Risk that Poultry Feed made with Corn—Potentially Contaminated with Eurasian-North American Lineage H5N2 HPAI Virus from Wild Migratory Birds—Results in Exposure of Susceptible Commercial Poultry
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EXECUTIVE SUMMARY

Background and Overview

Corn may sometimes be stored uncovered on the ground in large piles, exposed to the open environment. Open storage may allow for possible contamination with Eurasian-North American (EA/NA) lineage H5N2 highly pathogenic avian influenza (HPAI) virus in feces from wild migratory waterfowl shedding the virus. During the recent HPAI outbreak in Minnesota and Iowa, the observation of corn piles contaminated with wild bird feces raised concerns about the possibility that contaminated corn may be a potential pathway of HPAI virus introduction and spread, in the event that contaminated corn is used in commercial poultry feed in the upcoming fall or spring seasons.

To evaluate the risk of introduction of EA/NA H5N2 HPAI virus into commercial poultry through contaminated corn, we qualitatively estimated the likelihood that a corn pile could become contaminated by wild migratory birds during the fall and early spring seasons while corn is stored on the ground. Using dose response models, we then estimated the probability that a batch of commercial poultry feed containing a small quantity of EA/NA H5N2 HPAI virus contaminated feces would result in the transmission of HPAI virus to susceptible poultry. We also considered the effect of raw ingredient processing during feed production on the reduction of HPAI virus levels, and evaluated the risk of transmission through pelleted and mash feed separately, as well as mash feed treated with formaldehyde.

Risk managers were also concerned about the impact of breaches in biosecurity at feed mills and feed storage bins on the farm and the risk of contamination of finished feed. We evaluated potential pathways of contamination of finished feed by passerine or perching birds in order to estimate the likelihood that finished feed becomes contaminated. We then used dose response models to estimate the probability of HPAI virus transmission to poultry resulting from contamination by a small quantity of HPAI virus contaminated feces.

Analysis and Results

The likelihood that a corn pile becomes contaminated with EA/NA H5N2 HPAI virus when corn is stored on the ground depends on the species of wild birds attracted to corn piles, the frequency with which they feed from the piles, and the HPAI prevalence in those wild bird species. As of April 29, 2015, Minnesota State Department of Natural Resources officials reported that there had been no findings of the H5N2 HPAI virus in 2,216 tests of waterfowl fecal matter. The surveillance strategy objective was to test 3,000 environmental samples from around the State. As of June 15th, there had been no reports of isolation of this virus in wild migratory waterfowl in Minnesota. Considering the uncertainty in the prevalence of EA/NA H5N2 HPAI virus in migratory waterfowl in Minnesota, and the ability of the virus to persist in the environment under
cooler wetter conditions, we estimate that there is a very low to low\(^1\) likelihood that a corn pile will become contaminated with EA/NA H5N2 HPAI virus in feces from wild migratory birds during the fall and spring seasons.

**Pelleted Feed**

Pelleted feed is a complete feed fed to meat-type turkeys generally beginning at 2 to 4 weeks of age. The production of pelleted feed involves two main thermal processes: 1) steam conditioning of raw feed ingredients to produce a cereal mash suitable for pellet extrusion, and 2) the pellet extrusion process where a further increase in temperature is achieved due to friction and pressure. Direct data on the inactivation of HPAI virus in poultry feed ingredients representative for the time and temperature ranges of the pelleting processes are not available. We used thermal inactivation parameters for HPAI virus estimated from chicken meat or liquid egg as a proxy, based on World Organization for Animal Health (OIE) requirements for inactivation in these products. We also elicited input from experts in pelleted feed production to estimate the impact of steam treatment on raw ingredients on the reduction of HPAI virus levels on corn used in pelleted feed. The results of our analysis predict a high probability of HPAI virus inactivation, even when only the mash conditioning process is considered. Our simulation model estimates that steam treatment of raw ingredients in mash prior to pelleting results in more than a 99 percent chance of a greater than 20 log\(_{10}\) EID\(_{50}\)/g inactivation. The probability of infection of a susceptible poultry flock, given exposure to a load of pelleted feed made with corn contaminated with HPAI virus was predicted to be 0 in all of the 20,000 iterations. Based on these results, the risk of HPAI virus transmission through the pelleted feed pathway was estimated to be negligible.

**Mash Feed**

**Untreated Mash Feed**

Mash feed may be fed as a starter diet to meat-type turkeys or broilers up until 2 to 4 weeks of age, or as a complete feed to breeder birds and table-egg layers. In contrast to the production of pelleted feed, only minimal heat is generated during the production of mash feed during the process of grinding corn. In this case, HPAI virus contamination present on corn would result in a direct transmission pathway to poultry. However, some corn used in mash feed is dried in vertical driers to reduce moisture content. We considered the impact of drying on the reduction of HPAI virus titers in feces on corn. Using a single-hit dose response model, we predicted that there would be a 35 percent chance (mean 35 percent, 95 percent P.I. 0 to 100 percent) of HPAI virus transmission to susceptible poultry. This prediction assumes that a small quantity of contaminated corn is incorporated into a batch of mash feed, during the cooler wetter weeks where corn is stored on the ground (i.e., under favorable conditions where there would be minimal virus inactivation in feces on corn in the environment). The wide prediction interval is indicative of the uncertainty in the model inputs for this scenario, primarily due to limited data on HPAI virus inactivation in dry feces at corn drier temperatures. Although the probability of exposure is

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\(^1\) The chances of this event happening range from being very unlikely that the event would occur to the event is unlikely, but does occur.
reduced by drying, there is insufficient evidence that the risk would be mitigated through the drying process in all cases. Corn that is not dried would have a higher probability of transmission.

**Mash Feed Treated with Formaldehyde**

Formaldehyde products are not consistently used in the manufacturing of commercial poultry feed. One limitation on the use of these products is cost, which restricts use mostly to the treatment of feed for primary layer breeders (genetic stock). Because no direct data on inactivation of HPAI virus on feed ingredients by formaldehyde treatment were available, we used data on inactivation of other pathogens (i.e., *Salmonella*) in poultry feed to develop model parameters. We then used exploratory scenario analysis to estimate the reduction in the probability of HPAI virus transmission in mash feed products treated with formaldehyde, given our uncertainty in the efficacy of these products on HPAI virus and the variability due to inconsistent application under field conditions.

Some studies have concluded that pathogens in feed (i.e., *Salmonella*) may not be completely eliminated during the treatment of feed ingredients, if the application of formaldehyde is uneven, or if the initial pathogen challenge rates are too high. Based on limited data published in the scientific literature, we estimated a 2 to 8 $\log_{10}$ EID$_{50}$/gram reduction in HPAI virus levels in mash feed containing contaminated corn attributed to formaldehyde treatment. Using this range to address the uncertainty and variability in the degree of HPAI virus inactivation in treated feed, our simulation models predict that if freshly contaminated corn is incorporated into a batch of mash feed treated with formaldehyde, there would be a 3 percent chance (mean 3 percent, 95 percent P.I. 0 to 17 percent) of HPAI virus transmission to susceptible poultry. In some cases, mash feed treated with formaldehyde may lower the risk of HPAI transmission. However, formaldehyde treatment may not completely eliminate the possibility of a new introduction.

Overall, we estimate there is a very low to low likelihood that feeding mash feed made from contaminated corn could be responsible for the introduction of a new case of HPAI virus during the fall or early spring. This risk estimate considers the low likelihood that a corn pile will become contaminated due to the very low estimated prevalence of EA/NA H5N2 HPAI virus in wild migratory waterfowl. Although we predict that mash feed may be responsible for a rare introduction of EA/NA H5N2 HPAI virus, it is very unlikely that mash feed is a major pathway of HPAI virus spread. Diverting corn stored on the ground for use in pelleted feed may be a reasonable risk mitigation measure considering the high cost of formaldehyde products, as the risk of HPAI virus transmission to poultry through pelleted feed is estimated as negligible.

**Contamination of Finished Feed by Perching Birds**

Risk managers were also concerned about the impact of breaches in biosecurity at feed mills and feed storage bins on the farm. To estimate the risk of transmission of EA/NA H5N2 HPAI virus from birds that gain access to stored feed, we evaluated potential pathways of contamination of finished feed by wild birds. Passerine or perching birds such as sparrows, European starlings, and grackles were implicated in local area spread of HPAI viruses in past outbreaks. Although measures can be taken to prevent access to feed, there is a possibility that passerine birds may gain access to finished feed in the event that a storage bin lid or cover is not secure. We estimated that the likelihood of contamination of finished feed with HPAI virus by passerine birds at the
feed mill and on the farm is very low to low, considering the very low estimated HPAI prevalence of infection in these birds, and biosecurity measures taken to exclude entry. An exponential dose response model predicts a high probability of HPAI virus transmission in the event that feed becomes contaminated with small amounts of HPAI virus contaminated passerine bird feces.

Direct data on HPAI virus inactivation in wild bird feces present on feed treated with formaldehyde products were not available, so we were not able to estimate the degree of virus reduction on the surface of finished feed due to feed treatment. HPAI virus titers in feces from infected passerine birds can be as high as 10^4 EID_{50}/gm, so any treatment applied would need to result in a 3 log or greater inactivation so as to have a low likelihood of infection. Improving biosecurity measures for feed storage at the feed mill or on the farm would likely be a more cost effective risk management strategy given the high cost of feed treatment, and the very low to low overall estimated risk of HPAI transmission by passerine birds.

**Important Limitations in this Analysis**

We used an exploratory analysis approach to address a few key areas of uncertainty. Data are not currently available on the prevalence or quantity of wild bird feces that might be present on corn. Consequently, we estimated the risk of HPAI virus transmission through feed by assuming that a small quantity of fecal contamination could potentially be present on a corn pile. We elicited input from three experts on the operation of poultry feed mills in order to estimate the degree of HPAI virus inactivation due to steam conditioning of raw ingredients and heat generated during pelleting. As of June 15th, limited data were available on the degree of inactivation of pathogens in feed or on feed by products containing formaldehyde. Direct data on the degree of HPAI virus reduction due to formaldehyde treatment would help reduce the uncertainty in our estimates. Experts at Kemin, the manufacturer of Sal CURB®, a formaldehyde-based feed disinfectant used within the poultry industry for the control of *Salmonella* in feed and feed ingredients, shared published data from experimental trials on Porcine Epidemic Diarrhea virus in swine feed and *Salmonella* in expanded poultry diets. Due to time constraints, we were unable to utilize these data to refine our model parameters for the current analysis. Finally, we estimated the dose response for the EA/NA H5N2 HPAI virus in turkeys from the latest available data from Agriculture Research Service, Southeast Poultry Research Laboratory. These data may not be representative of the currently circulating virus, as it may have become more host adapted to poultry making it more likely that HPAI virus transmission could occur at lower doses.
SCOPE JUSTIFICATION

During the recent HPAI virus outbreaks in Minnesota and Iowa, risk managers were concerned about biosecurity practices related to storage of feed ingredients and finished feed. Specifically, the observation of corn piles stored on the ground contaminated with wild bird feces raised concerns about the possibility that contaminated corn may be a potential pathway of HPAI virus introduction and spread. Risk managers were also concerned that feed treatments containing formaldehyde used to reduce pathogen load may not be sufficient to disinfect feed due to variability in application of these products in the field. Finally, the impact of breaches in biosecurity at feed mills and feed storage bins on the farm by wild birds was perceived to be contributing to HPAI virus spread.

The scope of this risk assessment was defined in order to address these specific risk management questions. This risk assessment is not intended to evaluate the risk that feed, feed delivery, or the feed truck driver results in HPAI virus spread between poultry premises. Likewise, the assessment does not evaluate the risk of passerine birds entering the barn. Passerine birds that enter the barn may contaminate feed, water or litter. Preventing wild bird access to the live production area is a structural and operational biosecurity issue.

BACKGROUND INFORMATION ON THE PRODUCTION OF COMMERCIAL POULTRY FEED

Overview of the Production of Commercial Poultry Feed

The commercial poultry industry formulates rations based on a least-cost feed formulation approach in order to achieve the lowest feed cost-per-unit of salable product (15). Corn and soybean meal are usually the lowest-cost sources of energy and are extensively used in the United States (15). Milo, wheat, barley, and oats are also being used but are inferior to corn in their relative value (15). Grain by-products such as corn gluten, bran, and wheat processing by-products are also utilized (15). Cottonseed meal may be used to replace up to 50 percent soybean meal in grower poultry diets (15). Fish and meat meals (animal by-products, poultry meal, blood meal, hydrolyzed poultry feathers) may also be used as protein and amino acid sources (15). Supplemental lipids (up to 5 percent) are incorporated to increase energy utilization (15). Yellow corn in combination with alfalfa meal or corn gluten meal are often used as a source of xanthophyll for yellow coloration of the skin (15). Rations are supplemented with sources of the major minerals, calcium, and phosphorous as well as salt (15). Non-nutritive additives including treatments such as formaldehyde and organic acids, antibiotics, arsenicals, nitrofurans, antiparasitic compounds, antioxidative, and antifungal compounds (15). Grits in the form of oyster or clam shells, limestone, or gravel pebbles may be added to facilitate grinding of feed in the gizzard (15).

Harvesting and Storage Conditions for Corn Used in the Production of Commercial Poultry Feed

In order to maintain grain quality from the field to the point-of-sale, on-farm drying and storage of corn is carefully monitored (50). Treatment of grain soon after harvest determines the storability and influences the quality as delivery of the product may be weeks, months, or years
after harvest (50). All equipment used to handle, dry, and store corn is cleaned prior to harvest to reduce mold and insect infestations (50). Measures are taken around storage bins to prevent rodent and insect harborage and infestation (50).

**Drying**

Corn may be dried on- or off-the-farm. Corn is dried in bin dryers, column dryers, or a combination of the two (50). Each system uses different amounts of heat and airflow, but regardless of the type used, high-moisture corn should be dried to 16 percent moisture within 24 hours and cooled to the outside air temperature within 48 hours after harvest to avoid storage losses (50). Prolonged heating results in dry matter loss, whereas high-moisture corn is subject to the development of mold. Number 2 yellow corn is usually marketed at 15.0 or 15.5 percent wet bases and all corn should be dried to 13.0 percent moisture for longer term storage (if not sold before warm weather the following spring) (50). High temperature 120 to 240° F (48 to 115.6° C) in-bin column dryers are used for high moisture corn and drying times are usually between a half-hour and two hours (50). Outside air may be used to dry corn in bin-dryers for corn with low moisture at harvest (16 to 18 percent). Outside air with the addition of heat is used as corn moisture increases in bin-dryers. Temperatures of 120 to 240° F (48 to 115.6° C) are needed when moisture exceeds 28 percent (50).

