in our analysis. We used a chicken air intake rate of 1.2 m^3/day in order to convert the concentration into dose per chicken per day.

Appendix Table 3. Data points from Spekreijse et al. (2012) used to parameterize an exponential dose response model through maximum likelihood methods.

Trial	Concentration EID50/m3	Dose	Number infected out of 14 birds
Trial 2 day 4	2.3	2.38	0
Trial 2 day 5	2	2.08	0
Trial 2 day 6	1.5	1.58	1
Trial 2 day 9	2.2	2.28	0
Trial 2 day 10	2.8	2.88	0
Trial 4 day 2	2.5	2.58	0
Trial 4 day 3	2.9	2.98	0
Trial 4 day 4	3.5	3.58	0
Trial 4 day 5	3.2	3.28	0
Trial 4 day 6	4.1	4.18	0
Trial 4 day 7	3.9	3.98	0
Trial 4 day 8	3.5	3.58	0
Trial 4 day 9	4	4.08	0
Trial 4 day 10	3.7	3.78	0
Trial 4 day 13	3.5	3.58	2
Trial 4 day 14	3.7	3.78	0
Trial 1 day 2	2.1	2.18	0
Trial 1 day 3	2.9	2.98	0
Trial 1 day 4	3.1	3.18	0
Trial 1 day 5	2.6	2.68	0
Trial 1 day 6	2.8	2.88	0
Trial 1 day 7	2.9	2.98	0
Trial 1 day 8	2.7	2.78	0
Trial 3 day 2	2.4	2.48	0
Trial 3 day 4	3.05	3.12	0
Trial 3 day 5	2.7	2.78	0
Trial 3 day 6	3.1	3.18	0
Trial 3 day 8	3.51	3.59	0
Trial 3 day 9	2.75	2.83	0

Trial 3 day 10	3.28	3.36	0
Trial 3 day 13	2.91	2.99	0
Trial 3 day 14	3.50	3.58	0

The parameter for exponential distribution *r* was estimated to be $2.517*10^{-6}/EID_{50}$. There is considerable uncertainty regarding the parameters as well as the shape of the dose response model for the aerosol route, given the limited data (Appendix **Appendix Figure 3**). In particular, as aerosol may represent a very low concentration exposure for a large number of birds, the risk estimate would be considerably lower if there existed a threshold dose below which the probability of an exposed bird becoming infected is zero. The exponential dose response model is a "single hit" model without a threshold dose.



Appendix Figure 3 Exponential dose response model for aerosol transmission in chickens.

Appendix 3: AI Virus Survival on Various Substrates

Appendix Table 4 and **Appendix Table 5** summarize the results of studies documenting survival on various substrates (poultry feces, glass, metal etc.). Based on these data, moisture content appears to be a major determinant of the survival time of avian influenza viruses in both feces and on plastic/metal surfaces. We conclude that, if sufficiently dried, AI virus within poultry feces is likely inactivated within 2 days, and virus present on a metal or plastic surface (buggies, carts) would be inactivated within 1 day under room temperature and humidity conditions conducive to drying.

Appendix Table 4. Summary of experimental studies on survival of avian influenza virus on various substrates for HPAI inactivation in dried substances or under conditions that facilitate drying.*

Virus	Substrate	Survival	Humidity	Temperature	Reference
H5N1	Chicken feces	Not detected at 2 days	30-42% humidity	22°C	Wood et al. (2010) [133]
H5N2	Dried feces from AI-infected hens	Contained viable virus for 1 day	Stored in open vials	25°C	Beard et al. (1984) [21]
H5N1	Chicken manure	Lost infectivity at 24 hours	Not specified	25°C	Chumpolbanchorn et al. (2006) [37]
H5N1	Dried chicken feces	Nondetectable after 1 day	Not specified	25°C	Shortridge et al. (1998) [22]
H5N1	Glass, galvanized metal	No detection at 1 day	30-89% humidity (tested at both low and high RH)	22-23°C	Wood et al. (2010) [133]
H1N1	Tyvek, surgical mask, wood desk, N95 respirator, gloves	No detection at one day except on gloves	55%	25°C	Sakaguchi (2010) [134]
H1N1 (pan-demic)	Plastic, pine, steel, cloth	No detection of viable virus by one day	23-24%	17-21°C	Greatorex (2011) [135]

*Low moisture: Inoculated substrate was kept at low humidity (<70% RH), dried prior to testing, and/or stored in conditions conducive to the maintenance of low moisture content (e.g., storage in open vials)

Appendix Table 5. Summary of experimental studies on survival of avian influenza virus on various substrates for HPAI inactivation in moist substances or under conditions not conducive to drying. **

Virus	Substrate	Survival	Humidity	Temperature	Reference
H5N1	Chicken feces	Not detected at 4 days	91% humidity	22-23°C	Wood et al. (2010) [133]
H5N2	Feces from infected hens	Contained viable virus for 2 days	Stored in closed vials	25°C	Beard et al. (1984) [21]
H13N7	Steel, plastic	Inactivated by 6 days	stored in a cabinet,	Room temperature	Tiwari et al. (2006) [41]
H7N1	Egg-shell, PVC, metal (tin)	Inactivated by 15 days	50-84% humidity	17-25°C	Vrtlak and Kapitancik (1967) [136]
H7N1	Wood, burlap, grain, mixed feed	Inactivated by 8 days	50-84% humidity	17-25°C	Vrtlak and Kapitancik (1967) [136]

**High moisture: Inoculated substrate was kept at high humidity (>70% RH), not dried prior to testing, and/or stored in conditions conducive to the maintenance of higher moisture content (e.g., storage of moist feces in closed vials).

