An Assessment of the Risk Associated with the Movement of Nonpasteurized Liquid Egg (NPLE) and Its Products Into, Within, and Outside of a Control Area during a Highly Pathogenic Avian Influenza Outbreak

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

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1. Abbreviations and Definitions

AHPA	Animal Health Protection Act
APHIS	Animal and Plant Health Inspection Service (USDA)
AI	Avian influenza
CEAH	Centers for Epidemiology and Animal Health
CFR	Code of Federal Regulations
GMP	Good Manufacturing Practices
FDA	Food and Drug Administration
FSIS	Food Safety Inspection Service
HA	Hemagglutinin
HACCP	Hazard Analysis and Critical Control Point
HPAI	Highly pathogenic avian influenza
HPNAI	Highly pathogenic notifiable avian influenza
LE	Liquid egg
LPAI	Low pathogenic avian influenza
NA	Neuraminidase
NAHEMS	National Animal Health Emergency Management System
NPLE	Nonpasteurized liquid egg
OIE	World Organisation for Animal Health (formerly Office International des Epizooties)
PLE	Pasteurized liquid egg
RRT-PCR	Real-time Reverse Transcription Polymerase Chain Reaction
U.S.	United States of America
USDA	United States Department of Agriculture
VS	Veterinary Services (USDA: APHIS)

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

Continuous inspection
: Continuous inspection requires that the FSIS inspector be on the premises of the egg products processing facility whenever pasteurization equipment is operating.

Control area

A control area, consisting of an infected zone and a buffer surveillance zone, will be established to ensure the rapid and effective containment of the disease. Initially, the entire State, Commonwealth, Tribal Nation or territory may be declared a control area and subject to movement restrictions until appropriate surveillance and epidemiological evidence has been evaluated and the extent of the outbreak is known. All susceptible bird and other livestock movement will be stopped for a period long enough to determine the scope of the disease outbreak. The potential modes of transmission of HPAI will be 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.

Egg

The shell egg of the domesticated chicken. Shell eggs of turkeys, ducks, geese, and guineas are outside the scope of this assessment.

Infected zone

In an outbreak of HPAI, the infected zone will initially encompass the perimeter of all presumptive or confirmed positive premises and include as many of the contact premises as the situation logistically or scientifically requires. 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.

In an outbreak of HPAI, the infected zone initially will encompass the perimeter of all presumptive or confirmed positive premises and include as many of the contact premises as the situation requires logistically or scientifically. The boundary of the infected zone initially should be at least 2 miles (3 kilometers) beyond the perimeters of the presumptive or confirmed infected premises. The boundaries may be modified (either expanded or reduced) by Incident Command as new information becomes available. The actual distance in any one direction is determined by factors such as known characteristics of the virus, environmental conditions (terrain, weather, wind), the pattern of animal density and movements, the distribution and movements of susceptible wild and feral livestock, processing activities (livestock and products), and the effect on non-risk commodities. Boundaries of the infected zone can be modified when tracing and surveillance results become available and other listed factors become better defined.

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 nonpasteurized liquid egg from a premises or a geographic area described in a quarantine order.

Negligible Risk

For this risk analysis, the term "negligible risk" means there is a very low likelihood that moving nonpasteurized liquid egg will cause infection in another premise. The specific magnitude cannot be determined, as there is no evidence that this product has ever served as a transmission pathway. In quantitative terms, this is a likelihood that moving this product will result in infection of another premises is less than 1/1,000,000. This particular likelihood is used as it is consistent with other common meanings for the term, as discussed in Appendix 15. The determination of "negligible risk" suggests that allocating additional resources to mitigate this risk may not be a cost-effective use of resources (depending on circumstances).

Nonpasteurized liquid egg

Shell eggs that have been washed, sanitized and broken and converted to liquid egg which has not been subjected to pasteurization.

Pasteurization

The process of subjecting each particle of egg product to heat in order to destroy harmful viable microorganisms, including highly pathogenic avian influenza virus.

2. Executive Summary

This document assesses the risk that the movement of nonpasteurized liquid egg (NPLE) during a highly pathogenic avian influenza (HPAI) outbreak in the poultry egg industry in the United States will result in HPAI infection on another poultry premises. This assessment includes movement into, within, and outside of egg breaking facilities that are under continuous FSIS inspection.

This risk assessment is a joint effort between the Egg Products Industry working group, the University of Minnesota's Center for Animal Health and Food Safety, and USDA-APHIS-VS-CEAH to support permits for moving NPLE safely and in a timely fashion during an outbreak.

The purpose of this risk assessment is to identify risk pathways associated with the movement of NPLE, and assess the likelihood of carrying HPAI virus off of an infected premises and causing infection on another poultry premises despite all standard preventive measures as well as preventive measures implemented during an outbreak. This risk assessment will ultimately provide the framework necessary for decision makers to:

a) Quickly assess the effectiveness of preventive measures as they pertain specifically to the egg breaking process and movement of nonpasteurized liquid egg.
b) Consider implementing a permit system, which would allow uninfected egg breaking facilities that are under continuous FSIS inspection to move NPLE into, within, and outside of the control area during an HPAI outbreak.

To address these objectives, we first estimated the likelihood and the level of NPLE contamination with virus present in the internal contents and on the shell surface of eggs from an infected but undetected flock. We then evaluated the risks associated with post-breaking handling, storage and transportation of NPLE. In particular, this document assesses:

a) Risk of NPLE contamination from HPAI virus within the egg contents resulting in infection of a susceptible flock.
b) Risk of cross-contamination of NPLE via virus on the shell surface of washed and sanitized eggs resulting in infection of a susceptible flock.
c) Risk of vehicle or driver transporting NPLE resulting in HPAI infection of a susceptible flock.

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 this product.

Overall Finding and Conclusion

The risk that movement of nonpasteurized liquid egg into, within, and outside of a control area during a highly pathogenic avian influenza outbreak would cause an HPAI outbreak in another poultry production premises in the United States is *negligible*.

Unless major failures or significant deviations occur, this document concludes that the risk of NPLE being released during the egg breaking, post-breaking handling and storage operations and causing an HPAI outbreak in a susceptible flock is negligible. Similarly, if movement controls and all other recommended preventive and biosecurity measures described below are strictly followed, the risk of an HPAI outbreak in another poultry production premises due to the movement of commercial vehicles carrying NPLE products is negligible.

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.[1] In addition to compliance with such measures, 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 associated with the movement of the product in question is acceptable.[a]

Completing these types of risk assessments in a timely manner during an outbreak can be challenging. Risk assessments can take more time to conduct than the shelf-life of some of the perishable ingredients or products that need to be moved. In addition, the available storage capacity might be inadequate for holding the product while the risk assessment is being completed and may result in disposal of product. For some products, the risk may be evaluated before an outbreak occurs. Nonpasteurized liquid egg (NPLE) is one such product.

The purpose of this document is to determine the risk of disease spread due to the movement of NPLE produced from an undetected HPAI-positive flock in a control zone. Product from a known (i.e., detected) positive flock is not considered, as it is assumed that product will be restricted from movement.

This document assesses the risk associated with the movement of NPLE into, within, and outside of a control area during an HPAI outbreak. The facilities covered in this document are only those under continuous FSIS inspection. This assessment takes into consideration all applicable regulations, including preventive measures already in place, and additional preventive measures that will be implemented during an outbreak.[b] In accordance with USDA regulations, all NPLE must be sent to processing plants for pasteurization. Pasteurization has been shown to be effective in destroying the HPAI virus.[2]

This risk assessment does not guarantee that movement will be permitted during an HPAI outbreak. However, this document provides the framework necessary for decision makers to quickly assess the effectiveness of the preventive measures as they pertain specifically to transport of NPLE directly to pasteurization. This risk assessment will also allow decision makers to consider implementation of additional control measures which would allow NPLE movement for further processing into, within and outside of the control area during an HPAI outbreak.

[a] During an outbreak, APHIS conducts numerous product-specific risk assessments, taking into consideration all permit requirements and preventive measures currently in place.
[b] Normal day-to-day operations and preventive measures are in place via Good Manufacturing Practices (GMP), State regulations and Federal regulations as required by FSIS, FDA, and APHIS.

4. Scope

This assessment is applicable to FSIS-inspected NPLE processing facilities that may or may not have laying hens on-site and may or may not accept eggs from other egg producers.

This assessment is not intended to be a regulatory review of the effectiveness of FSIS and FDA programs. We did not directly evaluate their utility, but did review information related to other HPAI outbreaks and any potential connections to NPLE.

The focus of this risk assessment is on the risk that movement of NPLE will result in HPAI spread to other susceptible poultry. Although the risks to humans or wildlife associated with the production or movement of NPLE are critical concerns that should be addressed, they are outside the scope of the assessment. The draft national highly pathogenic avian influenza response plan has personnel safety measures towards mitigating the risk to humans. The disease spread risks associated with the byproducts of the egg breaking process such as eggshells or inedible eggs will be addressed in upcoming risk assessments.

5. 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 are making some assumptions to establish context and applicability. These assumptions are:

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

b) NPLE production facilities may have HPAI infection in their laying flocks but it has not yet been detected. If there was absolute certainty that HPAI infection was absent there would be no risk. If HPAI infection has been detected, however, it is assumed that Incident Command will shut down the production premises, movement of product will not be allowed, and any associated laying facilities will be depopulated. This situation also does not pose a risk associated with movement of product as the premises would cease production and be quarantined, depopulated, and cleaned and disinfected before resuming production.

c) The assessment is applicable to most (*but not all*) of the situations that may arise during an outbreak. As discussed in the movement section, permits to move NPLE may be issued for movement to slaughter/processing or for movement under conditions described on a movement permit. These conditions depend on the circumstances and cannot be known in advance. Therefore, the risk will depend on the circumstances and this assessment can only provide information and not generate recommendations.

6. HPAI Overview

6.1 Definition of Highly Pathogenic Notifiable Avian Influenza

HPAI is defined[c] in the Code of Federal Regulations, Title 9, Part 53, 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 part (a) of this definition, but has an amino acid sequence at the hemagglutinin cleavage site that is compatible with highly pathogenic avian influenza viruses.[d]

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.[3]

6.2 Agent and Host Range

Avian influenza (AI) virus is a heat-labile, single-stranded RNA virus of the *Orthomyxoviridae* family.[4-8] There are three antigenically distinct types of influenza viruses within the *Orthomyxoviridae* family: types A, B, and C. Types B and C are typically found only in humans. Influenza A viruses include all AI viruses and can infect a wide variety of animals including birds, pigs, horses, marine mammals, and humans.

The two surface glycoproteins of the influenza 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.[4,5,7-9] With regard to influenza A viruses, there are sixteen different subtypes of HA (H1 to H16) and nine different subtypes of NA (N1 to N9).[10]

Although a few viruses of relatively few subtype combinations have been isolated from mammalian species, all subtypes, in the majority of combinations, have been isolated from avian species.[4,5 11-13] While all bird species are thought to be susceptible to AI, some are more susceptible than others. Most AI viral infections in birds are subclinical or induce mild disease syndromes, consisting primarily of respiratory disease.[5,12,13] These viruses are designated as low pathogenic avian influenza (LPAI). A few isolates of H5 and H7 subtypes are very virulent and induce severe disease with morbidity and mortality rates reaching 100 percent in affected flocks.[4,9,12] These viruses are termed HPAI.

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.[14] Although other LPAI viruses may cause significant morbidity and production losses, they are not considered to be reportable diseases.

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[c] While there are other avian influenza types that are notifiable to OIE, this definition is used to maintain consistency with the APHIS HPAI Response Plan.

[d] The probability of not causing mortality is 4 percent. There have been 24 HPAI outbreaks since 1959 and only one was categorized as HPAI based solely on molecular criteria.

There are many examples of H5 and H7 virus isolates that are not pathogenic, so antigenic configuration alone does not determine pathogenicity.[9] Migratory waterfowl have yielded more influenza viruses than any other group, while turkeys and chickens have experienced the most substantial disease problems.[7,9]

6.3 Geographic Distribution of H5N1 HPAI

Since 2003, over 60 countries have reported H5N1 HPAI in domestic poultry and wildlife. The current list of all confirmed affected countries is maintained by the OIE.[15]

6.4 Susceptibility to Chemical and Physical Agents

AI viruses are easily inactivated by physical agents such as heat, extremes of pH, nonisotonic conditions, and dryness; however, their infectivity can be maintained for several weeks under moist, low temperature conditions. Infective virus can be retained in fecal matter anywhere from 9-82 days at 4 ºC[16-19] and 2 to 32 days at 15-22 ºC[16,20] with one report claiming survival of infectious material up to 70 days.[19]

Due to their lipid envelope, AI viruses are relatively sensitive to disinfection agents and inactivation by lipid solvents such as detergents.[9,21] EPA maintains a list of disinfectants with label claims for avian influenza viruses.[22] These products include halogens, aldehydes, quaternary ammoniums, phenols, alcohols, peroxides and some detergents. These label claims are for use on hard, non-porous surface, and in some instances removal of organic material is required for efficacious disinfection. Formalin and beta-propiolactone can be used to eliminate the infectivity of the viruses while preserving hemagglutinating and neuraminidase activity.

6.5 Transmission

Circumstantial evidence suggests that contact with migratory waterfowl, sea birds, or shore birds is a risk factor for introduction of virus into domestic poultry populations.[9,13,23-27] Since 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.[5,6,24] Once introduced into a flock, virus can be 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.[24]

6.6 Incubation Period

The incubation period can range from three to seven days depending on the isolate, dose of inoculums, species, and age of the bird.[27]

6.7 Clinical Signs

The absence, presence and severity of clinical signs of HPAI infection depends on the type of bird species affected. Infected wild and domestic ducks may be asymptomatic, whereas clinical signs in terrestrial birds are usually severe, resulting in high mortality.[28]

In poultry (chickens and turkeys), the clinical signs associated with HPAI include marked depression with ruffled feathers, lack of appetite, excessive thirst, decreased egg production, soft-shelled or misshapen eggs, respiratory signs (coughing and sneezing), and watery diarrhea.[27] Mature chickens frequently have swollen, cyanotic combs and wattles, and edema surrounding the eyes. The mortality rate can reach 100 percent, often within 48 hours of infection.

6.8 Gross Lesions

In mature birds, gross lesions 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.[27] 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 seven days.

6.9 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.[27] In the laboratory, 9- to 11-day-old embryonated chicken eggs are inoculated with swab or tissue specimens. If HPAI virus is the causative agent, the embryo will die within 48–72 hours. If the virus isolated is identified as influenza type A, its serologic identity (HA and NA type) is determined using a RT-PCR test.

6.10 Laboratory Specimens

AI viruses can be isolated from tracheal, oronasal, or cloacal swabs.[27] If large numbers of birds are to be sampled, swabs from up to five birds can be pooled in the same tube of brain and heart infusion broth. These specimens are then taken to USDA-approved laboratories where a RRT-PCR test is run.

6.11 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.[29] It also must be differentiated from heat exhaustion and severe water deprivation.

6.12 Control and Eradication

The overall goal for response to a highly contagious disease such as HPAI is to detect, control, and eradicate the agent as quickly as possible to return individual farms to normal production and regain disease-free status for the United States.[1] Control and eradication will rely on three basic principles:

a) Prevent contact between susceptible flocks and disease agents.

b) Stop the production of the agent by infected or exposed flocks.

c) Increase the disease resistance of susceptible flocks.

7. Background Information on Breaking Operations

7.1 Purpose

This portion of the risk assessment describes:

a) Major processes in egg breaking operations.

b) The minimum standards required for breaker egg product facility design, equipment specifications, and FSIS inspection requirements.

c) Equipment specifications and inspection requirements.

7.2 Background: Description of Egg Breaking Process

Shell eggs destined for breaking and becoming liquid eggs can be temporarily held at a warehouse or go directly to a breaking facility. Breaking facilities can be located on-site or off-site. It takes about 425,000 eggs to fill a single 50,000-pound liquid egg tanker.

There are two major breaking plant designs used in the industry and some that have features of both. These designs are known as "off-line" and "in-line" breaking.

Off-line breaking: In off-line breaking, the eggs are delivered to a centrally located facility that receives shell eggs for breaking from multiple farms. The eggs are held in bulk until presented for breaking.

In-line breaking: In-line operations are generally located at large farm complexes that may have up to 5 million hens. Eggs are collected from the individual barns and presented to the breaking operation via a collection of belts and conveyors. Some in-line operations are designed to produce washed and graded shell eggs for retail or food service customers; undersized, oversized, and under-grade eggs are diverted for breaking at that location or are shipped to an off-line plant for breaking. In contrast, other in-line facilities break only the eggs produced at that location. Some in-line operations have equipment arranged so that shell eggs from other locations can be merged into the breaking process, a practice called "side loading."

All processing plants use the same breaking process illustrated in Figure 1.

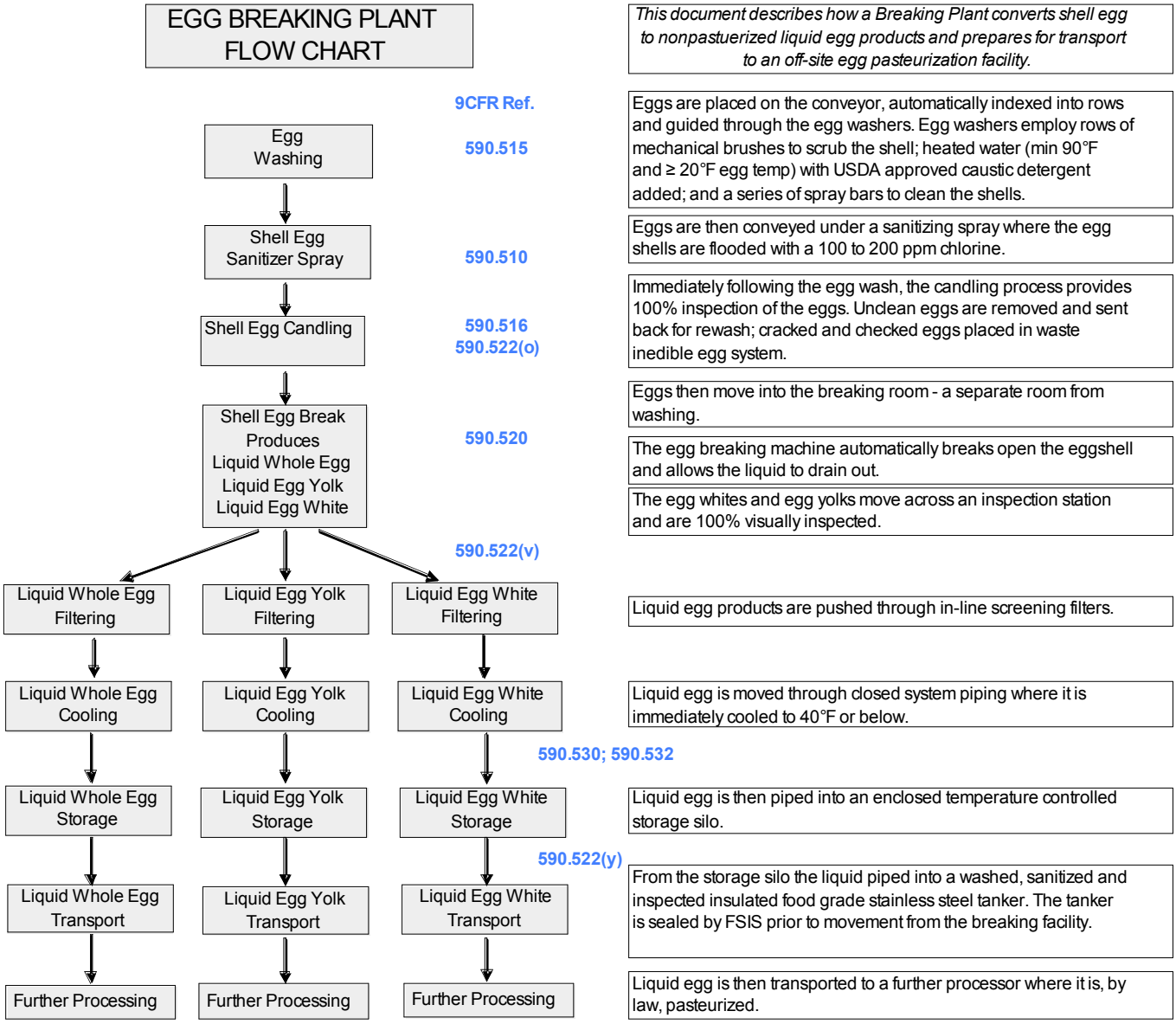

Figure 1. Diagram of Egg Breaking Operations

7.2.1 Major Processes in Egg Breaking Operations

The primary operations and steps under egg breaking are:

1. Washing the shell eggs.
2. Sanitization of the shell surfaces.
3. Candling/inspection of shell eggs.
4. Breaking the shell and separating the contents.
5. Screening and chilling liquid egg.
6. Storage and transport for further processing.

1. Washing the shell eggs

The initial phase of washing shell eggs occurs in an area known as a "transfer room." It is in this area where the shell eggs from off-line operations are removed from their bulk packaging (pallets, racks, and flats) and introduced via vacuum lifts to the egg washer conveyor. Eggs from in-line operations are received via conveyor belts and accumulated on egg washer conveyors for entry into the washing machines.

The egg conveyors then move the eggs into the egg washing machine where they are washed with detergents and rinsed with potable water containing an approved sanitizer prior to breaking. Egg cleaning operations are regulated by 9CFR590.515-516 (see Appendix 1)[30-32] to prevent contamination of the internal contents of the shell egg during washing. This is accomplished by prohibiting immersion of eggs in the wash water, defining minimum temperature differences between wash water and eggs, defining the temperature of the sanitizing rinse, and setting requirements for sanitation of the washing equipment and condition of the wash water.

2. Sanitization of shell surfaces

In accordance with 9CFR590.516 (Appendix 1),[31] cleaned and washed shell eggs are sanitized by rinsing with potable water containing a minimum of 100 ppm and a maximum of 200 ppm of chlorine. All surfaces of the eggshell come into contact with the sanitizing rinse. The sanitizing rinse remains in contact with the shell surface as it is transferred by conveying lines to the breaking room. Regulations require that the shell be sufficiently dry at the time of breaking so that the contents are not adulterated by free moisture on the shell.

3. Candling/inspection of shell eggs

Candling is a process in which eggs are passed over an intense light. Workers or specialized computerized detection equipment identify internal defects in shell eggs. Eggs with adhering dirt/soil are removed from the line for rewashing; eggs with internal defects or broken membranes are removed for disposal as inedible egg. The risks associated with the movement of inedible egg will be addressed in a future risk assessment.

4. Breaking the shell and separating the contents

It is a regulatory requirement that only clean eggs be presented to the breaker. As stipulated in 9CFR590.520 (Appendix 1),[33] breaking machines are located in a separate room from the transfer/washing processes. Breaking machines are mechanical devices designed to open individual shell eggs in a series of operations that allow control and inspection of each individual egg (Figure 2).

Individual eggs are picked up by the machine, the shell is broken, and the contents of each egg are secured in an individual "cup" for inspection. 9CFR590.522 (Appendix 1)[34] requires that each egg be evaluated for acceptance before being combined with other eggs in the process of producing liquid egg. The machines may be set up to produce liquid whole egg (natural proportions of yolk and white), yolk, and whites. Production of yolk and white is known as "separation" and results in streams of liquid consisting of egg yolks or egg whites.

Figure 2. Photo of a high-speed egg breaker with inspectors.

5. Screening and chilling liquid egg

Liquid egg is collected as separate streams of whole egg, whites, or yolk and pumped through screens that remove shell fragments and break down the native structures of the yolk membrane and egg white. The screening process

results in homogeneous fluids that are transferred via stainless steel-enclosed pipes through a heat exchanger where the temperature of the egg is reduced to ≤ 40°F prior to accumulation in chilled storage tanks. The NPLE is held in chilled and agitated storage tanks until transfer for further processing at the breaking location or to an off-site facility.

6. Storage and transport to further processing

As with the egg washing, inspection and egg breaking operations, NPLE is maintained under the supervision of FSIS inspectors within the breaking facility, during loading for transport, during transport to off-site locations, and when received at off-site locations.

The NPLE is transported under a FSIS seal in large insulated tank trucks or in smaller containers such as totes or plastic lined barrels. FSIS inspectors inspect the condition of the tanker or containers before receiving the NPLE, monitor the transfer of NPLE to transport tankers or containers, provide documentation that will accompany the NPLE in transport, and seal the tanker or transport vehicle. Transfers to tankers and/or other containers are accomplished using sanitary, enclosed transfer lines and pumps. At the receiving destination, FSIS inspectors are required to receive the NPLE, break the seal, and document the condition of the contents when received. Tankers are offloaded via flexible lines that connect the tank truck to pumps and stainless steel receiving lines and storage tanks. Totes are generally emptied by attaching a line with a pump to transfer the liquid contents in enclosed lines. Product in barrels may be emptied via pumps or barrel-dumps, and the NPLE is then handled via enclosed process lines.

7.2.2 Equipment Specifications and Inspection Requirements

All plants breaking eggs under 9CFR590.24 are required to have continuous inspection by a FSIS inspector (Appendix 1).[35] The inspector must be on-site whenever breaking equipment is operating unless specifically exempted.

The above portion of the risk assessment describes the typical conditions under which egg breaking occurs. When any processing industry operates equipment, there exists the possibility of process failure. Equipment failure can result in an improper washing of eggs, low chlorine concentration in sanitizer water, low pH, and other errors.

Refer to the previously completed risk assessment on liquid pasteurized eggs for more detail on industry standard GMPs and SOPs.[36]

8. Risk of HPAI Virus in Egg Contents Contaminating NPLE and Resulting in Infection of a Susceptible Flock

This portion of the risk assessment describes the risk that HPAI virus contaminated NPLE moved from an egg breaking facility infects susceptible poultry.

Risk of HPAI Virus in Egg Contents Contaminating NPLE and Resulting in Infection of a Susceptible Flock

- **Risk Factors:** Internal contents of eggs used for the production of NPLE contaminated with HPAI, late detection of HPAI infection in a flock, release of NPLE during handling/storage before pasteurization, movement of personnel between NPLE handling areas and the henhouse in inline pasteurization facilities.
- **Current Preventive Measures:** Good Manufacturing Practices (GMPs) and FDA requirements as outlined in 21CFR110.80; FSIS Sanitary processing measures as outlined in 9CFR590, including requirements for the inspection of egg products.
- **Additional Preventive Measures** (to be implemented by industry in conjunction with APHIS during an outbreak): Active surveillance of flock for clinical signs of illness, changes in feed and water intake, drop in egg production, and submission of a pooled sample of swabs from 5 randomly chosen birds among the daily mortality for RRT-PCR testing.
- **Overall Risk:** Negligible

8.1 Background Information

Natural outbreak and experimental studies have found HPAI H5N2 virus within the contents of eggs laid by infected chickens.[23] Therefore, there is a possibility that NPLE is contaminated with HPAI virus from the contents of the eggs used in its production. To estimate the degree of NPLE contamination from HPAI virus present in the egg contents, we need to estimate what proportion of eggs from an infected but undetected flock would be contaminated.

The proportion of contaminated eggs from an infected but undetected farm would depend on the HPAI prevalence at various time periods before infection is detected. A characteristic feature of HPAI infection in a flock is the exponential increase in prevalence and mortality over time. Consequently, the proportion of contaminated eggs expected from an infected, undetected farm during an outbreak would depend on the time taken to detect infection given the surveillance protocol followed during the outbreak. As

an extreme example, in the scenario of perfect surveillance, the number of contaminated eggs produced before detection would be zero.
The United Egg Producers/United Egg Association - USDA APHIS Veterinary Services Highly Pathogenic Avian Influenza Movement Control Model Plan (UEP/UEA – USDA APHIS VS Movement Control Model Plan) for use in an HPAI outbreak specifies active surveillance based on RRT-PCR testing (Appendix 11). We utilized a stochastic disease transmission model to estimate HPAI spread within the flock. The transmission model results were then used in conjunction with a simulation model of the active surveillance protocol to estimate the number of contaminated eggs that might be used in the production of NPLE that is moved before infection is detected in the flock.

The virus concentration[e] in a container of NPLE leaving an egg breaking facility is likely lower than the concentration of virus within individual eggs due to mixing with the contents from virus-free eggs. We performed further simulation analysis to estimate the concentration of virus in an outgoing tanker of NPLE. In the final portion of this chapter, we evaluate the potential pathways by which susceptible chicken may be exposed to NPLE and likelihood of the NPLE being infectious to chicken exposed through such pathways.

8.2 Evaluation of Risk

We evaluated the risk of HPAI virus in the internal contents of eggs from an infected undetected flock resulting in an infectious level of HPAI in the NPLE as the following constituent risks:

a) The risk of the internal contents of eggs from an infected and undetected flock being contaminated with HPAI virus.

b) The risk of the viral titer in a 48,000-lb tanker of NPLE being infectious to a chicken exposed to one ml of the NPLE.

8.2.1 The Number of Internally Contaminated Eggs from an HPAI Infected but Undetected Flock

We estimated this factor in three parts:

a) The likelihood that internal contents of eggs laid by an HPAI infected hen are contaminated.

b) The HPAI infection prevalence and the number of internally contaminated eggs at various time periods post infection of the flock.

c) The time to detect HPAI infection in the flock and the maximum daily number of contaminated eggs before detection when following the active surveillance protocol.

[e] By concentration of virus, we mean the infectivity titer of NPLE expressed as the number of 50 percent chicken egg embryo infectious doses per ml of NPLE (the number of ID_{50} doses in the container).

8.2.1.1 Likelihood that Internal Contents of Eggs Laid by an HPAI Infected Hen are Contaminated

The data on the virus recovery frequencies from egg yolk and albumen do not show a statistically significant difference between them.[18,37] For this assessment, we assumed that the virus recovery frequencies are the same for egg contents regardless of whether we are referring to egg yolk or albumen.