**Storage in enclosed bins**

Moisture and temperature are monitored throughout storage to prevent spoilage problems. Storage bins are monitored to ensure that no moisture buildup occurs during storage. Mold and insect activity are held in check when grain temperatures are held below 55° F (12.8° C), and the relative humidity in the airspace between corn kernels is below 65 percent (50). Corn in storage in September is stored at 65° F (18.3° C) and is cooled an additional 10 to 15° F each month during the fall. Aeration is used to equalize temperatures throughout the bin during storage to prevent moisture migration.

**Storage in the open environment**

Corn used in the production of commercial poultry feed may sometimes be stored uncovered on the ground in large piles, exposed to the open environment. Open storage may allow for the potential contamination of corn piles with feces from wild birds. In some cases, corn stored on the ground may be used without being dried.

**Poultry Feed Manufacturing Process**

Poultry diets are fed as either mash (ingredients are ground course, medium, or fine), pelletized (mash feeds that are cooked and then pelleted), or crumbles (produced by rolling pellets to break them into smaller particles) (15). Nearly all commercial meat-type broilers and turkeys are fed pelleted feed after 2 to 4 weeks of age, whereas nearly all table-egg layer and layer breeder operations feed mash diets, due primarily to the level of calcium carbonate (7 to 8 percent) required in the layer diet (pers. com., Industry Expert 1, May 18, 2015). However, some smaller locally owned cooperatives that source local ingredients may produce mash for use in turkeys. With the exception of corn, all other ingredients are post-processed (i.e., cooked or rendered) and
are handled in a closed-loop system, according to American Feed Industry Association guidelines.

**Corn as a feed ingredient**

In most poultry diets, corn is a major contributor of metabolizable energy (46). The U.S. feed industry usually uses grade Number 2 corn, which contains no more than 5 percent damaged kernels and 3 percent foreign material. Lower grades are often available due to adverse growing, harvesting, or storage conditions. For corn fed in mash diets, a uniform medium grind (0.7 to 0.9 mm) is preferred for digestibility.

**Pelleted feed**

Pelleted feeds are agglomerated feeds formed by extruding individual ingredients or mixtures by compacting and forcing them through die openings by a mechanical process (from the California Pellet Mill Co. website http://www.cpm.net/downloads/Animal%20Feed%20Pelleting.pdf). The pelleting process starts with a bin in which the mixture of mash is stored. From there, the mash will flow by gravity into the pellet mill. This machine is usually located on the ground or main work floor level. The pelleting process usually involves treating ground feed with steam and then passing the hot, moist mash through a die under pressure (46). The hot, extruded mash (pellets) flows by gravity into a cooler where it is held for three to six minutes while being cooled and dried by a flow of forced air. The air is drawn through the mass of pellets and passed into a dust-collecting device, such as a cyclone collector. The dust from the outlet of the collector is returned to the pellet mill to again be compacted into a pellet.

Steam conditioning of poultry diets requires a saturated steam, which consists mostly of vapor, as opposed to wet steam, which consists of free moisture (40). Saturated steam has been shown to increase mash temperature by 60° F (16° C) for every 1 percent moisture added. Steam temperature, on entry to the conditioner, needs to be about 212° F (100° C) in order to adequately condition the feed. Ideally, the temperature in the conditioner should be greater than 176° F (80° C) (40) to 180° F (82° C)(5). The presence of the appropriate level of moisture in the feed allows efficient transfer of heat and is critical in order to achieve gelatinization of starch. Typical meal moisture should approach 17 percent or 18 percent (5). The optimum retention (dwell) time for any particular conditioner is the amount of time that is required for heat and moisture to reach the center core of each feed particle in the ration. Retention times vary depending on the conditioner used (Table 1).

**Table 1. Retention time for different types of feed conditioners (40).**

<table>
<thead>
<tr>
<th>Equipment type</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single shaft conditioner</td>
<td>15 to 20 seconds</td>
</tr>
<tr>
<td>Double conditioner</td>
<td>40 to 45 seconds</td>
</tr>
<tr>
<td>Differential diameter speed</td>
<td>120 to 180 seconds</td>
</tr>
</tbody>
</table>

Optimum moisture content of feed is required for good pelleting (46). A range of 15 to 18 percent moisture is desirable, which results in “steam cooked” food. Pelleting at too low a temperature or with too little steam results in “shiny pellets” where the original mash is enclosed in a hard
capsule where the internal contents has not benefited from the cooking process (46). There are three types of coolers (horizontal, vertical, and counter flow) used to cool pellets after exiting the extruder to ambient temperature (5).

Mash feed
Mash feed is a uniform coarse-ground material, usually with no thermal processing (40). Mash is commonly fed in the table-egg layer industry and to breeder birds. Mash may also be fed to broilers or turkeys up to 2 to 4 weeks of age. However, some smaller cooperatives may feed mash feed to meat-type turkeys or broilers. With the exception of corn, all ingredients used in mash have been pre-processed. The only heat generated during the processing of mash is in the process of grinding corn. The majority of feed mills producing mash do not treat feed or feed ingredients for microbial control (i.e., they do not use formaldehyde based products) (pers. com., Industry Expert 1, May 18, 2015).

In the formulation of mash feed, corn is ground into a ground corn bin, batched into a mixer, blended for 160 to 210 seconds, conveyed to a load-out bin, and dropped into a feed truck or pneumatically blown into bins or large conveyors on farms with an on-farm-mixer that produces layer feed (pers. com., Industry Expert 2; May 20, 2015). Mash feed for layers is usually fed within 3 to 7 days of production, depending on their barn bin holding capacity.

ENTRY ASSESSMENT
An entry assessment determines the likelihood of a commodity (in this case poultry feed), being contaminated with a hazard (EA/NA lineage H5N2 HPAI virus), and describes the biological pathways necessary for the hazard to be introduced into a particular environment (flock of susceptible poultry). The entry assessment includes an estimation of the likelihood of each of the pathways occurring.

The entry assessment of this risk assessment evaluates the likelihood of EA/NA H5N2 HPAI virus being transmitted onto a commercial poultry premises via poultry feed contaminated with HPAI virus from wild bird feces. Contamination pathways include the use of contaminated corn stored in open piles on the ground by wild migratory waterfowl, or contamination of finished feed at the feed mill or on the farm by passerine (perching) birds.

Likelihood and Degree of Contamination of Commercial Poultry Feed with EA/NA H5N2 HPAI Virus from Wild Birds

Likelihood and degree of HPAI virus contamination of corn used in commercial poultry feed by wild migratory waterfowl

During the recent HPAI outbreak in Minnesota and Iowa, risk managers raised concerns that corn stored uncovered in piles on the ground may become contaminated with EA/NA H5N2 HPAI virus in feces from wild migratory waterfowl, resulting in the introduction and spread of HPAI virus into commercial poultry flocks during the upcoming fall and spring seasons. In this case, poultry feed made from HPAI virus contaminated corn would represent a direct exposure pathway. We consider factors such as the prevalence of avian influenza viruses in wild birds in Minnesota and the persistence of avian influenza viruses in the environment in feces under cool
wet conditions, in order to estimate the likelihood that a corn pile becomes contaminated with EA/NA H5N2 HPAI virus in wild bird feces. We then estimate the risk of transmission of the virus to poultry, assuming that a small quantity of HPAI virus contamination is present on corn before it is incorporated into poultry feed, in order to facilitate decision making regarding the risk of HPAI virus introduction and spread.

**Wild waterfowl as a source of environmental contamination**

Wild migratory waterfowl are natural reservoir hosts for type-A influenza viruses, known as avian influenza viruses (73). Disease is usually absent in most free-flying waterfowl species with avian influenza virus infection (73). Although migratory waterfowl are the primary source of introduction of low pathogenicity avian influenza viruses into domestic poultry, dispersal of HPAI viruses over longer distances by wild birds is thought to be a relatively recent phenomenon (73). Until the emergence of Asian lineage H5N1 HPAI viruses 18 years ago, there was little evidence that migratory waterfowl played a role in major geographic spread of HPAI viruses (3). Most findings of HPAI viruses in wild migratory waterfowl had been associated with local outbreaks in poultry (3).

The adaptation of the Asian lineage virus clades may represent a shift in the ecology of the virus. Typically, avian influenza viruses that become host adapted for efficient transmission in domestic poultry after introduction by wild birds do not transmit well after reintroduction back into waterfowl populations from poultry, as gallinaceous poultry are quite distant taxonomically from migratory waterfowl (72). Asian lineage H5N1 HPAI viruses identified in wild migratory waterfowl in Europe, the Middle East, northeastern Africa, and throughout Asia, demonstrate the potential for wide geographic spread of these virus clades (i.e., not all HPAI viruses) (11). However, there is still some uncertainty as to whether or not the Asian H5N1 HPAI viruses isolated from wild birds are “hitch hiker viruses” that were introduced after contact with infected poultry, or are the result of sustained transmission in wild bird populations (11, 22, 35, 57).

The novel properties of recently introduced EA/NA lineage H5N2 HPAI virus may be the result of the recombination of North American avian influenza viruses with Asian lineage H5N1 HPAI virus in wild birds. It is currently unknown whether this virus strain transmits efficiently among wild migratory waterfowl. Therefore, there is considerable uncertainty regarding prevalence estimates for the EA/NA lineage H5N2 HPAI virus in waterfowl in the United States, given the available surveillance data.

As of April 29, 2015, officials from Minnesota State Department of Natural Resources’ reported there had been no findings of the EA/NA H5N2 HPAI virus in 2,216 tests of waterfowl fecal matter. The surveillance strategy goal was to test 3,000 samples from around the State. Results from this study were not available at the time of this analysis. As of June 15th, there were also no reports of isolation of this virus in wild migratory waterfowl in Minnesota from wild bird surveys.

Prevalence estimates of avian influenza viruses in waterfowl may provide insight into the prevalence of the EA/NA H5N2 HPAI virus in wild birds. In a recent study, the mean estimated prevalence of avian influenza viruses in wild migratory waterfowl (all species) during the months of September through November was estimated from samples collected in Minnesota, Wisconsin, and North Dakota from 15 watersheds over 4 years (pers. com., Ryan Miller, April 30th,
Most samples were collected during a single year from hunter surveys in the fall or summer banding season. Bird-level prevalence estimates for avian influenza viruses ranged from ~9.75 percent (95 percent Confidence Interval; 6.5 to 12 percent) in September, to ~8 percent (95 percent Confidence Interval; 3 percent to 11 percent) in November (pers. com., Ryan Miller, April 30th, 2015).

The mean estimated prevalence of avian influenza viruses in wild migratory waterfowl (all species) during the months of February through June ranged from 0 percent (February through May) to ~2 percent (95 percent Confidence Interval; 0 percent to 5 percent) in June (pers. com., Ryan Miller, April 30th, 2015).

Variability in the prevalence of EA/NA H5N2 HPAI virus in wild migratory waterfowl at different times of the year would affect the likelihood that a pile of corn becomes contaminated. In the event that the EA/NA H5N2 HPAI virus is efficiently transmitted – similar to transmission and maintenance of other avian influenza viruses among their natural reservoir hosts – the prevalence of this virus in waterfowl would be similar to seasonal estimates of avian influenza virus prevalence in wild birds. Without direct data on the prevalence of EA/NA H5N2 HPAI virus during the time that corn is stored on the ground, it is difficult to estimate the likelihood that a corn pile becomes contaminated during this time period due to the uncertainty in the prevalence of this virus strain in wild migratory waterfowl.

HPAI virus titers in feces of wild migratory waterfowl
Inoculation studies in 2–week-old Mallard ducks via intranasal administration of $10^6 \text{ log EID}_{50}$ with 10 different HPAI virus strains resulted in variable levels of cloacal shedding over a 14-day period (56). Highest fecal titers were typically observed at day four post inoculation for both H5 and H7 subtypes. Mallards infected with H5 HPAI virus subtypes generally shed at lower levels than those infected with H7 HPAI virus subtypes. The EA/NA H5N2 HPAI virus was not evaluated in this study, so direct estimates of the degree of shedding for this virus were not available. HPAI viral titers in duck feces in this study ranged from $10^2 \text{ EID}_{50}$ to $10^8 \text{ log EID}_{50}$ per gram of feces.

Estimation of the degree or amount of HPAI virus contamination on corn piles
There is considerable uncertainty in the estimation of the quantity of HPAI virus contamination that might be present on corn stored in uncovered piles that might be used in commercial poultry feed. Currently, data are not available on the quantity of HPAI virus contaminated wild bird feces on corn piles. Ducks may excrete 7.5 to 10 kg of feces-per-year, and geese excrete 12.5 to 15 kg (87). Assuming 15 kg per year as an upper bound results in approximately 41 grams of feces excreted per day. For the purposes of this analysis, we assumed that a small quantity of HPAI virus contaminated feces could be present on corn piles (2, 5, and 50 grams of feces for wild migratory waterfowl).

HPAI virus persistence in moist feces
The HPAI virus shed by infected birds may be protected environmentally by accompanying organic material that shields the virus particles from physical and chemical inactivation. Specific environmental conditions such as cool and moist conditions increase survival times in organic media and on surfaces. For example, the 1983 Pennsylvania H5N2 HPAI virus remained viable in
liquid poultry manure for 105 days in winter under freezing conditions and 35 days at 4° C (9, 23). Asian H5N1 HPAI virus persisted for 13 days under high relative humidity at 4° C (90).

**HPAI virus survival on surfaces at various temperatures**

Table 2 and Table 3 summarize the results of studies documenting survival on various substrates (poultry feces, glass, metal etc.). Based on these data, moisture content appears to be a major determinant of the survival time of avian influenza viruses. Even under moist conditions, virus did not survive on surfaces longer than a few days when temperature ranged from 17 to 25 ° C. In general, higher humidity and cooler temperatures permit virus survival in moist substrates over longer periods of time.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Substrate</th>
<th>Survival</th>
<th>Humidity</th>
<th>Temperature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1</td>
<td>Chicken feces</td>
<td>Not detected at 2-days</td>
<td>30-42% humidity</td>
<td>22-23°C</td>
<td>(90)</td>
</tr>
<tr>
<td>H5N2</td>
<td>Dried feces from AI-infected hens</td>
<td>Contained viable virus for 1 day</td>
<td>Stored in open vials</td>
<td>25° C</td>
<td>(8)</td>
</tr>
<tr>
<td>H5N1</td>
<td>Chicken manure</td>
<td>Lost infectivity at 24 hours</td>
<td>Not specified</td>
<td>25° C</td>
<td>(16)</td>
</tr>
<tr>
<td>H5N1</td>
<td>Dried chicken feces</td>
<td>Nondetectable after 1 day</td>
<td>Not specified</td>
<td>25° C</td>
<td>(68)</td>
</tr>
<tr>
<td>H5N1</td>
<td>Glass, galvanized metal</td>
<td>No detection at 1 day</td>
<td>30-89% humidity (tested at both low and high relative humidity)</td>
<td>22 to 23°C</td>
<td>(90)</td>
</tr>
<tr>
<td>H1N1</td>
<td>Tyvek, surgical mask, wood desk, N95 respirator, gloves</td>
<td>No detection at one day except on gloves</td>
<td>55%</td>
<td>25° C</td>
<td>(63)</td>
</tr>
<tr>
<td>H1N1 (pandemic)</td>
<td>Plastic, pine, steel, cloth</td>
<td>No detection of viable virus by one day</td>
<td>23-24%</td>
<td>17-21° C</td>
<td>(27)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Virus</th>
<th>Substrate</th>
<th>Survival</th>
<th>Humidity</th>
<th>Temperature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1</td>
<td>Chicken feces</td>
<td>Not detected at 4 days</td>
<td>91% humidity</td>
<td>22-23°C</td>
<td>(90)</td>
</tr>
</tbody>
</table>

Table 3. Summary of experimental studies on survival of avian influenza virus on various substrates for HPAI inactivation studies in moist substances or under conditions not conducive to drying. **
### Evaluation of the likelihood and degree of EA/NA H5N2 HPAI virus contamination on corn

Estimates of the prevalence of EA/NA H5N2 HPAI virus in migratory waterfowl and data on the quantity of fecal contamination on corn piles were not available for this analysis. Recent surveys of waterfowl in Minnesota in late winter and early spring have not identified EA/NA H5N2 HPAI virus in cloacal samples taken from waterfowl.