Appendix 4: 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 HPAI infected day-old chicks are moved from operations in the Control Area when the preventive measures from the SBS Plan are strictly followed. 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".[137] 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".[138] 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.[139] Overall, various definitions of "low risk" have been used as appropriate in different situations.

Negligible to Low Risk

When there is a substantial 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 through the risk pathway is unlikely but does occur.

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

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

Appendix 5: 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 likelihood of infection of day-old chicks at the hatchery through the specific 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.[140-143] 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.[144]

Use in Agricultural Risk Analysis

The use of risk analysis for imports of agricultural products became mandatory with the adoption of the SPS Agreement[e] in 1995.[f] 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).[145] The OIE has published standards and guidance for conducting risk analysis, but has not formally defined "negligible" in a quantitative sense.[1,146] 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."[97,149,150]

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 contexts."[151,143,152] 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.

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

[f] Risk analysis is also required for moving animals and animal products during a HPAI outbreak (Kwon *et al*., 2005)

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."[1] 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. These opinions by the court system are also consistent with U.S. views expressed in WTO trade disputes.

Appendix 6: 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 Table 6** provides examples of qualitative scales used in risk assessments elsewhere and **Appendix Table 7** lists adjectives to describe likelihoods considered appropriate by the OIE. The likelihood scale used in this assessment is defined by **Appendix Table 8**.

Appendix Table 6. 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 Table 7. Terms used as adjectives to qualify likelihood estimates considered appropriate by the OIE[1].

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 Table 8. 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

11. References

1. World Organization for Animal Health (OIE). *Handbook on Import Risk Analysis for Animals and Animal Products.* Vol 1. 2 ed. Paris, France: The Wold Organisation for Animal Health (OIE); 2010.
2. USDA:NASS. Hatchery Production: 2010 Summary. 2011.
3. USDA:APHIS:VS:NAHMS. Poultry 2010: Structure of the U.S. Poultry Industry. 2011.
4. USDA:APHIS:VS:CEAH, UMN:CAHFS, Broiler:Sector:Working:Group. *An Assessment of the Risk Associated with the Movement of Broiler Hatching Eggs Into, Within, and Out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak.* Fort Collins, CO October 2012.
5. Bell DD, Weaver WD. *Commercial Chicken Meat and Egg Production.* 5th ed. Norwell, Massachusetts: Kluwer Academic Publishers; 2002.
6. USDA :APHIS :VS: CEAH: NAHMS. *Poultry 2010: Reference of Health and Management Practices on Breeder Chicken Farms in the United States, 2010.* Fort Collins, CO August 2011 2011.
7. Swayne DE. *Avian Influenza.* First ed. Ames, Iowa: Blackwell Publishing; 2008.
8. Perdue ML, Suarez DL, Swayne DE. Avian Influenza in the 1990s. *Avian and Poultry Biology Reviews.* 2000 2000;11(1):1-20.
9. Clifford JR. Veterinary Services Memorandum No. 565.14; Reporting Confirmed Findings of Low Pathogenic Notifiable Avian Influenza (LPNAI) (H5 and H7 Subtypes) to the World Organization for Animal Health (OIE) and to Trading Partners. In: VS Management Team DVS, ed2006.
10. Gaidet N, Cattoli G, Hammoumi S, Newman SH, Hagemeijer W, Takekawa JY, Cappelle J, Dodman T, Joannis T, Gil P. Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. *PLoS pathogens.* 2008;4(8):e1000127.
11. Senne D, Suarez D, Stallnecht D, Pedersen J, Panigrahy B. Ecology and epidemiology of avian influenza in North and South America. *Developments in biologicals.* 2006;124:37.
12. Swayne DE. Understanding the complex pathobiology of high pathogenicity avian influenza viruses in birds. *Avian Diseases.* 2007;51(s1):242-249.
13. Boyce WM, Sandrock C, Kreuder-Johnson C, Kelly T, Cardona C. Avian influenza viruses in wild birds: A moving target. *Comparative Immunology, Microbiology and Infectious Diseases.* 2009;32(4):275-286.
14. Alexander DJ. An overview of the epidemiology of avian influenza. *Vaccine.* 2007 Jul 26 2007;25(30):5637-5644.
15. Stallknecht DE, Brown JD. Wild birds and the epidemiology of avian influenza. *Journal of Wildlife Diseases.* 2007;43(3 Supplement):S15-S20.
16. Hinshaw V, Webster R, Easterday B, Bean W. Replication of avian influenza A viruses in mammals. *Infection and Immunity.* 1981;34(2):354-361.
17. Englund L, Klingeborn B, Mejerland T. Avian Influenza-a Virus Causing an Outbreak of Contagious Interstitial Pneumonia in Mink. *Acta Veterinaria Scandinavica.* 1986 1986;27(4):497-&.
18. Hall JS, Bentler KT, Landolt G, Elmore SA, Minnis RB, Campbell TA, Barras SC, Root JJ, Pilon J, Pabilonia K. Influenza infection in wild raccoons. *Emerging infectious diseases.* 2008;14(12):1842.