Table 1 summarizes previous outbreak and laboratory study data on the fraction of eggs from HPAI infected hens that are internally contaminated. In these data, none of the eggs laid on the first day post infection were contaminated. Given this temporal effect, we assumed that eggs laid during the first 19 hours after infection are not contaminated and eggs laid after the first 19 hours are all contaminated. This threshold distribution corresponds to egg contamination option 3 of the draft FSIS interagency risk assessment model.[38]

Table 1. Summary of data on the fraction of internally contaminated eggs from HPAI infected layers.

Study	*Percent contaminated eggs*	*Time of detection*	*HPAI strain*	*Source*
1	8% (3/37)	Last egg	Lab study (H5N2)	Bean *et al.* (1985)[24]
2	35% (15/42)	From 3rd day post infection onwards	Lab study (H5N2)	Beard *et al.* (1984)[18]
3	7-57% for different flocks	Not shown	(H5N2) Natural Outbreak	Cappucci *et al.* (1985)[23]
4	45%	Day 2 and day 3 post infection	Lab study (H5N2)	Swayne and Suarez (2008)[37]

8.2.1.2 HPAI Infection Prevalence and the Fraction of Internally Contaminated Eggs at Various Time Points Post Infection of the Flock

We used an infectious disease transmission model to estimate the prevalence of HPAI infection that might occur in a typical 100,000-bird layer house over time and the prevalence of infection in pools of daily mortality over time. The deterministic version of the transmission model was developed as part of an interagency risk assessment conducted by FSIS in collaboration with FDA and APHIS.[39] The disease transmission model is an extension of the Reed-Frost Susceptible-Latent-Infectious-Died (or Removed) model to include more infectious states. The model estimates the number of susceptible, infected, and dead birds and the number of contaminated eggs every 6 hours post-infection.

The inherent variability in the time course of HPAI spread within a flock can have a significant impact on the time to detection of the infection. For instance, if the mortality

due to HPAI infection is low on a particular day, then the likelihood of detecting infection when following a surveillance protocol based on daily mortality sampling is reduced. Data from the H7N7 outbreak in the Netherlands showed a relatively high variance in the daily mortality among various infected flocks.[40] We developed a stochastic version of the transmission model to incorporate the effects of variability in spread of HPAI in a flock. Details of the disease transmission model are in Appendix 2.

We estimated the latently infected and infectious periods for the transmission model based upon experimental studies of HPAI inoculated hens.[41] In the transmission model, the expected latently infected period is 13.8 hours and the expected infectious period is 25.8 hours. An important parameter for the transmission model is the effective contact rate: this is defined as the number of birds an infectious chicken comes into contact with that could become infected per a specified time period. The effective contact rate has been estimated using a variety of approaches such as experimental studies in the laboratory, empirical estimates, HPAI outbreak data, and simulation studies in the literature.[42-46] We used an effective contact rate of 2 chickens/6-hour time period considering the contact rate estimates from the literature for caged layers. We discuss the impact of the uncertainty in the effective contact rate estimate and perform sensitivity analysis for our estimate in section 8.3. Given a rate of 2 chickens/6 hours, the basic reproductive number (R_0) for our model is 8.6. Finally, we used the data presented in section 8.2.1.1 to estimate the number of contaminated eggs from an infected flock. The main assumptions of the disease transmission model are summarized below. Details concerning our estimates are included in Appendix 2.

(i) Assumptions

- *The effective contact rate for disease transmission estimated from HPAI outbreak data from the Netherlands and Thailand (2 birds/6 hours) is applicable for layer flocks (i.e. a flock of birds contained in a single layer house) in the US.* Differences in the layer management practices between the United States and other countries, and the characteristics of the HPAI strain causing the outbreak may result in a different contact rate than that used in this assessment. Sensitivity analysis with respect to the contact rate (presented in section 8.3) indicates that our risk estimates are robust and conservative with respect to the range of contact rates estimated in the literature for HPAI infection in caged layers.
- *No reduction in the egg laying rate due to HPAI infection in a hen.* Previous outbreak studies have reported a more than 30 percent drop in egg production rate in H7N7 or H5N2 HPAI infected flocks.[24,40,47] If this is the case, then a drop in egg production could provide an early indication of HPAI infection. Whether such a drop in egg production rate would occur with infection with the more virulent Asian H5N1 strains is not clear, therefore we took a conservative approach and assumed that there is no drop in egg production rate.
- *A flock size of 100,000 is typical in the industry.* A greater flock size would likely result in higher normal mortality and a decreased chance of detecting HPAI infection within a given time. We consider the flock size of 100,000 as a conservative estimate given that the USDA APHIS Layer 1999 data indicates that

the mean and median flock sizes are less than 83,000 hens (see Appendix 2 for further details).[48]

(ii) Simulation Results:

The above model was coded in Excel using Visual Basic for Applications and @RISK software.[f] An effective contact rate of 2 chickens/6-hour time period and a flock size of 100,000 layers (contained within a single layer house) were used as input parameter values. We conducted simulations for 6000 iterations with Latin Hypercube sampling. The daily mortality results from the transmission model are presented in Table 2. Table 3 shows the estimated number of contaminated eggs from the output of the disease transmission model.

Table 2. Daily mortality predicted by the transmission model in 100,000 bird layer house starting with one infected bird.

	Daily Mortality					
Parameter type	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Deterministic	0	1	5	40	296	2,150
Stochastic mean	0	1	5.2	40	297	2,146
Stochastic 90% probability interval	0	1	1-10	12-78	93-479	684-3,408

Table 3. Number of internally contaminated eggs predicted by the transmission model in a 100,000 bird layer house starting with one infected bird.

	Estimated Daily Number of Contaminated Eggs					
Parameter type	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Stochastic mean	0.175	1.5	11.9	87	639	4,374
Stochastic 90% probability interval	0-1	0-3	4-22	28-167	204-1,217	1,491-8,000

8.2.1.3 The Time to Detect Infection and the Maximum Daily number of Contaminated Eggs under the Active Surveillance Protocol

In the targeted active surveillance described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan, swabs from 5 randomly selected birds among the daily mortality sample are pooled together and tested via RRT-PCR each day. The number of

[f] @RISK version 4.5.3 Copyright © 2004, Palisade Corporation.

days to detect infection under this protocol depends on the variability in the normal mortality independent of HPAI and the variability in the mortality due to HPAI. We used outputs from the transmission model in conjunction with a simulation model of the active surveillance protocol to estimate the number of days to detect infection in a flock.

According to the UEP/UEA – USDA APHIS VS Movement Control Model Plan, eggs or egg products from flocks within the control area will be allowed to move with a permit only after the flock tests negative with RRT-PCR testing as described above.[g] This protocol implies that if infection from a flock is detected on a particular day by RRT-PCR testing, then the eggs or egg products from the flock on that day would not be moved from the premises. Therefore, for this analysis we assumed that eggs produced on the day on which infection is detected are not relevant for risks associated with movement of NPLE. Given this assumption, we defined the maximum daily fraction of contaminated eggs as the highest daily fraction of contaminated eggs among all the days starting from the day the flock is infected to one day before the infection in the flock is detected.

The simulation model of the active surveillance protocol can best be explained by using a scenario tree (Figure 3). The number of diseased birds present in the 5 randomly chosen birds from the daily mortality group follows a hypergeometric distribution, (hypergeometric (*M, n, D*)), where *M* is the total mortality, *D* is the mortality due to HPAI and $n = 5$ is the sample size.[h] Given this protocol, there is a 95 percent chance of including at least one diseased bird in the pooled sample if the HPAI prevalence among dead birds is greater than 39 percent. The pooled sample of 5 swabs from the selected birds is tested via RRT-PCR. The sensitivity of this test is estimated to be 86.5 percent,

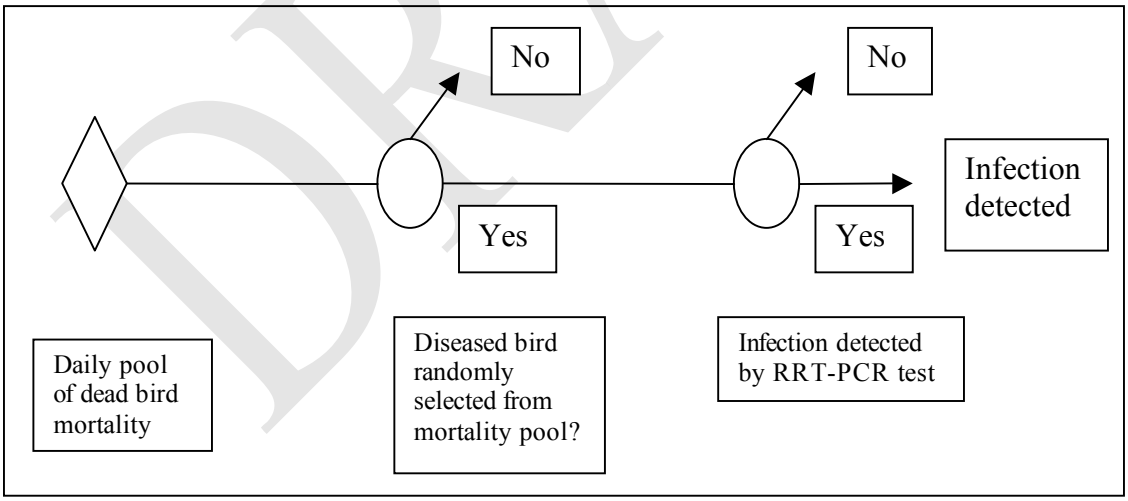

Figure 3. A scenario tree analysis illustrating the probability of reporting at least one test-positive bird via RRT-PCR.

[g] Such holding time for eggs or egg products awaiting negative RRT –PCR results may increase the risk that storage capacity is exceeded leading to disposal of product.

[h] Although some dead birds might be missed (not detected) while counting mortality each day, this will not impact the probability of detecting HPAI infection under the active surveillance protocol provided that all dead birds are equally likely to be missed regardless of whether they have HPAI or not.

so there is a 13.5 percent chance that infection will not be detected even when the pooled sample contains an HPAI-positive bird. We modeled the process of RRT-PCR testing as a simple Bernoulli trial.

(i) Assumptions

- Apart from active surveillance of mortality via RRT-PCR, other clinical indicators of HPAI infection such as a drop in egg production and decreased feed intake are not considered towards detecting infection.
- The sampling of daily mortality is random i.e., swabs from dead birds with clinical signs are not more likely to be included in the pooled sample tested via RRT-PCR.
- Weekly mortality data was used to estimate the normal daily mortality.

(ii) Model Inputs

The main model input parameters for this model are summarized in Table 4.

Table 4. Key input parameters for the simulation model to estimate the number of days to detect infection in a flock when the active surveillance protocol is followed.

Input Parameter	*Value*	*Unit*	*Source*
Flock size	100,000	Birds	Average flock size (FSIS risk assessment)[38]
Normal daily mortality independent of HPAI infection	Empirical distribution mean 28 (95 percent CI 26.4-29.6), std. dev. 33	Birds/ day	Unpublished Data from 27 layer flocks for the entire production cycle, Agri Stats, Inc.[49] (See Appendix 2 for details)
RT-PCR test sensitivity	86.5%	--	Elvinger *et al*.(2007)[50]; Dr Erika Spackman, pers. comm., 2007

(iii) Model Results

Number of days to detect HPAI after the first chicken is infected:

The distribution of the number of days to detect HPAI infection from simulated output is shown in Figure 4. The mean time to detection was 3 days. However, as shown in Figure 4, there is a 6.5 percent chance that ≥ 5 days are required to detect infection. One reason for the late detection of HPAI (≥ 4 days) is a high normal mortality rate. A high normal mortality rate implies that a swab from a bird that dies due to HPAI is less likely to be included in the random sample for the pooled specimens sent for RRT-PCR testing. Another factor leading to late detection is the low sensitivity (86.5%) of the RRT-PCR test.

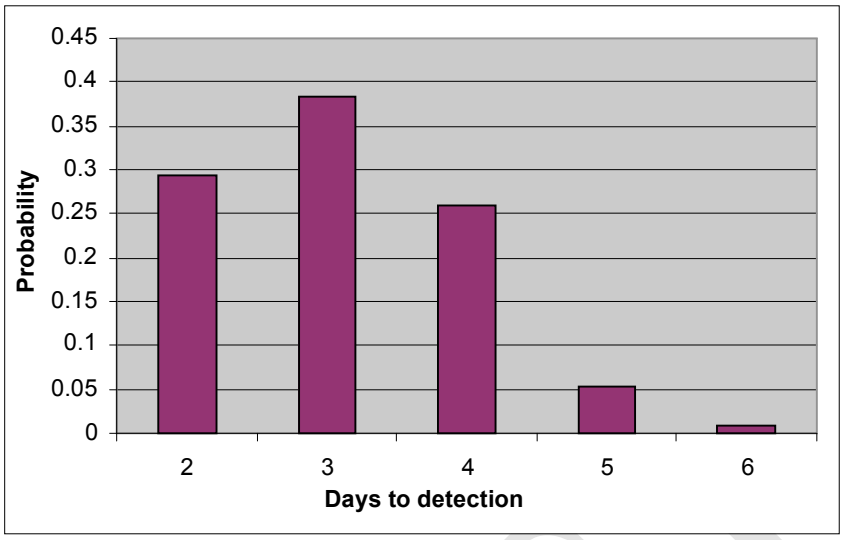

Figure 4. Simulated number of days to detect HPAI in a flock of 100,000 layers with active surveillance via RRT-PCR testing.

Maximum number of contaminated eggs per day from an infected but undetected flock:

The expected maximum number of contaminated eggs produced per day from a 100,000 layer flock prior to detection was 12 contaminated eggs/day (90 percent probability interval 0-40). However, in 5 percent of the simulation iterations, 40-1209 contaminated eggs were produced prior to detection. Typically, the cases in the simulation results exhibiting a greater number of contaminated eggs produced prior to detection were associated with late detection of infection.

8.2.2 Estimated HPAI Viral Titer in a 48,000-lb Tanker of NPLE Leaving an Egg Breaking Facility that Received Eggs from an Infected, Undetected Layer House

In this section, we analyze the impact of mixing of NPLE from contaminated and virus-free eggs to estimate the resulting viral titer in an outgoing tanker of NPLE. The numbers of contaminated eggs were obtained from the output of the disease transmission model. In the following, we first summarize the data on the viral titers in egg contents and then estimate the final viral titer in NPLE.

8.2.2.1 HPAI Titers in Egg Contents

Studies on the HPAI virus titers in egg contents are summarized in Table 5. We did not model the HPAI viral titers in egg yolk and albumen separately as the data on differences between their viral titers is limited (sample size is three in Bean *et al.*[24]). We assumed a uniform distribution, Uniform ($10^{3.6}$, 10^{5}) EID_{50}/ml, for the HPAI viral titer in the contents of a contaminated egg. Here, EID_{50} refers to the 50 percent chicken embryo infectious dose.

Table 5. Summary of data on the HPAI viral titer within the egg contents.

Source	*Sample*	*Viral titer (EID_{50}/ml)*	*HPAI Strain*
Swayne and Beck (2004)[i],[51]	egg contents	$10^{4.9}$	H5N2
Bean et al (1985)[24]	yolk	$10^{3.6}$	H5N2
Bean et al (1985)[24]	albumen	$10^{5.6}$	H5N2
Beard et al (1984)[18]	egg contents	$>10^{4.0}$	H5N2
Swayne and Suarez (2007)[37]; *Swayne et al (2008)*[52]	yolk	$10^{3.0}$	H5N2
Swayne and Suarez (2007)[37]; *Swayne et al (2008)*[52]	albumen	$10^{3.6}$	H5N2

8.2.2.2 Estimated Viral Titer of NPLE while Considering the Mixing of Internal Contents from Contaminated and Virus-Free Eggs used in its Production

We utilized a simulation model to estimate the final viral titer in an outgoing tanker of NPLE. For this model, we assumed that the daily egg production from a single 100,000-bird henhouse would be loaded into one tanker and mixed with liquid egg from other houses on the same or different premises. To consider the possibility that the tanker may not be filled to its capacity, we also analyzed an alternate scenario where the tanker is only 25 percent full. Another key assumption is that the NPLE in the tanker has been mixed thoroughly and the viral titer is homogeneously distributed throughout the tanker. This assumption is based on the fact that agitators in typical NPLE refrigeration and storage systems would facilitate mixing. The main inputs for the model are summarized in Table 6.

Table 6. Simulation model inputs to estimate the final viral titer in a tanker of NPLE.

Input parameter	*Value*	*Unit*	*Source*
Volume of egg	64	ml	7CFR56[j] (Based on average of extra large and jumbo egg volume)[53]
Egg content density	1.030	g/ml	Rahn and Paganelli (1989)[54]
Viral titer in contaminated egg	Uniform (3.6-5)	Log EID_{50}/ml	Section 8.2.3.a
Percent of tanker capacity filled	Uniform (80-100)	percentage	Assumption
Weight capacity of tanker	48,000	pounds	Assumption

[i] The reported virus titer is from unpublished data (M. Brugh) cited within this reference.

Simulation Results:

The amount of infectivity present in one ml of NPLE in a tanker is typically about one hundredth of that needed to cause infection in chickens. We performed a simulation using @RISK for 5,000 iterations with Latin hypercube sampling. The mean viral titer in the NPLE from the simulation results was 1.15 EID_{50}/ml (90% probability interval 10^{-3}- 3.71 EID_{50}/ml). If the tanker was only 25 percent full, then the mean viral titer in the NPLE from simulation results was 4.3 EID_{50}/ml (90% probability interval 10^{-3}- 12 EID_{50}/ml). It should be noted that the 50 percent chicken infectious dose (CID_{50})[k] was more than 100 EID_{50} for most HPAI strains studied in Swayne and Slemons (2008).[55] These results indicate that the probability of infection is small even if a chicken were exposed to the NPLE by some pathway, as the amount of infectivity present is low.

8.2.3 The Risk that Susceptible Poultry on another Premises are Infected via Exposure to HPAI Virus Contaminated NPLE

8.2.3.1 The risk that susceptible poultry are exposed to HPAI virus contaminated NPLE transported from the premises

The NPLE produced at egg breaking facilities is transported in tankers or containers sealed by FSIS to other continuously FSIS inspected processing facilities for pasteurization or heat treatment. Movement of NPLE has never been implicated as a cause of secondary spread of HPAI to susceptible poultry, making the pathways by which susceptible poultry may be exposed to contaminated NPLE transported from infected premises theoretical. Nevertheless, evaluating such potential pathways may be worthwhile considering the uncertainty associated with them.

One potential pathway for secondary HPAI spread to susceptible poultry is through wild birds infected from NPLE spilled during loading, unloading or transportation. The wild birds could then access and expose backyard flocks to HPAI virus. We find that NPLE handling processes significantly mitigate this risk as NPLE is typically pumped to or from storage tanks in enclosed lines, reducing the likelihood of spillage. If a spill occurs, standard operating procedures and GMPs include cleaning and disinfection of surfaces, so cross-contamination into other areas is also mitigated. It is highly unlikely that NPLE spills during transportation given that it is transported in sealed containers. Finally, wild birds are not considered to pose a significant risk of secondary spread of AI among domestic poultry.[55,56] We thus consider the risk of HPAI spread to susceptible poultry by this pathway as negligible.

Pasteurization facilities to which NPLE is transported to can be categorized as off-line (no hens on the premises) and in-line facilities (hens on the premises). In off-line pasteurization facilities, there is no opportunity for NPLE to be exposed to a susceptible flock. If NPLE were transported to an in-line operation for pasteurization, there is a possibility that NPLE could be released into the facility during handling or storage before pasteurization, then be transmitted into the henhouse via personnel or pests. Current

[j] 7CFR56 is the Agricultural Marketing Service voluntary grading of shell eggs program.
[k] CID_{50} refers to a dose of HPAI virus that infects 50 percent of the chicken exposed to it. The CID_{50} is frequently expressed as the number of EID_{50} contained in it.

preventive measures include Federal regulations and industry good manufacturing practices (GMPs) aimed at preventing microbial contamination of egg products (9CFR590 and 21CFR110). Most FSIS-inspected egg processing plants also follow additional precautions such as inspection of non-product contact zones, mid-shift clean ups, and environmental microbial testing.[57] In the following subsections, we evaluate the likelihood that NPLE is exposed to susceptible poultry at pasteurization plants given the aforementioned preventive measures.

a) Likelihood of release of NPLE into processing facilities during pre-pasteurization handling and storage.

   At in-line pasteurization facilities, NPLE is usually transferred from incoming trucks to holding tanks via enclosed pipes, reducing the chance of spillage (industry GMPs). Relevant requirements from 9CFR590 include:

   - Tanks and vats holding liquid egg are to be of approved construction, fitted with covers, and are to be located in rooms maintained in sanitary conditions.
   - Surface coolers and liquid egg holding tanks must be covered while in use.
   - Liquid egg holding vats are required to be cleaned after each use.

   Considering these processes and regulatory requirements for sanitary operation of liquid egg handling and storage equipment, we conclude that the probability of release of NPLE into a processing facility during handling and storage is low if all the relevant regulations and GMPs are strictly followed.

b) Likelihood that susceptible chickens are exposed to NPLE released into the facility via cross-contamination by personnel or pests.

   NPLE accidentally released into in-line pasteurization facilities should be removed via cleaning and sanitizing. Relevant sanitary measures from 9CFR590 and 21CFR110 include:

   - Processing rooms are to be clean and free of flies, insects, rodents, refuse, odors and waste materials.
   - Equipment and utensils are to be cleaned and sanitized at the start of processing.

   A survey of 77 egg processing plants found that 82 percent of the plants inspected non-product contact zones daily and more than 90 percent have cleanup shifts and mid-shift cleanups.[57]

   Industry experts have stated that in in-line operations personnel are dedicated to working in specific portions of the plant such as egg processing and henhouse operations. During an HPAI outbreak we expect that there will be increased awareness and implementation of biosecurity and sanitary measures for personnel. We conclude that the likelihood of personnel transmitting NPLE accidentally released in pasteurization facilities to the henhouse is very low.

Egg processing rooms are required to have a positive air pressure system relative to the henhouse,[1] and must have opening that prevent the entrance of flies or other insects (9CFR590.520(d)(1); 9CFR590.500). These requirements will reduce the likelihood of contamination by flies. Furthermore, as mentioned previously, 9CFR590 and 21CFR110 require processing rooms or any portion of the plants where egg products are handled or stored to be kept insect and rodent free. Doors and windows leading into rooms where edible product is processed are required to be of solid construction and fitted with self closing devices (9CFR590.500). Considering these sanitary measures for preventing pests from entering the egg processing rooms, we conclude that the likelihood of pests transmitting any HPAI virus contaminated NPLE accidentally released in pasteurization facilities to the henhouse is negligible providing that that all applicable FSIS regulations are followed.

In summary, for susceptible poultry to be exposed to HPAI virus contaminated NPLE through cross-contamination during pre-pasteurization handling and storage, NPLE must first be released into the processing room, then people or pests must transfer the released HPAI virus contaminated NPLE to the henhouse. Based on the above discussion, the likelihood of both of these events occurring independently is low. We thus conclude that the likelihood that susceptible poultry are exposed to HPAI virus contaminated NPLE at in-line pasteurization facilities is negligible.

Even if susceptible poultry were exposed to HPAI virus from NPLE through cross-contamination via personnel, the amount of exposure is likely to be small. The rationale for the low amount of exposure is that if a large amount of NPLE contaminated a person's hands or clothes, it would be easily detected and preventive action would be taken. Furthermore, the exposure to susceptible chickens through this pathway would have to occur via the oral route as there is no opportunity for NPLE to be aerosolized during handling or storage before pasteurization. In the following section, we estimate the probability of infection in chickens exposed to one mL of HPAI virus contaminated NPLE. We used one ml of NPLE as a conservative estimate of NPLE exposure to a chicken.

8.2.3.2 Estimating the Probability of Infection in a Chicken Exposed to one ml of Contaminated NPLE from an Outgoing Tanker via Dose Response Analysis

In this section, we used a dose response analysis to estimate the probability of infection in chickens exposed to NPLE at in-line pasteurization facilities via the pathways evaluated in the previous section. As mentioned above, we used one ml of NPLE as a conservative estimate of NPLE exposure to a chicken.

Virus characterization studies of various HPAI strains found 50 percent chicken infectious doses (CID_{50}) in the range of 2-3.9 log EID_{50} via the intranasal route.[55,58,59] From the above data, we decided to use a logistic distribution with a mean of 2.80 and a standard deviation of 0.43 log EID_{50} as the distribution of CID_{50} for HPAI.

[1] Federal regulations require this direction of airflow in blending and packaging rooms for pasteurized products as well as breaking rooms.

We used the single parameter exponential dose response model which has been used to model avian influenza and other viruses such as FMD.[60,61] The parameter *p* of the exponential dose response model represents the probability that a dose of one EID_{50} infects an exposed chicken. We calculated *p* from the CID_{50} value and obtained its distribution via simulation. Finally, we calculated the distributions for the probabilities of infecting a chicken at various doses of HPAI. Figure 5 shows the mean and the two sided 90 percent probability interval for the probability of infection at various doses.

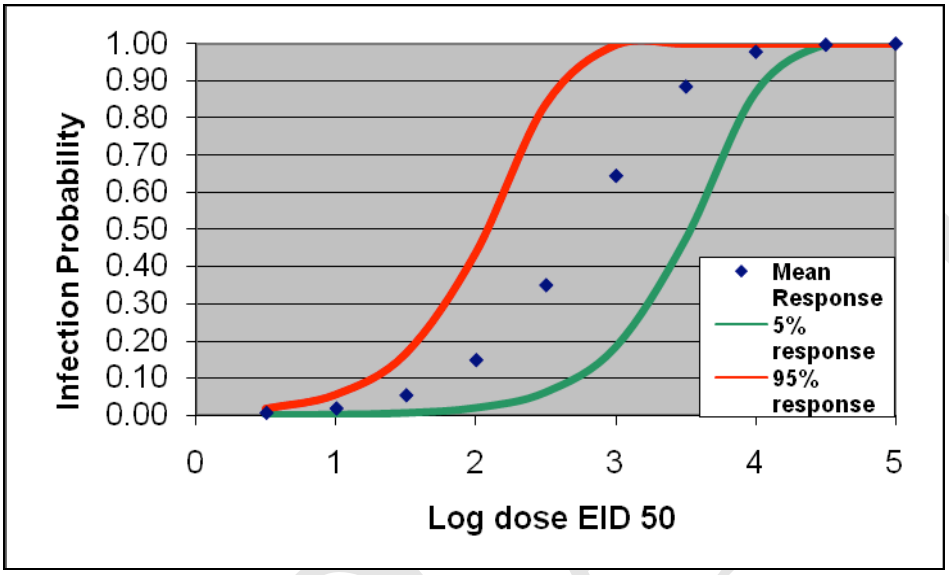

Figure 5. Dose response curve for chicken exposed to HPAI virus.

Given the above dose response relationship, we analyzed whether a chicken exposed to one ml of NPLE from an outgoing tanker containing product with a viral titer of 1.15-4.5 EID_{50}/ml would become infected. The mean probability of infection from simulation was 0.2 percent (90 percent interval 10^{-4} percent-0.6 percent). If the outgoing NPLE tanker was only 25 percent full, given the higher viral titer due to decreased dilution, the mean probability of infection would be 0.6 percent (90 percent interval 10^{-3}-2 percent). Also note that the exponential dose response model is relatively linear at doses for which the probability of infection is very low. For example, if the amount of exposure to NPLE were two ml of instead of one ml, then the expected probability of infection in the exposed chicken would be approximately 0.4 percent (details of the exponential dose response model are in Appendix 3).

Overall, as determined in section 8.2.3.1, it is highly unlikely that susceptible poultry would be exposed to NPLE in any case. Given the estimated viral titer in HPAI virus contaminated NPLE, the dose response analysis shows that the expected probability of infection in an exposed chicken would be less than 0.6 percent in a tanker ≥ 25 percent full. Considering the very low likelihood that susceptible chickens are exposed to NPLE and the low probability of infection in an exposed chicken, we conclude that the risk of susceptible poultry being infected via to exposure to HPAI virus contaminated NPLE transported from an infected but undetected facility is negligible.

8.3 Sensitivity Analysis for the Effective Contact Rate

We conducted a sensitivity analysis on the effective contact rate because there is less certainty associated with its estimation compared to other transmission parameters. An increase in the effective contact rate would have two competing effects that would impact the number of contaminated eggs produced from a flock before detection. The first effect is that a higher contact rate would result in a faster rate of disease spread and a higher mortality. It follows that the higher mortality rate would likely result in a reduced time to detection of infection in the flock. The second effect is that a greater number of infected chickens would result over a given time with a higher effective contact rate. This would likely result in a higher number of HPAI virus contaminated eggs being produced.

For the sensitivity analysis, we performed 6000 simulation iterations of the disease transmission and the active surveillance models for the effective contact rates of 1, 2 and 4 birds/6 hours. The contact rate of 4 birds/6 hours can be considered an upper bound estimate for caged layers as predicted by the simulation model of Savill *et al*.[46] As detailed in Appendix 2, the contact rates from HPAI outbreak data were mostly less than 2-birds/6 hours. From our simulation results, the expected maximum daily number of contaminated eggs with contact rates 1, 2 and 4 was 5 (90 percent probability interval 0-17), 12 (90 percent probability interval 0-40) and 30 (90 percent probability interval 0-62), respectively.