In the event that the EA/NA H5N2 HPAI virus is efficiently transmitted among waterfowl—similar to transmission and maintenance of other avian influenza viruses among their natural reservoir hosts—the prevalence would be similar to seasonal estimates of avian influenza virus prevalence in wild birds. From field survey data, the prevalence of avian influenza viruses in wild migratory waterfowl is estimated to be highest during the fall harvest season (pers. com., Ryan Miller, April 30th, 2015). Prevalence estimates for avian influenza viruses in spring and early summer months are much lower (0 to 2 percent) (pers. com., Ryan Miller, April 30th, 2015).

The timing of the presence of migratory waterfowl when corn is stored outside is an important consideration. Wild birds have been observed feeding on uncovered corn piles in the fall and winter months. Corn piles could also become contaminated as large flocks of migratory birds fly over the area during migration. Wild migratory waterfowl may not be present in early spring in Minnesota due to the variability in weather conditions, as migratory pulses are pushed back by winter storms (pers. com., Ryan Miller, April 30th, 2015).

According to one industry expert, corn is harvested beginning in September and is used until the following September, where it is typically binned at 15.5 percent moisture (pers. com., Industry Expert 2, May 20th 2015). Corn piled outside is typically used first to reduce handling, since it has been exposed to the elements of rain and snow (pers. com., Industry Expert 2, May 20th 2015). Generally, corn piles are picked up no later than January due to spoilage issues (pers. com., Industry Expert 2, May 20th 2015). However, corn piled on the ground contaminated with bird feces was observed during field investigations during the most recent outbreak.

It is possible that HPAI virus could survive in wet feces for several weeks in storage piles where moisture and higher humidity are present. Data from previous HPAI outbreaks and from experimental studies indicate that longer periods of virus survival are possible under cooler, wetter storage conditions.

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Duration of Survival</th>
<th>Storage Conditions</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N2</td>
<td>Feces from infected hens</td>
<td>Contained viable virus for 2-days</td>
<td>Stored in closed vials, 25°C</td>
<td>(8)</td>
</tr>
<tr>
<td>H13N7</td>
<td>Steel, plastic</td>
<td>Inactivated by 6 days</td>
<td>Stored in a cabinet, Room temperature</td>
<td>(81)</td>
</tr>
<tr>
<td>H7N1</td>
<td>Egg-shell, PVC, metal (tin)</td>
<td>Inactivated by 15 days</td>
<td>50-84% humidity, 17-25°C</td>
<td>(85)</td>
</tr>
<tr>
<td>H7N1</td>
<td>Wood, burlap, grain, mixed feed</td>
<td>Inactivated by 8 days</td>
<td>50-84% humidity, 17-25°C</td>
<td>(85)</td>
</tr>
</tbody>
</table>

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

---

**Table Legend:**
- **H5N2**: Type of virus
- **25°C**: Storage temperature
- **(8)**: Prevalence estimate
- **Steel, plastic**: Material used for inactivation
- **Room temperature**: Storage condition
- **(81)**: Prevalence estimate
- **(85)**: Prevalence estimate

**Table Notes:**
- H5N2 feces from infected hens contained viable virus for 2 days stored in closed vials at 25°C.
- H13N7 feces from infected hens were inactivated by 6 days and stored in a cabinet at room temperature.
- H7N1 feces from infected hens were inactivated by 15 days and stored in a cabinet at 50-84% humidity and 17-25°C.
- H7N1 feces from infected hens were inactivated by 8 days and stored in a cabinet at 50-84% humidity and 17-25°C.

---

**Additional Information:**
- **Precautions:** To minimize the risk of contamination, storage conditions should be controlled to maintain lower humidity levels and cooler temperatures.
- **Recommendations:** Regular monitoring of corn piles and prompt disposal of contaminated material are crucial to prevent virus survival and spread to susceptible commercial poultry.
We considered several factors when estimating the likelihood that a corn pile becomes contaminated with EA/NA H5N2 HPAI virus in wild bird feces including:

- The uncertainty in the prevalence of EA/NA H5N2 HPAI virus in wild birds in Minnesota given the current surveillance data
- The widely varying seasonal prevalence of avian influenza viruses in waterfowl
- Inefficient transmission of HPAI viruses in migratory waterfowl that are host adapted in poultry
- The ability of the virus to persist for several weeks in feces in the environment under conditions that favor virus survival

Considering the uncertainty and variability in HPAI prevalence in wild migratory waterfowl, we qualitatively estimate that there is a very low to low (Table D-3) likelihood that a corn pile becomes contaminated with a small quantity of EA/NA H5N2 HPAI virus contaminated feces during the fall and spring seasons. For the purposes of this analysis, we assumed that 5, 25, or 50 grams of HPAI virus contaminated feces could be present on a corn pile used for poultry feed during the weeks with cooler and wetter environmental conditions in the fall and spring. We also varied the HPAI virus titer levels in duck feces (2, 4, or 6 EID₅₀/g) based on experimental data from HPAI virus inoculation studies in 2-week-old mallards (56) to account for the variability in HPAI virus titers in feces.

**Likelihood that HPAI virus on contaminated corn is not inactivated to very low levels during the process of producing commercial poultry feed**

Control measures applied in the animal feed industry to control pathogens, such as *Salmonella*, may also be beneficial in reducing the levels of avian influenza virus in finished feed. High temperatures are used to pasteurize feed ingredients during processing of some feeds. Thermal destruction during the processing steps of pelleting or extrusion is the most critical control step for destruction or reduction of *Salmonella* and other pathogenic microorganisms in pelleted feed. We briefly review pertinent guidelines related to the manufacture of commercial poultry feed that could impact avian influenza virus survival if implemented at the feed mill.

**Regulation of animal feed and animal feed ingredients**

The Federal Food, Drug, and Cosmetic Act (the Act) is the basic Federal statute under which the FDA regulates food and drugs. In the Act, the term “food” is defined as food for man or other animals, and includes animal feed. The Animal Feed Safety System (AFSS) is the FDA’s program for animal feed aimed at protecting human and animal health by ensuring production and distribution of safe feed. Feed is defined as both feed ingredients and mixed animal feed intended for food-producing and non-food-producing animals (e.g., pet animals). The AFSS includes oversight of feed production, including manufacture, labeling, storage, distribution and use of all feed at all stages of production, whether at commercial or non-commercial establishments.

Component C – Process Control for the Production of Feed Ingredients and Mixed Feed is administered partly through the Association of Animal Feed Control Officials (AAFCO). Process control is a systematic approach designed to ensure feed safety through the identification and use
of appropriate controls during the manufacturing, packaging, storage, and distribution of feed ingredients and mixed feed. The AAFCO publishes Feed Manufacturing Guidance for the animal feed industry.

**Salmonella Control Guidelines recommended for use in commercial poultry feed manufacturing**

The American Feed Industry Association, *Salmonella Control Guidelines* ([http://ucfoodsafety.ucdavis.edu/files/172958.pdf](http://ucfoodsafety.ucdavis.edu/files/172958.pdf)) provided recommended guidelines on how to best effectively control *Salmonella* in feed and feed ingredients (4). However, these guidelines only apply to processes related to feed production at the feed mill. Storage conditions of feed ingredients prior to arrival at the mill, and storage conditions of finished feed after it leaves the mill are outside the scope of the AFIA guidelines.

**Process control – pelleting and extrusion**

High temperatures are used to pasteurize feed ingredients during processing. Thermal destruction during the processing steps of pelleting or extrusion is the most critical control step for destruction or reduction of *Salmonella* and other pathogenic microorganisms. Thermal death time curves have been established for *Salmonella* and for avian influenza viruses. According to AFIA guidelines for inactivation of *Salmonella*, one second of moist heat (22 percent moisture at an initial starting point of 10⁶ log population) at 77°C will inactivate *Salmonella* as long as these temperatures are reached throughout the pellet. These guidelines require that pellet mills and extrusion equipment should be carefully monitored to insure proper operation with respect to temperature. In a pelleting process, the duration of exposure to a temperature level of 71.1°C was reported to be approximately 20 seconds in one study (49).

**Steam treatments**

Heating with steam before pelleting as a means of destroying *Salmonella* is an option if equipment is available (49). The longest practical retention time in a heat conditioner under commercial conditions is about 90 seconds (49). Steam-conditioning feed to reduce or eliminate *Salmonella* has been evaluated by several investigators (32).

**Formaldehyde**

Although heat treatment is the most effective control method used to inactivate feed pathogens, chemical treatments such as organic acids alone or in combination with formaldehyde are sometimes used. FDA-approved formaldehyde products utilized at approved use rates and applications with ingredients and processed feeds can be effective in reducing *Salmonella* and other microbial contamination. These chemicals may also have a positive residual effect in finished feed in the reduction of contamination, and they may help reduce contamination of processing equipment (Appendix A. Code of Federal Regulations Title 21, Volume 6).

One commercial product (Termin-8 Anitox Corp.) is a formaldehyde liquid applied as a spray on raw ingredients during the mixing process (150 to 120 seconds mix time) (pers. com., Industry Expert 1, May 18, 2015). To reduce occupational exposure, the product is applied in an enclosed environment. One limitation on the use of this product is its cost ($1.50 per pound; $8 to $9 per ton of treated feed), which limits its use to the treatment of feed for primary breeders (pers. com., Industry Expert 1, May 18, 2015).
Another industry expert notes that formaldehyde is fed at 2 to 6 lbs. / ton of complete feed, using a 33 percent solution (pers. com., Industry Expert 2, May 20, 2015). Most use it for Salmonella control at 2 lbs. / ton due to the cost, because many believe there is little improvement past this 2 lbs. level. Breeders may be fed a higher level, but rarely 6 lbs. / ton due to cost constraints (pers. com., Industry Expert 2, May 20, 2015).

**Organic acids**

Several treatments have been proposed for reduction of Salmonella in feed (32). Elimination of Salmonella from feed by organic acids such as propionic acid in low concentrations has been evaluated as an alternative to heat (49). The addition of 0.2 percent propionic acid in combination with heat in an experimental trial of feed inoculated with Salmonella was shown to achieve a 250 fold reduction in numbers of Salmonella (49). Some formaldehyde products also contain organic acids. The presence of organic acids in these products and their impact on HPAI virus inactivation was not considered in this analysis.

**Heat treatments**

Some feed mills manufacture feed without the use of formaldehyde or any other chemical treatment. This may be due to cost, or to reduce occupational exposure of feed-mill employees to chemical vapors. One mill that does not use formaldehyde reports that feed is heated to 190° F (87.8° C) for 4 to 5 minutes with 17 percent moisture and that no feed under 185° F (85° C) enters the cooler (88).

**Evaluation of the likelihood that HPAI virus is not inactivated to very low levels during the production of commercial poultry feed**

In this part of the analysis, we consider heat treatments used in the production of pelleted and mash feeds and the use of formaldehyde in mash feed on the degree of reduction in HPAI virus concentration in raw ingredients. Failure to inactivate HPAI virus or to reduce it to low levels in feed ingredients increases the risk of possible transmission, given that it is present.

**Simulation model of HPAI virus inactivation due to heat treatments**

We developed a simulation model to estimate the degree of thermal inactivation of HPAI virus achieved through processes used in the production of pelleted and mash feed. Details of the model are provided in Appendix B. Simulation Model of HPAI Virus Inactivation in Pelleted Feed.

**Pelleted feed**

The production of pelleted feed involves two main thermal processes: 1) steam conditioning of raw feed ingredients (including ground corn) to produce a mash suitable for pellet extrusion, and 2) the pellet extrusion process where a further increase in temperature is achieved due to friction and pressure. Direct data on the inactivation of HPAI virus in poultry feed ingredients representative of the time and temperature ranges for the pelleting processes are not available. We used thermal inactivation parameters for HPAI virus estimated from chicken meat or liquid egg as a proxy, based on OIE requirements for inactivation in these products (Table 4). We also elicited expert opinion on the operational parameters for commercial feed mills producing pelleted feed in order to estimate the degree of HPAI virus reduction during this process.
Expert opinion on pellet mill operation

There are limited data available in the scientific literature on the effectiveness of pelleting on the reduction of pathogens in poultry feed (36, 37). In order to estimate the degree of HPAI virus reduction during pelleting, we elicited expert opinion on operational parameters for commercial feed mills producing pelleted feed.

Industry Expert 1:

Target terminal exit temperature for steam conditioned mash ingredients prior to pellet extrusion is 185° F (range 170° to 190° F).

- Entry temperature of ingredients (in May) is approximately 80° F (note, this is for Delmarva, USA).
- Conditioners vary in dimensions (8 to 20 feet in length and 24 to 36 inches diameter) and are typically filled to 60 percent capacity.
- 45-second retention time is typical for ingredients in the conditioner. The time at maximum temperature is typically less than one-half to one-third the total retention time (15 to 20 seconds) with three to four seconds being the shortest retention time.
- The pelleting process adds additional heat due to friction and pressure applied during extrusion (6° to 10° F gain in temperature). Mash entering at 185° F will exit at a pellet temperature near 195° F.

Industry Expert 2:

- Pelleting occurs at temperatures ranging from 170° to 200° F in a normal mill, depending on many parameters (17 percent moisture) and frictional heat at pelleting.
- Expanders reach 220° F pelleting temperature.
- Dry pellet extrusion is an academic interest and is not practiced in the commercial industry.