19. Cardona CJ, Xing Z, Sandrock CE, Davis CE. Avian influenza in birds and mammals. *Comparative Immunology, Microbiology and Infectious Diseases.* 2009;32(4):255-273.
20. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HHJ. Survival of influenza viruses on environmental surfaces. *J Infect Dis.* 1982 Jul 1982;146(1):47-51.
21. Beard CW, Brugh M. Laboratory Studies on the Pennsylvania Isolates of Avian Influenza (H5N2) in Specific Pathogen-Free Chickens. *Journal of the American Veterinary Medical Association.* 1984 1984;185(3):340.
22. Shortridge KF, Zhou NN, Guan Y, Gao P, Ito T, Kawaoka Y, Kodihalli S, Krauss S, Markwell D, Murti KG, Norwood M, Senne D, Sims L, Takada A, Webster RG. Characterization of Avian H5N1 Influenza Viruses From Poultry in Hong Kong. *Virology.* Dec 20 1998 1998;252(2):331-342.
23. Swayne DE, Eggert D, Beck JR. Reduction of high pathogenicity avian influenza virus in eggs from chickens once or twice vaccinated with an oil-emulsified inactivated H5 avian influenza vaccine. *Vaccine.* 2012.
24. Moses H, Brandly C, Jones EE, Jungherr E. The isolation and identification of fowl plague virus. *Am. J. Vet. Res.* 1948;9:314-328.
25. Starick E, Werner O. Detection of H7 Avian Influenza Virus Directly From Poultry Specimens. *Avian Diseases.* 2003 2003;47:1187-1189.
26. Birnbaum NG, O'Brien B. Methods for Inactivation of Avian Influenza Virus in the Environment. 1st ed. Ames, Iowa: Wiley-Blackwell; 2008:391-405.
27. De Benedictis P, Beato MS, Capua I. Inactivation of Avian Influenza Viruses by Chemical Agents and Physical Conditions: A Review. *Zoonoses and Public Health.* 2007 2007;54:51-68.
28. Lombardi ME, Ladman BS, Alphin RL, Benson ER. Inactivation of avian influenza virus using common detergents and chemicals. *Avian Dis.* 2008 Mar 2008;52(1):118-123.
29. Fichtner GJ. The Pennsylvania/Virginia experience in eradication of avian influenza (H5N2). *Avian Diseases.* 2003:33-38.
30. Songserm T, Jam-on R, Sae-Heng N, Meemak N. Survival and Stability of HPAI H5N1 in Different Environments and Susceptibility to Disinfectants. *In: Abstracts of the Office International des Epizooties/Food and Agricultural Organization International Conference on Avian Influenza.* 7-8 April, 2005 2005.
31. Webster RG, Yakhno M, Hinshaw VS, Bean WJ, Murti KG. Intestinal influenza: replication and characterization of influenza viruses in ducks. *Virology.* 1978 Feb 1978;84(2):268-278.
32. Yamamoto Y, Nakamura K, Yamada M, Mase M. Persistence of avian influenza virus (H5N1) in feathers detached from bodies of infected domestic ducks. *Applied and environmental microbiology.* 2010;76(16):5496-5499.
33. Lu H, Castro AE, Pennick K, Liu J, Yang Q, Dunn P, Weinstock D, Henzler D. Survival of Avian Influenza Virus H7N2 in Spf Chickens and Their Environments. *Avian Diseases.* 2003 2003;47:1015-1021.
34. Brown JD, Swayne DE, Cooper RJ, Burns RE, Stallknecht DE. Persistence of H5 and H7 avian influenza viruses in water. *Avian Dis.* 2007 Mar 2007;51(1 Suppl):285-289.
35. Stallknecht DE, Shane SM, Zwank PJ, Senne DA, Kearney MT. Avian Influenza-Viruses From Migratory and Resident Ducks of Coastal Louisiana. *Avian Diseases.* Apr-Jun 1990 1990;34(2):398-405.