The simulation models presented in sections 8.2.2 and 8.2.3 show that contents from approximately 65 contaminated eggs must be included in a tanker of NPLE in order to obtain a 1 percent probability of infection in a chicken exposed to one ml of NPLE. This result implies that the expected probability of infection in an exposed chicken is less than 1 percent when the effective contact rate is within the range of 1- 4 birds/6 hours.

Based upon the above sensitivity analysis we conclude that,

1) The number of contaminated eggs from an infected, undetected flock of 100,000 birds (within a single layer house) following the active surveillance protocol will increase with the effective contact rate. The effective contact rate of 2-birds/6 hours used in our assessment is higher than the estimates from HPAI outbreak data in the literature and is conservative.

2) The probability of infection in a chicken exposed to one ml of HPAI contaminated NPLE from an outgoing tanker is less than 1 percent for the range of effective contact rates reported in the literature for HPAI infections in caged layers.

8.4 Conclusion

The entire risk pathway for infection of a susceptible flock with HPAI virus contaminated NPLE is summarized as follows:

a) The number of contaminated eggs from an infected but undetected flock varies with the time of disease detection (i.e. the level of surveillance effort).

Consequently, the level of HPAI virus contamination of NPLE would vary with the time of disease detection.

b) Our simulation models predict that the maximum number of contaminated eggs that would be used for producing NPLE on a given day that may be moved from the premises before detection of infection is 12 (90 percent PI 0-40) when the active surveillance protocol described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan is followed.

c) The likelihood that susceptible poultry are exposed to NPLE that is transported to in-line pasteurization facilities through is negligible if all the applicable regulations and biosecurity practices are strictly followed. The likelihood that susceptible poultry are exposed to NPLE transported to off-line pasteurization facilities is negligible.

d) Given the estimated number of contaminated eggs from an infected but undetected flock and considering the dilution of the viral titer in NPLE due to mixing with the product from virus-free eggs, we estimated that the chance of infection in a chicken exposed to one ml of NPLE is 0.2 percent (90 percent interval 10^{-4}-0.6 percent).

e) Considering the very low likelihood that susceptible chicken are exposed to NPLE and the low probability of infection in an exposed chicken, we conclude that the risk of NPLE contamination with HPAI virus from the contents of eggs from an infected, undetected flock resulting in infection of a susceptible flock is negligible.

In our analysis, we considered RRT-PCR as the sole means for detecting infection. However, in practice, clinical signs such as increased mortality, decreased egg production and supplemental PCR testing may lead to earlier detection. An important caveat is that some deviation from modeling results is to be expected depending on the specific characteristics of the HPAI strain causing the outbreak (e.g., longer infectious period), variation in the flock size and layer management practices.

9. Risks due to Cross-contamination of NPLE via HPAI Virus on the Shell Surface of Washed and Sanitized eggs

This portion of the risk assessment will describe:

a) The risk of egg surface contamination with HPAI virus.

b) The risk of inadequate washing, sanitization and inspection of eggs resulting in a secondary HPAI outbreak due to cross-contamination of NPLE during the egg breaking process.

Risk of HPAI infection in a susceptible flock due to cross-contamination of NPLE via virus on the shell surface of washed and sanitized eggs

- **Risk Factors:** Egg shell outer surface contaminated with HPAI virus; egg washing, inspection and sanitizing steps do not remove the HPAI virus.
- **Current Preventive Measures:** Good Manufacturing Practices (GMPs) and FDA requirements as outlined in 21CFR110.80 (Appendix 8); FSIS measures as outlined in 9CFR590, which covers requirements for the inspection of egg products and facilities sanitation; 9CFR590.510, 515, 516, 520 and 522 (Appendices 2-5, 9) which describe shell egg washing, inspection and sanitizing.
- **Additional Preventive Measures** (to be implemented in the event of an outbreak): None needed
- **Overall Risk:** Negligible

9.1 Background Information

This section of the risk assessment evaluates the risk of HPAI virus on a shell egg surface surviving processing and contaminating NPLE. The eggshell may carry the virus inherently or be cross-contaminated with feces or respiratory secretions. Precedents for HPAI viruses surviving on shell egg surfaces include:

a) Isolation of H5N2 from shell egg surfaces of eggs obtained from an infected flock during the 1983-1984 Pennsylvania outbreaks.[23]

b) Isolation of H5N2 from shell surfaces of eggs from infected hens in laboratory studies.[18,37,62]

Current Federal regulations (9CFR590) require all shell eggs be washed, sanitized and inspected prior to presentation into the breaking machine. These regulations are aimed at reducing the eggshell contamination with *Salmonella* and other microbial agents. Avian influenza virus is an enveloped virus that exhibits marked sensitivity to disinfectants,[63] therefore the current Federally mandated sanitization methods likely result in some

inactivation of HPAI virus on the shell egg surface. The degree of inactivation is dependent on specific operational conditions including the pH of the wash water and sanitizing rinse, contact times of the wash and rinse steps, and organic load. These conditions are not specified in the Federal regulations; therefore industry practices yield varying degrees of virus inactivation.

In this section, we first estimate the degree of viral inactivation from existing preventive measures utilizing data from inactivation studies of HPAI and other viruses. We then utilize HPAI dose response data to assess whether cross-contamination of NPLE from shell egg surfaces during egg breaking causes a significant increase in the risk of a secondary HPAI outbreak.

9.2 Current Preventive Measures

Preventive measures considered in this risk assessment are those specified in 9CFR590 regarding shell egg washing, inspection and sanitizing prior to breaking.

As described in section 7.2.1, all eggs presented for breaking must first be washed, inspected and sanitized prior to entering the breaking process. The washing operation utilizes a combination of heat, pH, detergent action, contact time and mechanical agitation to accomplish the removal of soil from the shell egg surface. Detergents used in the washing process must be USDA approved and labeled for such use by the EPA. In addition, the wash temperatures must reach a minimum temperature of 90°F (9CFR590.515, Appendix 1).[32] Unclean eggs are disposed of or rewashed per 9CFR590.510 (Appendix 1).[64]

All shell eggs must be sanitized prior to entering the breaking machine. USDA FSIS mandated and monitors use of a spray rinse containing an approved sanitizer of 100–200 ppm available chlorine or its equivalent (9CFR590.516, Appendix 1). After sanitization, shell eggs are inspected for interior and exterior defects. This process is performed for removal of any defects such as unclean eggs, eggs with interior defects, misshapen shells (an indicator of disease), and broken shells.

9.3 Evaluation of Risk

We address the overall risk of contaminating NPLE during the breaking process with HPAI remaining on washed, sanitized shell egg surfaces as the following individual risks.

a) The risk of HPAI being present on the eggshell surface.

b) The risk of HPAI on the eggshell surface not being removed during the wash step.

c) The risk of HPAI virus on the eggshell surface not being deactivated or removed during the shell sanitizing step.

d) The risk of HPAI virus on eggshell surface causing an additional outbreak via cross-contamination of NPLE during egg breaking.

9.3.1 The Risk of HPAI being Present on the Eggshell Surface

A study by Cappucci *et al.*[23] recovered HPAI virus from the shell egg surfaces of eggs obtained from naturally infected chickens during the 1983–84 Pennsylvania-Virginia outbreaks. HPAI virus could be isolated from 20 percent (90 percent confidence interval 12-35 percent) of the shell egg samples from three infected flocks. Laboratory studies with H5N2 virus suggest the timing and frequency of contamination on the eggshell surface of eggs from infected hens is similar to that of yolk and albumen contamination. Specifically, in these data, eggs laid on the first day post-inoculation weren't contaminated and 30-45 percent of the eggs laid by infected hens were contaminated.[18,37] Considering these laboratory studies, we used the disease transmission model presented in Chapter 8 to estimate the number of eggs with a contaminated shell surface from an infected flock prior to detection.

Data on the viral titer on the eggshell surface is limited. Based on recent unpublished data (Swayne 2008)[52], we used $10^{3.6}$ EID_{50}/eggshell as the viral load on the eggshell surface before washing and sanitization.

9.3.2 The Risk of HPAI on the Eggshell Surface not being Removed During the Wash Step

In a typical commercial egg washer, eggs are passed through conveyer rollers while being cleaned with brushes and sprayed with recycled wash water containing an approved cleaning agent.[m] Regulations concerning egg washing operations (9CFR590.515, Appendix 1) require the wash water temperature to be above 32.2°C and be replaced once every 4 hours. During the washing process, there is build up of egg contents, manure, dirt and microbes in the recycled water. A higher wash water pH is preferable for reducing *Salmonella* contamination.[65] Typically, the wash water pH is in the range of 10-11.5 and the total dissolved solids are greater than 2 grams/L.[66] Although avian influenza virus might be inactivated at high pH with sufficient contact time,[67] it is unclear whether the pH of 10-11.5 in the egg washing process would cause any inactivation of HPAI virus with the short contact time (typically less than one minute). Lu *et al.*[16] found no inactivation of LPAI H7N2 virus at pH 10 and 12 with contact times of 5, 10, and 15 minutes.

HPAI virus can be inactivated by the detergents and alkalis in the approved egg washing compounds.[21] Detergents act on the lipid components of enveloped virus via their surfactant property.[68] A recent article reported a 2 to 3 log factor reduction in the surface viral titer of LPAI virus H7N7 after ten minutes of treatment with a laundry detergent (4-6 grams/L) and peroxide.[69] Alkalis can inactivate influenza virus by denaturing proteins. Abe *et al.*[70] found more than a 3-log reduction of avian influenza virus with an alkali after a contact time of 30 minutes. However, the effectiveness of alkalis can decrease in the presence of organic matter.

[m] The current approval process for cleaners and sanitizers is included as a letter from the National Supervisor of Shell Eggs (Appendix 13).

Direct data on virus inactivation under the typical operational conditions encountered in the egg washing process (pH, organic load, detergent concentration, etc.) is not available. A recent study indicates commercial washing procedures are successful in removing a significant proportion of the *Enterobacteriaceae* (higher than 50 percent).[71] Knape *et al*.[66] found a 10 to100 factor reduction of aerobic plate counts with egg washing. Musgrove *et al.*[72] found that *Salmonella* was recovered more frequently from unwashed eggs (in 15.8 percent of eggs compared with 8.3 percent in washed eggs). Given the disinfectant activity of detergents on enveloped viruses like HPAI and the empirical evidence of the reduction of bacterial contamination in commercial egg washing operations, it is reasonable to postulate that a 0.5 to 1 log inactivation of HPAI virus is achieved through egg washing.

9.3.3 The Risk of HPAI Virus on the Eggshell Surface not being Deactivated or Removed during the Shell Sanitizing Step

9.3.3.1 Egg Sanitization using Chlorine Sanitizers

FSIS regulations in 9CFR590.516 (Appendix 1) require all shell eggs be spray rinsed with potable water containing an approved sanitizer with a chlorine concentration between 100-200 ppm or its equivalent. In addition, the sanitizing rinse is required to be at a temperature greater than 32°C. The sanitizing activity of chlorine is dependent upon operational conditions such as chlorine concentration, organic load, temperature, pH, and contact time. Among these conditions, only the chlorine concentration range and sanitizing spray temperature are specified by FSIS regulations. The variability in the other conditions such as pH and contact time may result in varying degrees of inactivation.

We developed an @RISK simulation model to incorporate the uncertainties of these conditions into our estimate of HPAI virus reduction on shell egg surfaces due to chlorine sanitizer spray. The model is presented below with additional details on the model design included as Appendix 5.

(i) Definitions:

Exposure time (t) - The time for which the eggshell surface is exposed to the sanitizing rinse.

Ct values (Ct) - The chlorine concentration (*C*) multiplied by exposure time *(t)*.[n] *Ct* is a frequently used measure of exposure to disinfecting agents.

Computed Ct values - The output *Ct* values from our simulation of the sanitization process.

Chlorine decay rate (k) - The exponential decay rate constant for chlorine concentration. We consider that the sanitizing rinse chlorine concentration decays exponentially due to the organic matter on the eggshell surface.

[n] *Ct* values indicate the chlorine concentration (in mg/L, or ppm) and time (in minutes).[117] *Ct* values can reflect various combinations of chlorine concentration and time and can be considered equivalent. For example, a *Ct* value of 10 could indicate an exposure of 10 minutes to a 1-ppm concentration or an exposure of 1 minute to a 10-ppm concentration.

(ii) Assumptions:

- Eggs have been washed and cleaned prior to sanitization, leaving no soil or feces on the shell egg surface.
- HPAI virus is present on the shell egg surface.[o]
- Eggs are in contact with the sanitizing spray for 1 to 8 seconds.[p]
- Chlorine concentration is maintained between 100-200 ppm.
- Hepatitis A virus is less sensitive to inactivation by chlorine than HPAI virus.
- The *Ct* values and chlorine decay rates are reasonable values to use for egg processing operations.

(iii) Simulation Model and Parameters:

In the simulation model, we first estimated a probability distribution for *Ct*, using exposure times provided by industry experts and chlorine concentration specified in regulations. We then compared *Ct* values from the simulation output with values required to achieve a 1000 factor inactivation as reported in various sources.[73] The values used for the key parameters of the simulation model are provided below. Details of the simulation model are provided in Appendix 5.

Parameter	*Uniform distribution range*	*Source*
Initial chlorine concentration (C_o)	100 – 200 ppm	9CFR590.516
Exposure time (t)	1 – 8 seconds	Expert opinion[74]
Chlorine decay (k)	1.24 – 2.33 min^{-1}	Rice *et al.*, 2007[73]

(iv)
Simulation Results:

1) The chlorine concentration time Ct:

The mean *Ct* value from the simulation was 11.9 mg-min/L, falling between 4.2 and 19.9 mg-min/L with 90 percent probability (Figure 6).

2) Degree of HPAI virus inactivation with computed *Ct* values:

The chlorine activity for a given *Ct* is dependent on the pH and temperature. Higher pH values cause the less potent ionic form of chlorine (OCl^-) to predominate in solution, making virus inactivation less efficient (*Ct* value increases). Conversely, higher

[o]The amount of virus present was not specified in the model, as there has been no research on this issue. However, HPAI has been detected on shell eggs.[23]
[p] The 8-second value is considered by an industry expert to be a minimum value.[74]

temperatures increase virus inactivation efficiency, decreasing the *Ct* value. As previously mentioned, the sanitizing rinse temperature is required to be above 32°C.

During shell egg sanitization, the effective shell egg surface pH would be between the pH of wash water (typically higher than 10) and the pH of the sanitizer spray. We model the scenarios where the shell egg surface is at neutral pH or high pH separately as follows.

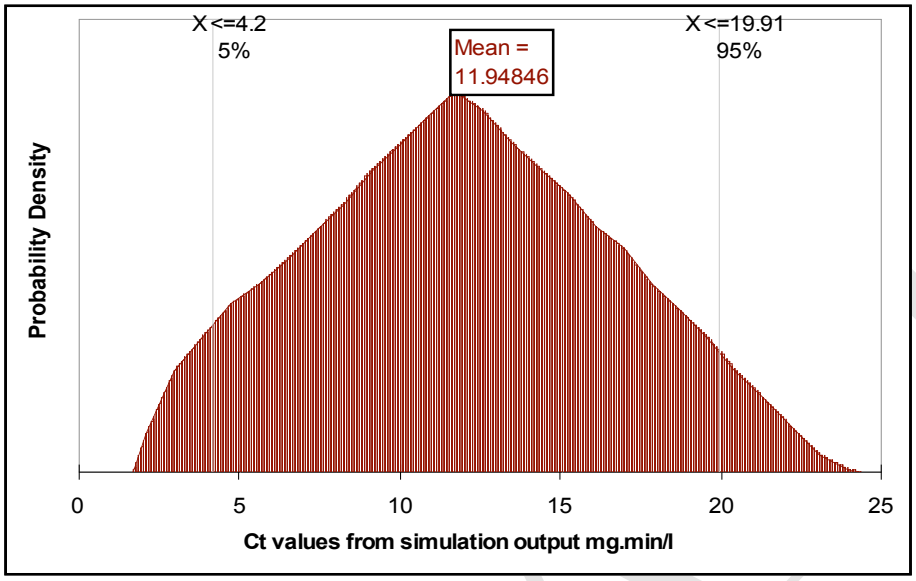

Figure 6. Distribution of computed *Ct* values from simulation output.

SCENARIO A: Neutral pH, chlorine sanitizer

For a chlorine sanitizer at pH 7 to 8, all simulated *Ct* values exceed previously reported *Ct* values for chlorinated sanitizers[73] that achieved a 3-log inactivation of HPAI virus at 5°C. On average, the *Ct* from simulation was greater than that required for 3-log inactivation by a factor of 24. The simulated *Ct* values are also higher than those reported for 4-log inactivation of other viruses such as Hepatitis A (a more chemical resistant, non-enveloped virus[75]) at pH 6 and 5°C. Furthermore, the required *Ct* values at the sanitizing rinse temperature of 32°C would likely be lower than those from the above experiments conducted at 5°C. These results indicate a 3-log inactivation of virus is achieved when the effective pH is less than 8.

SCENARIO B: High pH, chlorine sanitizer

The effectiveness of chlorine as a sanitizer can decrease significantly at pH greater than 10. To our knowledge, experimental data concerning chlorine inactivation of HPAI virus at pH ≥ 10 are not available. We used the following approaches to evaluate HPAI virus inactivation high pH: 1) Using correction factors for pH and temperature to extrapolate from the experimental data on HPAI virus at pH 7-8, and 2) Using data on Hepatitis A virus at high pH as a proxy for HPAI virus.

Effect of high pH on Ct value:
From studies of chlorine inactivation of other viruses,[75,76,77] the *Ct* value required for inactivation of viruses at a pH of 10 is 5 to 20 times higher than that required at a pH of 7 or 8.

Effect of high temperature on Ct value:
Experimental data concerning chlorine inactivation of HPAI virus at 32°C is also not available to our knowledge. However, the EPA guidance manual (1991)[95] suggests a 2-log decrease in *Ct* values for inactivation of other viruses for every 10°C increase in temperature. For example, a *Ct* value of 1 mg-min/L is sufficient to achieve a 4 log inactivation of Hepatitis A at 25°C and pH 10,[77] whereas a Ct value above 20 mg-min/L was required for a 3-log inactivation of Hepatitis A virus at 5°C and pH 10.[78] As Hepatitis A is a non-enveloped virus exhibiting less sensitivity to chlorine inactivation than the enveloped HPAI virus, we utilized available Hepatitis A virus inactivation data as a conservative estimate for HPAI virus inactivation when subjected to similar conditions.

Using the above correction factors and Hepatitis A virus as a proxy for HPAI virus, we conclude that the *Ct* value required for 3-log inactivation of HPAI virus at 25°C and a pH of 10 is between 1-5 mg-min/L. These *Ct* values are roughly 1 to 5 times higher than the reported *Ct* values for chlorine inactivation of HPAI virus in experiments performed at 5°C and pH 8.[73]

3) Computed *Ct* values compared to required *Ct* values:

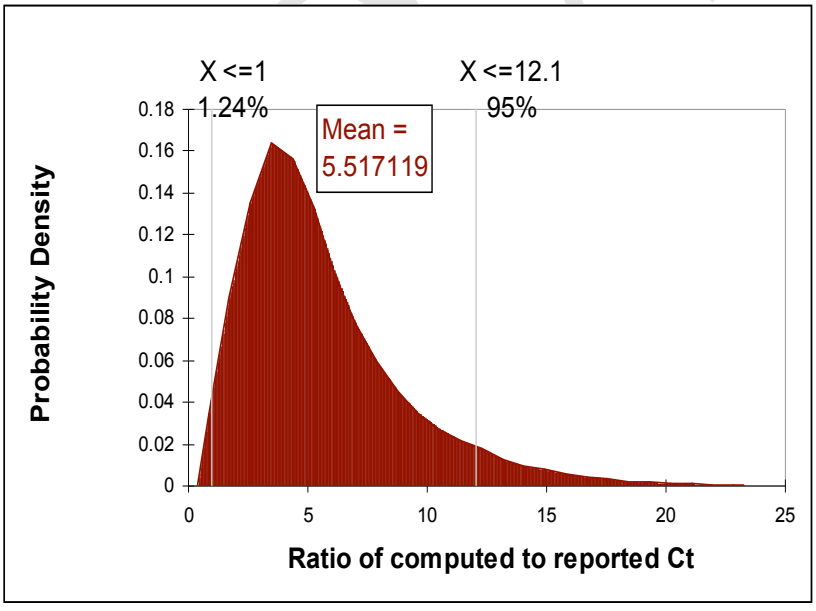

Figure 7. Ratio of computed and required *Ct* values.

We calculated the ratio between the computed *Ct* values (results section 1) and the *Ct* values required for 3-log inactivation of HPAI virus at pH 10, 25°C (results section 2), and simulated a distribution of the values (Figure 7). The simulation results show that

there is a 97 percent probability that the computed *Ct* value is higher than that required for 3-log inactivation of HPAI virus (i.e. the ratio is > 1), and on average the computed *Ct* value is 5 times higher than the *Ct* value required for 3-log inactivation of HPAI virus in the high pH conditions reflective of industry conditions.

9.3.3.1 Egg Sanitization Using Non-Chlorine Sanitizers

As required under 9CFR590.516 (Appendix 1), all shell eggs that are used for breaking must be spray rinsed with potable water containing an approved sanitizer with a chlorine concentration between 100-200 ppm or its equivalent. Although chlorine compounds are most commonly used in industry, the FDA has also approved several non-chlorine compounds such as quaternary ammonium and iodine compounds. As of the writing of this risk assessment, FSIS does not review new compounds for use in official processing plants but rather demands that the compounds used be unaltered since their initial approval.[q] Non-chlorine sanitizers should thus be cleared through FSIS and deemed acceptable alternatives to 100-200 ppm chlorine. We do not model the use of non-chlorine sanitizers in this assessment.

9.3.3.1 Summary

Experimental data testing HPAI virus H5N1 inactivation in allantoic fluid at neutral sanitizer pH (7-8) shows the viral load on the eggshell to be reduced by a factor of at least 1,000 (a 3-log reduction).[73] Efficacy of HPAI virus reduction in conditions reflective of industry (pH 10 sanitizer, high temperature) has not been tested to our knowledge. At a sanitizer pH of 10, utilizing data on inactivation of other indicator viruses, we conclude that there is a 95 percent chance that a 1000 factor inactivation of HPAI virus on eggshells is achieved.

9.3.4 The Risk of HPAI Virus on Eggshell Surface Causing an Additional Outbreak via Cross-contamination of NPLE during Egg Breaking

In this section, we discuss the risk of a secondary HPAI outbreak via NPLE contaminated with HPAI virus from eggshells during breaking operations. Although a plausible pathway for chickens to be exposed to NPLE has not been identified and there are no reports in the literature that NPLE has spread HPAI, we conservatively assume that such a pathway exists for the purposes of this analysis.

Based on some recent unpublished data (Swayne 2008),[52] we used $10^{3.6}$ EID_{50}/eggshell as the viral load on the eggshell surface. Any virus introduced into the NPLE due to cross-contamination from eggshell surfaces is likely diluted several fold due to mixing with the internal contents of virus-free eggs. Given a scenario where HPAI virus from the shell surfaces of all contaminated eggs from a potentially infected flock are mixed into a quarter tanker containing NPLE (1,133 gallons); and given an original virus load of $10^{3.6}$ EID_{50}/eggshell (without any virus inactivation due to sanitization), cross-contamination from the egg shell surface would represent less than 0.1 EID_{50}/ml increase in the viral titer of the NPLE. As shown in the dose response relationship presented in Chapter 8, this

[q]The current approval process for cleaners and sanitizers is included as a letter from the National Supervisor of Shell Eggs (Appendix 13).

viral load increase represents a negligible increase in the probability of causing infection. If the initial HPAI virus load of $10^{3.6}$ EID_{50}/eggshell were reduced by 3-logs via washing and sanitization, then by using similar calculations, cross-contamination from egg shell surfaces would increase the viral titer in a truckload of NPLE by less than 10^{-5} EID_{50}/ml.[r]

From this analysis, considering the dilution of any virus introduced into NPLE via cross-contamination from eggshells during breaking, the risk of a secondary HPAI outbreak due to cross-contamination during breaking is negligible even if washing and sanitization are not effective in inactivating the virus. The 3-log reduction due to washing and sanitization would further reduce the risk of a secondary HPAI outbreak due to cross contamination from eggshells during breaking to negligible levels.

9.4 Conclusion

The likelihood that there will be sufficient infective HPAI virus particles present on eggshells after washing and sanitization to cause an additional HPAI outbreak through cross-contamination of NPLE during the breaking process is negligible. Based on the simulation, there is a 95 percent chance that the chlorine concentrations specified in 9CFR590.516 (Appendix 1)[31] inactivate HPAI virus by a factor of 1,000.

[r] This is an increase of less than 1/100,000.

10. Risk of Vehicles Carrying NPLE from an Infected but Undetected Premises Resulting in HPAI Spread to another Poultry Premises

In this chapter, we evaluate the risk of HPAI spread to another poultry premises via cross-contamination from the vehicle or the truck driver transporting the NPLE.

Risk of Vehicles Carrying NPLE from an Infected, Undetected Premise Resulting in HPAI Spread to another Poultry Facility

• **Risk Factors:** Contamination of transport vehicle and/or tanker, inadequate cleaning and/or disinfection, cross-contamination during movement, and failure of biosecurity practices for personnel.

• **Current Preventive Measures:** Tankers inspected and sealed on-site by FSIS personnel as outlined in 9CFR590.410. Requirement that the grounds and facility of a food processing plant be kept in a sanitary condition to prevent the contamination of food as outlined in 9CFR590 and 21CFR110.20.

• **Additional Preventive Measures** (to be implemented by industry during an outbreak; see Appendix 14 for suggested protocols)**:** Cleaning and disinfection of the truck/tanker exterior and interior after unloading NPLE, and personnel biosecurity requirements.

• **Overall Risk:** Negligible

10.1 Background Information

A review of the scientific literature and response planning documents indicate that movements of contaminated equipment, vehicles and personnel between poultry premises is the primary means of spreading HPAI.[79,80] Feed and rendering vehicles were associated with the risk of spread of AI virus due to their movement among poultry farms and congregation at common facilities.[80] However, there have been no reports of vehicles transporting NPLE causing infection at another poultry premises.[25,81-84] Vehicles transporting egg products from processing facilities use different docking areas with a greater separation from the henhouse compared to the docks used by henhouse operations and related vehicles such as feed and rendering trucks.

Nevertheless, the potential risk of cross-contamination when vehicles transport NPLE to a pasteurization facility with poultry on the premises needs to be evaluated. Existing preventive measures include Federal regulations and industry GMPs. In the event of an HPAI outbreak, various movement and control measures will be implemented by various State and Federal authorities as well as by the egg industry.

In this chapter, we evaluate the existing and planned preventive measures for their ability to reduce the risk of HPAI virus spreading from the vehicle or driver to susceptible poultry.

10.2 Preventive Measures

10.2.1 Current Preventive Measures

FSIS regulations require that NPLE may only be shipped for pasteurization or heat treatment in sealed cars or trucks with an accompanying certificate. Existing preventive measures applicable for such movement include Federal regulations and industry GMPs.

General regulatory requirements for FSIS-inspected premises and movements involving them are specified in 9CFR325.1 (Appendix 12), 9CFR590.410, and 9CFR590.500 (Appendix 1). The following bullet points summarize these regulations:

a) All NPLE must be sent to processing plants for pasteurization (see Appendix 9).

b) All vehicles hauling liquid egg products that enter a FSIS-continuously-inspected plant are inspected by FSIS regulatory officials for seal integrity, leaks, the presence of labels and correct documentation. For all conveyances leaving the premises, the following applies:

- For outgoing tankers carrying NPLE, the surfaces with which the egg comes into contact are inspected by FSIS personnel as specified in 9CFR325.1 and 9CFR590.410, and the tanker is sealed and labeled to insure integrity.
- For other vehicles carrying packaged NPLE, industry personnel generally inspect interiors.

c) Means of conveyance must be clean and free of any potential contaminants.

d) All egg products must bear an official label before leaving FSIS-inspected premises, and containers moving bulk shipments between FSIS-inspected premises must be sealed (9CFR590.410).

10.2.2 Preventive Measures during an Outbreak

In the event of an HPAI outbreak, various movement and control measures will be implemented by local, State and/or Federal authorities as part of the disease control effort.[79,85,86] Vehicle cleaning and disinfection procedures are included in these measures.

APHIS and the egg product industry recognize the potential risk from vehicles/conveyances and the importance of preventing this risk. The UEP/UEA – USDA APHIS VS Movement Control Model Plan thus instigates regulation of the movement of vehicles transporting egg products during an outbreak (Appendix 11).[87]

The UEP/UEA – USDA APHIS VS Movement Control Model Plan includes cleaning and disinfecting all vehicles transporting egg products that move into,[s] within or outside of a control area. The cargo interior and exterior of the movement vehicle must be cleaned before a permit is issued. In addition, tires and wheel wells of vehicles moving NPLE must be cleaned and disinfected before leaving premises within a control area. The driver will not be allowed outside of the cab or the cab interior must also be cleaned and disinfected. Cleaning and disinfection requires the use of an EPA approved disinfectant[88] with efficacy against AI virus[t] following a standard protocol that will require vehicle interior and exterior cleaning and disinfection. The movement control plan does not require vehicles to be cleaned and disinfected on the premises, but it does require that cleaning and disinfection be done before a movement permit is issued.

Specific protocols for cleaning and disinfection exist and new ones may need to be developed depending on the circumstances. For example, vehicle cleaning and disinfection guidelines are given in the November 2005 Draft National Animal Health Emergency Management System (NAHEMS) Cleaning and Disinfection Operational Guidelines from the USDA,[86] FSIS Safety and Security Guidelines,[89] and published literature.[90-92]

10.3 Evaluation of Risk

The potential risks associated with the transportation of NPLE are:

a) Risk of the vehicle or the driver leaving an NPLE facility being contaminated with HPAI virus.

b) Risk of release of NPLE due to "splash" while filling trucks or during transport due to poorly seated seals.