Industry Expert 3:

Varying amounts of time is needed to dry corn (reduce the internal moisture content). Corn entering the drier at 21 percent moisture that is dried to 15.5 percent may be exposed to heat for 20 to 45 minutes. Corn at 17.5 percent moisture dried to 15.5 percent may only spend 15 minutes in the drier. However, not all corn stored on the ground is dried before grinding.

- Temperatures for vertical driers range from 225° to 325° F (110° C to 162.8° C). The delta over ambient temperature is 25° F (from corn harvested at 80° F) and 90° F for cold corn harvested below freezing.
- Retention times in the steam conditioner vary from 10 seconds as the shortest retention time; 15 seconds is typical in most operations; and up to 4 minutes in some operations.
- Injection of superheated steam at 250° to 280° F. Steam condenses to 212° F on the surface of cool ingredients, but it takes 5 to 10 seconds for the steam to cool to 212° F.
HPAI virus on the surface of corn particles would be exposed to superheated steam temperatures.

- Cold environmental conditions mean that cold corn enters the process. This makes it difficult to achieve ideal mash temperatures in the winter in cold climates. 30° F corn ingredients in the winter may only reach 160° F preconditioning mash temperature.

- However, there is a greater increase in pelleting temperatures with cooler mash ingredients as they are more lubricious. So 160° F mash would yield 210° F pellet; whereas, 185° F mash would yield 205° F pellets.

- Pellets spend from 7 to 12 minutes retention time in the cooler in order to go from 205° F pellet temperature to 10° F degree above ambient air temperature.

- Pellet throughput times vary due to the diameter area ratio. Minimum 4 seconds—maximum 15 seconds—most likely 6 seconds in the die. Die temperature ranges from 230° to 250° F (110° to 121.1° C). The delta is 10° to 40° F.

**Table 4. Survival of AI viruses in chicken meat**

<table>
<thead>
<tr>
<th>Temp</th>
<th>Time to Inactivation</th>
<th>Amount Left/Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 °C</td>
<td>28-34 s (D value)</td>
<td>Time to reduce titer by 90% (from 7.5–8 log/ml)</td>
<td>(80)</td>
</tr>
<tr>
<td>70° C</td>
<td>&lt;40 seconds to lose infectivity (ramped up from ambient temperature, 25° C – 70° C)</td>
<td>10^4.3 EID 50 (detection limit) reduction from 10^6.8 to 10^4.3 [thermo cycler took 40 seconds to reach 70° C, some virus was detected at 70° C but after a 5 second treatment, no virus was detected]</td>
<td>(71)</td>
</tr>
<tr>
<td>7° C</td>
<td>5.5 s</td>
<td>Predicted for 11 log reduction</td>
<td>(80)</td>
</tr>
<tr>
<td>73.9° C</td>
<td>0.8 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70° C</td>
<td>0.2 s (D value)</td>
<td>Time to reduce titer by 90%</td>
<td>(79)</td>
</tr>
<tr>
<td>74° C</td>
<td>0.03 s (D value)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The model also considers the increased inactivation rate at pelleting temperatures that are higher than the baseline temperatures for which D-values were estimated in the available data. A D-value is the time required to cause a 1-log reduction in HPAI virus concentration or amount at the baseline temperature. Specifically the reduction in D-value with temperature is approximated by using a conservative Z-value estimate. The details of the model parameters are provided in the Appendices.

HPAI virus has shown to be inactivated at a few seconds at temperatures around 70° C in poultry products such as meat and liquid egg in experimental laboratory studies (75). The results of our analysis indicate a high probability of inactivation even when the mash conditioning process alone is considered (more than a 99 percent chance of above 20-log EID₅₀/g inactivation).
Mash feed

A similar model to evaluate HPAI virus inactivation on corn used in mash feed is described in Appendix C. Simulation Model of HPAI Virus Inactivation in Mash Feed. In some cases, corn is dried to 15.5 percent moisture before grinding. Higher moisture corn (21 percent moisture), may require 20 to 45 minutes in a vertical drier, and lower moisture corn (17.5 percent moisture) may require 15 minutes of drying to achieve 15.5 percent moisture prior to grinding. In our analysis, we consider that there may be some inactivation of HPAI virus in wild bird feces if a drying process is used prior to production of mash feed. The model used for estimating HPAI virus inactivation through the drying process is similar to the pelleted feed model. In this case, although the dryer air temperature is high, we conservatively used the corn temperature in the dryer (the delta over ambient temperature was 25° F from corn harvested at 80° F, and 90° F for cold corn harvested below freezing) to predict HPAI virus inactivation. We note that the dryer corn temperature is much lower compared to pelleting temperatures.

There are limited data available on the inactivation of HPAI viruses at temperatures representative of those used to dry corn. In Lu et al., (2003), H7N2 LPAI virus in field chicken manure lost infectivity in 15 to 20 minutes when heated to 56° C (133° F) in a water bath, and 35 hours when heated to 28° to 30° C (82° to 86° F). In Elving et al., (2012), 1.7 hours at 45° C (113° F) was required for inactivation of H7N1 HPAI virus in compost material consisting of a manure and straw mixture.

Simulation model results predict that inactivation with the drying process had an approximate 90 percent prediction interval of 0.27 to 8 log EID<sub>50</sub> reduction in HPAI virus titers. This large prediction interval is due to the uncertainty in the D-values of HPAI virus at drying temperatures. Specifically, the D-value estimates from two different published studies on the degree of inactivation of HPAI virus in dried material at higher temperatures have varied widely in this temperature range (20, 48). In this case, although the probability of exposure would be reduced, there is insufficient evidence that the risk would be mitigated through the drying process in all cases.

Not all corn stored on the ground requires drying before grinding. In this case, there would be little inactivation of HPAI virus on corn that is freshly contaminated, as the grinding process produces very little heat. We did not repeat simulation modeling of heat effects on undried corn.

**HPAI virus inactivation by formaldehyde treatment**

We were unable to identify studies on the inactivation of avian influenza viruses in poultry feed by formaldehyde treatment. However, formaldehyde is used as a fumigant in hatcheries, and in disinfectants and has been effective at inactivation of avian influenza viruses in these applications. Also, industry guidelines for the inactivation of Salmonella in poultry products are considered adequate to inactive HPAI virus in poultry products, so it is reasonable to expect similar degrees of inactivation of HPAI virus in feed treated with formaldehyde.

**Susceptibility of avian influenza viruses to formaldehyde used in fumigation and disinfectants**

Formaldehyde fumigation has long been used for sanitizing hatching eggs in incubators. Commonly used methods to produce formaldehyde gas are mixing formalin solution with potassium permanganate and by heating paraformaldehyde powder using a hotplate. In general,
formaldehyde fumigation is most effective at a relative humidity of 60 to 80 percent and a temperature of 20° to 24° C. Formaldehyde gas rapidly loses its efficacy at low temperatures or in a very dry atmosphere (7, 53).

Formaldehyde has been demonstrated to be effective against enveloped viruses and bacterial spores with its site of action being cell membranes, enzymes, and nucleic acids. However, its activity is reduced in the presence of organic matter (19). Table 5 summarizes results on the degree of inactivation of various microorganisms with formaldehyde fumigation.

Disinfection formulations where formaldehyde solution (formalin) is mixed with other agents such as quaternary ammonium (e.g., DC&R® Disinfectant Spray, Neogen® Corporation) were found to be effective against avian influenza viruses with a contact time of 10 minutes (48). A 0.2 percent formalin solution was shown to inactivate 4 HA units (approximately $10^5$ EID$_{50}$) of HPAI H5N1 virus in solution within a contact time of 15 minutes (67).

### Table 5. Degree of inactivation of various microorganisms with formaldehyde fumigation

<table>
<thead>
<tr>
<th>Organism</th>
<th>Operational conditions</th>
<th>Degree of inactivation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine reproductive respiratory syndrome on live haul trailers</td>
<td>Contact time 30 minutes at 20° C</td>
<td>RNA detected post fumigation; half of sentinel pigs infected</td>
<td>(19)</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> spores</td>
<td>Formaldehyde gas produced by heating, 1 hour contact time</td>
<td>1 log in 0.5 hours and approximately 2 logs in 1 hour</td>
<td>(1)</td>
</tr>
<tr>
<td>Newcastle disease virus</td>
<td>Paraformaldehyde at 30 minutes at 60 percent relative humidity and 23° C</td>
<td>8.5 log</td>
<td>(77)</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> spores</td>
<td>Paraformaldehyde at 60 percent humidity and 23° C, 1 hour contact time</td>
<td>5 log</td>
<td>(77)</td>
</tr>
<tr>
<td>General bacterial counts on eggs</td>
<td>Formaldehyde gas 20 minute contact time</td>
<td>2 log</td>
<td>(89)</td>
</tr>
</tbody>
</table>

**Susceptibility of avian influenza viruses to formaldehyde used to inactivate pathogens in feed ingredients**

We reviewed the scientific literature in order to estimate the degree of reduction of HPAI virus in poultry feed treated with formaldehyde. A summary of studies is provided in Table 6.

Wales *et al.*, (2010) reviewed the efficacy of formaldehyde and other chemical compounds delivered in feed against *Salmonella* occurring in feed (86).

- Formaldehyde, applied as a fumigant gas to chick feed (5 minutes exposure with mixing) reduced artificial *Salmonella* contamination to low levels immediately after treatment and to undetectable levels 12 hours later.
• Relatively concentrated formaldehyde (formalin 0.5 to 1 percent volume/weight) was shown to be rapidly effective in rendering inoculated S. Typhimurium undetectable in feed.

• 1 percent of a combined formaldehyde propionic acid/terpene product (giving an inclusion rate of around 0.3 percent weight/weight formaldehyde) was an effective treatment for fishmeal artificially contaminated with 103 cfu/g Salmonella, such that a starter ration prepared from it did not infect chicks.

• At inclusion levels of 0.25 to 0.70 percent formaldehyde was associated with 1 to 2 log10 unit reductions in natural Salmonella contamination of oilseed meals within 48 hours.

• When a formaldehyde/propionic acid product was added at 1 to 2 percent to artificially contaminated animal protein meals, contamination of 10^2 to 10^4 cfu/g was eliminated within 24 hours.

Table 6. Literature review on the effects of formaldehyde (formalin) on microorganism growth in animal feed and animal health status

<table>
<thead>
<tr>
<th>Animal species/type of feed studied</th>
<th>Targeted microorganisms in the feed</th>
<th>Dietary concentration of formaldehyde used in the feed</th>
<th>Log Colony Forming Units (CFUs)/g of feed at the beginning of study period</th>
<th>Log Colony Forming Units (CFUs)/g of feed at the end of study period</th>
<th>Effects of concentrations on species studied</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mink (Neovison vison) feed</td>
<td>Gram positive, Gram negative bacteria</td>
<td>550 ppm</td>
<td>10.5^2</td>
<td>10.25^2 (low and stable)</td>
<td>None observed</td>
<td>(47)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1100 ppm</td>
<td>10^2</td>
<td>8.5^2 (decrease)</td>
<td>Adverse^2</td>
<td></td>
</tr>
<tr>
<td>Poultry feed^4</td>
<td>Salmonella enteriditis S. typhimurium</td>
<td>0.3%^4</td>
<td>-</td>
<td>Log_{10} reduction &gt;3</td>
<td>Not studied</td>
<td>(86)</td>
</tr>
<tr>
<td>Broiler chicks</td>
<td>Not studied</td>
<td>2.5 ml/kg of feed</td>
<td>-</td>
<td>-</td>
<td>None observed</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ml/kg</td>
<td>-</td>
<td>-</td>
<td>Less pronounced</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ml/kg</td>
<td>-</td>
<td>-</td>
<td>Adverse^3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 ml/kg</td>
<td>-</td>
<td>-</td>
<td>Adverse^3</td>
<td></td>
</tr>
<tr>
<td>Cockerels</td>
<td>Not studied</td>
<td>2.5 ml/kg</td>
<td>-</td>
<td>-</td>
<td>None observed</td>
<td>(42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ml/kg</td>
<td>-</td>
<td>-</td>
<td>None observed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ml/kg</td>
<td>-</td>
<td>-</td>
<td>None observed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 ml/kg</td>
<td>-</td>
<td>-</td>
<td>Adverse</td>
<td></td>
</tr>
<tr>
<td>Japanese quails</td>
<td>Not studied</td>
<td>2.5 ml/kg</td>
<td>-</td>
<td>-</td>
<td>None observed</td>
<td>(41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ml/kg</td>
<td>-</td>
<td>-</td>
<td>Adverse</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ml/kg</td>
<td>-</td>
<td>-</td>
<td>Adverse</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 ml/kg</td>
<td>-</td>
<td>-</td>
<td>Adverse</td>
<td></td>
</tr>
</tbody>
</table>

1 37% aqueous solution (formalin)
2 Numbers in this table are approximates because the study depicted the trends in a graph and did not provided exact numbers
3 Deleterious effects of kits survival at birth, hematologic parameters, body weight, quality of fur
4 Liquid: Mixture of formalin, propionic acid, terpenes, and surfactant
5 Decrease body weight and feed intake. Symptoms included depression, somnolence, staggering gait, and necrotic and ulcerative areas on GI mucosa
Wales et al. (2010) also noted that there can be considerable variability in the efficacy of the use of formaldehyde to inactivate pathogens in poultry feed. The decontamination of feed ingredients and compound feedstuffs using chemical agents needs to take account of likely initial contamination rates, and their use may provide adequate protection provided that the challenge level is not too great (86). Treatments at recommended application rates may fail if application is uneven or if existing environmental contamination exceeds the protection threshold (86). Few studies have been performed that have assessed efficacy of chemical treatments under controlled laboratory or field conditions (86). Many studies show substantial variation in efficacy (86). Errors in application can be attributed to mechanical failure of equipment, blocked nozzles, and electrostatic or sedimentation effects. Miscalculation of dose rates lead to release of microbial contamination due to inconsistency in application (86).

Time after harvest until use is also an important consideration when estimating the degree of reduction of HPAI virus contamination in formaldehyde treated feed. Freshly contaminated corn piles would be more likely to have a higher initial titer of HPAI virus contamination compared to corn that has been stored for several months. This estimation is supported by data from 1983 H5N2 Pennsylvania HPAI outbreak where virus has been shown to survive for 105 days in wet feces in cold wet conditions.

From the previous section, we estimated there was a very low to low likelihood (Table D-3) that a small amount of HPAI virus contaminated bird feces could be present on corn piles used in the production of poultry feed during the cooler wetter weeks when corn is stored outside on the ground (fall and spring seasons). Formaldehyde treatment of feed during processing is an optional control measure according to industry guidelines, and is used mostly in mash feed fed to breeder birds. Some smaller cooperatives may use local ingredients in the production of mash feed (pers. com., Industry Expert 1, May 18, 2015), which may not be treated with formaldehyde. Mash feed may also be fed to younger meat-type birds such as turkey pouls and broiler chicks up until 2 to 4 weeks of age.

