Journal of Food Protection 88 (2025) 100478

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Modeling the Impact of Between-lot and Within-lot Variability in *Listeria monocytogenes* Contamination on Risk Reduction From Sampling Ready-to-eat Foods

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ARTICLE INFO

Keywords:
Contamination patterns
Listeria monocytogenes
Microbial risk assessment
Sampling plans
Web applications

ABSTRACT

Microbiological sampling and testing are widely utilized in food safety risk management. We developed risk assessments to quantify the impact of various sampling plans on the risk of invasive listeriosis to consumers. We used the FDA-iRISK® tool and adapted available process, consumption, and dose-response modules of published *L. monocytogenes* risk assessments to predict cases per billion servings from consumption of ready-to-eat foods. We also developed an *ad hoc* quantitative risk assessment application using R (the "FDA-LmQRA App") to evaluate more complex scenarios and provide additional metrics. Data and model inputs included the prevalence and levels of *L. monocytogenes*, sampling plan parameters, and operating characteristic curve data. We derived prevalence and between-lot distributions from data from market basket surveys of *L. monocytogenes* in ready-to-eat foods in the U.S. and examined two assumptions for the within-lot contamination: a lognormal distribution, or a heterogeneous distribution with a defined proportion of exceptional (higher level) contamination in addition to a single lognormal distribution. We found that testing each lot using 2-class plans (e.g., $n = 5$ or 10, $m = 0/25$ g or 0/5 g, and $c = 0$) or 3-class mixed plans (e.g., $n = 5$ or 10, $m = 0/25$ g or 0/5 g, $M = 20$ CFU/g or 100 CFU/g, and $c = 1$) and replacing positive lots by noncontaminated lots predicted quantifiable, but relatively low, risk reduction. The risk estimates were highly influenced by the variability of the between-lot concentration distribution as well as the presence of exceptional contamination for the within-lot contamination. In the presence of exceptional contamination, a 3-class mixed plan ($c = 1$) was predicted to have comparable performance based on risk estimates to a 2-class plan (corresponding n and m but $c = 0$). Results from this study may inform the choice of sampling plans to optimize sampling and testing strategies for reducing listeriosis associated with ready-to-eat foods.

Sampling and testing finished products for foodborne pathogens plays an important role in a food safety risk management system (van Schothorst et al., 2009; FSIS, 2014; Scott et al., 2015; Buchanan & Schaffner, 2015; FDA, 2017; Farber et al., 2021). While it is well-recognized that the safety of food is principally ensured by effective preventive control measures throughout the food chain, microbiological sampling and testing are utilized, for example, to verify whether operations are under control, determine acceptability of individual lots of food, gather baseline or targeted surveillance data, inform traceback in epidemiological investigations, and monitor environmental pathogens or indicators (Scott & Chen, 2016). There is a long-standing interest in better understanding the performance of different sampling strategies. Several tools (ICMSF, 2020; FAO/WHO, 2024; Pouillot et al., 2024a) are available to evaluate lot acceptance, and some have considered both between-lot and within-lot variability in pathogen contamination on sampling performance regarding lot acceptance. However, even though sampling and testing are widely utilized in food safety risk management, the impact of sampling plans evaluated within a quantitative risk assessment on predicted risk to the consumer is seldom studied.

Guidance on "hold and test" procedures for *L. monocytogenes* in ready-to-eat (RTE) foods usually includes sampling plans recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 2011). For example, in the FDA draft guidance for industry on the control of *L. monocytogenes* in RTE foods (FDA, 2017) and the FSIS compliance guideline on RTE meat and poul-

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https://doi.org/10.1016/j.jfp.2025.100478
Received 1 November 2024; Accepted 5 March 2025
Available online 10 March 2025
0362-028X/Published by Elsevier Inc. on behalf of International Association for Food Protection.

try products (FSIS, 2014), the ICMSF sampling plans "cases 10–15" (2-class plans with 5–60 samples) are potential options to consider for product lot acceptance testing, depending on the conditions of the food and the susceptibility of the subpopulation for which the food is intended. While it is well-recognized that sampling and lot rejection *per se* may have a limited impact on risk reduction due to typically infrequent sampling and other limitations in finished product sampling, an important impact derives from the indirect effect of sampling through incentivizing changes in production management (FAO/WHO, 2016).