36. Stallknecht DE, Shane SM, Kearney MT, Zwank PJ. Persistence of Avian Influenza-Viruses in Water. *Avian Diseases.* Apr-Jun 1990 1990;34(2):406-411.
37. Chumpolbanchorn K, Suemanotham N, Siripara N, Puyati B, Chaichoune K. The effect of temperature and UV light on infectivity of avian influenza virus (H5N1, Thai field strain) in chicken fecal manure. *Southeast Asian J Trop Med Public Health.* 2006 Jan 2006;37(1):102-105.
38. Nazir J, Haumacher R, Ike A, Stumpf P, Böhm R, Marschang RE. Long-term study on tenacity of avian influenza viruses in water (distilled water, normal saline, and surface water) at different temperatures. *Avian Diseases.* 2010;54(s1):720-724.
39. Nazir J, Haumacher R, Ike AC, Marschang RE. Persistence of avian influenza viruses in lake sediment, duck feces, and duck meat. *Applied and environmental microbiology.* 2011;77(14):4981-4985.
40. Beato MS, Capua I, Alexander DJ. Avian influenza viruses in poultry products: a review. *Avian Pathology.* 2009;38(3):193-200.
41. Tiwari A, Patnayak DP, Chander Y, Parsad M, Goyal SM. Survival of two avian respiratory viruses on porous and nonporous surfaces. *Avian Dis.* 2006 Jun 2006;50(2):284-287.
42. Guan J, Chan M, Grenier C, Wilkie D, Brooks B, Spencer J. Survival of avian influenza and Newcastle disease viruses in compost and at ambient temperatures based on virus isolation and real-time reverse transcriptase PCR. *Avian Diseases.* 2009;53(1):26-33.
43. Muhmmad K, Das P, Yaqoob T, Riaz A, Manzoor R. Effect of Physico-chemical Factors on Survival of Avian Influenza Virus (H7N3 Type). *International Journal of Agriculture & Biology.* 2001 2001;3(4):416-418.
44. Terregino C, Beato MS, Bertoli E, Mancin M, Capua I. Unexpected heat resistance of Italian low-pathogenicity and high-pathogenicity avian influenza A viruses of H7 subtype to prolonged exposure at 37 C. *Avian Pathology.* 2009;38(6):519-522.
45. Davidson I, Nagar S, Haddas R, Ben-Shabat M, Golender N, Lapin E, Altory A, Simanov L, Ribshtein I, Panshin A. Avian influenza virus H9N2 survival at different temperatures and pHs. *Avian Diseases.* 2010;54(s1):725-728.
46. Negovetich NJ, Webster RG. Thermostability of subpopulations of H2N3 influenza virus isolates from mallard ducks. *Journal of virology.* 2010;84(18):9369-9376.
47. Homme P, Easterday B. Avian influenza virus infections. I. Characteristics of influenza A/turkey/Wisconsin/1966 virus. *Avian Diseases.* 1970:66-74.
48. Alexander DJ. The Epidemiology and Control of Avian Influenza and Newcastle-Disease. *Journal of Comparative Pathology.* Feb 1995 1995;112(2):105-126.
49. Ssematimba A, Hagenaars TJ, de Jong MCM. Modelling the Wind-Borne Spread of Highly Pathogenic Avian Influenza Virus between Farms. *PLoS ONE.* 2012;7(2):e31114.
50. Ypma RJF, Jonges M, Bataille A, Stegeman A, Koch G, van Boven M, Koopmans M, van Ballegooijen WM, Wallinga J. Genetic data provide evidence for wind-mediated transmission of highly pathogenic avian influenza. *Journal of Infectious Diseases.* 2012.
51. Kreager K. Avian Influenza Control Philosophies in the Layer and Layer Breeder industries. *Avian Diseases.* 2003:344-348.
52. Ssematimba A, Hagenaars T, de Wit J, Ruiterkamp F, Fabri T, Stegeman J, de Jong M. Avian influenza transmission risks: Analysis of biosecurity measures and contact structure in Dutch poultry farming. *Preventive Veterinary Medicine.* 2012.
53. Stegeman J, Bouma A. Epidemiology and control of avian influenza. 2004.

54. Canadian Food Inspection Agency. *Comprehensive Report on the 2004 Outbreak of High Pathogenicity Avian Influenza (H7N3) in the Fraser Valley of British Columbia, Canada.* June 2004 2004.
55. Swayne DE, Slemons RD. Using mean infectious dose of high- and low-pathogenicity avian influenza viruses originating from wild duck and poultry as one measure of infectivity and adaptation to poultry. *Avian Dis.* 2008 Sep 2008;52(3):455-460.
56. Spekreijse D, Bouma A, Stegeman JA, Koch G, de Jong MCM. The effect of inoculation dose of a highly pathogenic avian influenza virus strain H5N1 on the infectiousness of chickens. *Veterinary Microbiology.* 2011;147(1–2):59-66.
57. Kitajima M, Huang Y, Watanabe T, Katayama H, Haas C. Dose–response time modelling for highly pathogenic avian influenza A (H5N1) virus infection. *Letters in applied microbiology.* 2011;53(4):438-444.
58. Schijven F, Teunis PFM. Quantitative Risk Assessment of Avian Influenza Virus Infection via Water. 2006.
59. Kwon Y, Swayne D. Different routes of inoculation impact infectivity and pathogenesis of H5N1 high pathogenicity avian influenza virus infection in chickens and domestic ducks. *Avian Diseases.* 2010;54(4):1260-1269.
60. Swayne DE, Beck JR. Experimental Study to Determine if Low-Pathogenicity and High-Pathogenicity Avian Influenza Viruses Can Be Present in Chicken Breast and Thigh Meat Following Intranasal Virus Inoculation. *Avian Diseases.* Mar 2005 2005;49(1):81-85.
61. Purchase H. Experiments on the viability of the virus of fowl-plague under trade conditions. *Vet. Rec.* 1931;11:644-648.
62. Sergeev AA, Demina OK, Pyankov OV, Pyankova OG, Agafonov AP, Kiselev SA, Agranovski IE, Sergeev AA, Shikov AN, Shishkina LN, Safatov AS, Sergeev AN. Infection of Chickens Caused by Avian Influenza Virus A/H5N1 Delivered by Aerosol and Other Routes. *Transboundary and Emerging Diseases.* 2012:no-no.
63. Spekreijse D, Bouma A, Koch G, Stegeman A. Quantification of dust-borne transmission of highly pathogenic avian influenza virus between chickens. *Influenza and Other Respiratory Viruses.* 2012:no-no.
64. Yee KS, Carpenter TE, Farver TB, Cardona CJ. An evaluation of transmission routes for low pathogenicity avian influenza virus among chickens sold in live bird markets. *Virology.* 2009;394(1):19-27.
65. Elbers ARW, Fabri THF, De Vries TS, De Wit JJ, Pijpers A, Koch G. The Highly Pathogenic Avian Influenza a (H7N7) Virus Epidemic in the Netherlands in 2003 - Lessons Learned From the First Five Outbreaks. *Avian Diseases.* Sep 2004 2004;48(3):691-705.
66. Swayne DE, Suarez DL. Highly Pathogenic Avian Influenza. *Revue Scientifique Et Technique De L Office International Des Epizooties.* Aug 2000 2000;19(2):463-482.
67. Bouma A, Claassen I, Natih K, Klinkenberg D, Donnelly CA, Koch G, van Boven M. Estimation of transmission parameters of H5N1 avian influenza virus in chickens. *PLoS Pathog.* 2009 Jan 2009;5(1):e1000281.
68. Pfeiffer J, Pantin-Jackwood M, To TL, Nguyen T, Suarez DL. Phylogenetic and biological characterization of highly pathogenic H5N1 avian influenza viruses (Vietnam 2005) in chickens and ducks. *Virus Res.* 2009 Jun 2009;142(1-2):108-120.
69. Van Der Goot JA, Koch G, De Jong MCM, Van Boven M. Quantification of the Effect of Vaccination on Transmission of Avian Influenza (H7N7) in Chickens. *Proceedings of the*