At the time of this analysis, data were not available on the degree of reduction in feed pathogens due to the use of formaldehyde products in poultry feed in the United States. In one recent experimental study (14), up to a 4 log reduction was observed in fish meal inoculated with Salmonella treated with a commercial product containing formaldehyde and propionic acid product at a treatment dose of 10 kg per ton. It is important to note that this dosage is much higher than the dose used in feed in the United States according to industry experts (2 to 6 lbs per ton of complete feed, pers. com., Industry Expert 2, May 20, 2015). We also note that the application of formaldehyde may lead to variability in the reduction of pathogens in feed in some cases due to inconsistencies in application methods (86). Therefore, given that enveloped viruses are more susceptible to disinfectants when compared with bacterial spores—and that formalin has been shown to be effective against avian influenza—we estimate that the likelihood that HPAI virus would not be inactivated to very low levels in commercial poultry feed is very low to low (Table D-3). We estimate 2- to 8-log EID₅₀/gram of feces of inactivation of HPAI virus in poultry feed ingredients treated with products containing formaldehyde. This range reflects our uncertainty in the efficacy of this product, given the data currently available.
Likelihood that Commercial Poultry Feed becomes Cross Contaminated with EA/NA Lineage H5N2 HPAI Virus by Wild Birds (Passerine or Perching Birds) during Handling and Storage

Members of the order Passeriformes (passerine or perching birds) such as sparrows, European starlings and grackles, have been implicated in local area or neighborhood spread of HPAI viruses in past outbreaks. Although migratory waterfowl may have access to the external environment immediately surrounding poultry houses on a farm, passerine birds may gain direct access to poultry houses and have close contact with poultry. In this part of the analysis, we consider the potential role of passerines in secondary spread of HPAI virus between farms (Figure 1). Specifically, we examine pathways associated with contamination of poultry feed at the feed mill or during delivery or on-farm storage as a mechanism of introduction and spread to other operations.

Passerine birds in and around the commercial poultry environment

In comparison to wild waterfowl, passerines occupy a distinctly separate niche in and around the farm environment (21). Within the environment near and around commercial poultry farms, some members of the order are more likely than others to have direct or indirect contact with poultry due to food choice or nesting behavior. Some common passerines observed on poultry farms may remain in the local area year-round as residents, or may migrate short distances (a few hundred kilometers) to more southern regions during winter.

Table 7. Behavioral characteristics of several members of the order Passeriformes that impact the potential role of transmission of HPAI virus in environments on the farm around poultry houses.

<table>
<thead>
<tr>
<th>Common name and species</th>
<th>Migration</th>
<th>Habitat</th>
<th>Nesting behavior</th>
<th>Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Grackle (Quiscalus quiscula)</td>
<td>Resident or short distance migrant</td>
<td>Agricultural fields; feedlots; woodland; forest edges; marshes</td>
<td>Nearly always in scattered trees, rarely in barns</td>
<td>Omnivorous; seeds (agricultural grains)</td>
</tr>
<tr>
<td>House Sparrow (Passer domesticus)</td>
<td>Resident</td>
<td>Closely associated with people and their buildings</td>
<td>Prefers structures; eaves or walls of buildings</td>
<td>Grains and seeds (livestock feed)</td>
</tr>
<tr>
<td>European Starling (Sturnus vulgaris)</td>
<td>Resident or short distance migrant</td>
<td>Countryside near human settlements; feed in fields</td>
<td>Trees; buildings; structures</td>
<td>Focus on insects and invertebrates; also eat fruits, berries, grains (livestock feed)</td>
</tr>
<tr>
<td>House Finch (Haemorhous mexicanus)</td>
<td>Resident or short distance migrant</td>
<td>Farms; parks; urban centers; backyards</td>
<td>In or near buildings; trees</td>
<td>Plant materials almost exclusively; millet, milo etc.</td>
</tr>
</tbody>
</table>

Potential role of passerine birds in secondary spread of HPAI viruses

A review of the scientific literature provides evidence that passerine birds can harbor AI viruses, including HPAI viruses, but their role in the natural epidemiology of AI is considered to be only minor (58). Since its appearance, Asian lineage HPAI H5N1 virus clades have demonstrated the
unique ability among HPAI viruses to infect a wide variety of species, including wild birds. Small perching birds such as sparrows, European starlings, and grackles commonly frequent the poultry farm environment and thus have shown the potential to serve as biological or mechanical vectors of Asian H5N1 HPAI virus, or as so-called bridge species in its transmission (13). The potential pathway for EA/NA H5N2 HPAI virus transmission via passerine birds include infection or contamination of passerine birds on an infected poultry farm with subsequent secondary transmission through environmental contamination within an uninfected poultry house.

**Figure 1. Pathways for potential cross contamination of commercial poultry feed at a feed mill or stored on-farm via secondary HPAI virus spread by wild passerine birds.**

**Surveillance for avian influenza viruses in passerine birds**

Isolation of AI viruses from species of the order Passeriformes has been infrequent, indicating the passerine birds likely do not represent an important reservoir of AI viruses, but evidence suggests that passerine birds may play a role in perpetuation and transmission of AI viruses in areas of intense poultry production (58).

- In a 1995 survey to establish disease freedom for poultry operations during an outbreak of HPAI H5N2 virus in Mexico, serological evidence of infection of three passerine birds (species not specified) to an H5N2 serotype was reported (84). However, a LPAI H5N2 virus had been circulating in poultry in 11 Mexican States prior to the outbreak; it is ambiguous as to which virus resulted in the exposure.
• In a survey of wild birds in 1988, 2.9 percent of avian influenza viruses isolated were from Passeriformes (as a proportion of 21,318 total samples from all species), the second highest isolation rate compared with members of Anseriformes (15.2 percent). (70)

• In a sero-survey of passerine birds in the U.S. state of Georgia from 1999 to 2009, 0 of 234 birds from 25 different species tested positive for AI antibodies (12).

• No isolations of avian influenza viruses were reported from 83 cloacal swabs collected from 4 adult and 79 juvenile reed warblers (Acrocephalus scirpaceus) in 1993, despite proximity to aquatic habitats of known avian influenza reservoir species (18).

• During active surveillance of Passeriformes for H5N1 HPAI virus in Mongolia from 2005 to 2011, 0 of 80 live-bird, fecal and sick-bird samples were positive (26). However, no passerine birds were sampled during five H5N1 HPAI virus wild bird outbreak investigations.

• In 2006, out of 8,961 Passeriformes sampled via RT-PCR in Europe, 1 (0.01 percent) was H5N1 HPAI virus positive and 8 (0.09 percent) were LPAI virus positive (30).

• On Helgoland Island in the North Sea in 2001, 543 migrating passerine birds of different species all tested negative for AIV subtypes H5 and H7 (65). Virus detection using virus isolation in SPF chicken eggs was done on conjunctival, choanal cleft and cloacal swabs.

• In a summary of 3 studies from 1979 to 1980, in which a total of 11 passerine species were tested, AIV isolation was reported from 17 out of 586 birds (70).

• From a total of 670 cloacal swabs from 37 different species of migratory passerine birds in Slovenia from 2004 to 2006, there was one positive rRT-PCR in the only common starling (Sturnus vulgaris) tested, but virus isolation was unsuccessful (62).

• Cloacal samples from 1,300 tree sparrows (Passer montanus) in China in 2011 yielded no AIV, while 94 of 800 were serologically positive for H5N1, and zero of 800 were seropositive for H7 (29).

• From 2004 to 2007 in China, RT-PCR on 7,320 cloacal swabs, tissue or fecal samples from 33 Passeriforme species identified 0.36 percent to be H5N1 positive; 1.09 percent of tree sparrows were positive (44).

• 30 percent of 155 passerine birds from 12 species were AI virus positive via RT-PCR on cloacal and/or oropharyngeal swabs in a 2007 study in Slovakia (28). 3 of 6 swallows (Hirundo rustica) tested positive. The authors speculate the higher than typically reported prevalence may be due to the increased sensitivity of nested RT-PCR used in this study.

**Surveillance of passerine birds for HPAI virus in the vicinity of HPAI outbreaks in poultry**

While passerine birds have not been directly implicated in the spread of HPAI virus to poultry in previous outbreaks, passerine birds infected with HPAI virus have been observed near poultry outbreaks.

• In Pakistan in 2007, four wild crows were found to be Asian H5N1 HPAI virus positive following outbreaks in backyard poultry and zoo birds (22).
• Among 22 birds found dead, including chickens, one large-billed crow (*Corvus macrorhynchus*) was infected with Asian H5N1 HPAI virus in Hong Kong in 2009 (22).

• In a 1985 H7N7 HPAI virus outbreak in chickens in Australia, an antigenically closely related strain was isolated from starlings on the affected farm, and serologic evidence of H7N7 virus infection was found in sparrows.

• In Jalisco, Mexico in 2012, 81,000 general surveillance samples in an H7N3 HPAI virus outbreak region yielded one positive common grackle (*Quiscalus quiscula*) and one positive barn swallow (39).

• A chickadee recovered in Ramsey County MN and delivered on June 10th, 2015 to a wildlife rehabilitation center later tested positive for avian influenza (52). Although highly pathogenic H5 was diagnosed, the diagnostic laboratory was unable to determine the exact virus strain. Complete information about the circumstances surrounding the submission were not available so exactly where the bird became infected is unknown.

**HPAI experimental transmission studies in passerines**

In Perkins *et al.* (2003a) inoculation of house finches, house sparrows, and starlings with 0.05 ml of inoculum containing $10^6 \log \text{ELD}_{50}$ HPAI H5N1 (A/chicken/Hong Kong/220/97 (chicken/Honk Kong)) demonstrated that this clade of virus was highly pathogenic for house finches with disseminated infection typical of HPAI viruses in domestic poultry (58). In contrast to finches, inoculation of sparrows and starlings did not result in mortality and only transient morbidity was observed in a few of the sparrows. The authors concluded that the chicken/Hong Kong HPAI virus demonstrated distinctive grades of virulence among the passerine species studied. Data on the extent and duration of viral shedding were not reported.

<table>
<thead>
<tr>
<th>Common name and species</th>
<th>Morbidity</th>
<th>Mortality</th>
<th>Gross lesions</th>
<th>Presence of antigen in tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>House finches (<em>Carpodacus mexicanus</em>)</td>
<td>Severe depression and neurologic dysfunction</td>
<td>High; moribund or dead in 2 days</td>
<td>Mild or absent</td>
<td>Testicles and pancreas most severely affected; infrequent to rare in most other tissues</td>
</tr>
<tr>
<td>House sparrows (<em>Passer domesticus</em>)</td>
<td>Mild transient depression; no mortality</td>
<td>No gross lesions</td>
<td></td>
<td>Brain and testicles</td>
</tr>
<tr>
<td>European starlings (<em>Sternus vulgaris</em>)</td>
<td>Neither disease nor mortality</td>
<td>No gross lesions</td>
<td></td>
<td>Not observed in any tissues</td>
</tr>
</tbody>
</table>

Nestorowicz *et al.* (1987) infected house sparrows and starlings with $10^5 \log \text{EID}_{50}$ of an isolate of a HPAI H7N7 from chickens (A/Chicken/Victoria/1/85) via the oral/tracheal and nasal cleft route. (54) Uninfected birds were placed in contact with infected birds of the same species. Transmission to starlings was observed.
Table 9. Summary of the experimental transmission of H7N7 HPAI virus in house sparrows and starlings by Nestorowicz et al. (1987)

<table>
<thead>
<tr>
<th>Common name</th>
<th>Mortality</th>
<th>Virus isolation</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starlings</td>
<td>All inoculated birds died within 48 hrs. p.i.</td>
<td>Not reported</td>
<td>Contact birds died within 4 days of being placed with infected birds</td>
</tr>
<tr>
<td>Sparrows</td>
<td>30% mortality rate; Isolated from all tissues from birds that died within 2 days p.i.</td>
<td>Not transmitted to uninfected contact birds</td>
<td></td>
</tr>
</tbody>
</table>

In sparrows inoculated with four different H5N1 HPAI virus strains, mortality was 66 to 100 percent, oropharyngeal and cloacal titers were as high as 4.7 and 4.1 log₁₀ EID₅₀/ml, respectively, at 4 DPI, and there was no same-species contact transmission (31). Mortality was 0 percent in European starlings, maximum cloacal titer was 3.8 log₁₀ EID₅₀/ml at 2 DPI, and there was only one unduplicated instance of contact transmission. Oropharyngeal and cloacal titers were very low in pigeons (Columbia spp.), and their mortality was 0 percent. The authors inferred that sparrows may act as intermediate hosts for transmission to both poultry and mammals, but the lack of contact transmission and high mortality preclude them serving as a reservoir species for H5N1 HPAI virus. While starlings may also act as intermediate hosts, the authors concluded the low contact transmission rate likely indicates they could not serve as an H5N1 HPAI virus reservoir. Pigeons, while not passerine, were determined likely to play a minor role in the ecology of H5N1 HPAI virus.

Brown et al. (2009) found similar mortality rates (60-100 percent at 10^2 to 10^6 EID₅₀ inoculum/bird) and maximum oropharyngeal titers (4.2 log₁₀ TCID₅₀/ml) in house sparrows (Passer domesticus) inoculated with A/whooper swan/Mongolia/244/05 HPAI H5N1, but maximum cloacal titers were significantly (P=0.002) lower than oropharyngeal (13). While 40 percent of pigeons (Columbia livia) inoculated with the highest dose of H5N1 died, they and the survivors only briefly shed virus and at low titers. All pigeons in the low- and medium-dose groups survived and remained AI virus free. These authors concluded that sparrows could play a role in AI virus transmission in an outbreak, though more likely via contamination of the environment and feed, due to their predominantly oropharyngeal shedding, or via chickens scavenging their infected carcasses.

Two studies with the H5N1 HPAI virus strain A/chicken/Hong Kong/220/97 resulted in no mortality and infrequent histopathology lesions in house sparrows and European starlings (58, 59). While mortality in house finches (Carpodacus mexicanus) averaged 44 percent, histopathology lesions were absent to mild and viral antigen rare in the nasal cavity and gastrointestinal tract. The authors were not able to draw any definitive conclusions regarding the role of these species as biological vectors.

In another study, experimentally A/duck/Laos/25/06 H5N1-infected house sparrows shed virus for several days and rapidly contaminated their drinking water (25). On the other hand, inoculated chickens shed undetectable levels of virus into their water troughs, despite high oropharyngeal and cloacal shedding, due to rapid disease progression. These authors concluded that sparrows
may be unlikely to become infected by chickens under normal field conditions in an H5N1 HPAI virus outbreak. They also inferred that the behavior of infected sparrows may be a determining factor in their potential as intermediate H5N1 HPAI virus hosts via viral shedding.