The ICMSF developed a spreadsheet (standard program 10; ICMSF, 2020) that reports the arithmetic mean or the geometric mean concentration corresponding to a specified probability of detecting contamination in a lot (e.g., 95%) as a measure for sampling plan performance, where a lognormal distribution (i.e., normal distribution of $\log_{10}$ counts) is assumed for the within-lot distribution of the target organism. Farber et al. (2021) used the arithmetic mean concentration as the performance measure to compare the stringency of various sampling plans and to explore various approaches to manage *L. monocytogenes* in RTE foods, specifically those the investigators deemed "low risk." In a recent study (Pouillot et al., 2024a), we illustrated how the "operating characteristic" curve (OC curve) and the performance of sampling plans differ with and without consideration of "exceptional contamination" (higher level contamination) events, where the within-lot contamination includes exceptional contamination beyond what is expected by a single lognormal distribution. The ability of a sampling plan to reject a contaminated food lot is substantially influenced by the underlying within-lot distribution.

Whether and the degree to which sampling and testing RTE foods – along with subsequent removal of contaminated product lots from consumption – might reduce the risk associated with contamination of *L. monocytogenes* in RTE foods requires the consideration of both within- and between-lot distributions. In this study, we integrated data and assumptions on both within- and between-lot distributions of *L. monocytogenes* in RTE foods to quantify the performance of sampling plans in the context of a quantitative risk assessment. We aimed to address the question: What is the risk of illness from RTE foods that do not support the growth of *L. monocytogenes* before and after various sampling scenarios? We discussed the impact of sampling in the broader context of the relative risk between foods that do and those that do not support growth. The specific objectives of this study were to: (*i*) generate risk estimates using model inputs that included the OC curve to relate the probability of accepting a lot at a given contamination level and for a given sampling plan, (*ii*) generate risk estimates by specifically modeling within-lot and between-lot variability in *L. monocytogenes* contamination in the RTE food supply, and (*iii*) identify key factors and assumptions that may explain the differences in risk estimate with and without exceptional contamination. We utilized the peer-reviewed FDA-iRISK tool and OC curve outputs from the ICMSF spreadsheet and developed a new tool, FDA-LmQRA App that can consider more complex situations and provide additional metrics. We discuss the implications of our results on effective sampling strategies for risk management.

Materials and methods

Risk assessment framework and models. We developed risk assessment models based on a well-established quantitative microbial risk assessment framework (FAO/WHO, 2021a, 2021b), to evaluate the impacts of between-lot and within-lot variability in *L. monocytogenes* contamination on risk reduction from sampling RTE foods with different sampling plans. We used both the FDA-iRISK® tool (FDA/JIFSAN/RSI, 2021a) and developed a new *ad hoc* risk assessment tool, the FDA-LmQRA App, to create models. FDA-iRISK is a peer-reviewed risk modeling tool that enables users to conduct fully quantitative, fully probabilistic risk assessments of food-safety hazards. The FDA-LmQRA App is an application we developed using R (R Development Core Team, 2024) that is specific for *L. monocytogenes* with greater flexibility (see below); it is available at https://foodsafetyrisk.org/listeria/. We used FDA-iRISK to create a range of simpler risk scenarios, while using the FDA-LmQRA App for more in-depth modeling with consideration of exceptional contamination (see below). We adapted relevant process, consumption, and dose-response modules of published quantitative risk assessments for *L. monocytogenes* (FDA/FSIS, 2003; Pouillot et al., 2015; FDA/JIFSAN/RSI, 2021a, 2021b; Pouillot et al., 2024b) and added new components to quantify the impact of sampling and testing on the risk of invasive listeriosis from consumption of RTE foods that do not support growth.

Description of sampling plans. We used the ICMSF descriptions for 2-class and 3-class mixed plans (ICMSF, 2020). A 2-class sampling plan involves testing for the presence/absence of a target pathogen, where the total number of samples n (e.g., $n = 5$), the microbiological limit m (e.g., $m = 0$ CFU/25 g), and the acceptable number of samples c exceeding the microbiological limit are defined. In a typical 2-class plan, $c = 0$ (i.e., none of the n samples can have unsatisfactory test results or be positive for *L. monocytogenes*). A 3-class mixed plan has two microbiological limits; a qualitative limit (e.g., $m = 0$ CFU/25 g) and a quantitative limit M, the maximum count that can be acceptable on any sample (e.g., $M = 100$ CFU/g). A lot would be rejected if, among the total number n of samples tested, more than c samples have unsatisfactory test results, i.e., more than c samples are in the marginal region (between m and M) or one or more samples exceed M. The performance of a sampling plan can be expressed as an "arithmetic mean concentration" (ICMSF, 2020; Farber et al., 2021), where a product lot having the calculated arithmetic mean concentration or greater will be rejected at least 95% of the time, assuming the within-lot contamination is a lognormal distribution with a given standard deviation (s.d.). A sampling plan is part of a microbiological criterion, which also has other components such as the testing method, the size of analytical unit, and actions to be taken when a criterion is not met (van Schothorst et al., 2009; ICMSF, 2020).