National Academy of Sciences of the United States of America. Dec 13 2005 2005;102(50):18141-18146.
70. Bos ME, Van Boven M, Nielen M, Bouma A, Elbers AR, Nodelijk G, Koch G, Stegeman A, De Jong MC. Estimating the day of highly pathogenic avian influenza (H7N7) virus introduction into a poultry flock based on mortality data. *Vet Res.* 2007 May-Jun 2007;38(3):493-504.
71. Van Der Goot JA, De Jong MCM, Koch G, Van Boven M. Comparison of the Transmission Characteristics of Low and High Pathogenicity Avian Influenza a Virus (H5N2). *Epidemiology and Infection.* Oct 2003 2003;131(2):1003-1013.
72. Mannelli A, Ferrè N, Marangon S. Analysis of the 1999–2000 highly pathogenic avian influenza (H7N1) epidemic in the main poultry-production area in northern Italy. *Preventive Veterinary Medicine.* 2006;73(4):273-285.
73. Busani L, Valsecchi MG, Rossi E, Toson M, Ferrè N, Pozza MD, Marangon S. Risk factors for highly pathogenic H7N1 avian influenza virus infection in poultry during the 1999–2000 epidemic in Italy. *The Veterinary Journal.* 2009;181(2):171-177.
74. Boender GJ, Hagenaars TJ, Bouma A, Nodelijk G, Elbers AR, de Jong MC, van Boven M. Risk maps for the spread of highly pathogenic avian influenza in poultry. *PLoS Comput Biol.* 2007 Apr 20 2007;3(4):e71.
75. Sharkey KJ, Bowers RG, Morgan KL, Robinson SE, Christley RM. Epidemiological consequences of an incursion of highly pathogenic H5N1 avian influenza into the British poultry flock. *Proceedings of the Royal Society B: Biological Sciences.* January 7, 2008 2008;275(1630):19-28.
76. Nettles VF, Wood JM, Webster RG. Wildlife Surveillance Associated With an Outbreak of Lethal H5n2 Avian Influenza in Domestic Poultry. *Avian Diseases.* 1985 1985;29(3):733-741.
77. Henzler D, Kradel D, Davison S, Ziegler A, Singletary D, DeBok P, Castro A, Lu H, Eckroade R, Swayne D. Epidemiology, production losses, and control measures associated with an outbreak of avian influenza subtype H7N2 in Pennsylvania (1996-98). *Avian Diseases.* 2003;47(s3):1022-1036.
78. Dybing JK, Schultz-Cherry S, Swayne DE, Suarez DL, Perdue ML. Distinct Pathogenesis of Hong Kong-Origin H5n1 Viruses in Mice Compared to That of Other Highly Pathogenic H5 Avian Influenza Viruses. *Journal of Virology.* Feb 2000 2000;74(3):1443-1450.
79. Shapiro D, Stewart-Brown B. Farm biosecurity risk assessment and audits. *Avian influenza.* 2009:369-390.
80. World Organisation for Animal Health (OIE). *Terrestrial Animal Health Code.* 16 ed. Paris, France: World Organisation for Animal Health; 2007.
81. Lu HG, Dunn PA, Wallner-Pendleton EA, Hertzler DJ, Kradel DC, Liu JB, Shaw DP, Miller P. Investigation of H7N2 Avian Influenza Outbreaks in Two Broiler Breeder Flocks in Pennsylvania, 2001-02. *Avian Diseases.* Jan-Mar 2004 2004;48(1):26-33.
82. Brugh M, Johnson DC. Epidemiology of Avian Influenza in Domestic Poultry. *Avian Diseases.* 1986 Proceedings (2003) 1986;47(Special Issue: Second International Symposium on Avian Influenza):177-186.
83. Leibler JH, Carone M, Silbergeld EK. Contribution of company affiliation and social contacts to risk estimates of between-farm transmission of avian influenza. *PLoS ONE.* 2010;5(3):e9888.