The UEP/UEA – USDA APHIS VS Movement Control Model Plan contains provisions for movement controls and cleaning and disinfection during an outbreak. These plans are similar to ones developed to control the Exotic Newcastle Disease (END) outbreak in California[55] and were found to be effective in that situation.

There is no evidence to date that vehicles moving NPLE have been contaminated with or contributed to the spread of HPAI virus during an outbreak. The majority NPLE is destined to further processing plants which mostly do not have poultry on the premises.[57] Vehicles transporting egg products from processing facilities use different docking areas with greater separation from the henhouse compared to the docks used by henhouse operations and related vehicles, such as feed and rendering trucks, etc. Specifically, even in in-line egg processing facilities, the egg product-related docks are generally separated from the henhouse by multiple processing rooms such as the egg washing room and the

[s] Vehicles carrying egg products into a control area will be subject to cleaning and disinfection when they arrive at their delivery points.

[t] The EPA web page referenced in the text states the following: "Although there are no antimicrobial products registered specifically against the H5N1 subtype of **avian influenza A** viruses, EPA believes based on available scientific information that the currently registered **avian influenza A** products, when applied in strict accordance with the label directions, will be effective against the H5N1 strain."

breaking room. Furthermore, regulations require the egg breaking room to be at filtered positive air pressure limiting the potential for any hypothetical risk via aerosol contamination (9CFR590.520). Finally, the movement control plan requires cleaning and disinfection of the cargo interior, exterior, tires and wheel wells of the transportation vehicles.

Drivers moving NPLE generally do not have direct contact with live bird production and thus have limited opportunity to become contaminated with HPAI virus. The Draft Summary of the National Highly Pathogenic Avian Influenza Response Plan[1] outlines personal protective equipment (PPE) and biosecurity procedures to mitigate the risk of drivers carrying the HPAI virus off of an infected or contaminated site. The UEP/UEA – USDA APHIS VS Movement Control Model Plan requires that the driver not be allowed outside the cab or the cab interior must also be cleaned and disinfected. The Incident Command and movement permitting system along with FSIS provide for review and compliance in addition to industry biosecurity measures in practice.

There may be a pathway for NPLE to escape the cleaned and disinfected tanker through spillage at loading, unloading, or through improper sealing of the dome lid on top of the tanker. Spillage at loading or unloading, while being a potentially significant amount (>500 pounds), would occur in a controlled environment. Here, NPLE is already exposed to the environment due to the dome lid having to be opened during this process. Standard operating procedures and GMPs in the event of any spill include cleaning and disinfection of surfaces, so cross-contamination into other areas is also mitigated. Furthermore, the live bird production area is segregated from the loading and/or unloading areas, so there is no exposure to live bird production.

Lastly, if spillage during transport were to occur due to an unsecured dome lid, spillage would likely occur over the entire length of the trip. Spillage would be in the form of egg splash into the dome area which has a containment dike. Once egg enters the dike, it would then drip down the sides of the tanker. The egg dries or freezes to the sides of the tanker when this occurs. Due to heightened awareness within the industry in response to the U.S. Department of Homeland Security's recent focus on food security, this type of splash due to human error is extremely rare. When there is evidence of spillage from an unsecured dome lid, an additional investigation into the wholesomeness of the egg prior to unloading occurs, including interviews with the driver. The amount of egg lost is generally very small (<100 pounds) and could be determined through the normal truck scaling/weighing process. In a worst-case scenario, if a tanker were to lose 1 percent or 480 pounds over the course of a 60-mile trip, this would be 8 pounds per mile.

From chapter 8, the estimated virus titer in NPLE from an infected and undetected flock is relatively low and less than 1 percent of the chickens exposed to the NPLE would be infected. From the above discussion, the chance that a susceptible flock would be exposed to NPLE via transportation vehicle or the driver is unlikely. Therefore, we conclude that the overall risk of a susceptible flock getting infected due to cross-contamination via vehicle or driver transporting the NPLE is negligible.

10.4 Conclusion

Most vehicles and drivers transporting NPLE from a processing facility will not come into contact with susceptible species. The UEP/UEA – USDA APHIS VS Movement Control Model Plan has provisions for cleaning and disinfection of the cargo interior and exterior of the truck, the driver, and the cab interior. Lastly, spillage (which is extremely rare) from a vehicle will either occur in a controlled environment not exposed to live production or will occur over the course of several miles in a very small amount so that exposure is diluted. The likelihood that the movement of a vehicle carrying NPLE would mechanically transmit HPAI virus is negligible if the applicable regulations and the UEP/UEA – USDA APHIS VS Movement Control Model Plan is followed.

11. Summary

The objective of this risk assessment was to evaluate the risk that the movement of nonpasteurized liquid egg (NPLE) during a highly pathogenic avian influenza (HPAI) outbreak in the poultry egg industry in the United States will result in HPAI infection on another poultry premises. The assessment is applicable to FSIS-inspected NPLE processing facilities that may or may not have laying hens on-site and may or may not accept eggs from other egg producers.

To estimate the risk of HPAI spread to susceptible poultry via contaminated NPLE from an infected, undetected premises, we first estimated the concentration of HPAI virus infectivity in a tanker of NPLE leaving the premises. We then evaluated potential pathways by which susceptible poultry may be exposed to NPLE transported from the infected, undetected premises. Finally, we estimated the probability of infection in a chicken exposed to NPLE given the estimated concentration of HPAI virus infectivity in the NPLE.

The HPAI prevalence in an infected flock changes over time, increasing exponentially until detection. The number of contaminated eggs from an infected but undetected flock and the resulting levels of viral contamination that might occur in NPLE vary with the time to disease detection (i.e. the level of surveillance effort). Our simulation models predict that the number of contaminated eggs used for producing NPLE that may be moved from the premises before infection is detected is 12 (90 percent probability interval 0-40) when the active surveillance protocol described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan is followed.

Given the estimated number of internally contaminated eggs from an infected but undetected flock and considering the dilution of the viral titer in NPLE due to mixing with the product from virus-free eggs, we estimated that the mean HPAI viral titer in the a tanker of NPLE is 1.15 EID_{50}/ml (90% probability interval 10^{-3}- 3.71 EID_{50}/ml).

We also evaluated whether HPAI virus from the shell surface of washed, sanitized eggs would present a risk for HPAI spread via cross-contamination of NPLE during breaking. We found that the egg washing and sanitizing procedures as specified in 9CFR590.516 would inactivate HPAI virus on the eggshell surface by a factor of 1,000. In addition, considering the dilution of any virus introduced into NPLE from eggshells, the risk of a secondary HPAI outbreak due to cross-contamination from the shell surface of contaminated eggs is negligible even if washing and sanitization are not effective in inactivating the virus.

Movement of NPLE has never been implicated as a cause of secondary spread of HPAI to susceptible poultry. When NPLE is transported to off-line pasteurization facilities, there is no plausible pathway for exposure of NPLE to susceptible poultry. For the scenario where NPLE is transported to an in-line pasteurization facility, we evaluated the possibility that NPLE accidentally released into the facility during handling/storage prior to pasteurization could be transmitted to the henhouse via personnel or pests. We found

that the likelihood that susceptible poultry are exposed to HPAI virus contaminated NPLE at in-line pasteurization facilities is very low if all applicable regulations described in 9CFR590 and 21CFR110 as well as good manufacturing practices are strictly followed. We estimated that the probability of infection in a chicken exposed to one ml of NPLE orally is 0.2 percent (90 percent interval 10^{-4}-0.6 percent) based upon the estimated HPAI viral titer in NPLE and dose response analysis.

Considering the very low likelihood that susceptible chickens are exposed to NPLE and the low probability of infection in an exposed chicken, we conclude that the risk of NPLE contamination from HPAI virus within the egg contents resulting in infection of a susceptible flock is negligible.

Most vehicles and drivers transporting NPLE from a processing facility will not come into contact with susceptible species. The UEP/UEA – USDA APHIS VS Movement Control Model Plan has provisions for cleaning and disinfection of the cargo interior and exterior of the truck, the driver, and the cab interior. The risk that the movement of a vehicle carrying NPLE would mechanically transmit HPAI virus is negligible if the applicable regulations and the UEP/UEA – USDA APHIS VS Movement Control Model Plan is followed. The estimates of key variables and probabilities for the risk assessment are summarized in Table 7.

Table 7. Estimates of key variables and probabilities for the risk that movement of NPLE from an HPAI infected but undetected flock will result in infection of a susceptible flock.

Parameter Description	*Value*
Maximum daily number of contaminated eggs used to produce NPLE that may be moved from an infected but undetected flock following the active surveillance protocol.	12 (90 percent PI 0-40) eggs/day
Expected HPAI viral titer in a 48,000 lb tanker of NPLE leaving an egg breaking facility.	1.15 EID_{50}/ml (90% probability interval 10^{-3}- 3.71 EID_{50}/ml)
Degree of HPAI virus inactivation with egg washing and sanitizing operations as specified in 9CFR590.	1000 factor or 3-log reduction with 95 percent probability
Estimated amount of increase in the viral titer in a 48,000 lb tanker of NPLE due to cross-contamination from shell surface of washed and sanitized eggs.	less than 10^{-5} EID_{50}/ml
Risk of a secondary HPAI outbreak due to cross-contamination from the shell surface of washed and sanitized eggs.	negligible
Likelihood that susceptible poultry are exposed to HPAI virus contaminated NPLE moved from the egg breaking facility.	negligible
Probability of infection in a chicken exposed to one ml of NPLE via the oral route.	0.2 percent (90 percent interval 10^{-4}-0.6 percent)
Risk of NPLE contamination with HPAI virus from the contents of eggs from an infected but undetected flock resulting in infection of a susceptible flock.	negligible
Risk that the movement of a vehicle carrying NPLE from an infected but undetected flock would mechanically transmit HPAI virus.	negligible

12. Overall Conclusions

The objective of this assessment was to estimate the risk that the movement of nonpasteurized liquid egg products into, within, and outside of a control area during a highly pathogenic avian influenza outbreak in the poultry industry in the United States will result in HPAI infection of another poultry premise. With respect to the major component risks that were analyzed, this document concludes the following:

a) If the active surveillance protocol described in the movement control protocol is followed, the probability that nonpasteurized liquid egg made with eggs from an infected but undetected flock is infectious to an exposed chicken is 0.2 percent (90 percent interval 10^{-4}-0.6 percent).

b) The risk of NPLE contamination with HPAI virus from the contents of eggs from an infected but undetected flock resulting in infection of a susceptible flock is *negligible*.

c) Risk of cross-contamination of nonpasteurized liquid egg via virus on the shell surface of washed and sanitized eggs resulting in infection of a susceptible flock is *negligible.*

d) If movement controls and all other recommended preventive and biosecurity measures described herein are strictly followed, the risk of an HPAI outbreak in another poultry production premises due to the movement of commercial vehicles carrying nonpasteurized liquid egg is *negligible*.

It is concluded that the overall risk of moving nonpasteurized liquid egg into, within, and outside of a control area during an HPAI outbreak is *negligible*.

However, it should be remembered that:

a) It was assumed that the processing facility is in compliance with applicable laws and regulations.

b) This assessment is based on current (December 2008) information and will need to be reviewed and revised as circumstances warrant.

c) The assessment aids, but does not replace, the judgment of on-scene officials.

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Appendix 1. Selected portions of 9CFR590

Bold-type sections are included in this Appendix

Title 9: Animals and Animal Products
PART 590—INSPECTION OF EGGS AND EGG PRODUCTS
(EGG PRODUCTS INSPECTION ACT)

Scope of Inspection

§ 590.20 Inspection in accordance with methods prescribed or approved.
§ 590.22 Basis of service.
§ 590.24 Egg products plants requiring continuous inspection.
§ 590.26 Egg products entering or prepared in official plants.
§ 590.28 Other inspections.

Exemptions

§ 590.100 Specific exemptions.
§ 590.105 Suspension or termination of exemptions.

Identifying and Marking Product

§ 590.410 Shell eggs and egg products required to be labeled.
§ 590.411 Requirement of formulas and approval of labels for use in official egg products plants.
§ 590.412 Form of official identification symbol and inspection mark.
§ 590.414 Products bearing the official inspection mark.
§ 590.415 Use of other official identification.
§ 590.417 Unauthorized use or disposition of approved labels.
§ 590.418 Supervision of marking and packaging.
§ 590.419 Reuse of containers bearing official identification prohibited.

Sanitary, Processing, and Facility Requirements

§ 590.500 Plant requirements.
§ 590.502 Equipment and utensils; PCB-containing equipment.
§ 590.504 General operating procedures.
§ 590.506 Candling and transfer-room facilities and equipment.
§ 590.508 Candling and transfer-room operations.
§ 590.510 Classifications of shell eggs used in the processing of egg products.
§ 590.515 Egg cleaning operations.
§ 590.516 Sanitizing and drying of shell eggs prior to breaking.
§ 590.520 Breaking room facilities.
§ 590.522 Breaking room operations.
§ 590.530 Liquid egg cooling.
§ 590.532 Liquid egg holding.
§ 590.534 Freezing facilities.
§ 590.536 Freezing operations.
§ 590.538 Defrosting facilities.
§ 590.539 Defrosting operations.
§ 590.540 Spray process drying facilities.
§ 590.542 Spray process drying operations.
§ 590.544 Spray process powder; definitions and requirements.
§ 590.546 Albumen flake process drying facilities.
§ 590.547 Albumen flake process drying operations.
§ 590.548 Drying, blending, packaging, and heat treatment rooms and facilities.
§ 590.549 Dried egg storage.
§ 590.550 Washing and sanitizing room or area facilities.
§ 590.552 Cleaning and sanitizing requirements.
§ 590.560 Health and hygiene of personnel.

Scope of Inspection

§ 590.24 Egg products plants requiring continuous inspection.

No plant in which egg products processing operations are conducted shall process egg products without continuous inspection under these regulations, except as expressly exempted in §590.100.

§ 590.26 Egg products entering or prepared in official plants.

Eggs and egg products processed in an official plant shall be inspected, processed, marked, and labeled as required by these regulations. Egg products entering an official plant shall have been inspected, processed, marked, and labeled as required by these regulations.

Appendix 1: Selected portions of 9CFR590 (continued)

Exemptions

§ 590.100 Specific exemptions.

The following are exempt to the extent prescribed as to the provision for continuous inspection of processing operations in section 5(a) of the Act: *Provided,* That the conditions for exemption and provisions of these regulations are met:

(a) [Reserved]

(b) Subject to the approval of the Administrator as provided in §§590.600 through 590.670, the processing of egg products without continuous inspection at any plant where the facilities, sanitation, and operating procedures are the same as are required in this part for official plants and where the eggs received or used in the manufacture of egg products contain no more restricted eggs than are allowed by the official standards for U.S. Consumer Grade B shell eggs, and the egg products processed at such plant;

(c)–(d) [Reserved]

(e) The processing and sale of egg products by any poultry producer from eggs of his own flock's production when sold directly to a household consumer exclusively for use by such consumer and members of his household and his nonpaying guests and employees;

(f) [Reserved]

(g) The processing in nonofficial plants, including but not limited to bakeries, restaurants, and other food processors, without continuous inspection, of certain categories of food products which contain eggs or egg products as an ingredient, and the sale and possession of such products: *Provided,* That such products are manufactured from inspected egg products processed in accordance with this part or from eggs containing no more restricted eggs than are allowed in the official standards for U.S. Consumer Grade B shell eggs;

[36 FR 9814, May 28, 1971, as amended at 40 FR 20057, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 63 FR 69971, Dec. 17, 1998]

Identifying and Marking Product

§ 590.410 Shell eggs and egg products required to be labeled.

(a) All shell eggs packed into containers destined for the ultimate consumer shall be labeled to indicate that refrigeration is required, e.g., “Keep Refrigerated,” or words of similar meaning.

(b) Containers and portable tanks of edible egg products, prior to leaving the official plant, shall be labeled in accordance with §§590.411 through 590.415 and shall bear the official identification shown in Figure 2 of §590.412 or Figure 3 or 4 of §590.415. Bulk transport shipments of liquid pasteurized egg products to nonofficial outlets need not be sealed. Bulk shipments of liquid egg products transported from one official plant to another shall be sealed and accompanied by an official certificate.

[40 FR 20058, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 63 FR 45675, Aug. 27, 1998]

§ 590.414 Products bearing the official inspection mark.

Egg products which are permitted to bear the inspection mark shall be processed in an official plant from edible shell eggs or other edible egg products and may contain other edible ingredients. The official mark shall be printed or lithographed and applied as a part of the principal display panel of the container but shall not be applied to a detachable cover.

§ 590.415 Use of other official identification.

Other official identification as shown in this section shall be printed or lithographed and applied as a part of the principal display panel, but shall not be applied to a detachable cover. The plant number may be omitted from the identification if applied elsewhere on the container's principal display panel or other prominent location and preceded by the letter “P” or the word “plant”. Such products shall meet all requirements for egg products which are permitted to bear the official inspection mark shown in §590.412, except for pasteurization, heat treatment, or other such methods of treatment approved by the Administrator. Such products shall not be released into consuming channels until they have been subjected to pasteurization, heat treatment, or other approved methods of treatment.

(a) All **nonpasteurized** egg products, except as provided in paragraph (b) of this section, shipped from an official plant in packaged form shall be marked with the identification set forth in Figure 3 of this section. After pasteurization or treatment, the product may bear the official inspection mark as shown in §590.412.

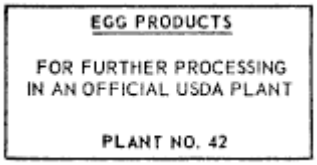


FIGURE 3.

(b) All **nonpasteurized** egg products, containing 10 percent or more added salt, shipped from an official plant in packaged form to an acidic dressing manufacturer shall be marked with the identification set forth in Figure 4 of this section.

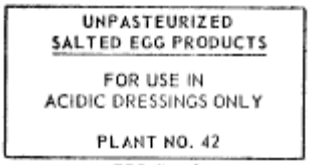


FIGURE 4.

[36 FR 9814, May 28, 1971; 36 FR 10841, June 4, 1971. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 60 FR 49170, Sept. 21, 1995]

Sanitary, Processing, and Facility Requirements

§ 590.500 Plant requirements.

(a) The plant shall be free from objectionable odors, dust, and smoke laden air.

(b) The premises shall be free from refuse, rubbish, waste, and other materials and conditions which constitute a source of odors or a harbor for insects, rodents, and other vermin.

(c) The buildings shall be of sound construction and kept in good repair to prevent the entrance or harboring of vermin.

(d) Rooms shall be kept free from refuse, rubbish, waste materials, odors, insects, rodents, and from any conditions which may constitute a source of odors or engender insects and rodents. Materials and equipment not currently needed shall be handled or stored in a manner so as not to constitute a sanitary hazard.

(e) Doors and windows that open to the outside shall be protected against the entrance of flies and other insects. Doors and windows serving rooms where edible product is exposed shall be so designed and installed to prevent the entrance of dust and dirt. Doors leading into rooms where edible product is processed shall be of solid construction and such doors, other than freezer and cooler doors, shall be fitted with self-closing devices.

(f) Doors and other openings which are accessible to rodents shall be of rodent-proof construction.

(g) There shall be an efficient drainage and plumbing system for the plant and premises. Drains and gutters shall be properly installed with approved traps and vents. The sewage system shall have adequate slope and capacity to readily remove waste from the various processing operations. Floor drains shall be equipped with traps, and constructed so as to minimize clogging. In new or remodeled construction the drainage systems from toilets and laboratories shall not be connected with other drainage systems within the plant.

(h) The water supply (both hot and cold) shall be ample, clean, and potable, with adequate pressure and facilities for its distribution throughout the plant or portion thereof utilized for egg processing and handling operations and protected against contamination and pollution. A water report, issued under the authority of a

State or municipal health agency, certifying to the potability of the water supply shall be obtained by the applicant and furnished to the Administrator whenever such report is required by the Administrator.

(i) The floors, walls, ceiling, partitions, posts, doors, and other parts of all structures shall be of such materials, construction, and finish to permit their ready and thorough cleaning. The floors and curbing shall be watertight.

(j) Each room and each compartment in which any shell eggs or egg products are handled or processed shall be so designed, constructed, and maintained to insure processing and operating conditions of a clean and orderly character, free from objectionable odors and vapors, and maintained in a clean and sanitary condition.

(k) Every precaution shall be taken to exclude dogs, cats, and vermin (including, but not being limited to, rodents and insects) from the plant, or portion thereof utilized in which shell eggs or egg products are handled or stored.

(l)(1) There shall be a sufficient number of adequately lighted dressing rooms and toilet rooms, ample in size, conveniently located and separated from the rooms and compartments in which shell eggs or egg products are handled, processed, or stored. The dressing rooms and toilet rooms shall be separately ventilated, and shall meet all requirements as to sanitary construction and equipment.

(2) The following formula shall serve as a basis for determining the toilet facilities required:

Persons of same sex	Toilet bowls required
1 to 15, inclusive	1
16 to 35, inclusive	2
36 to 55, inclusive	[1]3
56 to 80, inclusive	[1]4
For each additional 30 persons in excess of 80	[1]1

[1]Urinals may be substituted for toilet bowls but only to the extent of one-third of the total number of bowls stated.

(m) Lavatory accommodations (including, but not being limited to, hot and cold running water, single service towels, and soap which does not impart an odor which interferes with accurate evaluation of the product) shall be placed at such locations in the plant to assure cleanliness of each person handling any shell eggs or egg products. The hand washing facilities in the processing areas shall be operated by other than hand operated controls and the drains shall be trapped and connected to the plumbing system.

(n) Suitable facilities for cleaning and sanitizing utensils and equipment shall be provided at convenient locations throughout the plant.

(o) Refuse rooms shall be provided for the accumulation and storage of shells, trash, and other refuse. They shall be separate rooms completely enclosed without doorways opening into breaking rooms or rooms where egg products or packaging materials are handled or stored and have concrete floors with approved drains, facilities for cleaning, and an approved exhaust system vented to the outside. Alternative systems of handling shells, trash, and other refuse may be approved by the Administrator when such systems adequately contain all refuse and provide equivalent sanitary methods for the handling and removal of refuse.

[36 FR 9814, May 28, 1971; 36 FR 10841, June 4, 1971, as amended at 40 FR 20059, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, as amended at 45 FR 23641, Apr. 8, 1980. Redesignated at 46 FR 63203, Dec. 31, 1981]

§ 590.502 Equipment and utensils; PCB-containing equipment.

(a) Equipment and utensils used in processing shell eggs and egg products shall be of such design, material, and construction as will:

(1) Enable the examination, segregation, and processing of such products in an efficient, clean, and satisfactory manner;

(2) Permit easy access to all parts to insure thorough cleaning and sanitizing. So far as is practicable, all such equipment shall be made of metal or other impervious material which will not affect the product by chemical action or physical contact.

(b) Except as authorized by the Administrator, in new or remodeled equipment and equipment installations, the equipment and installation shall comply with the applicable 3–A or E–3–A Sanitary Standards and accepted practices currently in effect for such equipment.

(c) New or replacement equipment or machinery (including any replacement parts) brought onto the premises of any official plant shall not contain liquid polychlorinated biphenyls (PCBs) in concentrations above 50 parts per million by weight of the liquid medium. This provision applies to both food processing and nonfood processing equipment and machinery, and any replacement parts for such equipment and machinery. Totally enclosed capacitors containing less than 3 pounds of PCBs are exempted from this prohibition.

[36 FR 9814, May 28, 1971. Redesignated at 42 FR 32514, June 27, 1977, as amended at 45 FR 68919, Oct. 17, 1980. Redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 60 FR 49170, Sept. 21, 1995]

§ 590.504 General operating procedures.

(a) Operations involving processing, storing, and handling of shell eggs, ingredients, and egg products shall be strictly in accord with clean and sanitary methods and shall be conducted as rapidly as practicable. Pasteurization, heat treatment, stabilization, and other processes shall be in accord with this part and as approved by the Administrator. Processing methods and temperatures in all operations shall be such as will prevent a deterioration of the egg products.

(b) Shell eggs and egg products processed in official plants shall be subjected to constant and continuous inspection throughout each and every processing operation. Any shell egg or egg product which was not processed in accordance with these regulations or is not fit for human food shall be removed and segregated.

(c) All loss and inedible eggs or egg products shall be placed in a container clearly labeled “inedible” and containing a sufficient amount of approved denaturant or decharacterant, such as FD&C brown, blue, black, or green colors, meat and fish by-products, grain and milling by-products, or any other substance, as approved by the Administrator, that will accomplish the purposes of this section. Shell eggs shall be crushed and the substance shall be dispersed through the product in amounts sufficient to give the product a distinctive appearance or odor. Notwithstanding the foregoing, and upon permission of the Inspector, the applicant may hold inedible product in containers clearly labeled inedible which do not contain a denaturant if such inedible product is denatured or decharacterized prior to shipment from the official plant: Provided, That such product is properly packaged, labeled, segregated, and inventory controls are maintained. In addition, product shipped from the official plant for industrial use or animal food need not be denatured or decharacterized, provided, that such product is properly packaged, labeled, segregated, and inventory controls are maintained, and that such product is shipped under Government seal and certificate and received at the destination location by an inspector or grader as defined in this part.

(d) The inspector may, prior to receipt of laboratory results for salmonella, or for other reasons such as labeling as to solids content, permit egg products to be shipped from the official plant when he has no reason to suspect noncompliance with any of the provisions of this part. However, such shipments shall be made under circumstances which will assure the return of the product to the plant for reprocessing, relabeling, or under such other conditions as the Administrator may determine to assure compliance with this part.

(e) Pasteurizing, stabilizing, or drying operations shall start as soon as practicable after breaking to prevent deterioration of product, preferably within 72 hours from time of breaking for egg products other than whites which are to be desugared.

(f) Each person who is to handle any exposed or unpacked egg products or any utensils or container which may come into contact with egg product, shall wash his hands and maintain them in a clean condition.

(g) No product or material which creates an objectionable condition shall be processed, stored, or handled in any room, compartment, or place where any shell eggs or egg products are processed, stored or handled.

(h) Only germicides, insecticides, rodenticides, detergents, or wetting agents or other similar compounds which will not deleteriously affect the eggs or egg products when used in an approved manner and which have been approved by the Administrator, may be used in an official plant. The identification, storage, and use of such compounds shall be in a manner approved by the Administrator.

(i) Utensils and equipment which are contaminated during the course of processing any shell eggs or egg products shall be removed from use immediately and shall not be used again until cleaned and sanitized.

(j) Any substance or ingredient added in the processing of any egg products shall be clean and fit for human food.

(k) Packages or containers for egg products shall be of sanitary design and clean when being filled with any egg products; and all reasonable precautions shall be taken to avoid soiling or contaminating the surface of any package or container liner which is, or will be, in direct contact with such egg products. Only new containers or used containers that are clean, in sound condition and lined with suitable inner liners shall be used for packaging edible egg products. Fiber containers used without liners require the approval of the Administrator.

(l) Egg products shall be inspected to determine the wholesomeness of the finished product.

(m) Egg products shall be processed in such a manner as to insure the immediate removal of blood and meat spots, shell particles, and foreign materials.

(n) Utensils and equipment, except drying units, powder conveyors, sifters, blenders, and mechanical powder coolers shall be clean and sanitized at the start of processing operations. Equipment and utensils shall be kept clean and sanitary during all processing operations.

(o) Egg products prior to being released into consuming channels shall be pasteurized in accordance with §590.570 except that dried whites prepared from nonpasteurized liquid shall be heat treated in accordance with §590.575.

(1) To assure adequate pasteurization, egg products shall be sampled and tested for the presence of salmonella. Sampling for the presence of salmonella shall be in accordance with §590.580 and product found to be salmonella positive shall be reprocessed, pasteurized, and analyzed for the presence of salmonella, or denatured.

(2) Nonpasteurized or salmonella positive egg product may be shipped from an official plant only when it is to be pasteurized, repasteurized, or heat treated in another official plant. Shipments of products from one official plant to another for pasteurization, repasteurization, or heat treatment shall be in sealed cars or trucks with an accompanying certificate stating that the product is not pasteurized or is salmonella positive. If nonpasteurized or salmonella positive products are to be stored in other than the official plant facilities, the inspector at the consignee's and consignor's plants shall be given full knowledge of the disposition of the product, including warehouse inventory receipts, until such time as product is pasteurized, repasteurized, or heat treated. The containers of such nonpasteurized or salmonella positive product shall be marked with the identification mark shown in Figure 3 of §590.415.

(3) Notwithstanding the provision of paragraph (o)(2) of this section, nonpasteurized salted egg products containing 10 percent or more salt added may be shipped from an official plant directly to a manufacturer of acidic dressings only under the following provisions:

(i) Before such shipment is made, the manufacturer of the acidic dressing shall apply in writing and receive permission from the Administrator to receive and use unpasteurized egg products. The applicant shall sign a written statement containing the specification for the treatment of the nonpasteurized egg product in a manner that will insure that viable salmonella microorganisms are destroyed, and such processing treatment shall be approved by the Administrator prior to use.

(ii) Product shall be shipped under seal from the official plant, accompanied by an official USDA certificate stating that the product is nonpasteurized and for use in acidic dressings only.

(iii) The applicant shall acknowledge receipt of each shipment by indicating on the reverse side of the USDA certificate. “The quantity of nonpasteurized egg product stated on this certificate was received at _____,” the blank being filled in with the name and address of the receiving company and the date and signature of the person completing the form. The certificate shall be returned to the USDA inspector at the origin plant.