Assumptions for within-lot distributions. To evaluate sampling performance and to determine the acceptability of individual product lots, we assumed, in the first step, that the within-lot pathogen distribution was lognormal with a s.d. of 0.8 $\log_{10}$ CFU/g, which is also the assumption we used to obtain OC curve for the sampling plans as inputs for the risk assessment developed in FDA-iRISK (see more details below). The classical assumption of a single lognormal distribution of the within-lot concentration of the target pathogen is widely used to evaluate the acceptability of individual product lots (ICMSF, 2002, 2020; FAO/WHO, 2016; van Schothorst et al., 2009; Scott et al., 2015; Farber et al., 2021). However, the within-lot contamination pattern may be more complex, beyond a single lognormal distribution. In our parallel study (Pouillot et al., 2024a), we assumed heterogeneous distributions for within-lot contamination with exceptional contamination beyond a single lognormal distribution. Here, we evaluated the impact of both assumptions for the within-lot distribution – either a single lognormal distribution, or lognormal distribution plus exceptional contamination events – on the predicted risk reduction from sampling.

Prevalence and between-lot level distributions. We derived between-lot prevalence and level distributions based on data from a large interagency market basket survey of RTE foods in the U.S. (herein referred to as the FDA-ARS-FSIS Survey; Luchansky et al., 2017), where over 27,000 samples were collected at retail stores. Data from the survey show that prevalence, based on the detection of *L. monocytogenes* in 25 g units, are estimated to be 0.148% (95% CI 0.064–0.291%) for dairy commodities, 0.445% (95% CI 0.300–0.634%) for produce commodities, 0.573% (95% CI 0.296–0.998%) for seafood commodities, and 0.612% (95% CI 0.419–0.862%) for other commodities (Table S1, see supplemental materials). Samples collected in the FDA-ARS-FSIS Survey represented products across

many lots in the food supply (the study design involved weekly sample collection at large national chain supermarkets and independent grocery stores in four states over 100 weeks). The estimated prevalence at unit level, e.g., 0.148% for dairy commodities, is the overall marginal prevalence from a mixture of between- and within-lot data. In the absence of additional data, we made a simplifying assumption that the within-lot prevalence at the unit level was the same for all lots. Based on this assumption and the survey data, we determined that 100% of lots for each of these commodity categories would include at least one positive sample for product lots of 1,135 kg each, using a published methodology to derive equivalent prevalence according to different unit sizes (Chen et al., 2018). It is, however, conceivable that only a fraction of the lots in the survey were contaminated. We tested an alternative scenario with a prevalence of contaminated lots of 10% (vs. 100%) with a corresponding prevalence preserving the overall prevalence observed in the survey (e.g., 1.48% contaminated units within a lot for dairy commodities instead of 0.148%).

The FDA-ARS-FSIS Survey reported *L. monocytogenes* levels in positive samples varied from <0.036 MPN/g (limit of enumeration) to 6.14 $\log_{10}$ CFU/g. Using the method reported previously by Chen et al. (2003), we considered the amount of a sample taken for both screening and enumeration and estimated a level of 0.030 MPN/g for a 25 g sample testing positive but below the limit of enumeration. We used data from the FDA-ARS-FSIS Survey to derive between-lot concentration distributions for all food commodity groups except the dairy commodity group that has a relatively small number of positive samples and a large variability in the *L. monocytogenes* level. For this group, we combined these data with data from a study conducted by the National Food Processors Association (the NPFA Survey) that is an earlier market basket survey of similar design and scope (Gombas et al., 2003), to derive the between-lot concentration distribution. The between-lot distributions were based on overall frequency distributions (Fig. S1) and the prevalence of contamination (based on 25 g unit, the analytical sample size) for each group, assuming a log-normal within-lot distribution with a s.d. of 0.8 $\log_{10}$. Furthermore, the *L. monocytogenes* level was truncated at a maximum of 6.14 $\log_{10}$ CFU/g to represent foods that do not support growth between retail and consumption. We estimated that the between-lot distribution of the $\log_{10}$ of the arithmetic mean was Normal(1.17, 1.39) $\log_{10}$ CFU/g for seafood, Normal(-0.34, 1.83) $\log_{10}$ CFU/g for produce and other commodities, and Normal(-2.47, 3.81) $\log_{10}$ CFU/g for dairy commodities (Table S1). These distributions represent a range of seafood, dairy, fresh produce, and other commodities sampled in the FDA-ARS-FSIS Survey and in the NFPA survey, where a stratified sampling plan with consideration of consumption and several other factors was used to select products for testing (see more details in Table S1).