84. Vieira AR, Hofacre CL, Smith JA, Cole D. Human contacts and potential pathways of disease introduction on Georgia poultry farms. *Avian Diseases.* 2009;53(1):55-62.
85. Ansari SA, Sattar SA, Springthorpe VS, Wells GA, Tostowaryk W. Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *J Clin Microbiol.* 1988 Aug 1988;26(8):1513-1518.
86. Bidawid S, Malik N, Adegbunrin O, Sattar SA, Farber JM. Norovirus cross-contamination during food handling and interruption of virus transfer by hand antisepsis: experiments with feline calicivirus as a surrogate. *J Food Prot.* 2004 Jan 2004;67(1):103-109.
87. Grayson ML, Melvani S, Druce J, Barr IG, Ballard SA, Johnson PD, Mastorakos T, Birch C. Efficacy of soap and water and alcohol-based hand-rub preparations against live H1N1 influenza virus on the hands of human volunteers. *Clin Infect Dis.* 2009 Feb 1 2009;48(3):285-291.
88. Longworth N, Mourits M, Saatkamp H. Economic Analysis of HPAI Control in the Netherlands I: Epidemiological Modelling to Support Economic Analysis. *Transboundary and Emerging Diseases.* 2012.
89. Wood J, Webster R, Nettles V. Host range of A/Chicken/Pennsylvania/83 (H5N2) influenza virus. *Avian Diseases.* 1985:198-207.
90. Mcquiston JH, Garber LP, Porter-Spalding BA, Hahn JW, Pierson FW, Wainwright SH, Senne DA, Brignole TJ, Akey BL, Holt TJ. Evaluation of Risk Factors for the Spread of Low Pathogenicity H7N2 Avian Influenza Virus Among Commercial Poultry Farms. *Javma-Journal of the American Veterinary Medical Association.* Mar 1 2005 2005;226(5):767-772.
91. Swayne DE. Epidemiology of Avian Influenza in Agricultural and Other Man-Made Systems. *Avian influenza.* 2008:59-85.
92. Axtell RC. Poultry integrated pest management: status and future. *Integrated Pest Management Reviews.* 1999;4(1):53-73.
93. Brugh M, Johnson DC. Epidemiology of Avian Influenza in Domestic Poultry. *Avian Diseases.* 2003;47:177-186.
94. Wilson D, Schmidtmann E, Richard R, Lehman R. Isolation of avian influenza from insects. Paper presented at: Arbovirus Research in Australia-Proceedings 4th Symposium1986.
95. Sawabe K, Hoshino K, Isawa H, Sasaki T, Hayashi T, Tsuda Y, Kurahashi H, Tanabayashi K, Hotta A, Saito T, Yamada A, Kobayashi M. Detection and isolation of highly pathogenic H5N1 avian influenza A viruses from blow flies collected in the vicinity of an infected poultry farm in Kyoto, Japan, 2004. *Am J Trop Med Hyg.* 2006 Aug 2006;75(2):327-332.
96. Sawabe K, Hoshino K, Isawa H, Sasaki T, Kim KS, Hayashi T, Tsuda Y, Kurahashi H, Kobayashi M. Blow Flies Were One of the Possible Candidates for Transmission of Highly Pathogenic H5N1 Avian Influenza Virus during the 2004 Outbreaks in Japan. *Influenza Research and Treatment.* 2011;2011:8.
97. Sawabe K, Tanabayashi K, Hotta A, Hoshino K, Isawa H, Sasaki T, Yamada A, Kurahashi H, Shudo C, Kobayashi M. Survival of avian H5N1 influenza A viruses in Calliphora nigribarbis (Diptera: Calliphoridae). *J Med Entomol.* Jul 2009;46(4):852-855.

98. Wanaratana S, Panyim S, Pakpinyo S. The potential of house flies to act as a vector of avian influenza subtype H5N1 under experimental conditions. *Med Vet Entomol.* Mar;25(1):58-63.
99. Nielsen AA, Skovgard H, Stockmarr A, Handberg KJ, Jorgensen PH. Persistence of low-pathogenic avian influenza H5N7 and H7N1 subtypes in house flies (Diptera: Muscidae). *J Med Entomol.* May;48(3):608-614.
100. Tsuda Y, Hayashi T, Higa Y, Hoshino K, Kasai S, Tomita T, Kurahashi H, Kobayashi M. Dispersal of a blow fly, Calliphora nigribarbis, in relation to the dissemination of highly pathogenic avian influenza virus. *Japanese journal of infectious diseases.* 2009;62(4):294-297.
101. Wanaratana S, Amonsin A, Chaisingh A, Panyim S, Sasipreeyajan J, Pakpinyo S. Experimental assessment of house flies as vectors in avian influenza subtype H5N1 transmission in chickens. *Avian Diseases.* 2013.
102. James M, Harwood R. The house fly and its relatives. *Herm s Medical Entomology. McMillan Company. 6th edition, London, England.* 1969:249-265.
103. Stafford KC. *Fly management handbook A guide to biology, dispersal, and management of the house fly and related flies for farmers, municipalities, and public health officials.* Vol Bulletin 1013: The Connecticut Agricultural Experiment Station, New Haven; 2008.
104. Winpisinger KA, Ferketich AK, Berry RL, Moeschberger ML. Spread of Musca domestica (Diptera: Muscidae), from two caged layer facilities to neighboring residences in rural Ohio. *Journal of medical entomology.* 2005;42(5):732-738.
105. Selleck PW, Arzey G, Kirkland PD, Reece RL, Gould AR, Daniels PW, Westbury HA. An Outbreak of Highly Pathogenic Avian Influenza in Australia in 1997 Caused by an H7n4 Virus. *Avian Diseases.* 2003 2003;47:806-811.
106. Senne D, Holt T, Akey B. An overview of the 2002 outbreak of low-pathogenic H7N2 avian influenza in Virginia, West Virginia and North Carolina. *Frontis.* 2005;8:41-47.
107. Schofield L, Ho J, Kournikakis B, Booth T. *Avian Influenza Aerosol Sampling Campaign in the British Columbia Fraser Valley, 9-19 April 2004.* March 2005 2005.
108. Forman AJ, Parsonson IM, Doughty WJ. The Pathogenicity of an Avian Influenza-Virus Isolated in Victoria. *Australian Veterinary Journal.* Sep 1986 1986;63(9):294-296.
109. Van Der Goot JA, Koch G, De Jong MCM, Van Boven M. Transmission Dynamics of Low- and High-Pathogenicity a/Chicken/Pennsylvania/83 Avian Influenza Viruses. *Avian Diseases.* 2003 2003;47:939-941.
110. Zhang P, Tang Y, Liu X, Peng D, Liu W, Liu H, Lu S, Liu X. Characterization of H9N2 influenza viruses isolated from vaccinated flocks in an integrated broiler chicken operation in eastern China during a 5 year period (1998-2002). *J Gen Virol.* 2008/12/1 2008;89(12):3102-3112.
111. Tsukamoto K, Imada T, Tanimura N, Okamatsu M, Mase M, Mizuhara T, Swayne D, Yamaguchi S. Impact of different husbandry conditions on contact and airborne transmission of H5N1 highly pathogenic avian influenza virus to chickens. *Avian Dis.* 2007 Mar 2007;51(1):129-132.
112. Spekreijse D, Bouma A, Koch G, Stegeman JA. Airborne transmission of a highly pathogenic avian influenza virus strain H5N1 between groups of chickens quantified in an experimental setting. *Vet Microbiol.* Apr 22 2011;152(1-2):8.
113. Weber TP, Stilianakis NI. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *J Infect.* 2008 Nov 2008;57(5):361-373.