(iv) The acidic dressing manufacturer shall maintain processing records indicating the use of each shipment of unpasteurized salted product and the code lots of acidic dressing into which it was processed. Records of

the pH and the acidity expressed as percent acetic acid of each code lot shall be maintained. The records shall also demonstrate that the acidic dressing was held 72 hours prior to shipment. These records shall be maintained for 2 years and shall be available for inspection by a representative of the Department.

(v) Each container of salted egg product shipped from the official plant shall be labeled as required in §590.411, and shall bear the words "Caution—this egg product has not been pasteurized or otherwise treated to destroy viable salmonella microorganisms," and shall bear the official identification shown in figure 4 of §590.415.

(p) Air which is to come in contact with product or with product contact surfaces shall come from approved filtered outside air sources.

(q) All liquid and solid waste material in the official plant shall be disposed of in a manner approved by the Administrator to prevent product contamination and in accordance with acceptable environmental protection practices.

[36 FR 9814, May 28, 1971, as amended at 37 FR 6658, Apr. 1, 1972; 40 FR 20059, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 47 FR 745, Jan. 7, 1982; 60 FR 49170, Sept. 21, 1995]

§ 590.510 Classifications of shell eggs used in the processing of egg products.

(a) The shell eggs shall be sorted and classified into the following categories in a manner approved by the National Supervisor:

(1) Eggs listed in paragraph (d) of this section.

(2) Dirty.

(3) Leakers as described in paragraph (c)(2) of this section.

(4) Eggs from other than chicken; duck, turkey, guinea, and goose eggs.

(5) Other eggs—satisfactory for use as breaking stock.

(b) Shell eggs having strong odors or eggs received in cases having strong odors shall be candled and broken separately to determine their acceptability.

(c) Shell eggs, when presented for breaking, shall be of edible interior quality and the shell shall be sound and free of adhering dirt and foreign material, except that:

(1) Checks and eggs with a portion of the shell missing may be used when the shell is free of adhering dirt and foreign material and the shell membranes are not ruptured.

(2) Eggs with clean shells which are damaged in candling and/or transfer and have a portion of the shell and shell membranes missing may be used only when the yolk is unbroken and the contents of the egg are not exuding over the outside shell. Such eggs shall be placed in leaker trays and be broken promptly.

(3) Eggs with meat or blood spots may be used if the spots are removed in an acceptable manner.

(d) All loss or inedible eggs shall be placed in a designated container and be handled as required in §590.504(c). Inedible and loss eggs for the purpose of this section and §590.522 are defined to include black rots, white rots, mixed rots, green whites, eggs with diffused blood in the albumen or on the yolk, crusted yolks, stuck yolks, developed embryos at or beyond the blood ring state, moldy eggs, sour eggs, any eggs that are adulterated as such term is defined pursuant to this part, and any other filthy and decomposed eggs including the following:

(1) Any egg with visible foreign matter other than removable blood and meat spots in the egg meat.

(2) Any egg with a portion of the shell and shell membranes missing and with egg meat adhering to or in contact with the outside of the shell.

(3) Any egg with dirt or foreign material adhering to the shell and with cracks in the shell and shell membranes.

(4) Liquid egg recovered from shell egg containers and leaker trays.

(5) Open leakers made in the washing operation.

(6) Any egg which shows evidence that the contents are or have been exuding prior to transfer from the case.

(e) Incubator reject eggs shall not be brought into the official plant.

[36 FR 9814, May 28, 1971, as amended at 40 FR 20059, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981]

§ 590.515 Egg cleaning operations.

(a) The following requirements shall be met when washing shell eggs to be presented for breaking:

(1) Shell egg cleaning equipment shall be kept in good repair and shall be cleaned after each day's use or more frequently if necessary.

(2) The temperature of the wash water shall be maintained at 90 °F or higher, and shall be at least 20 °F warmer than the temperature of the eggs to be washed. These temperatures shall be maintained throughout the cleaning cycle.

(3) An approved cleaning compound shall be used in the wash water. (The use of metered equipment for dispensing the compound into solution is recommended.)

(4) Wash water shall be changed approximately every 4 hours or more often if needed to maintain sanitary conditions and at the end of each shift. Remedial measures shall be taken to prevent excess foaming during the egg washing operation.

(5) Replacement water shall be added continuously to the wash water of washers to maintain a continuous overflow. Rinse water and chlorine sanitizing rinse may be used as part of the replacement water. Iodine sanitizing rinse may not be used as part of the replacement water.

(6) Waste water from the egg washing operation shall be piped directly to drains.

(7) The washing operation shall be continuous and shall be completed as rapidly as possible. Eggs shall not be allowed to stand or soak in water. Immersion-type washers shall not be used.

(8) Prewetting shell eggs prior to washing may be accomplished by spraying a continuous flow of water over the eggs in a manner which permits the water to drain away, or by other methods which may be approved by the Administrator.

(b) Shell eggs shall not be washed in the breaking room or any room where edible products are processed.

[36 FR 9814, May 28, 1971, as amended at 40 FR 20059, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 60 FR 49170, Sept. 21, 1995]

§ 590.516 Sanitizing and drying of shell eggs prior to breaking.

(a) Immediately prior to breaking, all shell eggs shall be spray rinsed with potable water containing an approved sanitizer of not less than 100 ppm nor more than 200 ppm of available chlorine or its equivalent. Alternative procedures may be approved by the Administrator in lieu of sanitizing shell eggs washed in the plant.

(b) Shell eggs shall be sufficiently dry at time of breaking to prevent contamination or adulteration of the liquid egg product from free moisture on the shell.

[60 FR 49170, Sept. 21, 1995]

§ 590.520 Breaking room facilities.

(a) The breaking room shall have at least 30 foot-candles of light on all working surfaces except that light intensity shall be at least 50 foot-candles at breaking and inspection stations. Lights shall be protected with adequate safety devices.

(b) The surface of the ceiling and walls shall be smooth and made of a water-resistant material.

(c) The floor shall be of water-proof composition, reasonably free from cracks or rough surfaces, sloped for adequate drainage, and the intersections with walls and curbing shall be impervious to water.

(d) Ventilation shall provide for:

(1) A positive flow of outside filtered air through the room;

(2) Air of suitable working temperature during operations.

(e) There shall be provided adequate hand washing facilities which are easily accessible to all breaking personnel, an adequate supply of warm water, clean towels or other facilities for drying hands, odorless soap, and containers for used towels. Hand washing facilities shall be operated by other than hand operated controls.

(f) Containers for packaging egg products are not acceptable as liquid egg buckets.

(g) A suitable container conspicuously identified shall be provided for the disposal of rejected liquid.

(h) Strainers, filters, or centrifugal clarifiers of approved construction shall be provided for the effective removal of shell particles and foreign material, unless specific approval is obtained from the National Supervisor for other mechanical devices.

(i) A separate drawoff room with a filtered positive air ventilation system shall be provided for packaging liquid egg product, except product packaged by automatic, closed packaging systems.

[36 FR 9814, May 28, 1971, as amended at 37 FR 6659, Apr. 1, 1972. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981]

§ 590.522 Breaking room operations.

(a) The breaking room shall be kept in a dust-free clean condition and free from flies, insects, and rodents. The floor shall be kept clean and reasonably dry during breaking operations and free of egg meat and shells.

(b) All breaking room personnel shall wash their hands thoroughly with odorless soap and water each time they enter the breaking room and prior to receiving clean equipment after breaking an inedible egg.

(c) Paper towels or tissues shall be used at breaking tables, and shall not be reused. Cloth towels are not permitted.

(d) Breakers shall use a complete set of clean equipment when starting work and after lunch periods. All table equipment shall be rotated with clean equipment every 21/2 hours.

(e) Cups shall not be filled to overflowing.

(f) Each shell egg shall be broken in a satisfactory and sanitary manner and inspected for wholesomeness by smelling the shell or the egg meat and by visual examination at the time of breaking. All egg meat shall be reexamined by a person qualified to perform such functions before being emptied into the tank or churn, except as otherwise approved by the National Supervisor.

(g) Shell particles, meat and blood spots, and other foreign material accidentally falling into the cups or trays shall be removed with a spoon or other approved instrument.

(h) Whenever an inedible egg is broken, the affected breaking equipment shall be cleaned and sanitized.

(i) Inedible and loss eggs as defined in §590.510 apply to this section.

(j) The contents of any cup or other liquid egg receptacle containing one or more inedible or loss eggs shall be rejected.

(k) Contents of drip trays shall be emptied into a cup and smelled carefully before pouring into liquid egg bucket. Drip trays shall be emptied at least once for each 15 dozen eggs or every 15 minutes.

(l) Edible leakers as defined in §590.510(c)(2) and checks which are liable to be smashed in the breaking operation shall be broken at a separate station by specially trained personnel.

(m) Ingredients and additives used in, or for, processing egg products, shall be handled in a clean and sanitary manner.

(n) Liquid egg containers shall not pass through the candling room.

(o) Test kits shall be provided and used to determine the strength of the sanitizing solution. (See §§590.515(a)(9) and 590.552.)

(p) Leaker trays shall be washed and sanitized whenever they become soiled and at the end of each shift.

(q) Shell egg containers whenever dirty shall be cleaned and drained; and shall be cleaned, sanitized, and drained at the end of each shift.

(r) Belt-type shell egg conveyors shall be cleaned and sanitized approximately every 4 hours in addition to continuous cleaning during operation. When not in use, belts shall be raised to permit air drying.

(s) Cups, knives, racks, separators, trays, spoons, liquid egg pails, and other breaking equipment, except for mechanical egg breaking equipment, shall be cleaned and sanitized at least every 21/2 hours. This equipment shall be cleaned at the end of each shift and shall be clean and sanitized immediately prior to use.

(t) Utensils and dismantled equipment shall be drained and air dried on approved self-draining metal racks and shall not be nested.

(u) Dump tanks, drawoff tanks, and churns shall be cleaned approximately every 4 hours. All such equipment and all other liquid handling equipment, unless cleaned by acceptable cleaned in-place methods, shall be dismantled and cleaned after each shift. Pasteurization equipment shall be cleaned at the end of each day's use or more often if necessary. All such equipment shall be clean and shall be sanitized prior to placing in use.

(v) Strainers, clarifiers, filtering and other devices used for removal of shell particles and other foreign material shall be cleaned and sanitized each time it is necessary to change such equipment, but at least once each 4 hours of operation.

(w) Breaking room processing equipment shall not be stored on the floor.

(x) Metal containers and lids for other than dried products shall be thoroughly washed, rinsed, sanitized, and drained immediately prior to filling. The foregoing sequence shall not be required if equally effective measures approved by the National Supervisor in writing are followed to assure clean and sanitary containers at the time of filling.

(y) Liquid egg holding vats and containers (including tank trucks) used for transporting liquid eggs shall be cleaned after each use. Such equipment shall be clean and sanitized immediately prior to placing in use.

(z) Tables, shell conveyors, and containers for inedible egg product shall be cleaned at the end of each shift.

(aa) Mechanical egg breaking machines shall be operated at a rate to maintain complete control and accurately inspect and segregate each egg to insure the removal of all loss and inedible eggs. The machine shall be operated in a sanitary manner.

(1) When an inedible egg is encountered on mechanical egg breaking equipment, the inedible egg and contaminated liquid shall be removed. The machine shall be cleaned and sanitized, or contaminated parts replaced with clean ones in the manner prescribed by the Administrator for the type of inedible egg encountered and the kind of egg breaking machine.

(2) Systems for pumping egg liquid directly from egg breaking machines shall be of approved sanitary design and construction, and designed to minimize the entrance of shells into the system and be disconnected when inedible eggs are encountered. The pipelines of the pumping system shall be cleaned or flushed as often as needed to maintain them in a sanitary condition, and they shall be cleaned and sanitized at the end of each shift. Other pumping system equipment shall be cleaned and sanitized approximately every 4 hours or as often as needed to maintain it in a sanitary condition. All liquid egg pumped directly from egg breaking machines shall be reexamined, except as otherwise prescribed and approved by the Administrator.

(3) Mechanical egg breaking equipment shall be clean and sanitized prior to use, and during operations the machines shall be cleaned and sanitized approximately every 4 hours or more often if needed to maintain them in a sanitary condition. This equipment shall be cleaned at the end of each shift.

[36 FR 9814, May 28, 1971, as amended at 37 FR 6659, Apr. 1, 1972; 40 FR 20059, May 8, 1975; 40 FR 20941, May 14, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981]

§ 590.530 Liquid egg cooling.

(a) Liquid egg storage rooms, including surface coolers and holding tank rooms, shall be kept clean and free from objectionable odors and condensation. Surface coolers and liquid holding vats containing product

shall be kept covered while in use. Liquid cooling units shall be of approved construction and have sufficient capacity to cool all liquid eggs to the temperature requirements specified in this section.

(b) Compliance with temperature requirements applying to liquid eggs shall be considered as satisfactory only if the entire mass of the liquid meets the requirements.

(c) The cooling and temperature requirements for liquid egg products shall be as specified in Table I of this section.

Table I—Minimum Cooling and Temperature Requirements for Liquid Egg Products
[Unpasterurized product temperature within 2 hours from time of breaking]

Product	Liquid (other than salt product) to be held 8 hours or less	Liquid (other than salt product) to be held in excess of 8 hours	Liquid salt product	Temperature within 2 hours after pasteurization	Temperature within 3 hours after stabilization
Whites (not to be stabilized)	55 °F. or lower	45 °F. or lower		45 °F. or lower	
Whites (to be stabilized)	70 °F. or lower	55 °F. or lower		55 °F. or lower	([1])
All other product (except product with 10 percent or more salt added)	45 °F. or lower	40 °F. or lower		If to be held 8 hours or less 45 °F. or lower. If to be held in excess of 8 hours, 40 °F. or lower	If to be held 8 hours or less, 45 °F. or lower. If to be held in excess of 8 hours, 40 °F. or lower.
Liquid egg product with 10 percent or more salt added			If to be held 30 hours or less, 65 °F. or lower. If to be held in excess of 30 hours, 45 °F. or lower	65 °F. or lower[2]	

[1]Stabilized liquid whites shall be dried as soon as possible after removal of glucose. The storage of stabilized liquid whites shall be limited to that necessary to provide a continuous operation.

[2]The cooling process shall be continued to assure that any salt product to be held in excess of 24 hours is cooled and maintained at 45 °F. or lower.

(d) Upon written request and under such conditions as may be prescribed by the National Supervisor, liquid cooling and holding temperatures not otherwise provided for in this section may be approved.

(e) Agitators shall be operated in such a manner as will minimize foaming.

(f) When ice is used as an emergency refrigerant by being placed directly into the egg meat, the source of the ice must be certified by the local or State board of health. Such liquid shall be dried. All ice shall be handled in a sanitary manner.

(g) Previously frozen egg or egg product cannot be added to liquid product for the purpose of complying with liquid cooling requirements.

[36 FR 9814, May 28, 1971. Redesignated at 42 FR 32514, June 27, 1977 and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 47 FR 745, Jan. 7, 1982; 60 FR 49170, Sept. 21, 1995]

§ 590.532 Liquid egg holding.

(a) Tanks and vats used for holding liquid eggs shall be of approved construction, fitted with covers, and located in rooms maintained in a sanitary condition. Notwithstanding the foregoing, tanks designed for installation partially outside of a room or building are acceptable, providing all openings into the tanks terminate in the processing room.

(b) Liquid egg holding tanks or vats shall be equipped with suitable thermometers and agitators.

(c) Inlets to holding tanks or vats shall be such as to prevent excessive foaming.

(d) Gaskets, if used, shall be of a sanitary type.

[36 FR 9814, May 28, 1971. Redesignated at 42 FR 32514, June 27, 1977, as amended at 45 FR 23641, Apr. 8, 1980. Redesignated at 46 FR 63203, Dec. 31, 1981]

Appendix 2. Estimating the Viral Concentration in a Transport Container of NPLE Made with Eggs from an Infected but Undetected Flock

Introduction

In this appendix, we provide the details of the simulation analysis for estimating the concentration of HPAI virus in an NPLE transport container leaving the processing facility. We conducted this simulation analysis in three parts. We first estimated the HPAI prevalence, the disease mortality and the fraction of internally contaminated eggs at various time points post infection of the flock using a stochastic disease transmission model. Next, we used a simulation model of the planned surveillance protocol in the event of an outbreak along with the disease transmission model results to estimate the number of days to detect infection and the maximum daily fraction of contaminated eggs before infection is detected. Next, we estimated the viral concentration in a transport container of NPLE, considering the mixing of the internal contents of contaminated and virus-free eggs from various flocks.

Modeling the Spread of HPAI in an Infected but Undetected Flock Using a Stochastic Disease Transmission Model

The disease transmission model simulates the spread of HPAI within a flock to estimate the HPAI prevalence and the disease mortality at various time points post infection of the flock. The deterministic version of the disease transmission model was developed by an FSIS-APHIS-FDA interagency group for use in various risk assessments. The deterministic version of the model is available online as part of a draft of the FSIS risk assessment[38] of the public health risks due to HPAI infection in poultry.

In the following, we first provide an overview of the deterministic disease transmission model and then describe the extension of the disease transmission model to consider the variability in the number of birds in various stages of infection.

Deterministic Disease Transmission Model

The disease transmission model is an extension of the Reed-Frost Susceptible-Latent-Infectious-Died (or Removed) model to include more infectious states. The model calculates the number of birds at various disease stages at discrete 6-hour time intervals following the infection in the flock. The primary advantage of having more infected stages is that differences in the probability of death and the infectivity of chickens infected for different durations can be considered. For example in the baseline scenario of the FSIS HPAI risk assessment, there are 7 infected stages: infected 1-hour (I1), infected 7 hours (I7), infected 37 hours (I37), in addition to the susceptible (S), and dead states.

As often assumed in HPAI transmission models, Vandergoot *et al.,*[43], Bouma *et al.,*[42] we assume frequency dependent transmission where each individual makes a fixed number of contacts with other individuals per unit time regardless of the population size. Alternatively, in density dependent transmission, the number of contacts each individual makes per unit time is dependent on the population size. In reality, the type of disease

transmission can depend on the mode of transmission such as fecal-oral or aerosol. Frequency dependent transmissions might be more appropriate for animals that are held at a fixed stocking density (such as caged layers) Bouma *et al.*[42] In addition, frequency dependent transmission models provided reasonable fits to the outbreak data as described in Bos *et al.*[45] and Tiensen *et al.*[44]

The latent state was not modeled explicitly in the transmission model. Instead it was considered that chickens infected for different periods have different chances of being infectious. For instance, in the baseline scenario, it was assumed that chickens in the first 6 hours post infection are all latently infected whereas chickens infected for more than 19 hours post infection are 100 percent infectious. Refer to draft FSIS HPAI risk assessment[38] for formal presentation of the mathematical equations related to the deterministic transmission model.

Stochastic version of the disease transmission model to consider additional variability

The draft FSIS HPAI risk assessment[38] utilized deterministic calculations to estimate the number of chickens in the susceptible, dead and various infectious stages. The number of susceptible chickens is *S*, the number of infectious birds is *I*, and the total number *(S+I)* is *N*. In the deterministic calculations, the number of susceptible birds in period *t*+1 is calculated as

$$S_{t+1} = S_t(e^{-\beta\frac{I_t}{N_t}})$$

Where β is the effective contact rate

As in various chain binomial models, we used the binomial distribution to introduce variability in the number of birds moving from the susceptible to the first stage of infection (equivalent to a latent stage for the parameters used in the baseline scenario of FSIS risk assessment). Let *p* be the probability that a susceptible bird in period *t* becomes infected in period *t+1*. We estimated *p* according to the following equation

$$p = (1 - e^{-\beta\frac{I_t}{N_t}})$$

Given *p*, we used the binomial(*S*, *p)* distribution to compute the number of birds moving from susceptible to the first infection stage (latent) between periods *t* and *t*+1. When the number of trials was greater than 1000, we used the Poisson approximation when $Sp < 15$. The Normal approximation to the Binomial distribution was used for other cases.

In the baseline scenario in the draft FSIS HPAI risk assessment, it was estimated that 40 percent of chickens in the 31st hour of infection would die in the next time period. We therefore used the binomial distribution with a 40 percent probability (*p*=0.40) to estimate the number of chicken in 31st hour of infection that would die by the next time period. The probabilities of transitioning between various possible states (infected 1-hour (I1),

infected 7 hours (I7), infected 37 hours (I37), susceptible (S), and dead states) from one period to the next are summarized in Appendix 2 Table 1.

Appendix 2 Table 1: Probability of a chicken being in a specific state in period (t+1) given its state in period (t) in the disease transmission model

	S_{t+1}	$I1_{t+1}$	$I7_{t+1}$	$I13_{t+1}$	$I19_{t+1}$	$I25_{t+1}$	$I31_{t+1}$	$I37_{t+1}$	Dead
S_t	$e^{-\beta\frac{I_t}{N}}$	$(1-e^{-\beta\frac{I}{N}})$	0	0	0	0	0	0	0
$I1_t$	0	0	1	0	0	0	0	0	0
$I7_t$	0	0	0	1	0	0	0	0	0
$I13_t$	0	0	0	0	1	0	0	0	0
$I19_t$	0	0	0	0	0	1	0	0	0
$I25_t$	0	0	0	0	0	0	1	0	0
$I31_t$	0	0	0	0	0	0	0	0.6	0.4
$I37_t$	0	0	0	0	0	0	0	0	1
Dead	0	0	0	0	0	0	0	0	1

Estimation of the disease transmission model parameters

(i) Expected length of latently infected period

Data on the latently infected period and mean time to death from chicken inoculated with HPAI H5N1 virus are summarized in Appendix 2 Table 2. To our knowledge, only a couple of studies, Bouma *et al.*[42] and Das *et al.*[41] estimated the latent period for HPAI H5N1 infected chicken. Bouma *et al.*[42] utilized Bayesian analysis and estimated a latent period of 0.24 (0.099-0.48) based on daily testing of artificially inoculated chickens (1 sample per day). Das *et al.*[41] tested tracheal and tissue (breast thigh and heart) samples of intranasal inoculated chickens every 6 hours. We decided to use this study for the estimation of latent and infectious periods as the sampling was done for every 6 hours compared to Bouma *et al.*[42] where the sampling was done on a daily basis and the latent period was approximated via statistical analysis.

In our model, the probability of being latently infected is 0, 0.6, and 0.7 for I1, I7 and I13 states respectively. The probability of being latently infected is zero for the states I19 to I37. Given these probabilities, the expected length of latently infected period is 13.8 hours.

(ii)Estimation of the mean time to death and the infectious period

The expected infectious period in our model is 25.8 hours. The expected time to death is 39.6 hours. We employed Das *et al.*[41] to estimate the mean time to death as the data had a greater precision with sampling at every 6 hours compared to most studies which report the mean time to death in days (see Table 2). Pfeiffer *et al.*[93] found a mean time to death between 36-48 hours. Other studies, shown in Table 2, have found the mean time to death to vary between 1-3 days.

Appendix 2 Table 2: Latently infected period, infectious period and the mean time to death for HPAI H5N1 infections in chicken from experimental studies

Source	Latent period	Infectious period
Bouma *et al.* (2009)[42]	6 hours	48 hours
Das *et al.* (2008) [41]	6-24 hours	12-36 hours
Source	Mean time to death	
Pfeiffer *et al.* (2009)[93]	36-48 hours	
Shortridge *et al.* (1998)[17]	2-3 days, most likely value was 2 days	
Lee *et al.* (2008)[94]	1-2 days	
Tsukomoto *et al.* (2007)[95]	2 days	

(iii) Effective contact rate and associated parameters

The rate of the exponential increase in prevalence from the disease transmission model is largely determined by the effective contact rate parameter (also referred to as the transmission parameter). The effective contact rate is defined as the number of birds an infectious chicken comes into contact with that is sufficient to transmit infection per unit time. To estimate the effective contact rate directly, we would require data on the number of susceptible, infectious and dead birds over time from an HPAI infected flock in conditions reflective of commercial operations. Such data is rare. Consequently, approximate methods have to be utilized. In the following, we summarize the estimates for the contact rate for HPAI in caged layers from the literature.

Several articles estimated the contact rate from experiments on transmission of HPAI infection between pairs of chickens consisting of an inoculated bird and a contact bird. The small population of just two birds is justified on the assumption of frequency dependent transmission where the transmission rate is not dependent on the population size. However, in practice, the contact rate might increase with the population when the population size is very low.[42] For instance, the contact rate might be higher if there are a greater number of chickens within a single cage. Furthermore, considering the impact of layer management practices such as airflow, feed delivery systems, and stocking density, extrapolating the contact rate from small scale experiments to that of a commercial flock might be unreliable.[43] Utilizing this approach Vandergoot *et al.*[43] estimated a contact rate of 33 birds/day for HPAI H7N7 infection in chickens. Recently, Bouma *et al.*[42] estimated the contact rate as 0.72 (0.42-1.2)/day for HPAI H5N1 infections in chicken.

A couple of studies have estimated the contact rate from data on the increase in daily mortality over time from HPAI infected flocks. Typically, such outbreak data is inadequate for estimating the contact rate directly and hence several assumptions or back calculations have to be made. Some of the issues associated with natural outbreak data are that the day when the flock was first infected, the number of infected birds at various points in time is not known. A couple of articles estimated the contact rate by back calculating the number of infected birds over time from the number of dead birds assuming specific values for latently infected and infectious periods. Using 2004 H5N1

epidemic data from Thailand, Tiensen *et al.*[44] estimated a contact rate of 2.30 birds/per day for laying hens and broiler chicken with a one day infectious period and no latent period. Based upon 2003 Netherlands H7N7 outbreak data, Bos *et al.*[45] estimated a contact rate of 4.50 birds per day (95 percent CI 2.68-7.57) with no latent period and a 4 day infectious period. In an alternate model in Bos *et al.*[45] (not the best fit) with a latent period of 1 or 2 days, the contact rate was 19.9 per day. Hence, inclusion of latent period can significantly impact the contact rate estimated from the data. Also, including the type of housing (caged or loose) did not have a significant influence on the model results.

Savill *et al.*[46] developed a simulation model of H5N1 infection spread in caged layers via feces or aerosol. In their model, the contact rate was 17 birds per day with an infectious period of 34.8 hours and a basic reproductive number R_0 of 25.

In summary, the effective contact rate typically is not directly estimable from the data without significant assumptions or approximations. There is considerable variation in the effective contact rate estimates from alternate approaches and from different models within the same approach. The contact rates from the literature ranged from 0.72 birds per day to 33 birds per day. Although, it is logically plausible that the contact rate is lower for caged layers, some recent studies of outbreak data report that the inclusion of housing type did not improve the fit of the data to model.[44,45] Given the significant uncertainty in the effective contact rate estimates, it is essential to choose a conservative value for the effective contact rate. Sensitivity analysis of the deterministic as well as the stochastic versions of the disease transmission models indicated that a higher contact rate might lead to greater number of contaminated eggs. In this respect, using a higher effective rate is more conservative. We conservatively assumed a contact rate of 2-birds/6-hour interval. We discuss the impact of uncertainty associated with the contact rate with sensitivity analysis later on in the appendix.

Given an expected infectious period of 25.8 hours, the basic reproductive number R_0 for our transmission model is 8.6. The expected generation interval (the mean duration between time of infection of a secondary infected hen and the time of infection of its primary infector is 16.8 hours.[96]

(iv) Frequency and Timing of contaminated eggs

To estimate the daily number of contaminated eggs, we used the egg production Option 3 of the disease transmission model as presented in the draft FSIS interagency risk assessment.[38] In this option, no contaminated eggs are laid before the first 19 hours post infection while all the eggs laid after 19 hours post infection are considered to be contaminated. This option corresponds well with empirical data from laboratory studies[18,37] that show that eggs laid on the first day post infection are not contaminated (see FSIS risk assessment[38] for details on transmission model).

Transmission model assumptions

The main assumptions of the disease transmission model are summarized below:

i. *The effective contact rate for disease transmission estimated from HPAI outbreaks in Netherlands and Thailand (2 birds/6 hours) is applicable for layer flocks in the US.* Differences in the layer management practices between the United States and other countries, and the characteristics of the HPAI strain causing the outbreak may result in a different contact rate than that used in this assessment. Sensitivity analysis with respect to the contact rate indicates that our risk estimates are robust and conservative with respect to the range of contact rates estimated in the literature for caged layers.

ii. *No reduction in the egg laying rate due to HPAI infection in a hen*
Previous outbreak studies have reported more than 30% drop in egg production rate H7N7 or H5N2 HPAI infected flocks[24,40,47]. However, whether such a drop in egg production rate would occur with infection with the more virulent Asian H5N1 strains is unclear. Therefore, it was conservatively assumed that there is no drop in the egg production rate.

iii. *A flock size of 100,000 represents the mean flock size currently in the industry.* A greater flock would likely result in higher normal mortality and decreased chance of detection in a given time. We consider the flock size of 100,000 as a conservative estimate given that the USDA APHIS Layer 1999 survey of layer farms in the U.S. indicates that the mean and median flock sizes are less than 83,000 hens.[48]

Simulation Results

The above model was coded in Excel using Visual Basic for Applications and @RISK software. An effective contact rate (β) of 2 chicken/6 hour time period and a flock size of 100,000 layers were used as input parameter values. We conducted simulations for 6000 iterations with Latin Hypercube sampling.

From Appendix 2 Table 3, we observe that the 90 percent probability intervals for the daily mortalities in various days are relatively wide. Outbreak data from Netherlands showed similarly high variance in the daily mortality.[40] Depending on normal flock mortality (mortality independent of HPAI infection), this variance in the daily mortality may result in increased uncertainty in the day HPAI is detected in the flock.