In A/chicken/Miyazaki/K11/2007 and A/chicken/Shimane/1/2010 H5N1-inoculated tree sparrows, mortality was 100 percent within 11 days (mean > 6 days), with oral swabs positive from 1-8 DPI and maximum titers of $10^{6.5}$ to $10^{7.3}$ EID$_{50}$/ml (92). While there was no intraspecies transmission among sparrows, 10 of 16 (62.5 percent) contact chickens died when housed with infected sparrows. Due to the prolonged viral shedding observed here, the authors conclude that tree sparrows have the potential to serve as biological vectors of H5N1 HPAI virus.

Twenty-three of 23 A/Cygnus cygnus/Germany/R65/2006 H5N1-inoculated stonechats (Saxiola torquata) died within 3 to 7 days, most with no clinical signs (38). Oropharyngeal shedding peaked at $10^3$ to $10^4$ TCID$_{50}$/ml on 3 to 6 DPI.

**Qualitative evaluation of the prevalence of EA/NA H5N2 HPAI virus in passerine birds during the upcoming fall and spring seasons**

Based on a review of the scientific literature, the prevalence of EA/NA H5N2 HPAI virus would be expected to be higher in passerine bird populations in contact with poultry in or near a HPAI control area during an outbreak. However, preliminary results from passerine bird surveys from birds on or near infected operations recently reported indicate that detection of HPAI virus in these birds is very rare. We are only aware of one other infected passerine bird reported in MN in June, an infected Chickadee.

The objective of the present analysis is to estimate the risk of introduction and spread of HPAI virus through feed by these birds during the upcoming fall or spring seasons. There is considerable uncertainty as to whether or not these viruses are maintained in an endemic state in passerine bird populations in MN and IA (i.e., circulating in passerine birds in the absence of active transmission in poultry). Therefore, we estimate that the prevalence of EA/NA H5N2 HPAI virus in passerine birds during the upcoming spring and fall seasons is very low. This estimate is a conservative upper bound for prevalence in passerine birds given the recent outbreaks in poultry in MN and IA, and the extremely low expected prevalence in these bird populations in the absence of active infection in poultry.

**Likelihood of contamination of feed ingredients or finished feed by wild passerine birds at the feed mill or during transport**

American Feed Industry Association (AFIA) guidelines, and industry good manufacturing practices, require that feed manufacturers take measures at the feed mill to prevent the contamination of raw feed ingredients and finished feed by pests such as rodents and wild birds (36). However, there is a possibility that passerine birds may gain access to feed ingredients or finished feed in the event that a storage bin lid or cover is not secure. Data are not available on the frequency of biosecurity breaches of feed storage bins at feed mills. However, given that there is a breach in biosecurity, we would expect a high visitation rate by passerine birds that are attracted to finished poultry feed that is easily accessible. Based on a review of the scientific literature on the surveillance of HPAI virus in passerine birds and limited preliminary surveillance data from the current outbreak, the prevalence of HPAI virus infection in these birds
is estimated to be very low. Assuming that feed mill managers are following these guidelines, we estimate that the likelihood of contamination of finished feed with HPAI virus by passerine birds at the feed mill is very low (Table D-3).

Finished feed is augured into the feed truck and transported to the farm in a closed system. Therefore, we estimate that there is a negligible likelihood (Table D-3) of contamination of feed by wild birds during the movement of finished feed from the feed mill to the farm.

**Likelihood of contamination of finished feed by wild passerine birds on the farm**

**Summary of observational studies that evaluate the role of passerine birds in on-farm disease transmission of pathogens to poultry**

European starlings and house sparrows are frequently located near poultry houses (17). During a field survey to estimate the incidence of bacterial pathogens in passerines near broiler houses, starlings were seen trying to gain entrance to all chicken houses on one farm, and a nest with young starlings was seen in the eaves of one house (17). Numerous droppings on the sides of the houses on another farm indicated that sparrows and starlings were attracted to the house and possibly trying to gain entrance.

In a survey of table-egg layer operations in California regarding pest management practices, producers ranked wild birds (passerines) as being somewhat more pestiferous on southern ranches (38) than on northern ones (30) when asked to rank pests in order of perceived importance (100 being most severe) (33).

Burns et al. (2012) counted wild birds near poultry farms in Ontario and British Columbia (Burns, 2012).

- Barn swallows (*Hirundo rustica*), rock doves (*Columba livia*) and European starlings (*Sturnus vulgaris*) were all observed entering poultry barns, which included broiler, broiler breeder, layer, and turkey production.

- Rock doves were observed entering barns the most frequently.

**Summary of studies evaluating the effectiveness of on-farm biosecurity measures taken to prevent access by wild birds**

Table-egg layer producers in California reported that exclusion was the most common practice for controlling wild birds in a survey of pest control strategies. Nearly half (48 percent) of caged layer producers reported taking no measures to exclude wild birds from buildings. Producers in Northern California were less likely (63 percent) to take measures than those in Southern California (41 percent). Although construction practices that prevent access are used, several producers reported that the effectiveness was dependent on maintaining buildings so that access points are not breached (33). Craven et al. (2000) notes that starlings have the ability to peck through plastic wire mesh on the sides of chicken houses (17).

**Estimation of the likelihood that finished feed becomes contaminated with EA/NA H5N2 HPAI virus by passerine birds on the farm**

Commercial poultry feed is typically stored in fully enclosed, weather tight bulk feed-storage bins on the farm. Field studies evaluating the role of passerine birds in on-farm disease transmission of poultry diseases indicate that passerine birds are common pests on poultry farms and are capable
of breaching biosecurity. Although data are not available on the rate of biosecurity breaches during feed storage on the farm, there is a possibility that passerine birds may gain access to finished feed in the event a storage bin lid or cover is not secure or during the process of filling the storage bin during delivery. Given that there is a breach in biosecurity, passerine birds would be attracted to easily accessible feed, so there would likely be a high visitation rate.

Based on a review of the scientific literature on the surveillance of HPAI virus in passerine birds, the prevalence of HPAI virus infection in these birds is estimated to be very low. Preliminary results from a survey of 419 passerine birds\(^2\) on 5 farms infected with H5N2 HPAI virus and 5 non-infected farms in Iowa indicates that mechanical transmission through contamination of the external surface of passerine birds is a possibility, although the likelihood is very low (1 external surface swab was positive by matrix gene RRT-PCR and submitted for further testing) (83). Data on the quantity of external contamination on the bird or the virus titer were not reported. There was an additional report of an infected chickadee in MN in June 2015, however, the exact origin of the infected bird could not be determined.

Given the potential for a high visitation rate by passerine birds in the event that biosecurity is breached due to the attraction of these birds to feed and the very low estimated prevalence of HPAI virus infection, we estimate that the likelihood of contamination of finished feed with HPAI virus by passerine birds on the farm is low (Table D-3).

**Estimation of the degree of HPAI virus contamination present on finished feed**

No data were available on the quantity of passerine bird feces contamination present on finished poultry feed in storage binds at feed mills or on farms. For the purposes of this analysis, we assumed a range for a small amount of contaminating fecal material that might be present on a batch of finished feed (1, 2 and 5 grams), given a breach in biosecurity. The HPAI virus titer in feces in wild passerine birds was assumed to be \(10^4\) EID\(_{50}/gm, based on literature review.

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\(^2\) 220 individual birds across 17 species on infected farms, 199 individual birds across 17 species on uninfected farms; only 77\% of samples from infected premises had been analyzed at the time of the preliminary report.
Risk that Contaminated Feed Results in HPAI Spread to Susceptible Poultry

Background information on contaminated poultry feed as a potential pathway for spread of HPAI virus

We were unable to identify epidemiologic studies that implicate poultry feed as a pathway for introduction or spread of HPAI virus. Several experimental feeding trials indicate that susceptible poultry can become infected by eating meat or blood containing HPAI virus at higher titer levels, but there is considerable uncertainty in the infectious dose required for transmission through this route. Therefore, feeding poultry feed contaminated with HPAI virus should be considered as a potential pathway of introduction.

HPAI virus dose response in chickens

Most experimental studies in chickens used intranasal inoculation as an entry point. For the intranasal route, the 50 percent chicken infectious dose ($\text{CID}_{50}$) for 10 HPAI strains varied between $10^{1.2}$ to $10^{4.7}$ EID$_{50}$ with a geometric mean of $10^{2.82}$ EID$_{50}$ (76). Most strains in this study had a mean CID$_{50}$ above $10^2$ EID$_{50}$ except for the HPAI H7N1. Other studies have also found similar estimates for the CID$_{50}$ through the intranasal route (69).

Single-hit dose response models (e.g., exponential) have been used for HPAI virus in chickens and mammals (43, 64). These models assume that each virion has the capacity to independently act and cause infection in the host. Dose response models enable us to estimate the probability of infection when a bird is exposed to a dose different from the 50 percent infectious dose. For example, given a CID$_{50}$ less than $10^{2.82}$ EID$_{50}$, a chicken exposed to 10 EID$_{50}$ would have a one percent chance of infection according to the single hit exponential dose response model.

Given limited data, there is a greater uncertainty regarding the infectious dose for other routes such as oral consumption of infected material. Kwon and Swayne (2010) found a substantially higher 50 percent infectious dose for HPAI H5N1 via oral consumption of chicken meat ($10^7$ EID$_{50}$) or drinking of contaminated water ($10^{6.7}$ EID$_{50}$) (45) compared to intranasal inoculation. However, in this study, a group of 3 to 5 chickens were fed contaminated meat with a single virus concentration, and details regarding the uncertainty in the estimates were not provided. The study also found higher infectious doses for the intra-gastric inoculation route by gavage ($10^{6.2}$ EID$_{50}$ for liquid and $10^{7.4}$ for meat EID$_{50}$) compared to the intranasal route. In Swayne and Beck (2005), feeding of finely chopped meat from chickens infected with H5N1 HPAI viruses at higher doses ($10^{7.8}$ EID$_{50}$/bird) resulted in transmission of H5N1 HPAI virus (74). However, feeding of HPAI H5N2 infected chicken breast or thigh meat to SPF chickens ($10^{3.5-3.6}$ EID$_{50}$/bird) did not produce infection. The authors reasoned that lack of direct exposure of respiratory tract (i.e. minced meat likely did not pass through the choanal cleft and contact nasal surfaces) could explain the lack of infection in H5N2 trials with lower doses. Moreover, a reference is made to a feeding trial by Purchase et. al. (1931), where 0.5g of blood fed to chickens resulted in HPAI transmission whereas feeding 5g of meat did not, suggesting that transmission is more likely if a feedstuff is conducive to passage into the nasal cavity (61). However, in the Purchase et al. study, the HPAI virus concentration in blood was not estimated and it may have been sufficient to cause infection via intra-gastric route.
RISK THAT POULTRY FEED MADE WITH CORN
—POTENTIALLY CONTAMINATED WITH EURASIAN-NORTH AMERICAN LINEAGE H5N2 HPAI VIRUS FROM WILD MIGRATORY BIRDS—
RESULTS IN EXPOSURE OF SUSCEPTIBLE COMMERCIAL POULTRY

Sergeev et al., (2012) found a CID$_{50}$ of $10^{3.9}$ EID$_{50}$ and $10^{5.2}$ EID$_{50}$ for oral inoculation and intragastric inoculation via gavage tube, respectively (66). The authors suggested contamination of the nasal mucosal membranes from the oral cavity via the choanal slit as a possible internal mechanism for transmission via the fecal oral route.

**HPAI virus dose response in turkeys**

Both intraocular and intranasal inoculation were used in an experimental study of infectious and lethal doses of two HPAI strains in turkeys (2). In this study, turkeys were inoculated with H5N1 and H7N1 strains and all birds shown to be infected died. The ID$_{50}$ and LD$_{50}$ were thus equal; the median was $10^{1}$ EID$_{50}$ for H5N1 and $10^{2.2}$ EID$_{50}$ for H7N1. Turkeys were found to be more susceptible than chickens by over 200-fold for H5N1 and over 100-fold for H7N1.

In another study, turkeys were inoculated with different doses of A/ostrich/Italy/984/2000 H7N1 HPAI by a combined intranasal/intraocular route (55). Although ID$_{50}$ and LD$_{50}$ were not explicitly measured, the latter can be extrapolated from their data and was shown to be both dose- and time-dependent. There was no mortality with $10^{3}$ EID$_{50}$ by 7 days post-inoculation (PI), while there was greater than 50 percent (4/6) mortality with $10^{6}$ EID$_{50}$ at 48 hours PI. At 72 hours PI, the LD$_{50}$ was $10^{2}$ EID$_{50}$, and it was $10^{2}$ EID$_{50}$ at both 96 and 120 hours PI.

In their studies using a highly poultry-adapted LPAI strain, Pillai et al., (2010) demonstrated a 50 percent lower ID$_{50}$ for turkeys ($10^{1.4}$ EID$_{50}$) than for chickens ($10^{2.6}$ EID$_{50}$) (60). They cautioned that virus strain, as well as genetic make-up of the study birds, may affect the minimum infectious dose, such that it may not be possible to generalize results from a few isolates in a certain breed of turkey. In addition, they point out that field conditions add the compounding factors of secondary infections and other influences that may compromise immune responses and result in increased morbidity and mortality.

As stated above, the infectious dose for turkeys through intranasal inoculation for HPAI viruses (H5N1 and H7N1) has been found to be 2 to 3 logs lower than that for chickens (2). Given a 50 percent chicken infectious dose of 5 to 6 log EID$_{50}$ for aerosol transmission from the dose response models, it is possible the turkey infectious dose is between 3 to 4 logs EID$_{50}$.

Transmission of LPAI to turkeys has been demonstrated via an estimated aerosol dose between 3 to 5 log EID$_{50}$ (34). Data from this experimental study suggests that the 50 percent aerosol infectious dose is close to or less than 3 to 5 log EID$_{50}$.

HPAI infection via the gastric route is not well documented in turkeys. In one small study, 50-day-old turkeys were inoculated directly into the esophagus with 2 grams of $10^{3.6}$ EID$_{50}$/0.1g HPAI H7N1 (for a total dose of $10^{4.9}$ EID$_{50}$) infective meat homogenate (82). Tracheal and cloacal swabs collected out to day 7 remained negative, as did serum samples out to day 21, and no clinical signs were observed. These results imply that the infective dose for HPAI via esophageal inoculation is likely more than 20 times $10^{3.6}$ EID$_{50}$. However, since the choanal eleft was bypassed, no inference can be made as to the infective dose with exposure that may occur through natural feeding process.
Route of entry and 50 percent infectious dose estimate used in this assessment

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

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

We asked experts for their opinion regarding the appropriate infectious dose (intranasal or intragastric) that best represents oral exposure in chickens, given the limited data on this aspect. Experts stated that it is reasonable to assume that transmission may occur if contaminated food or water were to pass through the choanal cleft into the nasal cavity. Therefore, due to the limited studies on exposure via natural feeding of contaminated materials and the associated uncertainty, we conservatively assumed that transmission of HPAI viruses through consumption of contaminated materials might occur with exposure to doses infectious for the intranasal route. Based on recent experimental studies performed at the Southeast Poultry Research Lab (SEPLRL), the intranasal infectious dose for Eurasian HPAI H5N2 in turkeys was estimated to be $10^{4.6}$ ($10^{3.6}$-$10^{5.2}$) EID$_{50}$.