114. Tellier R. Review of aerosol transmission of influenza A virus. *Emerg Infect Dis.* 2006 Nov 2006;12(11):1657-1662.
115. Dungan RS. BOARD-INVITED REVIEW: fate and transport of bioaerosols associated with livestock operations and manures. *J Anim Sci.* Nov 2010;88(11):3693-3706.
116. EPA. 40 CFR Part 51 Appendix W. November 2005.
117. EPA:OAQPS:AQAD. *AERMOD Implementation guide.* Research Triangle Park, N.C.2009.
118. Halvorson DA. Prevention and management of avian influenza outbreaks: experiences from the United States of America. *Rev Sci Tech.* 2009 Apr 2009;28(1):359-369.
119. USDA: APHIS: VS. National Animal Health Emergency Management System Guidelines: Cleaning and Disinfection (Draft). 2005:6-8.
120. Kramer A, Galabov AS, Sattar SA, Döhner L, Pivert A, Payan C, Wolff MH, Yilmaz A, Steinmann J. Virucidal activity of a new hand disinfectant with reduced ethanol content: comparison with other alcohol-based formulations. *Journal of Hospital Infection.* 2006;62(1):98-106.
121. Li H, Burns R. Aerosol. 2010.
122. Burns R, Li H, Moody L, Xin H, Gates R, Overhults D, Earnest J. Quantification of particulate emissions from broiler houses in the southeastern United States. 2008:unpaginated.
123. Roumeliotis TS, Dixon BJ, Van Heyst BJ. Characterization of gaseous pollutant and particulate matter emission rates from a commercial broiler operation part I: Observed trends in emissions. *Atmospheric Environment.* 2010;44(31):3770-3777.
124. Wathes CM, Holden MR, Sneath RW, White RP, Phillips VR. Concentrations and emission rates of aerial ammonia, nitrous oxide, methane, carbon dioxide, dust and endotoxin in UK broiler and layer houses. *British Poultry Science.* 1997/03/01 1997;38(1):14-28.
125. Li H. B turkey tom aerosol. 2008.
126. Takai H, Pedersen S, Johnsen JO, Metz JHM, Groot Koerkamp PWG, Uenk GH, Phillips VR, Holden MR, Sneath RW, Short JL, White RP, Hartung J, Seedorf J, Schröder M, Linkert KH, Wathes CM. Concentrations and Emissions of Airborne Dust in Livestock Buildings in Northern Europe. *Journal of Agricultural Engineering Research.* 1998;70(1):59-77.
127. Roumeliotis TS, Van Heyst BJ. Size fractionated particulate matter emissions from a broiler house in Southern Ontario, Canada. *Science of The Total Environment.* 2007;383(1–3):174-182.
128. REDWINE, #160, S. J, LACEY, E. R, MUKHTAR, S., CAREY, B. J. *Concentration and emissions of ammonia and particulate matter in tunnel-ventilated broiler houses under summer conditions in Texas.* Vol 45. St. Joseph, MI, ETATS-UNIS: American Society of Agricultural Engineers; 2002.
129. Banhazi TM, Seedorf J, Laffrique M, Rutley DL. Identification of the risk factors for high airborne particle concentrations in broiler buildings using statistical modelling. *Biosystems Engineering.* 2008;101(1):100-110.
130. Yamamoto Y, Nakamura K, Okamatsu M, Yamada M, Mase M. Avian influenza virus (H5N1) replication in feathers of domestic waterfowl. *Emerg Infect Dis.* 2008 Jan 2008;14(1):149-151.