Appendix 2 Table 3: Daily mortality predicted by the transmission model in a flock of 100,000 layers starting with one infected bird (I=1)

	Daily Mortality					
Parameter	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Deterministic	0	1	5	40	296	2150
Stochastic mean	0	1	5.2	39.99	297	2146
Stochastic two sided 90% interval	0	1	1-10	12-78	93-479	684-3408

We noted that the daily mortality values were quite sensitive to the time period in the day when the mortality was enumerated. Given that the unit time period in the transmission model is 6 hours, there are 4 time periods in the day. For instance, in the deterministic transmission model, the daily mortality for the 5th day is 66 if mortality is checked in the first 6-hour period of the day and 109 if mortality is checked on the second 6-hour period of the day. The sensitivity of the outputs shows the importance of considering the variability in the transmission model.

Simulation Model to Estimate the Time to Detect Infection and the Maximum Daily Fraction of Contaminated Eggs under the Active Surveillance Protocol

In the targeted active surveillance described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan, swabs from 5 randomly selected birds among the daily mortality sample are pooled together and tested via RRT-PCR each day. The number of days to detect infection under this protocol depends on the variability in the normal mortality independent of HPAI and the variability in the mortality due to HPAI. We used outputs from the transmission model in conjunction with the simulation model of the active surveillance protocol to estimate the number of days to detect infection in a flock.

According to the UEP/UEA – USDA APHIS VS Movement Control Model Plan, eggs or egg products from flocks within the control area will be allowed to move with a permit only after the flock tests negative with RRT-PCR testing as described above. This protocol implies that if infection from a flock is detected on a particular day by RRT-PCR testing, then the eggs or egg products from the flock on that day would not be moved. Therefore, for this analysis we assumed that eggs produced on the day on which infection is detected are not relevant for risks associated with movement of NPLE. Given this assumption, we defined the maximum daily fraction of contaminated eggs as the highest daily proportion of contaminated eggs among all the days starting from the day the flock is infected to one day before the infection in the flock is detected. In the following, we present the details of the simulation analysis of the active surveillance protocol.

Notation

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

$M^d(t)$ = mortality due to HPAI on day t (birds/flock of 100,000 layers).

$M^n(t)$ = normal mortality independent of HPAI on day t (birds/flock of 100,000 layers).

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

n = number of swabs in the pooled sample submitted for RRT-PCR testing each day (swabs).

$$X^{pool}(t) = \left\{ \begin{array}{c} 1 \text{ if a swab from HPAI mortality is included in pooled sample for RRT - PCR on day t} \\ 0 \text{ otherwise} \end{array} \right\}$$

$$X^{pcr}(t) = \left\{ \begin{array}{c} 1 \text{ if RRT - PCR test result for a sample submitted on day t is positive} \\ 0 \text{ otherwise} \end{array} \right\}$$

$$Y(t) = \left\{ \begin{array}{c} 1 \text{ if infection in the flock is detected on day t. Y(t) = 1 implies day t = td.} \\ 0 \text{ otherwise} \end{array} \right\}$$

Se = sensitivity of the RRT-PCR testing procedure.

E(t) = calculated number of HPAI contaminated eggs produced on day *t* (contaminated eggs/flock of 100,000).

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

Assumptions

- Apart from active surveillance of mortality via RRT-PCR, other clinical indicators of HPAI infection such as drop in egg production and decreased feed intake are not considered towards detecting infection.
- The sampling of daily mortality is random i.e., swabs from dead birds with clinical signs are not more likely to be included in pooled sample tested via RRT-PCR.
- Weekly mortality data was used to estimate the normal daily mortality. This assumption can result in some underestimation of the variability in the daily mortality.

Simulation Model

The number of diseased birds present in the 5 randomly chosen birds from the pool of daily mortality was assumed follow a hypergeometric distribution. Specifically,

$$X^{pool}(t) = HyperGeometric(M^t(t), n, M^d(t))$$

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

$$X^{pcr}(t) = \left\{ \begin{array}{c} 0 \ if \ X^{pool}(t) = 0 \\ Bernoulli(Se) \ if \ X^{pool}(t) = 1 \end{array} \right\}$$

The probability that infection is detected on a particular day *t*, $P(Y(t)=1)$ or equivalently $P(t=t_d)$ is given by

$$P\{Y(t)=1\} = P\left\{\sum_{k=1}^{k=t-1} X^{pcr}(k) < 1\right\} P\{X^{pcr}(t)=1\}$$

The maximum number of contaminated eggs/day before infection in the flock is detected is given by

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

Input Data

Flock size: 100,000 to represent the average flock size currently in the industry. The flock size of 100,000 is conservative as data from the USDA APHIS Layer 1999 survey indicate that the mean and median layer flock sizes are less than 83,000 hens. [48] Additionally, only 13.5 percent of the flocks had more than 120,000 hens.

Daily mortality due to HPAI in different days in an infected flock (**$M^d(t)$**): We estimated this parameter from the output of the disease transmission model.

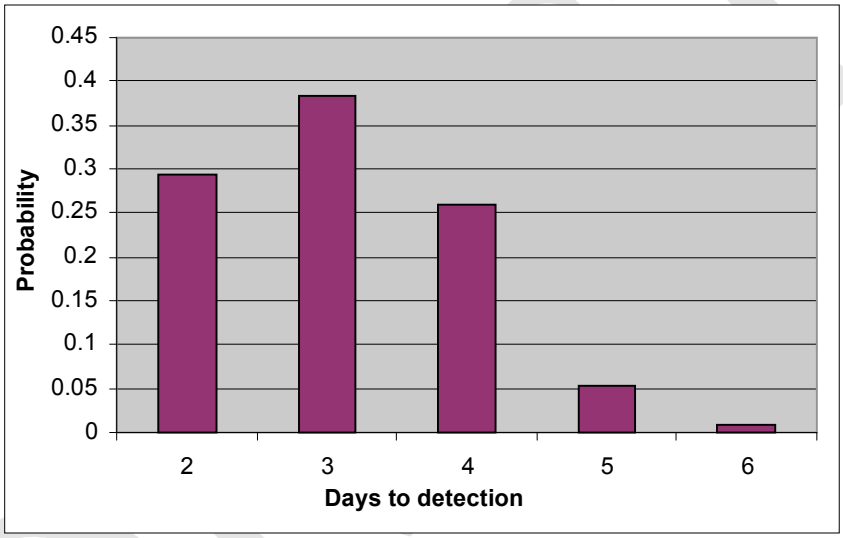
Normal mortality independent of disease (**$M^n(t)$**): Normal mortality data was provided by AgriStats Inc. through Iowa State University Veterinary Extension. The data consisted of weekly mortality numbers for 27 layer flocks for the entire production cycle (60-100 weeks). The data is from a fairly representative sample of 9 flocks each from the 3 major egg layer breeds in the US and 9 flocks each from 3 geographic regions (Iowa and Minnesota, California and, Pennsylvania). We calculated daily mortality in a flock of 100,000 layers based on the weekly mortality numbers, i.e, daily mortality = weekly mortality/7. The mean daily mortality rate was 28 deaths per 100,000 birds with a standard deviation of 33 deaths/100,000 birds. Given a sample size of 1801 the standard deviation of the expected mortality is 0.78. The 97.5th percentile for this data was 91, i.e., the P(number of deaths <=91)=0.975). We note a higher flock size would lead to a greater normal mortality and can result in increased time to detection and a greater number of contaminated eggs before detection. The flock size of 100,000 is conservative given the results of the USDA APHIA Layers 1999 survey (see above reasoning for flock size estimate).[48] For the simulation, we utilized an empirical distribution (sampled directly from data) as the sample size is relatively large.

Egg Production Rate: 0.7 eggs/hen/day[38]. AgriStats data described in the preceding section indicates that the egg production rate may vary from 0.6 eggs/hen/day to 0.9 eggs/hen/day depending on the age of the flock and other factors. The number of contaminated eggs from an infected but undetected flock is directly proportional to the egg production rate.

Sensitivity of the PCR testing (*Se):* Based on the published results for H7N2 LPAI [50]and personal communication, Dr. Erika Spackman, we used a sensitivity of 86.5 percent for the RRT-PCR testing.

Results

Number of days to detect HPAI after the first chicken is infected t_d: The distribution for t_d from the simulation is shown in Appendix 2 Figure 1. The mean t_d was 3 days. However, there is a 6.5 percent chance that 5 or more days are required to detect the infection. One of the reasons for the late detection of HPAI (>4 days) is high normal mortality rate. A high normal mortality implies that a swab from a chicken death due to HPAI is less likely to be included in the pooled sample for RRT-PCR testing. Another factor leading to the late detection is that the RRT-PCR is 86.5 percent sensitive and may therefore fail to detect some cases.



Appendix 2 Figure 1. Simulation output distribution for the number of days to detect HPAI in a flock of 100,000 layers with active surveillance via PCR testing.

Maximum number of contaminated eggs per day that may be moved prior to detection of infection in the flock E_{max} **:** The mean E_{max} from simulation was 12 contaminated eggs/day. The two sided 90 percent probability interval for E_{max} from simulation was 0-40 contaminated eggs/day. However, in 5 percent of the simulation iterations, 40-1209 contaminated eggs were produced prior to detection. Typically, these cases when a greater number of contaminated eggs were produced prior to detection in the simulation results were associated with late detection of infection.

Simulation Model to Estimate the HPAI Viral Concentration in a Transport container of NPLE Leaving the Premises

We developed a simulation model to estimate the resulting viral titer in an outgoing tanker of NPLE while considering mixing of NPLE from contaminated and virus- free eggs. For this model, we assumed that the daily egg production from a single 100,000-

bird henhouse would be loaded into one tanker and mixed with liquid egg from other houses on the same or different premises. To consider the possibility that the tanker is not filled completely or smaller NPLE transport containers are used, we also analyzed an alternate scenario where the tanker is only 25 percent full. Another key assumption is that the NPLE in the tanker has been mixed thoroughly and the viral titer is homogeneously distributed throughout the tanker. This assumption is based on the fact that agitators in typical NPLE refrigeration and storage systems would facilitate mixing.

Simulation Model

We calculated the virus titer in a tanker of NPLE as

$$\frac{\text{Total EID}_{50}\text{ of HPAI Virus in tanker}}{\text{Volume of NPLE in tanker}}$$

The total EID_{50} of HPAI virus in the tanker was inturn calculated according to the Equation below

$$\text{Total EID}_{50}\text{ in tanker} = (E_{max})(\text{Viral titer in egg contents})(\text{Egg volume})$$

The volume of NPLE in the outgoing tanker of NPLE was calculated as,

$$\text{Volume of NPLE in tanker} = \frac{(\text{Weight capacity of tanker})(\text{Fraction of tanker capacity filled})}{\text{Egg content density}}$$

The main inputs for the model are summarized in Appendix 2 Table 4

Appendix 2 Table 4. Inputs for the simulation model to estimate the final viral titer in a tanker of NPLE

Input parameter	*Value*	*Unit*	*Source*
Volume of egg	64	ml	AMS 56 (Based on average of extra large and jumbo egg volume)[53]
Egg content density	1.030	g/ml	Rahn and Paganelli (1989)[54]
Viral titer in contaminated egg	Uniform (3.6-5)	Log EID_{50}/ml	Section 8.2.3.a
Percent of tanker capacity filled	Uniform (80-100)	Percentage	Assumption
Weight capacity of tanker	48,000	pounds	Assumption

Results

We performed simulation using @RISK for 5,000 iterations with Latin hypercube sampling. The mean viral titer in the NPLE from the simulation results was 1.15 EID_{50}/ml (90% probability interval 10^{-3}- 3.71 EID_{50}/ml). Suppose the tanker was only 25 percent full, then the mean viral titer in the NPLE from simulation results was 4.3 EID_{50}/ml (90% probability interval 10^{-3}- 12 EID_{50}/ml).

Sensitivity Analysis with Respect to Effective Contact Rate

We selected the effective contact rate for sensitivity analysis considering the higher uncertainty associated with its estimates compared to other transmission parameters such as the latent and infectious periods that are more directly estimable from experimental studies. An increase in the effective contact rate would have two competing effects that impact the number of contaminated eggs produced from a flock before detection. First, given a higher contact rate, a greater number of chicken are expected to become infected and die with HPAI within a specific time. The higher HPAI mortality likely results in reduced time to detect infection in the flock. However, increased contact rate would also result in a greater number of infected chickens within a given time and thus potentially higher number of contaminated eggs.

For our sensitivity analysis, we performed 6000 simulation iterations of the disease transmission and the active surveillance models for the effective contact rates of 1, 2 and 4 birds/6 hours. The contact rate of 4 birds/6 hours can be considered an upper bound estimate for caged layers as predicted by the simulation model of Savill *et al.*[46] As detailed in appendix 2, the contact rates from HPAI outbreak data were mostly less than 2-birds/6 hours. The results shown in Appendix 2 Table 5 indicate that a higher effective contact rate may result in a greater number of contaminated eggs being moved before infection is detected in the flock.

Appendix 2 Table 5. Variation of *Emax* with the effective contact rate.

	Estimated highest number of contaminated eggs per day among all the days prior to detection from a flock of 100,000 layers		
Effective contact rate (birds/6 hours)	1	2*	4
Mean (eggs/day)	5	12	30
90% interval (eggs/day)	0-17	0-40	0-62
Max (eggs/day)	404	1209	6139

*We used this contact rate as the baseline for our analysis.

The simulation models to estimate the viral titer in a tanker of NPLE and the dose response model show that contents from approximately 65 contaminated eggs should be included in one tanker of NPLE in order to have a 1 percent probability of infection in a chicken exposed to one ml of NPLE. Considering the results from Appendix 2 Table 5, this result implies that the expected probability of infection in an exposed chicken is less than 1 percent when the effective contact rate is within the range of 1-4 birds/6 hours.

Based upon the above sensitivity analysis we conclude that,

1) The number of contaminated eggs from an infected undetected flock following the active surveillance protocol increases with the effective contact rate. The effective contact rate of 2-birds/6 hours used in our assessment is higher than the estimates from HPAI outbreak data in the literature and is conservative.

2) The probability of infection in a chicken exposed to 1 ml of NPLE from an outgoing tanker is less than 1 percent for the range of effective contact rates reported in the literature for HPAI infections in caged layers.

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Appendix 3. Estimating the likelihood that NPLE in Transport Container Leaving the Premises is Infectious to Exposed Chicken

The exponential dose response model

A dose response model is useful in estimating the probability of infection in a chicken exposed to a certain dose of virus. We used the single parameter exponential dose response model which has been used previously to model avian influenza and other viruses such as FMD.[60] The exponential model assumes that each unit dose of virus has an identical probability (p) of causing infection. The most commonly used dosage unit is the 50 percent embryo infectious dose or EID_{50}. The dose response curve is generated from data on the fraction of chickens developing infection given varying doses. A recent article by Swayne and Selmons[55] provides experimental data from eleven HPAI strains and also summarizes the relevant data in the literature. We used data from this article to fit the dose response curve.

Estimating the probability that a single embryo infectious dose of HPAI infects a chicken

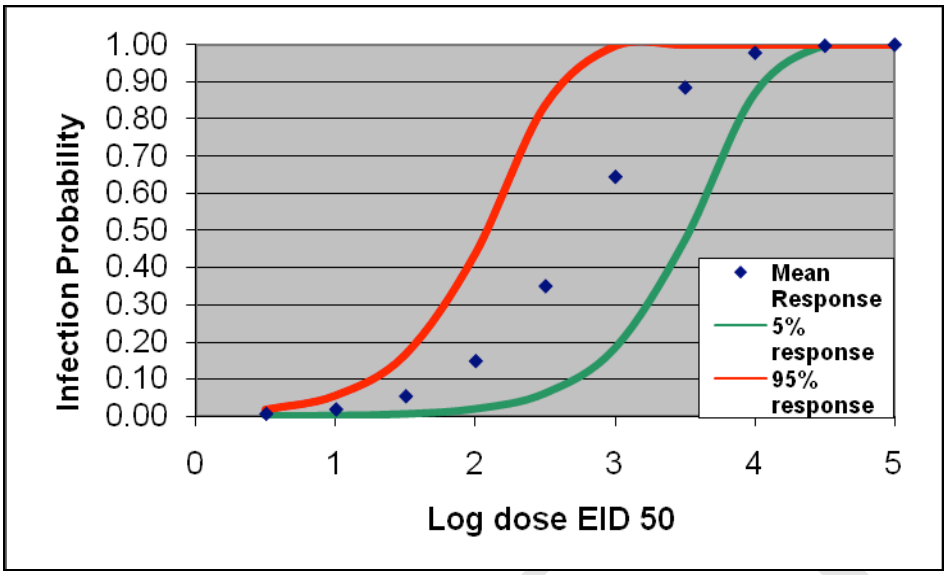
Data on infectivity of various HPAI strains is often presented as the 50 percent chicken infectious dose (CID_{50}). For example, a CID_{50} of 10^3 EID_{50} implies that a dose of 10^3 EID_{50} would infect 50 percent of exposed chickens. The CID_{50}'s of 17 HPAI strains reviewed in Swayne and Selmons [55] were mostly between 10^2 EID_{50} to $10^{3.9}$ EID_{50}. We utilized the @RISK to fit various distributions to this data. We decided to use a logistic distribution for this data based on Kolmogorov-Smirnov goodness of fit test.[97] We used logistic distribution with a mean of $10^{2.80}$, and a standard deviation of $10^{0.43}$ EID_{50} as the distribution of CID_{50} for HPAI. According to the exponential dose response model, the probability of infection in a chicken (P_{inf}) given a dose (D) is given by the following equation, where p is defined as the probability of 1 EID_{50} causing infection:

$$P_{\text{inf}} = 1 - e^{-pD}$$

Given a dose (D) equal to the CID_{50}, the probability (P_{inf}) is 0.5 by definition, and p can be derived as follows:

$$p = -\ln(0.5) \Big/ D$$

We utilized @RISK to simulate the distribution of CID_{50} and obtain the output distribution for the parameter p. Given the uncertainty in p via simulation, we also obtained the distributions for the probability of infection for various doses in the range $10^{0.5}$ to 10^7 EID_{50}. We used Latin Hypercube sampling with 50000 iterations for the simulation settings. The resulting dose response curve with the mean probability of infection at various doses and two sided 90 percent probability interval is shown in Appendix 3 Figure 1.



Appendix 3 Figure 1. Dose response curve for HPAI.

Estimating the Probability that a Chicken Exposed to 1 ml of NPLE from an Outgoing Tanker Develops HPAI Infection

Given an estimate of the parameter p of the dose response model, the probability (p_{inf}) of infection in a chicken exposed to a dose D (measured in EID_{50}) is given by,

$$P_{\inf} = 1 - e^{-pD}$$

We used the results from section 8.2.2 and appendix 2 to estimate the dose of virus D contained in 1 ml of NPLE from an outgoing tanker. We simulated utilizing @RISK with Latin Hypercube sampling for 5000 iterations. The mean P_{inf} from simulation 0.2 percent (90 percent interval 10^{-4}-0.6 percent). If the outgoing NPLE tanker is only 25 percent full, given the higher viral titer due to decreased dilution, the mean P_{inf} was 0.6 percent (90 percent interval 10^{-3}-2 percent).

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Appendix 4. Selected Portions of 21CFR110

TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (CONTINUED)

PART 110_CURRENT GOOD MANUFACTURING PRACTICE IN MANUFACTURING, PACKING, OR HOLDING HUMAN FOOD

Subpart A_General Provisions

Sec.
110.3 Definitions.
110.5 Current good manufacturing practice.
110.10 Personnel.
110.19 Exclusions.

Subpart B_Buildings and Facilities

110.20 Plant and grounds.
110.35 Sanitary operations.
110.37 Sanitary facilities and controls.

Subpart C_Equipment

110.40 Equipment and utensils.

Subpart D [Reserved]

Subpart E_Production and Process Controls

110.80 Processes and controls.
110.93 Warehousing and distribution.

Subpart F [Reserved]

Subpart G_Defect Action Levels

110.110 Natural or unavoidable defects in food for human use that present no health hazard.

Authority: 21 U.S.C. 342, 371, 374; 42 U.S.C. 264.

Source: 51 FR 24475, June 19, 1986, unless otherwise noted.

Subpart A_General Provisions

Sec. 110.3 Definitions.

The definitions and interpretations of terms in section 201 of the Federal Food, Drug, and Cosmetic Act (the act) are applicable to such terms when used in this part. The following definitions shall also apply:
(a) Acid foods or acidified foods means foods that have an equilibrium pH of 4.6 or below.
(b) Adequate means that which is needed to accomplish the intended purpose in keeping with good public health practice.
(c) Batter means a semifluid substance, usually composed of flour and other ingredients, into which principal components of food are dipped or with which they are coated, or which may be used directly to form bakery foods.
(d) Blanching, except for tree nuts and peanuts, means a prepackaging heat treatment of foodstuffs for a sufficient time and at a sufficient temperature to partially or completely inactivate the naturally occurring enzymes and to effect other physical or biochemical changes in the food.

(e) Critical control point means a point in a food process where there is a high probability that improper control may cause, allow, or contribute to a hazard or to filth in the final food or decomposition of the final food.
(f) Food means food as defined in section 201(f) of the act and includes raw materials and ingredients.
(g) Food-contact surfaces are those surfaces that contact human food and those surfaces from which drainage onto the food or onto surfaces that contact the food ordinarily occurs during the normal course of operations. ``Food-contact surfaces'' includes utensils and food-contact surfaces of equipment.
(h) Lot means the food produced during a period of time indicated by a specific code.
(i) Microorganisms means yeasts, molds, bacteria, and viruses and includes, but is not limited to, species having public health significance. The term ``undesirable microorganisms'' includes those microorganisms that are of public health significance, that subject food to decomposition, that indicate that food is contaminated with filth, or that otherwise may cause food to be adulterated within the meaning of the act. Occasionally in these regulations, FDA used the adjective ``microbial'' instead of using an adjectival phrase containing the word microorganism.
(j) Pest refers to any objectionable animals or insects including, but not limited to, birds, rodents, flies, and larvae.
(k) Plant means the building or premises or parts thereof, used for or in connection with the manufacturing, packaging, labeling, or holding of human food.
(l) Quality control operation means a planned and systematic procedure for taking all actions necessary to prevent food from being adulterated within the meaning of the act.
(m) Rework means clean, unadulterated food that has been removed from processing for reasons other than insanitary conditions or that has been successfully reconditioned by reprocessing and that is suitable for use as food.
(n) Safe-moisture level is a level of moisture low enough to prevent the growth of undesirable microorganisms in the finished product under the intended conditions of manufacturing, storage, and distribution. The maximum safe moisture level for a food is based on its water activity (a). An a will be considered safe for a food if adequate data are available that demonstrate that the food at or below the given a will not support the growth of undesirable microorganisms.
(o) Sanitize means to adequately treat food-contact surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance, and in substantially reducing numbers of other undesirable microorganisms, but without adversely affecting the product or its safety for the consumer.
(p) Shall is used to state mandatory requirements.
(q) Should is used to state recommended or advisory procedures or identify recommended equipment.
(r) Water activity is a measure of the free moisture in a food and is the quotient of the water vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature.

Sec. 110.5 Current good manufacturing practice.

(a) The criteria and definitions in this part shall apply in determining whether a food is adulterated (1) within the meaning of section 402(a)(3) of the act in that the food has been manufactured under such conditions that it is unfit for food; or (2) within the meaning of section 402(a)(4) of the act in that the food has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health. The criteria and definitions in this part also apply in determining whether a food is in violation of section 361 of the Public Health Service Act (42 U.S.C. 264).
(b) Food covered by specific current good manufacturing practice regulations also is subject to the requirements of those regulations.

Sec. 110.10 Personnel.

The plant management shall take all reasonable measures and precautions to ensure the following:
(a) Disease control. Any person who, by medical examination or supervisory observation, is shown to have, or appears to have, an illness, open lesion, including boils, sores, or infected wounds, or any other abnormal source of microbial contamination by which there is a reasonable possibility of food, food-contact surfaces, or food-packaging materials becoming contaminated, shall be excluded from any operations which may be expected to result in such contamination until the condition is corrected. Personnel shall be instructed to report such health conditions to their supervisors.
(b) Cleanliness. All persons working in direct contact with food, food-contact surfaces, and food-packaging materials shall conform to hygienic practices while on duty to the extent necessary to protect against contamination of food. The methods for maintaining cleanliness include, but are not limited to:
(1) Wearing outer garments suitable to the operation in a manner that protects against the contamination of food, food-contact surfaces, or food-packaging materials.
(2) Maintaining adequate personal cleanliness.
(3) Washing hands thoroughly (and sanitizing if necessary to protect against contamination with undesirable microorganisms) in an adequate hand-washing premises before starting work, after each absence from the work station, and at any other time when the hands may have become soiled or contaminated.
(4) Removing all unsecured jewelry and other objects that might fall into food, equipment, or containers, and removing hand jewelry that cannot be adequately sanitized during periods in which food is manipulated by hand. If such hand jewelry cannot be removed, it may be covered by material which can be maintained in an intact, clean, and sanitary

condition and which effectively protects against the contamination by these objects of the food, food-contact surfaces, or food-packaging materials.
(5) Maintaining gloves, if they are used in food handling, in an intact, clean, and sanitary condition. The gloves should be of an impermeable material.
(6) Wearing, where appropriate, in an effective manner, hair nets, headbands, caps, beard covers, or other effective hair restraints.
(7) Storing clothing or other personal belongings in areas other than where food is exposed or where equipment or utensils are washed.
(8) Confining the following to areas other than where food may be exposed or where equipment or utensils are washed: eating food, chewing gum, drinking beverages, or using tobacco.
(9) Taking any other necessary precautions to protect against contamination of food, food-contact surfaces, or food-packaging materials with microorganisms or foreign substances including, but not limited to, perspiration, hair, cosmetics, tobacco, chemicals, and medicines applied to the skin.
(c) Education and training. Personnel responsible for identifying sanitation failures or food contamination should have a background of education or experience, or a combination thereof, to provide a level of competency necessary for production of clean and safe food. Food handlers and supervisors should receive appropriate training in proper food handling techniques and food-protection principles and should be informed of the danger of poor personal hygiene and insanitary practices.
(d) Supervision. Responsibility for assuring compliance by all personnel with all requirements of this part shall be clearly assigned to competent supervisory personnel.

[51 FR 24475, June 19, 1986, as amended at 54 FR 24892, June 12, 1989]

…
Subpart B_Buildings and Facilities

Sec. 110.20 Plant and grounds.

(a) Grounds. The grounds about a food plant under the control of the operator shall be kept in a condition that will protect against the contamination of food. The methods for adequate maintenance of grounds include, but are not limited to:
(1) Properly storing equipment, removing litter and waste, and cutting weeds or grass within the immediate vicinity of the plant buildings or structures that may constitute an attractant, breeding place, or harborage for pests.
(2) Maintaining roads, yards, and parking lots so that they do not constitute a source of contamination in areas where food is exposed.
(3) Adequately draining areas that may contribute contamination to food by seepage, foot-borne filth, or providing a breeding place for pests.
(4) Operating systems for waste treatment and disposal in an adequate manner so that they do not constitute a source of contamination in areas where food is exposed. If the plant grounds are bordered by grounds not under the operator's control and not maintained in the manner described in paragraph (a) (1) through (3) of this section, care shall be exercised in the plant by inspection, extermination, or other means to exclude pests, dirt, and filth that may be a source of food contamination.
(b) Plant construction and design. Plant buildings and structures shall be suitable in size, construction, and design to facilitate maintenance and sanitary operations for food-manufacturing purposes. The plant and facilities shall:
(1) Provide sufficient space for such placement of equipment and storage of materials as is necessary for the maintenance of sanitary operations and the production of safe food.
(2) Permit the taking of proper precautions to reduce the potential for contamination of food, food-contact surfaces, or food-packaging materials with microorganisms, chemicals, filth, or other extraneous material. The potential for contamination may be reduced by adequate food safety controls and operating practices or effective design, including the separation of operations in which contamination is likely to occur, by one or more of the following means: location, time, partition, air flow, enclosed systems, or other effective means.
(3) Permit the taking of proper precautions to protect food in outdoor bulk fermentation vessels by any effective means, including:
(i) Using protective coverings.
(ii) Controlling areas over and around the vessels to eliminate harborages for pests.
(iii) Checking on a regular basis for pests and pest infestation.
(iv) Skimming the fermentation vessels, as necessary.
(4) Be constructed in such a manner that floors, walls, and ceilings may be adequately cleaned and kept clean and kept in good repair; that drip or condensate from fixtures, ducts and pipes does not contaminate food, food-contact surfaces, or food-packaging materials; and that aisles or working spaces are provided between equipment and walls and are adequately unobstructed and of adequate width to permit employees to perform their duties and to protect against contaminating food or food-contact surfaces with clothing or personal contact.

(5) Provide adequate lighting in hand-washing areas, dressing and locker rooms, and toilet rooms and in all areas where food is examined, processed, or stored and where equipment or utensils are cleaned; and provide safety-type light bulbs, fixtures, skylights, or other glass suspended over exposed food in any step of preparation or otherwise protect against food contamination in case of glass breakage.
(6) Provide adequate ventilation or control equipment to minimize odors and vapors (including steam and noxious fumes) in areas where they may contaminate food; and locate and operate fans and other air-blowing equipment in a manner that minimizes the potential for contaminating food, food-packaging materials, and food-contact surfaces.
(7) Provide, where necessary, adequate screening or other protection against pests.

Sec. 110.35 Sanitary operations.

(a) General maintenance. Buildings, fixtures, and other physical facilities of the plant shall be maintained in a sanitary condition and shall be kept in repair sufficient to prevent food from becoming adulterated within the meaning of the act. Cleaning and sanitizing of utensils and equipment shall be conducted in a manner that protects against contamination of food, food-contact surfaces, or food-packaging materials.
…
(c) Pest control. No pests shall be allowed in any area of a food plant. Guard or guide dogs may be allowed in some areas of a plant if the presence of the dogs is unlikely to result in contamination of food, food-contact surfaces, or food-packaging materials. Effective measures shall be taken to exclude pests from the processing areas and to protect against the contamination of food on the premises by pests. The use of insecticides or rodenticides is permitted only under precautions and restrictions that will protect against the contamination of food, food-contact surfaces, and food-packaging materials.