Estimation of the Risk that Pelleted Feed Containing Corn Contaminated with EA/NA H5N2 HPAI Virus Results in Exposure of Susceptible Poultry

In the entry assessment, we considered the impact of steam conditioning of raw feed ingredients (including ground corn) on the reduction of the amount of HPAI virus on corn contaminated with wild bird feces. Although direct data on the inactivation of HPAI virus in poultry feed ingredients representative of the time and temperature ranges for the pelleting process are not available, we used thermal inactivation parameters for HPAI virus from poultry products. Simulation modeling results indicate a high probability of inactivation of HPAI virus in raw feed ingredients, even when the mash conditioning process alone was considered (more than a 99 percent chance of above 20 log inactivation). When these results are incorporated in an exponential dose response model, the probability of infection of a commercial poultry flock, given exposure to a load of contaminated feed, was predicted to be 0 in all of the 20,000 iterations. We conclude that the risk of transmission of EA/NA H5N2 HPAI virus through pelleted feed that contains HPAI virus contaminated corn is negligible.

Estimation of the Risk that Mash Feed Containing Corn contaminated with EA/NA H5N2 HPAI Virus Results in exposure of Susceptible Poultry

From the entry assessment, simulation model results predicted that inactivation with the drying process had an approximate 90 percent prediction interval of 0.27 to 8 log EID$_{50}$ reduction in HPAI virus titers. This large prediction interval is due to the uncertainty in the D-values of HPAI virus at drying temperatures. Specifically, the D-value estimates from two different published
studies on the degree of inactivation of HPAI virus in dried material at higher temperatures have varied widely in this temperature range (20, 48).

Using an exponential dose-response model, we predict that contaminated corn dried in a vertical drier before being incorporated into mash feed would result in HPAI virus transmission 35 percent of the time (mean 35 percent, 95 percent P.I. 0 to 100 percent). This prediction means that on average, 35 out of 100 loads of contaminated mash feed would result in transmission of EA/NA H5N2 HPAI virus to poultry. Although drying reduces the probability of exposure, there is insufficient evidence that the risk would be mitigated in all cases.

Not all corn stored on the ground requires drying before grinding. No data were available on the frequency of use of corn stored on the ground that is not dried before being used in poultry feed. In this case, there would be little inactivation of HPAI virus on corn that is freshly contaminated, as the grinding process produces very little heat. Therefore, incorporation of undried corn into mash feed would result in a much higher risk of HPAI virus transmission compared with dried corn.

Formaldehyde products are not uniformly used in the manufacturing of commercial poultry feed. One limitation on the use of these products is cost, which limits their use mostly to the treatment of mash feed for primary layer breeders (genetic stock). According to industry experts, the majority of feed mills producing mash do not treat feed or feed ingredients for microbial control (i.e., they do not use formaldehyde), unless the feed is for layer breeders.

In the entry assessment, we estimated that the likelihood that HPAI virus would not be inactivated to very low levels in commercial poultry mash feed by formaldehyde is very low to low (Table D-3), based on a limited review of feed pathogen inactivation studies in the published literature. Further study is needed to reduce our uncertainty in the degree of inactivation. We estimated 2- to 8-log EID50/g inactivation of HPAI virus in poultry feed ingredients treated with formaldehyde. This range reflects our uncertainty in the efficacy of these products, given the data currently available. Our simulation models predict that if freshly contaminated corn is incorporated into a batch of mash feed treated with formaldehyde, there would be a 3 percent chance (mean 3 percent, 95 percent P.I. 0 to 17 percent) of HPAI virus transmission to susceptible poultry.

Although there is a high probability that mash feed made from corn contaminated with EA/NA H5N2 HPAI virus would result in transmission to poultry, the chances a pile of corn becomes contaminated has to be considered in the overall risk estimation. Considering the very low to low estimated likelihood (Table D-3) that a corn pile becomes contaminated, we estimate that it is very unlikely that feeding contaminated mash feed is a major pathway of HPAI virus spread. We qualitatively estimate that there is a very low to low likelihood (Table D-3) that feeding mash feed made from corn contaminated with EA/NA H5N2 HPAI virus by wild migratory waterfowl could be responsible for the introduction of a new case of HPAI virus.

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**Estimation of the Risk that Poultry Feed Contaminated with EA/NA H5N2 HPAI Virus by Wild Birds (Passerine or Perching birds) Results in Exposure of Poultry**

Certain species of wild passerine (perching) birds are implicated in secondary transmission of Asian lineage H5N1 HPAI virus in other countries. Although sustained transmission or long
distance geographic spread through these species has not been demonstrated, HPAI virus has been recovered from passerine birds around infected farms in several past outbreaks. We considered the possibility that passerine birds (e.g., sparrows, starlings, or grackles) may play a role in the secondary transmission of EA/NA H5N2 HPAI virus by contaminating finished feed at the feed mill or on the farm.

Surveillance survey data indicates that the prevalence of HPAI virus infection in these birds is lower than in migratory waterfowl. However, there is also the possibility of mechanical transmission of HPAI virus if plumage or feet become contaminated. Preliminary results from a survey of 419 passerine birds on 5 farms infected with H5N2 HPAI virus and 5 non-infected farms in Iowa indicates that mechanical transmission through contamination of the external surface of passerine birds is a possibility, although the likelihood is very low (1 external surface swab was positive by matrix gene RRT-PCR and submitted for further testing). No data were reported either on the quantity of external contamination on the bird or the virus titer. From the entry assessment, we estimated that the likelihood of contamination of finished feed with HPAI virus by passerine birds at the feed mill and on the farm is very low to low (Table D-3), considering the very low estimated HPAI prevalence of infection in these birds and biosecurity measures taken to exclude entry.

No data were available on the quantity of passerine bird feces contamination present on finished poultry feed in storage bins at feed mills or on farms. For the purposes of this analysis, we assumed a range for a small amount of contaminating fecal material that might be present on a batch of finished feed (1, 2 and 5 grams), given a breach in biosecurity. The HPAI virus titer in feces in wild passerine birds was assumed to be $10^4$ EID$_{50}$/gm, based on literature review.

In this case, our exponential dose response model predicts that HPAI virus transmission would occur in 15 out of 100 loads of feed in the event that feed becomes contaminated with 1 gram of HPAI virus contaminated passerine bird feces due to a breach in biosecurity, and 58 out of 100 loads of feed in the event that feed is contaminated with 5 grams of HPAI virus contaminated passerine bird feces.

Table 10. The probability of transmission of HPAI virus from finished feed contaminated with HPAI virus in feces from wild passerine birds

<table>
<thead>
<tr>
<th>Grams of wild bird feces (passerine birds) on finished poultry feed fed directly to poultry</th>
<th>Probability of infection of susceptible poultry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15%</td>
</tr>
<tr>
<td>2</td>
<td>29%</td>
</tr>
<tr>
<td>5</td>
<td>58%</td>
</tr>
</tbody>
</table>

$^3$ 220 individual birds across 17 species on infected farms, 199 individual birds across 17 species on uninfected farms; only 77% of samples from infected premises had been analyzed at the time of the preliminary report.
FDA-approved formaldehyde-based feed treatments are labelled for use in feed to reduce pathogen load and to prevent cross contamination of finished feed for up to 21 days. At the time of this analysis, specific data on the degree of reduction in HPAI virus load in bird feces on the surface of finished feed that has been treated with formaldehyde-based products were not available. Therefore, we did not evaluate the potential reduction in HPAI virus concentration in bird feces on the external surface of finished feed in the current analysis.

For the overall risk estimate, we consider the very low to low likelihood of contamination of finished feed with HPAI virus by passerine birds at the feed mill and on the farm. In conjunction with the high risk of transmission from the presence of a small amount of contaminated wild bird feces from dose response models, we qualitatively estimate that the risk that EA/NA H5N2 HPAI virus is spread to other commercial poultry operations by passerine birds is very low to low (Table D-3). The likelihood estimate of very low to low (Table D-3) reflects our uncertainty in the role of passerine birds in mechanical transmission of HPAI virus; the frequency of biosecurity breaches at the feed mill and on the farm; the prevalence of this virus in passerine birds on or near poultry operations during the upcoming fall and spring seasons; and the high risk of HPAI virus transmission given the presence of a small quantity of virus on potentially contaminated feed.

**Overall Conclusions**

The likelihood that a corn pile becomes contaminated with EA/NA H5N2 HPAI virus when corn is stored on the ground depends on the species of wild birds attracted to corn piles, the frequency with which they feed from the piles, and the HPAI prevalence in those wild bird species. As of April 29, 2015, Minnesota State Department of Natural Resources officials reported that there had been no findings of the H5N2 HPAI virus in 2,216 tests of waterfowl fecal matter. The surveillance strategy goal was to test 3,000 environmental samples from around the State. As of June 15th, there had been no reports of isolation of this virus in wild migratory waterfowl in Minnesota. Data on the quantity of feces contaminated with EA/NA H5N2 HPAI virus present on a corn pile or the frequency that corn piles were contaminated were not available for this analysis. Data from field studies on the prevalence of AI virus contamination among corn piles and the quantity of contaminated fecal material would help reduce the uncertainty in our risk estimates.

There is some chance that a batch of contaminated corn will be used in either pelleted feed, untreated mash feed, or mash feed treated with formaldehyde. We considered these possibilities as separate scenarios. Formaldehyde products are not consistently used in the manufacturing of commercial poultry feed. One limitation on the use of these products is cost, which restricts use mostly to the treatment of feed for layer breeders (genetic stock). The estimated risk of transmission of HPAI virus in untreated mash feed was high using dose response models under a scenario where a small quantity of contaminated wild bird feces is present in the corn pile. Although the probability of exposure would be reduced when corn is dried in a vertical drier, there is insufficient evidence that the risk of transmission would be mitigated through the drying process in all cases due to variability in corn drier temperature. Treating mash feed with formaldehyde reduced the risk of HPAI transmission. We estimated that the likelihood that HPAI virus would not be inactivated to very low levels in commercial poultry mash feed by formaldehyde is very low to low (Table D-3), based on a limited review of feed pathogen inactivation studies in the published literature. We estimated 2- to 8-log EID<sub>50</sub>/gram of feces of inactivation of HPAI virus in
poultry feed ingredients treated with products containing formaldehyde. This range reflects our uncertainty in the efficacy of this product given the data currently available, and the variability due to inconsistent application under field conditions. When the overall risk estimate includes the likelihood that wild birds contaminate a corn pile, the risk of introduction of HPAI virus through contaminated mash feed was estimated to be very low to low (Table D-3). Diverting corn stored on the ground for use in pelleted feed may be a reasonable risk mitigation measure, as the estimated risk of HPAI virus transmission to poultry through pelleted feed is negligible (Table D-3).

Finished feed could become contaminated by sparrows, starlings, and other perching birds if there is a break in biosecurity. In this case, there would be a high probability of HPAI virus transmission if birds are shedding virus in their feces. However, the estimated prevalence of HPAI virus infection in these birds is very low. There is also the possibility of mechanical transmission of HPAI virus if plumage or feet were to become contaminated. Preliminary results from a survey of 419 passerine birds on 5 farms infected with H5N2 HPAI virus and 5 non-infected farms in Iowa indicates that mechanical transmission through contamination of the external surface of passerine birds is a possibility, although the likelihood is very low (1 external surface swab was positive by matrix gene RRT-PCR and submitted for further testing). Data on the quantity of external contamination present on the bird or the virus titer were not reported. Direct data on HPAI virus inactivation in wild bird feces present on feed treated with formaldehyde products were not available, so we were not able to estimate the degree of virus reduction attributed to feed treatment. HPAI virus titers in feces from infected passerine birds can be as high as $10^4$ EID$_{50}$/gm, so any treatment applied would need to result in 3 log or more inactivation so as to have a low likelihood of infection. Improving biosecurity measures for feed storage at the feed mill or on the farm would likely be a more cost effective risk management strategy, given the high cost of feed treatment, and the very low to low (Table D-3) overall estimated risk of HPAI transmission by passerine birds.

REFERENCES

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4 220 individual birds across 17 species on infected farms, 199 individual birds across 17 species on uninfected farms; only 77% of samples from infected premises had been analyzed at the time of the preliminary report.
RISK THAT POULTRY FEED MADE WITH CORN
—POSSIBLY CONTAMINATED WITH EURASIAN-NORTH AMERICAN LINEAGE H5N2 HPAI VIRUS FROM WILD MIGRATORY BIRDS—
RESULTS IN EXPOSURE OF SUSCEPTIBLE COMMERCIAL POULTRY


27. Grea


52. Minnesota Department of Natural Resources Second confirmed case of avian influenza reported in wild birds. In. 2015.
Risk that Poultry Feed Made with Corn
—Potentially Contaminated with Eurasian-North American Lineage H5N2 HPAI Virus from Wild Migratory Birds—
Results in Exposure of Susceptible Commercial Poultry

APPENDIX A. CODE OF FEDERAL REGULATIONS TITLE 21, VOLUME 6

TITLE 21—FOOD AND DRUGS
CHAPTER I—FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER E—ANIMAL DRUGS, FEEDS, AND RELATED PRODUCTS

PART 573 -- FOOD ADDITIVES PERMITTED IN FEED AND DRINKING WATER OF ANIMALS

Subpart B--Food Additive Listing

Sec. 573.460 Formaldehyde.

The food additive formaldehyde may be safely used in the manufacture of animal feeds in accordance with the following conditions:

(a) The additive is used, or intended for use, to improve the handling characteristics of fat by producing a dry, free-flowing product, as follows:

(1) For animal fat in combination with certain oilseed meals, as a component of dry, nonpelletted feeds for beef and nonlactating dairy cattle.

(i) An aqueous blend of soybean and sunflower meals in a ratio of 3:1, respectively, is mixed with animal fat such that the oilseed meals and animal fat are in a ratio of 3:2. The feed ingredients are those defined by the "Official Publication" of the Association of American Feed Control Officials, Inc., 2003 ed., pp. 303, 308, and 309, which is incorporated by reference. The Director of the Office of the Federal Register approves this incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. You may obtain copies from the Assistant Secretary-Treasurer, Association of American Feed Control Officials Inc., P.O. Box 478, Oxford, IN 47971, or you may examine a copy at the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852, or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

(ii) Formaldehyde (37 percent solution) is added to the mixture at a level of 4 percent of the dry matter weight of the oilseed meals and animal fat. This mixture, upon drying, contains not more than 1 percent formaldehyde and not more than 12 percent moisture.
(iii) To assure the safe use of the additive, in addition to the other information required by the Federal Food, Drug, and Cosmetic Act (the act), the label and labeling of the dried mixture shall bear:

(A) The name of the additive.