131. Li H, Xin H, Burns RT, Hoff SJ, Harmon JD, Jacobson LD, Noll SL. Effects of bird activity, ventilation rate and humidity on PM10 concentration and emission rate of a turkey barn. 2008:unpaginated.
132. Maghirang RG, Manbeck HB, Roush WB, Muir FV. Air Contaminant Distributions in a Commercial Laying House. *American Society of Agricultural Engineers.* Sep-Oct 1991 1991;34(5):2171-2180.
133. Wood JP, Choi YW, Chappie DJ, Rogers JV, Kaye JZ. Environmental persistence of a highly pathogenic avian influenza (H5N1) virus. *Environmental Science & Technology.* 2010;44(19):7515-7520.
134. Sakaguchi H, Wada K, Kajioka J, Watanabe M, Nakano R, Hirose T, Ohta H, Aizawa Y. Maintenance of influenza virus infectivity on the surfaces of personal protective equipment and clothing used in healthcare settings. *Environmental health and preventive medicine.* 2010;15(6):344-349.
135. Greatorex JS, Digard P, Curran MD, Moynihan R, Wensley H, Wreghitt T, Varsani H, Garcia F, Enstone J, Nguyen-Van-Tam JS. Survival of Influenza A (H1N1) on Materials Found in Households: Implications for Infection Control. *PLoS ONE.* 2011;6(11):e27932.
136. Vrtlak O, Kapitancik B. Study of Fowl Plague Virus Resistance in Biological and Technical Material. *Bull. Off. Int. Epiz.* 1967 1967;67(7-8):969-988.
137. USDA: APHIS: PPQ. Guidelines for Pathway-Initiated Pest Risk Assessments. 2000.
138. US Department of Health and Human Services: Food and Drug Administration: Center for Veterinary Medicine. Guidance for Industry: Evaluating the Safety of Antimicrobial New Animal Drugs with Regards to Their Microbiological Effects on Bacteria of Human Health Concern. 2003;Document #152.
139. USDA: APHIS. Qualitative Risk Assessment for Potential Federal Noxious Weeds-Template. 2004.
140. Rodricks JV. Some attributes of risk influencing decision-making by public health and regulatory officials. *Am J Epidemiol.* 2001 Dec 15 2001;154(12 Suppl):S7-S12.
141. Fiori JM, Meyerhoff RD. Extending the threshold of regulation concept: de minimis limits for carcinogens and mutagens. *Regul Toxicol Pharmacol.* 2002 Apr 2002;35(2 Pt 1):210.
142. National Library of Medicine. IUPAC Glossary of Terms Used in Toxicology -R. 2009; http://sis.nlm.nih.gov/enviro/iupacglossary/glossaryr.html. Accessed 2/29/2008.
143. Peterson M. What Is a de Minimis Risk? *Risk Management: An International Journal.* 2002/04/01/print 2002;4(2):47-55.
144. U.S. House of Representatives, Committee on Commerce. *Section 405. Tolerances And Exemptions For Pesticide Chemical Residues.* July 23 1996 1996.
145. Technical Cooperation Department. Multilateral Trade Negotiations on Agriculture: A Resource Manual III. Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) and Agreement on Technical Barriers to Trade (TBT) - SPS Agreement: Provisions, Basic Rights and Obligations. 2000; http://www.fao.org/docrep/003/x7354e/X7354e02.htm#TopOfPage. Accessed 02/29/2008.
146. Murray TS, Groth AE, Weitzman C, Cappello M. Epidemiology and Management of Infectious Diseases in International Adoptees. *Clinical Microbiology Reviews.* Jul 2005 2005;18(3):510-+.

147. *Japan --Measures Affecting The Importation of Apples,* (WTO Dispute Settlement Body 2005).
148. *Ranchers Cattlemen Action Legal Fund United Stockgrowers of America v. United States Department of Agriculture*, 28-34 (United States Court of Appeals for the Ninth Circuit 2005).
149. Gruszczynski LA. The Role of Science in Risk Regulation under the SPS Agreement. *SSRN eLibrary.* 2006 2006.
150. World Trade Organization DSB. *Japan--Measures Affecting The Importation Of Apples: Report Of The Panel.* World Trade Organization; 15 july 2003 2003. WT/DS245/R.
151. Hrudey S, Krewski D. Is There a Safe level of Exposure to a Carcinogen? *Environmental Science & Technology.* 1995 1995;29(8):370A- 375A.
152. Graham JD. Historical perspective on risk assessment in the federal government: Biological Mechanisms and Quantitative Risk Assessment. *Toxicology.* 1995/9/1 1995;102(1-2):29-52.
153. Bryson N. Citrus, Clementines and Mad Cows: Judicial Standards of Review for Sanitary and Phytosanitary Decisions of USDA's Animal and Plant Health Inspection Service (APHIS). Paper presented at: International Conference on Sanitary & Phytosanitary (SPS) Risk Assessment Methodology; 9-11 August 2005, 2005; Washington, D.C.
154. Theil M. The role of translations of verbal into numerical probability expressions in risk management: a meta-analysis. *Journal of Risk Research.* 2002;5(2):10.
155. Behn RD, Vaupel JW. *Quick analysis for busy decision makers.* New York, New York, USA: Basic Books 1982.
156. Reynolds DL, Theil KW, Saif YM. Demonstration of rotavirus and rotavirus-like virus in the intestinal contents of diarrheic pheasant chicks. *Avian Dis.* Apr-Jun 1987;31(2):376-379.
157. World Organization for Animal Health (OIE). Guidelines for Import Risk Analysis. 2006; http://www.oie.int/eng/normes/mcode/en_chapitre_1.3.2.htm. Accessed July 3, 2007.
158. Fletcher W. The application of qualitative risk assessment methodology to prioritize issues for fisheries management. *ICES Journal of Marine Science: Journal du Conseil.* 2005;62(8):1576.