…
Subpart E_Production and Process Controls

Sec. 110.80 Processes and controls.

All operations in the receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, and storing of food shall be conducted in accordance with adequate sanitation principles. Appropriate quality control operations shall be employed to ensure that food is suitable for human consumption and that food-packaging materials are safe and suitable. Overall sanitation of the plant shall be under the supervision of one or more competent individuals assigned responsibility for this function. All reasonable precautions shall be taken to ensure that production procedures do not contribute contamination from any source. Chemical, microbial, or extraneous-material testing procedures shall be used where necessary to identify sanitation failures or possible food contamination. All food that has become contaminated to the extent that it is adulterated within the meaning of the act shall be rejected, or if permissible, treated or processed to eliminate the contamination.
(a) Raw materials and other ingredients. (1) Raw materials and other ingredients shall be inspected and segregated or otherwise handled as necessary to ascertain that they are clean and suitable for processing into food and shall be stored under conditions that will protect against contamination and minimize deterioration. Raw materials shall be washed or cleaned as necessary to remove soil or other contamination. Water used for washing, rinsing, or conveying food shall be safe and of adequate sanitary quality. Water may be reused for washing, rinsing, or conveying food if it does not increase the level of contamination of the food. Containers and carriers of raw materials should be inspected on receipt to ensure that their condition has not contributed to the contamination or deterioration of food.
(2) Raw materials and other ingredients shall either not contain levels of microorganisms that may produce food poisoning or other disease in humans, or they shall be pasteurized or otherwise treated during manufacturing operations so that they no longer contain levels that would cause the product to be adulterated within the meaning of the act. Compliance with this requirement may be verified by any effective means, including purchasing raw materials and other ingredients under a supplier's guarantee or certification.
(3) Raw materials and other ingredients susceptible to contamination with aflatoxin or other natural toxins shall comply with current Food and Drug Administration regulations and action levels for poisonous or deleterious substances before these materials or ingredients are incorporated into finished food. Compliance with this requirement may be accomplished by purchasing raw materials and other ingredients under a supplier's guarantee or certification, or may be verified by analyzing these materials and ingredients for aflatoxins and other natural toxins.
(4) Raw materials, other ingredients, and rework susceptible to contamination with pests, undesirable microorganisms, or extraneous material shall comply with applicable Food and Drug Administration regulations and defect action levels for natural or unavoidable defects if a manufacturer wishes to use the materials in manufacturing food. Compliance with this requirement may be verified by any effective means, including purchasing the materials under a supplier's guarantee or certification, or examination of these materials for contamination.
(5) Raw materials, other ingredients, and rework shall be held in bulk, or in containers designed and constructed so as to protect against contamination and shall be held at such temperature and relative humidity and in such a manner as to

prevent the food from becoming adulterated within the meaning of the act. Material scheduled for rework shall be identified as such.

(6) Frozen raw materials and other ingredients shall be kept frozen. If thawing is required prior to use, it shall be done in a manner that prevents the raw materials and other ingredients from becoming adulterated within the meaning of the act.

(7) Liquid or dry raw materials and other ingredients received and stored in bulk form shall be held in a manner that protects against contamination.

(b) Manufacturing operations. (1) Equipment and utensils and finished food containers shall be maintained in an acceptable condition through appropriate cleaning and sanitizing, as necessary. Insofar as necessary, equipment shall be taken apart for thorough cleaning.

(2) All food manufacturing, including packaging and storage, shall be conducted under such conditions and controls as are necessary to minimize the potential for the growth of microorganisms, or for the contamination of food. One way to comply with this requirement is careful monitoring of physical factors such as time, temperature, humidity, pH, pressure, flow rate, and manufacturing operations such as freezing, dehydration, heat processing, acidification, and refrigeration to ensure that mechanical breakdowns, time delays, temperature fluctuations, and other factors do not contribute to the decomposition or contamination of food.

(3) Food that can support the rapid growth of undesirable microorganisms, particularly those of public health significance, shall be held in a manner that prevents the food from becoming adulterated within the meaning of the act. Compliance with this requirement may be accomplished by any effective means, including:

(i) Maintaining refrigerated foods at 45 [deg] F (7.2 [deg] C) or below as appropriate for the particular food involved.

(ii) Maintaining frozen foods in a frozen state.

(iii) Maintaining hot foods at 140 [deg] F (60 [deg] C) or above.

(iv) Heat treating acid or acidified foods to destroy mesophilic microorganisms when those foods are to be held in hermetically sealed containers at ambient temperatures.

(4) Measures such as sterilizing, irradiating, pasteurizing, freezing, refrigerating, controlling pH or controlling that are taken to destroy or prevent the growth of undesirable microorganisms, particularly those of public health significance, shall be adequate under the conditions of manufacture, handling, and distribution to prevent food from being adulterated within the meaning of the act.

(5) Work-in-process shall be handled in a manner that protects against contamination.

(6) Effective measures shall be taken to protect finished food from contamination by raw materials, other ingredients, or refuse. When raw materials, other ingredients, or refuse are unprotected, they shall not be handled simultaneously in a receiving, loading, or shipping area if that handling could result in contaminated food. Food transported by conveyor shall be protected against contamination as necessary.

(7) Equipment, containers, and utensils used to convey, hold, or store raw materials, work-in-process, rework, or food shall be constructed, handled, and maintained during manufacturing or storage in a manner that protects against contamination.

(8) Effective measures shall be taken to protect against the inclusion of metal or other extraneous material in food. Compliance with this requirement may be accomplished by using sieves, traps, magnets, electronic metal detectors, or other suitable effective means.

(9) Food, raw materials, and other ingredients that are adulterated within the meaning of the act shall be disposed of in a manner that protects against the contamination of other food. If the adulterated food is capable of being reconditioned, it shall be reconditioned using a method that has been proven to be effective or it shall be reexamined and found not to be adulterated within the meaning of the act before being incorporated into other food.

(10) Mechanical manufacturing steps such as washing, peeling, trimming, cutting, sorting and inspecting, mashing, dewatering, cooling, shredding, extruding, drying, whipping, defatting, and forming shall be performed so as to protect food against contamination. Compliance with this requirement may be accomplished by providing adequate physical protection of food from contaminants that may drip, drain, or be drawn into the food. Protection may be provided by adequate cleaning and sanitizing of all food-contact surfaces, and by using time and temperature controls at and between each manufacturing step.

(11) Heat blanching, when required in the preparation of food, should be effected by heating the food to the required temperature, holding it at this temperature for the required time, and then either rapidly cooling the food or passing it to subsequent manufacturing without delay. Thermophilic growth and contamination in blanchers should be minimized by the use of adequate operating temperatures and by periodic cleaning. Where the blanched food is washed prior to filling, water used shall be safe and of adequate sanitary quality.

(12) Batters, breading, sauces, gravies, dressings, and other similar preparations shall be treated or maintained in such a manner that they are protected against contamination. Compliance with this requirement may be accomplished by any effective means, including one or more of the following:

(i) Using ingredients free of contamination.

(ii) Employing adequate heat processes where applicable.

(iii) Using adequate time and temperature controls.

(iv) Providing adequate physical protection of components from contaminants that may drip, drain, or be drawn into them.

(v) Cooling to an adequate temperature during manufacturing.
(vi) Disposing of batters at appropriate intervals to protect against the growth of microorganisms.
(13) Filling, assembling, packaging, and other operations shall be performed in such a way that the food is protected against contamination. Compliance with this requirement may be accomplished by any effective means, including:
(i) Use of a quality control operation in which the critical control points are identified and controlled during manufacturing.
(ii) Adequate cleaning and sanitizing of all food-contact surfaces and food containers.
(iii) Using materials for food containers and food- packaging materials that are safe and suitable, as defined in Sec. 130.3(d) of this chapter.
(iv) Providing physical protection from contamination, particularly airborne contamination.
(v) Using sanitary handling procedures.
(14) Food such as, but not limited to, dry mixes, nuts, intermediate moisture food, and dehydrated food, that relies on the control of a for preventing the growth of undesirable microorganisms shall be processed to and maintained at a safe moisture level. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
(i) Monitoring the of food.
(ii) Controlling the soluble solids-water ratio in finished food.
(iii) Protecting finished food from moisture pickup, by use of a moisture barrier or by other means, so that the of the food does not increase to an unsafe level.
(15) Food such as, but not limited to, acid and acidified food, that relies principally on the control of pH for preventing the growth of undesirable microorganisms shall be monitored and maintained at a pH of 4.6 or below. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
(i) Monitoring the pH of raw materials, food in process, and finished food.
(ii) Controlling the amount of acid or acidified food added to low-acid food.
(16) When ice is used in contact with food, it shall be made from water that is safe and of adequate sanitary quality, and shall be used only if it has been manufactured in accordance with current good manufacturing practice as outlined in this part.
(17) Food-manufacturing areas and equipment used for manufacturing human food should not be used to manufacture nonhuman food-grade animal feed or inedible products, unless there is no reasonable possibility for the contamination of the human food.

[51 FR 24475, June 19, 1986, as amended at 65 FR 56479, Sept. 19, 2000]

Sec. 110.93 Warehousing and distribution.

Storage and transportation of finished food shall be under conditions that will protect food against physical, chemical, and microbial contamination as well as against deterioration of the food and the container.

Appendix 5. Simulation Model to Evaluate the Effectiveness of the Chlorine Based Sanitizing Spray in Inactivating HPAI Virus

Overview

We developed an @RISK[98] simulation model to evaluate the potential degree of inactivation resulting from the sanitizing spray. A commonly used measure of exposure to disinfecting agents is *Ct* defined as the product of the average concentration C_a and exposure time *t*. In the simulation model, we first estimated a probability distribution for *Ct*, using exposure times t=1,…,8 seconds. We considered that chlorine concentration would exponentially decay (Equation 1) due to reaction with organic material on the eggshell surface.

$$C = C_o e^{-kt} \qquad (1)$$

Where *C* is the chlorine concentration at time *t,* C_0 is the initial chlorine concentration and *k* is the decay rate (min^{-1}). *Ct* can then be calculated as shown in equation (2).

$$Ct = \frac{C_o}{k}(1 - e^{-kt}) \qquad (2)$$

We compared *Ct* values from the simulation output with values required to achieve a 1000 factor inactivation as reported in various sources.[73,99]

Model Assumptions

a) Eggs have been washed and cleaned prior to sanitization, leaving no soil or feces on the shell egg surface.
b) HPAI virus is present on the shell egg surface[u]
c) Eggs are in contact with the sanitizing spray for 1 to 8 seconds
d) Chlorine concentration is maintained at 100-200 ppm
e) Hepatitis A virus is less sensitive to inactivation by chlorine compared to the enveloped HPAI virus.
f) The *Ct* values and chlorine decay rates given in the above reference are reasonable values to use for egg processing operations

Model Inputs

a) The exposure time *t* =time (minutes) that the eggshell is in contact with the sanitizing rinse. The exposure time is not specified by regulations and may vary according to conveyer belt speed and timing of sanitizing-rinse application. Industry experts suggest a minimum contact time of 8 seconds. We used a conservative distribution, *t*~Uniform (1/60-8/60), in minutes, for this parameter.

[u] The amount of virus present was not specified in the model, as there has been no research on this issue. However, HPAI has been detected on egg shells [9,23,62]

b) k is the exponential decay rate constant for chlorine concentration. This decay rate could be relatively low if washing is effective and all eggshells are relatively clean. We conservatively chose to utilize the k from experiments with chlorine demand from chicken egg aminoallantoic fluid.[73] We used Uniform (1.24, 2.33) (min^{-1})as the distribution for k.
c) Initial chlorine concentration C_0: We used Uniform (100, 200) mg/l as the distribution for initial chlorine concentration given that regulation mandates a chlorine concentration between 100-200 ppm.
d) Reported Ct: the Ct required for achieving a 3-log inactivation of HPAI virus as reported from various literature sources. The reported Ct depends on the temperature and effective pH of the sanitizing rinse. For neutral pH (7-8), we used the Ct value from Rice *et al*.[73] of 0.79 mg-min/L. For pH greater than 10, we used reported values based on hepatitis A inactivation at 25° C [77,99]. Uniform 1-5 mg-min/L was used as the distribution for reported Ct when sanitizing rinse pH is greater than 10.

Simulation Results

We used @RISK to simulate the above input distributions. We utilized Latin hypercube sampling with 50000 iterations. The mean Ct value from the simulation was 11.9 mg-min/L. The Ct value was greater than 4.2 mg-min/L with 95 percent confidence. For neutral pH(7-8) all simulated values were greater than the reported Ct implying that a 3-log inactivation would be achieved. For pH greater than 10, there is 98 percent probability that the simulated Ct is greater than the reported Ct for achieving 3-log inactivation of HPAI virus.

Appendix 6. Form PY203 Daily Report of Plant Operation.

UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
EGG PRODUCTS INSPECTION

DAILY REPORT OF PLANT OPERATION

NAME OF PLANT	ADDRESS OF PLANT		PLANT NUMBER
SIGNATURE OF INSPECTOR	INSPECTOR'S BADGE NUMBER	DATE	CODE DATE
PROCESSING OPERATIONS BREAKING FROM:_____A.M. TO:_____P.M. PASTEURIZATION FROM:_____A.M. TO:_____P.M.	INSPECTOR'S HOURS OF DUTY FROM:_____A.M. TO:_____P.M.		

INSTRUCTIONS: Give exact figures where applicable. Mark ✓ for "Yes" or "Satisfactory" and X or "No" or "Unsatisfactory".

TIME OF INSPECTION		TIME OF INSPECTION				
SANITATION/PROGRAM MONITORING		21. Organoleptic inspection and pour test.				
1. Cleanliness and sanitizing of equipment used for pasteurized liquid *(pipelines, gaskets, valves, pumps, etc.)*		22. Health and cleanliness of employees *(uniforms, hair nets, clean hands, etc.)*				
2. Cleanliness and sanitizing of equipment prior to start up.		23. Breaking or breaking machines operating in sanitary manner.				
3. Cleanliness of shell egg washers and conveyors.		24. Organoleptic examination of individual eggs.				
4. General sanitation of other areas		25. Breaking procedure when inedible is encountered *(segregate inedible, change equipment, wash hands, etc.)*				
5. Premises, receiving, and shipping areas.						
6. Refuse removal and disposal.		26. Segregation of leakers, dirties and loss for breaking.				
7. Restrooms and lunchrooms.		27. Denaturing and labeling of inedible eggs and liquid.				
8. CIP cleaning of pipelines and equipment.		28. Hydrogen peroxide test.				
9. a. Are breaking and packaging room, compressor, air filters, etc., satisfactory?		29. Sanitation - packaging room and equipment.				
		30. Product containers clean and sanitarily filled.				
9. b. Are air lines to product contact surfaces blown out and clean prior to use?		31. Container identification and labeling.				
		32. Accuracy of weighing product.				
10. Edible ingredient storage		33. Positive flow of air in processing and packaging rooms.				
11. Insecticides, rodenticides, etc., isolated from chemical compounds.		34. Processing rooms free from flies and odors.				
		35. Equipment clean and sanitized prior to use.				
12. Insecticides, rodenticides, and chemical compounds isolated from edible products.		36. Sanitation - breaking and processing rooms and equipment.				
		37. Shell strainers, egg filters efficient and cleaned.				
13. Package material storage.		38. Sanitation - transfer room, wash water and equipment.				
14. Freezers, clean, containers properly spaced, and air circulation adequate.		39. Show ppm of sanitizing spray for shell eggs				
		40. Show temperature of shell egg wash water.				
15. Is the exhaust system operable in the rest-room, transfer and refuse rooms?		41.				
		42.				
16. Tanker truck area.		43.				
17. Shell egg rooms and coolers.		44.				
18. Fly and rodent control inside and outside plant.		45.				
19. Verify Plant's Salmonella Surveillance Record		46.				
20. Verify Product Formulation/Refractometer		47.				

TEMPERATURES	TIME:				TIME:				TIME:				TIME:			
	WHOLE EGGS	YOLKS	WHITES	EGG PROD.	WHOLE EGGS	YOLKS	WHITES	EGG PROD.	WHOLE EGGS	YOLKS	WHITES	EGG PROD.	WHOLE EGGS	YOLKS	WHITES	EGG PROD.
UNPASTEURIZED LIQUID a. 2 hrs. after breaking - to be held under 8 hrs.																
b. 2 hrs. after breaking - to be held over 8 hrs.																
c. Held for shipment or processing																
PASTEURIZATION a. Recorder - controller																
b. Indicating thermometer																
c. Flow-diversion valve setting.																
d. Flow-rate per minute																
e. Holding time *(minutes)*																
PASTEURIZED LIQUID a. 2 hrs. after pasteurizing - to be held under 8 hrs.																
b. 2 hrs. after pasteurizing - to be held over 8 hrs.																
c. Held for shipment																
FREEZER OR LIQUID HOLDING ROOM																

REMARKS: *(Explain any deviations from above. Use reverse, if necessary.)*

PY FORM 203 (3/16/2005) PREVIOUS EDITIONS ARE OBSOLETE

PY FORM 203 (3/16/2005) REVERSE

EGGS PROCESSED	TOTAL OF SHELL EGGS (liquid equivalent) AND LIQUID DENATURED
30 - Doz Cases	lbs.

TYPE PRODUCT PRODUCED	PRODUCTION RECORD			FINAL INSPECTION *(Liquid or Frozen)*				TEST WEIGHING *(Finished Product)*			
	NO. CONTAINER	CONT MARKED WEIGHT	POUNDS	DATE DRILLED OR INSPECTED	NO. CONTAINER	TEMP.	CONDITION	NO. CONTAINER	GROSS	TARE	NET
Inedible											
TOTAL											

REMARKS

INDICATING THERMOMETER			
ACCURACY *(Quarterly)* ° F.		LAG *(Quarterly)* SECONDS	
RECORDER - CONTROLLER			
ACCURACY *(Quarterly)* ° F.	THERMOMETRIC LAG *(Quarterly)*	CUT-IN TEMPERATURE *(Monthly)* ° F.	CUT-OUT TEMPERATURE *(Monthly)* ° F.
FLOW-DIVERSION			
RESPONSE TIME *(Quarterly)*		DOES VALVE SEAT PROPERLY? *(Daily)* ☐ YES ☐ NO	
DOES FLOW LEAK PAST FORWARD FLOW SEAT WHEN OPERATING IN DIVERSION? *(Biweekly)* ☐ YES ☐ NO		RESPONSE TO MANUAL DIVERSION: *(Biweekly)*	

Appendix 7. PY159 Daily Report of Egg Drying Operations.

U.S. DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
EGG PRODUCTS INSPECTION

DAILY REPORT OF EGG DRYING OPERATIONS

PLANT NAME AND ADDRESS (Print)	PLANT NUMBER	DATES	VISIT NUMBER (Enter numerical visit number for this fiscal year)	CODE DATE

INSPECTOR'S SOCIAL SECURITY NUMBER	SIGNATURE(S) OF INSPECTOR(S)	INSPECTOR'S HOURS OF DUTY				PROCESSING OPERATIONS			
		FROM a.m.	TO a.m.	FROM p.m.	TO p.m.	DRYING FROM	DRYING TO	PASTEURIZATION FROM	PASTEURIZATION TO

INSTRUCTIONS: Give exact figures or method where applicable. Place a checkmark for "YES" or "SATISFACTORY" and an "X" for "NO" or "UNSATISFACTORY." Explain deviations on reverse side under "Remarks."

OPERATIONAL SANITATION CHECKLIST — TIME OF INSPECTION →								
1. Organoleptic inspection of liquid and powder?								
2. Packaging rooms, equipment, containers and liners and product sanitarily packaged?								
3. Are packaging and processing rooms, etc., air filtration systems and air flow satisfactory?								
4. Health and cleanliness of employees (*uniforms, hairnets, clean hands, food handling practices, etc.*)?								
5. Drier and processing rooms and equipment?								
6. Cleaning and sanitizing of tanker trucks?								
7. Container identification, labeling, and accuracy of weighing?								
8. Are openings closed, joints, gaskets, etc., sealed so unfiltered air is not drawn into drier system?								
9. Reconstituting, free flowing, adding and mixing ingredients?								
10. Pasteurizer and equipment used for pasteurized liquid (*pipelines, gaskets, valves, pumps, etc.*)?								
11. High pressure pumps, lines, valves, nozzles, cores, etc., and CIP cleaning of pipelines?								
12. Fly, rodent and odor control?								
13. Control, denaturing and labeling of inedible?								
14. Heat treatment room, spacing of product and air circulation?								
15. Other (*Specify*)								
16. Other (*Specify*)								

TEMPERATURES — TIME OF INSPECTION →									
PRODUCT →									
17. UNPASTEURIZED LIQUID a. Received - held under 8 hours									
b. Received - held over 8 hours									
c. Held for processing									
18. STABILIZATION									
19. PASTEURIZATION a. Recorder - Controller									
b. Indicating thermometer									
c. Flow-diversion valve setting									
d. Flow rate per minute									
e. Holding time (*minutes*)									
20. PASTEURIZED LIQUID a. Direct to drier									
b. To be held under 8 hours									
c. To be held over 8 hours									
d. Held for processing									
e. Other (*Specify*)									

INDICATING THERMOMETER

Accuracy	Thermometric response
°F	Seconds

RECORDER-CONTROLLER

Accuracy	Thermometric response
°F	°F

FLOW DIVERSION VALVE

Response Time	Does valve seat properly?
Seconds	☐ YES ☐ NO

Does flow leak past forward flow seat?

☐ YES ☐ NO

Response to Manual Diversion:

PY FORM 159 (5/97) REPLACES PY-159 (01-94), WHICH MAY BE USED UNTIL EXHAUSTED. Designed on Form Flow Software.

SANITATION CHECKLIST — TIME OF INSPECTION →		DRIER SANITATION	CLEANING METHOD	INSPECTION
21. Air lines to product contact surfaces blown out and clean prior to use		29. Drier, fans and ducts		
22. Edible ingredient storage		30. Augers, pneumatic system and valves		
23. Insecticides and chemicals isolated from edible products		31. Sifters and flexible connectors		
24. Package material and product storage		32. Bags and bag house		
25. General sanitation of other areas		33. Bag section(s) changed		
26. Rest rooms and lunchrooms		34. Drier air filtration system, fan and air ducts		
27. Premises, receiving and shipping areas		35. Sanitizing or preheating augers, pneumatic, sifter, etc.		
28. Refuse removal and disposal		36. Drier system openings and exhaust closed after cleaning and shutdown		
		37. Drier preheated _____ time _____ °F Intake _____ °F Exhaust _____		

TYPE PRODUCT PRODUCED	PRODUCTION RECORD			TEST WEIGHING (*Finished Product*)			
	NUMBER CONTAINERS	SIZE CONTAINERS	POUNDS	NUMBER CONTAINERS	GROSS	TARE	NET
INEDIBLE NONDENATURED							
INEDIBLE DENATURED							
TOTAL →							

REMARKS:

PRIVACY ACT STATEMENT:

The following statements are made in accordance with the Privacy Act of 1974 (5 USC 552a). Disclosure by you of your Social Security number is voluntary and is authorized under provisions of Executive Order 9397, dated November 22, 1943.

Purpose and Uses - The Social Security number will be used primarily to identify your records. The principal purpose of this form is to collect information needed to determine employee's on-the-job performance as well as employee/supervisory/plant performance on an area, regional and national level. It will also be used to develop percentages and statistics relating to grade standard tolerances. The use of the Social Security number is made necessary because of the large number of present and former Federal and State employees who have identical names and whose identities can only be distinguished by the Social Security number.

Effects of Nondisclosure - Failure to provide your Social Security number will result in an arbitrary identifier being assigned.

This program or activity will be conducted on a nondiscriminatory basis without regard to race, color, religion, national origin, age, sex, marital status, or disability.

PY FORM 159 (5/97) (REVERSE)

Appendix 8. Selected Portion of 9CFR590, Showing Official Identification Marks and Seals

(b) The inspection mark which is to be used on containers of edible egg products shall be contained within the outline of a shield and with the wording and design set forth in Figure 2 of this section, except the plant number may be preceded by the letter "P" in lieu of the word "plant". Alternatively, it may be omitted from the official shield if applied on the container's principal display panel or other prominent location and preceded by the letter "P" or the word "Plant".

FIGURE 1.

FIGURE 2.

[36 FR 9814, May 28, 1971, as amended at 40 FR 20008, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 60 FR 49170, Sept. 21, 1995]

§590.414 Products bearing the official inspection mark.

Egg products which are permitted to bear the inspection mark shall be processed in an official plant from edible shell eggs or other edible egg products and may contain other edible ingredients. The official mark shall be printed or lithographed and applied as a part of the principal display panel of the container but shall not be applied to a detachable cover.

§590.415 Use of other official identification.

Other official identification as shown in this section shall be printed or lithographed and applied as a part of the principal display panel, but shall not be applied to a detachable cover. The plant number may be omitted from the identification if applied elsewhere on the container's principal display panel or other prominent location and preceded by the letter "P" or the word "plant". Such products shall meet all requirements for egg products which are permitted to bear the official inspection mark shown in §590.412, except for pasteurization, heat treatment, or other such methods of treatment approved by the Administrator. Such products shall not be released into consuming channels until they have been subjected to pasteurization, heat treatment, or other approved methods of treatment.

(a) All nonpasteurized egg products, except as provided in paragraph (b) of this section, shipped from an official plant in packaged form shall be marked with the identification set forth in Figure 3 of this section. After pasteurization or treatment, the product may bear the official inspection mark as shown in §590.412.

FIGURE 3.

Appendix 9. Requirement of NPLE to be pasteurized

See the Egg Products Inspection Act, Section 1036— "Egg products inspected at any official plant under the authority of this chapter and found to be not adulterated shall be pasteurized before they leave the official plant, except as otherwise permitted by regulations of the Secretary…"

9CFR415—"…Such products shall meet all requirements for egg products which are permitted to bear the official inspection mark shown in Sec. 590.412 (Appendix 8),[39] except for pasteurization, heat treatment, or other such methods of treatment approved by the Administrator. Such products shall not be released into consuming channels until they have been subjected to pasteurization, heat treatment, or other approved methods of treatment.

Appendix 10. Inspection Under HACCP

HACCP-oriented food safety inspection changes the approach of FSIS to overseeing the safety of meat and poultry products. Under this new approach, FSIS will rely less on after-the-fact detection of product and process defects and more on verifying the effectiveness of processes and process controls designed to ensure food safety. FSIS will restructure its inspection procedures to determine that production systems prevent the production of unsafe meat and poultry products. FSIS will carry out various activities to ensure that industry HACCP systems meet the requirements of this rule, and are functioning as designed.

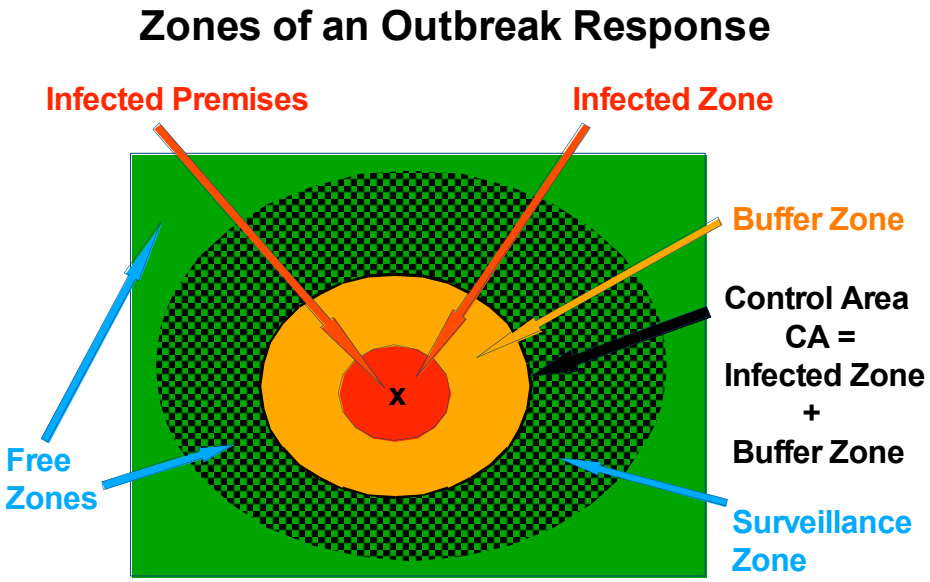
The establishment must comply with all regulatory requirements. The establishment must develop written plans/procedures for HACCP, SSOP, and E. coli testing. Any time inspection personnel determine that regulatory requirements have not been met, the noncompliance will be documented and appropriate enforcement action will be taken. Upon initiation, inspection personnel verify that the plan or procedure is apparently responding to all regulatory requirements. If a required feature is not included in the plan or procedure, the nonconformance is documented and enforcement action is taken.
Additional verification activities and noncompliance documentation are used to determine whether there has been a system failure. Enforcement is the action taken by inspection personnel when a failure has occurred.