(B) Adequate directions for use providing that the feed as consumed does not contain more than 25 percent of the mixture.

(2) For soybean and canola seeds and/or meals to which there may be added vegetable oil as a component of dry, nonpelleted feeds for beef and dairy cattle, including lactating dairy cattle.

(i) An aqueous blend of oilseed and/or meals, with or without added vegetable oil, in a ratio such that, on a dry matter basis, the final protein level will be 25 to 35 percent and the fat content will be 20 to 45 percent. The feed ingredients are those defined by the "Official Publication" of the Association of American Feed Control Officials, Inc., 2003 ed., pp. 301, 307, 308, and 309, which is incorporated by reference. The Director of the Office of the Federal Register approves this incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. You may obtain copies from the Assistant Secretary-Treasurer, Association of American Feed Control Officials Inc., P.O. Box 478, Oxford, IN 47971, or you may examine a copy at the Division of Dockets Management, Food and Drug Administration, 5630 Fishers lane, rm. 1061, Rockville, MD 20852, or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

(ii) Formaldehyde (37 percent solution) is added to the mixture at a level of 2.7 percent of the dry matter weight basis of the oilseeds and/or meals and the vegetable oil. This mixture, upon drying, contains not more than 0.5 percent formaldehyde and not more than 12 percent moisture.

(iii) To assure the safe use of the additive, in addition to the other information required by the act, the label and labeling of the dried mixture shall bear:

(A) The name of the additive.

(B) The statement, "This supplement is not to exceed 12.5% of the total ration. Dietary calcium and magnesium levels should be considered when supplementing the diet with fat."

(C) The minimum and maximum levels of crude fat must be guaranteed and must be between -5 percent and +5 percent of the analyzed fat content for each batch.

(b)(1) The food additive is formaldehyde (CAS No. 50-00-0; 37 percent
aqueous solution). It is used at a rate of 5.4 pounds (2.5 kilograms) per ton of animal feed or feed ingredient. It is an antimicrobial agent used to maintain complete animal feeds or feed ingredients 

Salmonella negative for up to 21 days.

(2) To assure safe use of the additive, in addition to the other information required by the Act, the label and labeling shall contain:

(i) The name of the additive.

(ii) A statement that formaldehyde solution which has been stored below 40 deg. F or allowed to freeze should not be applied to complete animal feeds or feed ingredients.

(iii) Adequate directions for use including a statement that formaldehyde should be uniformly sprayed on and thoroughly mixed into the complete animal feeds or feed ingredients and that the complete animal feeds or feed ingredients so treated shall be labeled as containing formaldehyde. The label must prominently display the statement: "Treated with formaldehyde to maintain feed 

Salmonella negative. Use within 21 days."

(iv) The labeling for feed or feed ingredients to which formaldehyde has been added under the provisions of paragraph (b)(1) of this section is required to carry the following statement: "Treated with formaldehyde to maintain feed 

Salmonella negative. Use within 21 days."

(3) To assure safe use of the additive, in addition to the other information required by the Act, the label and labeling shall contain:

(i) Appropriate warnings and safety precautions concerning formaldehyde.

(ii) Statements identifying formaldehyde as a poison with potentials for adverse respiratory effects.

(iii) Information about emergency aid in case of accidental inhalation.

(iv) Statements reflecting requirements of applicable sections of the Superfund Amendments and Reauthorization Act (SARA), and the Occupational Safety and Health Administration's (OSHA) human safety guidance regulations.

(v) Contact address and phone number for reporting adverse reactions or to request a copy of the Materials Safety Data Sheet (MSDS).

# Appendix B. Simulation Model of HPAI Virus Inactivation in Pelleted Feed

Table B1. Simulation model of thermal inactivation of HPAI virus through steam treatment of mash ingredients used in the pelleted feed production processes.

<table>
<thead>
<tr>
<th>Parameter symbol</th>
<th>Definition</th>
<th>Value, distribution or formula</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_b$</td>
<td>D-value is the time required to cause a 1 log reduction in HPAI virus concentration or amount at the baseline temperature</td>
<td>$26.8 \text{ sec at } 60^\circ C \text{ for whole egg; } 0.5 \text{ sec at } 70^\circ C \text{ for chicken meat}$</td>
<td>These are based on OIE pasteurization and cooking requirements to achieve a 7-log inactivation</td>
</tr>
<tr>
<td>$T_b$</td>
<td>Baseline temperatures at which D-value data is available</td>
<td>$60^\circ C \text{ for whole egg; } 70^\circ C \text{ for chicken meat}$</td>
<td>These are based on OIE pasteurization and cooking requirements to achieve a 7-log inactivation</td>
</tr>
<tr>
<td>$Z$</td>
<td>Z-value is the rise in temperature required to reduce the D-value by 1 log</td>
<td>$5^\circ C$</td>
<td>See the Eggshells and Inedible Egg Products risk assessment</td>
</tr>
<tr>
<td>$T_{pm}$</td>
<td>Pellet mash temperature(^\dagger)</td>
<td>Pert distributed (71.1, 85, 87.8 $^\circ C$)</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>$\tau_{pm}$</td>
<td>Time for which the pellet mash is maintained at the temperature $T_{pm}$</td>
<td>Pert distributed (3, 5, 80 sec)</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>$D_{pm}$</td>
<td>Time required to cause a 1 log reduction in HPAI virus concentration or amount at pellet mash temperature</td>
<td>$D_b/10^{(\frac{T_{pm}-T_b}{Z})}$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$C_{pm}$</td>
<td>Inactivation achieved due to steam conditioning of pellet mash</td>
<td>$\tau_{pm}/D_{pm}$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$G$</td>
<td>Grams of feces per load</td>
<td>Pert (2, 25, 50) distributed</td>
<td>Based on assumption</td>
</tr>
<tr>
<td>$H$</td>
<td>Concentration of HPAI virus in duck feces</td>
<td>Pert (2, 4, 8) EID$\text{50}/g$ (56)</td>
<td></td>
</tr>
<tr>
<td>$A$</td>
<td>Dose of HPAI virus in a load of corn/feed</td>
<td>$G * H/10^{C_{pm}}$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$r$</td>
<td>Exponential dose-response parameter, likelihood of exposure with a single EID$\text{50}$</td>
<td>$-\ln(0.5)/\text{TID}_{50} = 0.00001741$</td>
<td>A turkey infectious dose of $10^{4.6}$ EID$\text{50}$ was estimated from SEPRL data</td>
</tr>
<tr>
<td>$P_{pm}$</td>
<td>Probability of infection of at least one bird per load of pelleted feed</td>
<td>$1-\exp(-r*A)$</td>
<td>Calculated</td>
</tr>
</tbody>
</table>

\(^\dagger\) Conservatively estimated as 1/3 the total retention time in the steam conditioner based on expert opinion; this is the estimated time that particles achieve maximum temperature during steam conditioning.
### APPENDIX C. SIMULATION MODEL OF HPAI VIRUS INACTIVATION IN MASH FEED

Table C 1. Simulation model of thermal inactivation of HPAI virus in dried feces on the surface of corn dried in a vertical counter-flow drier before grinding for incorporation into mash feed.

<table>
<thead>
<tr>
<th>Parameter Symbol</th>
<th>Definition</th>
<th>Value, distribution or formula</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_b$</td>
<td>D-value is the time required to cause a 1 log reduction in HPAI virus concentration or amount at baseline temperature</td>
<td>252 sec at 45° C; 18,514 sec at 30° C</td>
<td>(20); and (48)</td>
</tr>
<tr>
<td>$T^b$</td>
<td>Baseline temperatures at which D-value data is available</td>
<td>45° C; 30° C</td>
<td>(20); and (48)</td>
</tr>
<tr>
<td>$Z$</td>
<td>Z-value is the raise in temperature required to reduce the D value by 1 log</td>
<td>5° C</td>
<td>See Eggshells and Inedible Egg Product risk assessment</td>
</tr>
<tr>
<td>$T^{dr}$</td>
<td>Corn temperature range*</td>
<td>Pert distributed (105° to 120° F).</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>$\tau^{dr}$</td>
<td>Time for which the corn is maintained at the temperature $T^{dr}$</td>
<td>Pert distributed (15, 20, 45 minutes)</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>$D^{dr}$</td>
<td>Time required to cause a 1 log reduction in HPAI virus concentration or amount at drying temperature</td>
<td>$D_b/10^{\frac{(T^{dr}-T^b)}{Z}}$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$C^{dr}$</td>
<td>Inactivation achieved due to drying of corn</td>
<td>$\tau^{dr}/D^{dr}$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$G$</td>
<td>Grams of feces per load</td>
<td>Pert (2, 25, 50) distributed</td>
<td>Based on assumption</td>
</tr>
<tr>
<td>$H$</td>
<td>Concentration of HPAI virus in duck feces</td>
<td>Pert (2, 4, 8) EID$_{50}$/g</td>
<td>(56)</td>
</tr>
<tr>
<td>$A$</td>
<td>Dose of HPAI virus in load</td>
<td>$G \times H/10^{C_{pm}}$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$r$</td>
<td>Exponential dose response parameter, likelihood of exposure with a single EID$_{50}$</td>
<td>$-ln(0.5)/TID_{50} = 0.00001741$</td>
<td>A turkey infectious dose of 10$^{4.6}$ EID$_{50}$ was estimated from SEPRL data</td>
</tr>
<tr>
<td>$p_{pm}$</td>
<td>Probability of infection of at least one bird per load of mash feed</td>
<td>$1-exp(r*A)$</td>
<td>Calculated</td>
</tr>
</tbody>
</table>

*Range of hot air temperatures in vertical corn driers used to dry corn to 15.5% moisture before grinding (225° to 325° F). Based on expert opinion, for 80° F corn harvested in summer, the delta was estimated to be 25° F. For corn harvested in winter (say 30° F), the delta was estimated to be 90° F.
APPENDIX D. QUALITATIVE LIKELIHOOD SCALE

This appendix defines the qualitative likelihood scale used to describe the probability of events in this risk assessment. Qualitative scales attach a specific narrative phrase which conveys a meaning to terms used to describe the likelihood of an event occurring. Generally, it is best to choose an expression where there is some evidence for a high degree of consensus for its interpreted meaning (78). For example, use of the narrative phrase “there is a high likelihood that the event will occur” has been interpreted as a probability that ranges from 0.60 to 0.97 (60 to 97 percent chance of occurrence) (10); and the expression likely has been interpreted to range from 0.63 to 0.77 (78). To date, there is no one universally accepted or utilized likelihood scale and the scales are customized as appropriate for specific assessments. The OIE handbook on qualitative risk analysis does not prescribe a specific likelihood scale although it provides examples for terms which might be used in likelihood scales such as low, negligible, high etc. (91) Table D-1

An example likelihood scale adapted from Standards Australia for qualitative risk assessment in fisheries management (24) provides an example of a qualitative scale used in risk assessments elsewhere and Table D-2 lists adjectives to describe likelihood considered appropriate by the OIE. The likelihood scale used in this assessment is defined by Table D-3.

Table D-1. An example likelihood scale adapted from Standards Australia for qualitative risk assessment in fisheries management (24)

<table>
<thead>
<tr>
<th>Category</th>
<th>Probability Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likely</td>
<td>It is expected to occur</td>
</tr>
<tr>
<td>Occasional</td>
<td>May occur sometimes</td>
</tr>
<tr>
<td>Possible</td>
<td>Some evidence to suggest this is possible here</td>
</tr>
<tr>
<td>Unlikely</td>
<td>Uncommon, but has been known to occur elsewhere</td>
</tr>
<tr>
<td>Rare</td>
<td>May occur in exceptional circumstances</td>
</tr>
<tr>
<td>Remote</td>
<td>Never heard of, but not impossible</td>
</tr>
</tbody>
</table>
Table D-2. Terms used as adjectives to qualify likelihood estimates, considered appropriate by the OIE. (91)

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

Table D-3. Qualitative likelihood scale used in this assessment.

<table>
<thead>
<tr>
<th>Category</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely High</td>
<td>The event is almost certain to occur</td>
</tr>
<tr>
<td>High</td>
<td>The event has a reasonable chance of occurring</td>
</tr>
<tr>
<td>Low</td>
<td>The event is unlikely but does occur</td>
</tr>
<tr>
<td>Very Low</td>
<td>It is very unlikely that the event will occur</td>
</tr>
<tr>
<td>Negligible</td>
<td>The likelihood that the event will occur is insignificant: not worth considering.</td>
</tr>
</tbody>
</table>
APPENDIX E. DECISION BY FDA ON USE OF FORMALDEHYDE GAS TO TREAT FEED CONTAMINATED BY HPAI VIRUS

Email correspondence on July 7, 2015 from Michael Henry, Animal Feed Safety Team, Division of Animal Feeds, Center for Veterinary Medicine, FDA to Nathan Birnbaum, USDA APHIS Veterinary Services, Surveillance Preparedness and Response Services, National Preparedness and Incident Coordination.

“We have reviewed the information on the proposed use of formaldehyde gas to treat feed ingredients contaminated HPAI virus. The agency would consider the application of products containing formaldehyde to eliminate viruses in feed as unapproved food additives. Although 21 CFR 573.460 allows for the food additive formaldehyde to be used in the manufacture of animal feeds, it only permits the use of formaldehyde under specific conditions and for the intended use listed below.

(a) The additive is used, or intended for use, to improve the handling characteristics of fat by producing a dry, free-flowing product,

(b) It is an antimicrobial agent used to maintain complete animal feeds or feed ingredients Salmonella negative for up to 21 days.

For formaldehyde to be legally used in the treatment of feed ingredients contaminated HPAI virus, an approved food additive petition is required.

However, this does not prevent the USDA and the firm (sic Kemin) from conducting experimental studies to evaluate the effectiveness of formaldehyde prevent HPAI virus in feeds, as long as feeds and animals used in these experiments are not used for animal and human food.”
Risk that poultry feed made with corn—potentially contaminated with Eurasian-North American lineage H5N2 HPAI virus from wild migratory birds—results in exposure of susceptible commercial poultry.

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RISK THAT POULTRY FEED MADE WITH CORN
—POTENTIALLY CONTAMINATED WITH EURASIAN-NORTH AMERICAN LINEAGE HSN2 HPAI VIRUS FROM WILD MIGRATORY BIRDS—
RESULTS IN EXPOSURE OF SUSCEPTIBLE COMMERCIAL POULTRY

Document #294.0515 cl