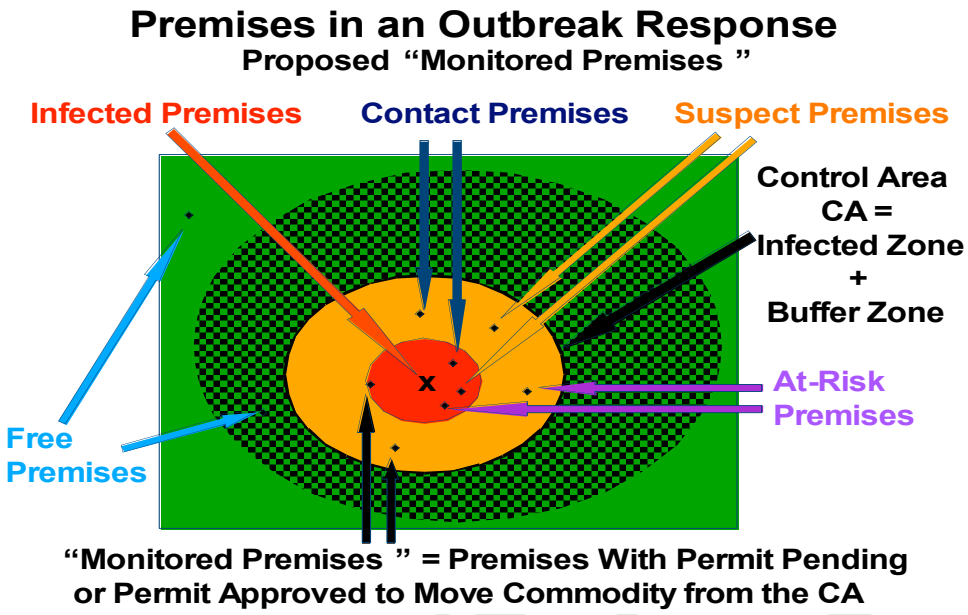
Appendix 11. United Egg Producers/United Egg Association Movement Control Model Plan

United Egg Producers/United Egg Association
USDA APHIS Veterinary Services

Highly Pathogenic Avian Influenza (HPAI)
Movement Control Model Plan: Commercial Layer Industry Operations

Protocol for the Movement of Liquid Egg Product, Further Processed Egg Products, Inedible Egg, Table Eggs and Broken Egg Shells, Egg-Type Hatching Eggs, and Day-Old Chicks Within, Out of, and Into a Defined "Control Area"





1. **Flocks that are determined to be "Infected" premises by epidemiological investigation and/or diagnostic testing:**

   a. **Definition of "Infected" premises:** "Infected" premises are premises where HPAI is presumed or confirmed to exist based on laboratory results and compatible clinical signs. All presumed positive premises and confirmed positive premises are classified as infected premises. In addition, all other premises that meet the current case definition for HPAI are classified as "Infected" premises.

   b. **Disposition of "Infected" premises:** "infected" premises are quarantined immediately, and all domesticated birds and other susceptible livestock are depopulated and disposed of in proper biosecure procedures. No movement of susceptible species or their products (e.g., shell eggs, hatching eggs, day old chicks, broken egg shells, unpasteurized liquid egg product, pasteurized egg products) will be allowed off the "Infected Premises" premises, except for disposal and must be moved under permit.

2. **Flocks that are determined to be "Contact" premises by epidemiological investigation:**

   a. **Definition of "Contact" premises: "**Contact" premises are premises with birds or other susceptible animals, *conveyances, or products* that have been exposed directly or indirectly to birds and other animals, products, materials, people, or aerosol from an "Infected" premises. The specific exposure factors to be considered must be appropriate to the epidemiology of HPAI. The commercial layer industry HPAI "Contact" premises" include the following direct or indirect contact sources:

i. Premises with susceptible birds exposed to poultry **manure** from an infected flock (virus in manure).
ii. Premises with susceptible birds exposed to **dead poultry** from an infected flock (virus in carcasses, etc).
iii. Premises with susceptible birds exposed to **live poultry** from an infected flock (virus in bird & secretions & excretions).
iv. Premises with susceptible birds exposed to **eggs or egg handling materials** from an infected flock (HPAI virus in and on egg).
v. Premises with susceptible birds with **unprotected exposure to equipment** that have been in contact with infected birds, manure, carcasses, or eggs. Unprotected means inadequate sanitation procedures for those items/people that come into contact with an infected flock.
vi. Premises with susceptible birds with **unprotected exposure to people** that have been in contact with infected birds, manure, carcasses, or eggs.
vii. Premises involved in depopulation of infected flocks.

b. Disposition of "Contact" premises: "Contact" premises will be quarantined and subject to strict biosecurity measures, daily monitoring of mortality in each house, and intensive surveillance for HPAI viruses in each house by RRT-PCR testing (see 5 below) for at least 42 days or until the Incident Commander is convinced that no HPAI is present on the premises,

i. Following complete epidemiological investigation, biosecurity assessments, and negative diagnostic testing for HPAI, "Contact" premises can be re-designated as "At-Risk" premises or "Monitored" premises.
ii. "Contact" premises with 75,000 hens or more will not be depopulated until a diagnosis of HPAI has been confirmed by case definition or diagnostic testing.
iii. "Contact" premises that are determined to be HPAI infected by case definition or diagnostic testing will be depopulated immediately.
iv. Movement from "Contact" premises is allowed by permit only.

3. **Flocks that are determined to be "Suspect" premises by epidemiological investigation:**

a. Definition of "Suspect" premises: "Suspect" premises are premises where birds or other susceptible livestock are under epidemiological investigation for a report of clinical signs compatible with HPAI, but the case definition for HPAI has not been met, and HPAI has not been detected or confirmed by diagnostic testing.

b. Disposition of "Suspect" premises": "Suspect" premises will be quarantined and subject to strict biosecurity measures, daily monitoring of mortality in each house, and surveillance for HPAI viruses in each house

by RRT-PCR testing (see 5 below), until the conditions are met to re-designate the "Suspect" premises as an "Infected" premises," a "Contact" premises, an "At Risk" premises, or a "Monitored" premises.

i. "Suspect" premises must have complete epidemiological investigation, biosecurity assessments, and test negative for HPAI before being re-designated a "Monitored" premises (see 6 below).
ii. "Suspect" premises with 75,000 hens or more will not be depopulated depopulated until a diagnosis of HPAI has been confirmed by case definition or diagnostic testing.
iii. "Suspect" premises that are determined to be HPAI infected by case definition or diagnostic testing will be depopulated immediately.
iv. Movement from "Suspect" premises is allowed by permit only.

4. **Flocks that are designated as "At-Risk" premises prior to epidemiological investigation:**

a. Definition of "At-Risk" premises: "At-Risk" premises are those premises in the Infected Zone or Buffer Zone that have susceptible animals, but none of those susceptible animals have clinical signs compatible with HPAI. "At-Risk" premises have not been subject to epidemiological investigation, biosecurity assessments, or diagnostic testing for HPAI to warrant a re-designation to "Contact" premises or "Monitored" premises.

b. Disposition of "At-Risk" premises: After complete epidemiological investigation, biosecurity assessments, and diagnostic testing for HPAI, "At-Risk" premises can be re-designated to "Contact" premises or " "Monitored" premises.

i. Movement from "At-Risk" premises is allowed by permit only.

5. **Flocks that are determined to be <u>"Monitored" premises</u> by epidemiological investigation:**

a. Definition of "Monitored" premises: "Monitored" premises are in close proximity to an infected flock, and are located in the Infected Zone or Buffer Zone, which comprise the Control Area. "Monitored premises" can objectively demonstrate that they are not to be designated as "Infected" premises, "Contact" premises, "Suspect" premises, or "At-Risk" premises, following complete epidemiological investigation, biosecurity risk assessments, and diagnostic testing for HPAI. "Monitored" premises objectively demonstrate:

i. "Monitored" premises objectively demonstrate that they do not meet the definitions for "Infected" premises, "Contact" premises, "Suspect"

premises or "At-Risk" premises by complete epidemiological investigation and questionnaire.

ii. "Monitored premises" objectively demonstrate that systematic biosecurity measures and precautions have been taken to protect the premises against HPAI.

iii. "Monitored" premises objectively demonstrate flock health parameters

iv. "Monitored" premises objectively demonstrate diagnostic testing negative for HPAI.

b. Disposition of "Monitored" premises:

i. Premises located within the Control Area must only be designated "Monitored" premises by the Incident Commander or their designee.

ii. The designation of "Monitored" premises during an actual incident by the Incident Commander or their designee will be facilitated and accelerated by biosecurity risk assessments conducted prior to the incident, by rapid epidemiological investigation and epidemiological questionnaire at the time of the incident, strategic placement of diagnostic sampling equipment prior to the incident, and tactical execution of diagnostic sample testing at the start of an incident.

iii. "Monitored" premises, depending upon their location, the actual incident circumstances, and epidemiological considerations of the actual outbreak, will be granted permits to move Liquid Egg Product, Further Processed Egg Products, Inedible Egg, Table Eggs and Broken Egg Shells, Egg-Type Hatching Eggs, and Day-Old Chicks Within, Out of, and Into a Defined "Control Area" at the discretion of the incident commander or their designee.

6. Determination of non-infected layer industry flocks in the Control Area.

a. The absence of infection will be documented by requiring chickens from flocks that are not exhibiting signs of the disease and that show no unexpected increase in mortality from each house on the farm to be tested each day and found to be negative by the real time reverse transcriptase – polymerase chain reaction (RRT-PCR) or other suitable procedure as determined by the Incident Command.

i. A minimum or five chickens from the daily mortality and/or from euthanized sick birds from each house (flock) will be placed in a leak proof container (e.g. heavy duty plastic garbage bag) each morning. Each container will be labeled with the farm of origin, house of origin, and the number of birds found dead in the house that day. The containers will be taken to a designated pick-up point, typically the public road closest to the premises.

Rationale: In a large commercial poultry house (100,000 layers) normal" mortality will be about 10 per day. A doubling of normal mortality to 20 due to HPAI (dead bird prevalence of 50 percent and flock prevalence of

0.04 percent) would be detected by sampling 5 dead birds. Historically, APHIS sampled 5 dead birds **per week** to monitor chicken houses in the END outbreak in CA and this plan requires daily monitoring. The proposed AI plan requires daily monitoring and will be 7 times more effective than the monitoring during the END outbreak. It is not unusual for mortality to fluctuate that much from day to day, so sampling dead/sick birds every day is likely more sensitive than monitoring weekly mortality (where a trend over 2 or 3 days might be observed before acting). It is reasonable to assume that 50 percent of the sick and dead birds (in a house that is infected with HPAI) would actually be shedding AI virus then a sample size of 5 birds would allow you to have 95% confidence of finding the virus in the sick or dead birds.

ii. A state or federal regulatory official or an individual authorized by the Incident Command will take a "oropharyngeal" swab from each chicken. Five oropharyngeal swabs will be pooled in a tube containing brain-heart infusion (BHI) broth. Sample pooling will be done on a per house basis. One BHI tube containing oropharyngeal samples (5 oropharyngeal swabs/BHI tube) will be submitted as directed by the Incident Command to an authorized State Veterinary Diagnostic Laboratory (VDL). These samples must be submitted on the day of sample collection by the state or federal regulatory official or an individual authorized by the Incident Command. The State VDL and the IC will establish the time of day by which samples must be submitted to an authorized VDL (example, by 12:30 pm). VDL personnel will perform RRT-PCR testing on these samples immediately upon receipt and electronically send test results to the Incident Command (IC) by the end of each day. The IC will report the test result information to the premises as soon as it is available.

7. Movement of liquid egg product, further processed egg products, inedible egg, table eggs and broken egg shells, egg-type hatching eggs, and day-old chicks from "Monitored" premises**.**

a. Movement of liquid egg product, table eggs, egg-type hatching eggs, further processed egg products, and broken egg shells *within and out of* a Control Area from "Monitored" premises will be allowed by permit for those flocks inside the control area testing negative (see Section 5 above) as follows, including any unsold inventories on hand:

i. USDA FSIS inspected pasteurized egg products, or precooked egg products produced by plants within a control area may move within or out of the Control Area by Permit (accompanied by documentation of origin of the products). The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

ii. Unpasteurized liquid egg product may move in officially FSIS sealed vehicles per 9 CFR Chapter III Part 590.410 from breaking operations within the Control Area directly to pasteurization plants located within or out of the Control Area by permit. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver willnot be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

iii. Inedible egg from graders and/or breaking plants in a Control Area may move by permit for pasteurization or to approved waste disposal sites within or outside the Control Area. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

iv, Washed and graded shell eggs destined for food service, retail marketing, further processing, or for breaking may be moved out of the Control Area by permit if they have been washed and sanitized using 100 – 200 ppm chlorine solution. The transport vehicle shall be sealed by farm or company personnel under the authorization of the Incident Command. Egg handling materials used in the transport of eggs to breaking or further processing plants must be destroyed at the plant or cleaned, sanitized (following accepted procedures) and returned to the premises of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

v. Nest run shell eggs (not washed and sanitized) must be moved directly for washing and grading, further processing, or to an off-line breaking operation. The transport vehicle shall be sealed by farm or company personnel under the authorization of the Incident Command. Egg handling materials must be destroyed at the destination plant or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

vi. Broken eggshells on the farm or from breaking plants, pasteurization plants, and/or further processing plants may be moved by permit. The transport vehicle shall be sealed by farm or company personnel under

the authorization of the Incident Command. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

vii. Hatching eggs from source flocks tested negative for AI virus by daily mortality sampling may be moved to hatcheries within the Control Area with a permit. Egg handling materials must be destroyed at the hatchery or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area

viii. Hatching eggs from "Monitored Premises" source flocks tested negative for AI virus by daily mortality sampling may be moved out of the Control Area by permit. The chicks must be placed under a "post-hatch" quarantine for 30 days. Egg handling materials must be destroyed at the premises of destination or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area. The State Veterinarian of the state of destination must be faxed a copy of the restricted movement permit within 24 hours of issuance, and a permit is required to move within and out of the Control Area.

ix. Day-old chicks from source flocks tested negative for AI virus by daily mortality sampling may be shipped by permit within or out of the Control Area and must be placed under a 30 day quarantine. The State Veterinarian of the State of destination must be faxed a copy of the restricted movement permit within 24 hours of issuance. Hatcheries may receive eggs that originate outside the Control Area (accompanied by documents showing the origin of the eggs and the AI negative status of the source flock) without a permit. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area.

x, The Incident Command or designate will evaluate and approve the risk assessment and risk mitigation procedures necessary to move products

by permit. A permit must be issued and seals placed on the vehicle by a state or federal regulatory official or a person authorized by the Incident Command. The Incident Command will authorize procedures to break the seals outside of the control area with proper documentation.

b. Movement of liquid egg product, shell eggs, broken egg shells, and hatching eggs *into* a Control Area will be allowed without permit under the following conditions:

   i. Pasteurized liquid egg product and unpasteurized liquid egg (and blends) from breaking plants and/or pasteurization plants outside a Control Area (and accompanied by documentation of origin) may move into pasteurization and/or further processing plants located in a Control Area without permit. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises in a Control Area, and a permit is required to exit the Control Area.
   ii. Shell eggs may move into breaking, grading, pasteurization, and/or further processing plants from outside Control Areas (accompanied by proof of origin) without a permit. Egg handling materials must be destroyed at the plant or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises within a Control Area, and a permit is required to exit the Control Area.
   iii. Broken egg shells may move into a Control Area (accompanied by proof of origin) without a permit. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises within a Control Area, and a permit is required to exit the Control Area.
   iii. Hatching eggs may move into a hatchery from outside Control Areas (accompanied by proof of origin and AI tested negative flocks without a permit. Egg handling materials must be destroyed at the plant or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The cargo interior and exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises within a Control Area, and a permit is required to exit the Control Area.

8. Determination of Release of Movement Restrictions

a. All premises within the Control Area will be eligible for release from movement restrictions as determined by the Incident Command when:

i. All infected flocks in a Control Area have been depopulated. All depopulated flock premises have been cleaned and disinfected. A minimum of 42 days have passed, or environmental sampling has proven HPAI virus negative status for the depopulated premises.

ii. All contact premises in a control area must have been depopulated or must have been monitored for 42 days.

9. Appendices.

a. APHIS CEAH - Egg Sector Working Group – University of MN CAHFS Pasteurized Liquid Egg Risk Assessment.

This plan has been written by egg industry and university personnel based on their knowledge of the egg industry. Standard Operating Procedures from the Exotic Newcastle Disease (END) outbreak were reviewed as a starting point for developing this plan.

Appendix 12. Selected Portions of 9CFR325.

Bold-type sections are included in this Appendix

Title 9: Animals and Animal Products
PART 325—TRANSPORTATION

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§ 325.1 Transactions in commerce prohibited without official inspection legend or certificate when required; exceptions; and vehicle sanitation requirements.

(a) No person shall sell, transport, offer for sale or transportation, or receive for transportation, in commerce, any product which is capable of use as human food unless the product and its container, if any, bear the official inspection legend as required under parts 316 and 317 of this subchapter or such product is exempted from the requirement of inspection under part 303 of this subchapter.

(b)(1) No carrier shall transport or receive for transportation in commerce (including transportation in the course of importation) and no person shall offer for transportation any carcass, part thereof, meat or meat food product until a certificate, if required for such transportation by this part, is made and furnished to the carrier in one of the forms prescribed in this part.

(2) Product imported into the United States may be transported and offerred or received for transportation if such product is conveyed in railroad cars, trucks or other means of conveyance, prior to inspection, to an authorized place of inspection, as provided in §327.6 of this part.

(c) No person, engaged in the business of buying, selling, freezing, storing, or transporting, in or for commerce, meat or meat food products capable of use as human food, or importing such articles, shall transport, offer for

transportation, or receive for transportation in commerce or in any State designated under §331.2 of this subchapter, any such meat or meat food product which is capable of use as human food and is not wrapped, packaged, or otherwise enclosed to prevent adulteration by airborne contaminants, unless the railroad car, truck, or other means of conveyance in which the product is contained or transported is completely enclosed with tight fitting doors or other covers for all openings. In all cases, the means of conveyance shall be reasonably free of foreign matter (such as dust, dirt, rust, or other articles or residues), and free of chemical residues, so that product placed therein will not become adulterated. Any cleaning compound, lye, soda solution, or other chemical used in cleaning the means of conveyance must be thoroughly removed from the means of conveyance prior to its use. Such means of conveyance onto which product is loaded, being loaded, or intended to be loaded, shall be subject to inspection by an inspector at any official establishment. The decision whether or not to inspect a means of conveyance in a specific case, and the type and extent of such inspection shall be at the Program's discretion and shall be adequate to determine if product in such conveyance is, or when moved could become, adulterated. Circumstances of transport that can be reasonably anticipated shall be considered in making said determination. These include, but are not limited to, weather conditions, duration and distance of trip, nature of product covering, and effect of restowage at stops en route. Any means of conveyance found upon such inspection to be in such condition that product placed therein could become adulterated shall not be used until such condition which could cause adulteration is corrected. Product placed in any means of conveyance that is found by the inspector to be in such condition that the product may have become adulterated shall be removed from the means of conveyance and handled in accordance with §318.2(d) of this subchapter.

[35 FR 15605, Oct. 3, 1970, as amended at 41 FR 23700, June 11, 1976; 47 FR 17274, Apr. 22, 1982; 56 FR 65180, Dec. 16, 1991]

§ 325.5 Unmarked inspected product transported under official seal between official establishments for further processing; certificate.

(a) Any product which has been inspected and passed may be transported from one official establishment to another for further processing without each article being marked with the official inspection legend, if it is so transported in a railroad car, motortruck, or other means of conveyance which is sealed by a Program employee with an official seal of the Department prescribed in §312.5(a) of this subchapter. Unless 25 percent or more of the contents of each car or other means of conveyance consists of product not marked with the inspection legend, transportation will not be permitted under this paragraph.

(b) When articles are offered for transportation under paragraph (a) of this section, the initial carrier shall require, and the shipper shall make in duplicate and deliver to the carrier, one copy of a certificate in the following form:[1]

[1] For convenience in filing, it is requested that these certificates be made on paper 51/2×8 inches in size.

Date _____, 19__Name of carrier_____
Establishment number of consignor_____
Point of shipment_____
Establishment number of consignee_____
Destination_____
Car number and initials_____
License number of other means of conveyance_____

I hereby certify that the following described product has been U.S. inspected and passed by the U.S. Department of Agriculture; and that it is not marked “U.S. inspected and passed,” but has been placed in the means of conveyance specified above under the supervision of an employee of the Meat and Poultry Inspection Programs of said Department, and the means of conveyance has been sealed by him with official U.S. Government seals Nos. __ and __.

Kind of product Amount and weight

_____
_____
_____
(Signature of shipper)(Address of shipper)

When paunches are offered for transportation under this paragraph, the initial carrier shall require, and the shipper shall make in duplicate and deliver to the carrier, one copy of a certificate in duplicate in the form set out in §**325**.5(b), appropriately modified. Certificates in this form or copies thereof need not be forwarded to any official or office of the Department, but the original of the certificate shall be retained by the carrier and a copy

shall be retained by the shipper in accordance with part 320 of this subchapter. If the shipper is also the carrier, he shall nevertheless execute and retain the certificate in accordance with part 320 of this subchapter.

(c) The signature of the shipper or his agent shall be written in full. This certificate may be stamped upon or incorporated in any form ordinarily used in the transportation of product. Certificates in this form or copies thereof need not be forwarded to any official or office of the Department. The original of the certificate required by this section shall be retained by the carrier and a copy shall be retained by the shipper in accordance with part 320 of this subchapter. If the shipper is also the carrier, he shall nevertheless execute and retain the certificate in accordance with part 320 of this subchapter.

§ 325.14 Certificates, retention by carrier.

All original certificates delivered to a carrier in accordance with this part shall be filed separate and apart from all its other papers and records or identified in such a manner as to be readily checked by Department employees. Every certificate required to be maintained under this part shall be retained for a period of 2 years after December 31 of the year in which the transaction has occurred.

§ 325.16 Official seals; forms, use, and breaking.

(a) The official seals required by this part shall be those prescribed in §312.5(a) of this subchapter.

(b) Except as provided in §**325**.18(b), official seal affixed under this part shall be affixed or broken only by Program employees, and no person other than a Program employee shall affix, detach, break, change, or tamper with any such seal in any way whatever. Commission of any such acts contrary to this regulation is a criminal offense.

§ 325.17 Loading or unloading products in sealed railroad cars, trucks, etc., en route prohibited; exception.

Unloading any product from an officially sealed railroad car, truck, or other means of conveyance containing any unmarked product or loading any product or any other commodity in the means of conveyance while en route from one official establishment to another official establishment is not permitted, except that product transported under §**325**.5 from one official establishment to another for further processing may be unloaded and stored in transit at any approved warehouse which is operated under the identification service provided under the regulations in part 350 of subchapter B of this chapter and which has railroad facilities or a receiving dock for unloading the product directly into such warehouse: *Provided,* That the product is stored in rooms which are of such size and type as will not result in adulteration or misbranding of the product: *And provided further,* That the product is transported to and from such warehouse, and under official seal as provided in §**325**.5 and stored in such rooms at such warehouse.

Appendix 13. Email from Roger Glasshoff, AMS National Supervisor Shell Eggs

Regarding list of approved egg cleaners and sanitizers:

7/23/08 to Camille Effler

The subject "List" was actually published by the Food Safety and Inspection Service of USDA. However, in 1997, FSIS notified the industry that, as an agency, it would no longer review for approval any compounds for cleaning, sanitizing, lubricating, marking, etc., for use in official processing plants. The Department discontinued publication of the "List" in 1998 and advised industry that a letter of guarantee must be provided musty be provided from the manufacturer of a compound indicating its acceptability as safe for the intended use. In 2001, FSIS stated that although a specific compound appears acceptable on the "1998 List", the industry must have a letter of guarantee that the compound formula has not been changed since approved previously by FSIS. Similarly, AMS established the policy of requiring the letter of guarantee for such compounds for use in plants operating under the Regulations Governing the Voluntary Grading of Shell Eggs. Some State programs that are responsible for the processing of eggs for human consumption (non-official plants) may still be referencing the "1998 List." Unfortunately, I do not have a list any longer.

The National Sanitation Foundation (NSF) provides compound manufacturers an approval process on a fee basis in accordance with FDA regulations and posts the approved compounds (with their intended use) on their website – www.nsf.org. The NSF also requires that a manufacturer confirm on an established frequency that the compound formulation has not changed. This practice assures that the NSF list of compounds remains current.

Appendix 14. Key Issues for Moving Nonpasteurized Liquid Eggs during an HPAI Outbreak

This document is intended to give emergency response staff a summary of issues to consider when assessing the risk of moving nonpasteurized liquid eggs during an HPAI outbreak. It summarizes, but does not replace, the information given in the complete risk assessment. Given the specific circumstances of the outbreak, it is likely that additional information that is not shown in the summary will be needed to complete the risk assessment.

1. Food Safety and Inspection Service (FSIS)
   - 1.1. FSIS contact for the premises: _____
   - 1.2. What is the FSIS overall opinion on bio-security at the premises?
   - 1.3. Is FSIS aware of any additional measures the premise has implemented in response to the outbreak?
   - 1.4. Has FSIS found any regulatory violations in the last six months that may have led to cross-contamination of products?
   - 1.5. Does FSIS know of any other issues that may affect the likelihood that HPAI may contaminate products at these premises?

2. Sources of eggs
   - 2.1. Does the premise receive eggs from off-site suppliers? If yes, answer questions 2.1.1- 2.1.4 below.
     - 2.1.1. What measures is the premise taking to exclude eggs from premises where HPAI is known to be present?
     - 2.1.2. How are communications with egg suppliers established, maintained, and used?
     - 2.1.3. Can the premises trace incoming shipments of eggs back to the suppliers?
     - 2.1.4. Have the premises received eggs from premises that were known to have HPAI present? If so, how were eggs disposed?
   - 2.2. Does the premise have layer operations on-site? If yes, answer questions 2.1.1- 2.1.4 below.
     - 2.2.1. Are there separate dedicated employees for the henhouse operations and the egg processing/breaking operations?
     - 2.2.2. Is there any additional segregation of duties between those working in the henhouse and the egg breaking operations in response to outbreak?
     - 2.2.3. Please list any additional bio-security practices for employees entering and leaving the henhouse in response to the outbreak.

3. Egg Breaking Process
   - 3.1. Please provide documentation for the cleaning and sanitization procedures, for the egg breaking and the liquid egg handling equipment in the egg breaking room.
   - 3.2. Please list any additional sanitary measures for personnel entering/leaving the egg breaking room in response to outbreak
   - 3.3. Has there been any malfunction of the breaking or handling equipment in the last six months resulting in the spillage of liquid egg product.
   - 3.4. Do the premises have any other comments or concerns related to egg breaking process during this outbreak?

3.5. Has the premises implemented any additional measures related to egg breaking and handling of NPLE?
3.6. If additional measures have been implemented, please provide documentation for them.

4. Handling and storage
   4.1. Please provide a copy of the premises' policies and procedures for handling and storage of liquid egg products at the premises.
   4.2. Please list the different types of liquid egg storage containers/tanks used in the plant.
   4.3. Has there been any malfunction of handling/chilling/storage equipment in the last six months resulting in the spillage of liquid egg product.
   4.4. If there have been malfunctions, what happened and what corrective actions were taken?
   4.5. Has the premises implemented any additional measures related to handling and storing egg products in response to the outbreak? If yes what are they?
   4.6. Please provide documentation of these additional measures.
   4.7. About how long do the premises hold nonpasteurized liquid before they are shipped to the customer?

5. Access Controls
   5.1. What access control measures are in place at the premises?
   5.2. What access control measures are used when there is no HPAI outbreak?
   5.3. What access control measures are used during an HPAI outbreak?
   5.4. Are these access control measures different? If so, how are they different?

6. Movement of Nonpasteurized Liquid Eggs
   6.1. Please provide a copy of the premise's policies and procedures for moving nonpasteurized liquid eggs.
   6.2. Are vehicles transporting pasteurized liquid eggs segregated from other vehicles on the premise's grounds?
   6.3. Has the premises implemented any additional measures related to moving nonpasteurized liquid eggs in response to the outbreak?
   6.4. Does the premise use the model movement plan developed by the egg products industry? (Note to reviewer: the model movement plan is in Appendix 11 of this assessment.)
   6.5. If additional measures have been implemented (besides those shown in the model movement plan), if yes provide documentation of these additional measures?
   6.6. Where does the premises clean and disinfect vehicles transporting liquid eggs?
   6.7. What procedures are followed and what disinfectant is used?

Are vehicles transporting liquid eggs that enter the premises' grounds cleaned and disinfected before loading or unloading cargo?

Appendix 15. The Use of "Negligible Risk" in this Assessment

Negligible Risk Defined for this Analysis:

For this risk analysis, the term "negligible risk" means there is a very low likelihood that moving nonpasteurized liquid egg products will cause infection in another poultry production premise. The specific magnitude cannot be determined, as there is no evidence that these products have ever served as a transmission pathway. In quantitative terms, this is defined as the likelihood of less than 1/1,000,000 that moving these products will result in infection in another premise. 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 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.[100] [101-104]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.[105]

Use in Agricultural Risk Analysis

The use of risk analysis for imports of agricultural products became mandatory with the adoption of the SPS Agreement[v] in 1995.[w] 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).[106] The OIE has published standards and guidance [107] [108]for conducting risk analysis, but has not formally defined "negligible" in a quantitative sense.[109] However, in a World Trade Organization trade dispute case,[110] negligible risk was considered to be a risk whose probability is very low,[111] 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."[112]

Policy Implications of a Quantitative Definition for Negligible

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,"[113] vague and inconsistent,[104] and has been "used and (abused) in various policy contexts."[114] However, use of this figure is meant to be a very rough

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

[w] Risk analysis is also required for moving animals and animal products during an HPAI oubreak.[118]

approximation and should not be given the same degree of certainty that may be applied when quantitative risk assessments can be used.

Negligible Risk as a Qualitative Measure for Agricultural Risk Analysis

The OIE has issued guidance that recommends using ”negligible” to mean “not worth considering; insignificant.”[107] 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.[115] 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.[116] These opinions by the court system are also consistent with U.S. views expressed in WTO trade disputes.