Predicting risk with and without sampling using FDA-iRISK and OC curves. We used the FDA-iRISK tool to create a risk assessment model with a wide range of scenarios to predict risk with and without sampling. The structure of a risk model developed in FDA-iRISK has been described in published technical documents (Chen et al., 2013; FDA/JIFSAN/RSI, 2021a).

Inputs for the risk assessment components include data and parameters for the process model, consumption model, and dose-response model (Table S2). The process model includes two stages (the initial condition stage and the sampling stage) to estimate the prevalence and levels of *L. monocytogenes* in the food at consumption. In this study, we did not consider growth between retail and consumption because our focus was on assessing the risk associated with the contamination in foods that do not support *L. monocytogenes* growth. The initial condition stage is defined by the prevalence of *L. monocytogenes* among lots, lot size, and the between-lot distribution of the $\log_{10}$ of the arithmetic mean. The sampling stage is defined by the OC curve, which is based on a within-lot distribution with a s.d. of 0.8 $\log_{10}$ and an arithmetic mean obtained through Monte Carlo simulation from the between-lot distribution, for a sampling plan of interest and the proportion of lots tested (default is 100% tested).

For the between-lot distribution, we used Normal (-2.5, 1.0) $\log_{10}$ CFU/g as a "baseline distribution" and several other plausible distributions to explore the impact of sampling and to compare different sampling plans when they are applied to test multiple lots in the food supply. Because the RTE food samples were collected from retail establishments from 100 sampling weeks over two years, the data and the overall frequency distributions (Fig. S1) represent the combined within-lot and between-lot variability in *L. monocytogenes* levels in the U.S. RTE food supply during the survey. In the baseline scenario, each lot is 1,135 kg (10 thousand units of 0.25 lb), and the prevalence is 100% among lots. We developed alternative scenarios where the mean of the $\log_{10}$ distribution of the arithmetic mean was -3.5, -1.5, or -0.5 $\log_{10}$ CFU/g, representing other plausible or potential between-lot distributions in the risk assessment developed in FDA-iRISK. We obtained OC curves from the ICMSF spreadsheet (ICMSF, 2020), using within-lot s.d. of 0.8 $\log_{10}$, for various 2-class and 3-class mixed plans, and used the OC curves as inputs in the risk assessment model to determine the impact of sampling on the risk estimate.

Built-in features in FDA-iRISK, e.g., the Sampling (OC Curve) feature and others, allow us to define a sampling stage in the process model by using the OC curve, which will prescribe a posttest adjustment in the between-lot concentration distribution in the RTE foods. More specifically, FDA-iRISK generates outputs for the prevalence and level of *L. monocytogenes* in the lots subject to testing, where the probability of rejection (for a given concentration value from the between-lot distribution) is estimated as prescribed by the OC curve, and this probability is used to adjust the prevalence weights for the concentration values, essentially resulting in an adjustment in the between-lot distribution across lots subject to sampling and testing (FDA/JIFSAN/RSI, 2021a). As implemented in the process model, the OC curve relates the probability of accepting a lot at a given arithmetic mean contamination level and for a given sampling plan, where the arithmetic mean concentration of *L. monocytogenes* in the lot is drawn from the between-lot distribution. The baseline scenario included inputs of 1 billion servings of 113.4 g (0.25 lb) for consumption, and the model predicted risk (the number of cases per billion servings) for the 65+ subpopulation with no known underlying condition by using the corresponding dose-response curve from Pouillot et al., 2015 (Table S2). We selected 1 billion servings for consumption because this was in a relevant range of consumption for the U.S. population and 65+ subpopulation (FDA/FSIS, 2003; FDA, 2022; see more details in Table S2). In alternative scenarios, we used different inputs for consumption (50 g serving size) and dose-response (the marginal across all population subgroups dose-response curve) to predict risk for the whole population to show that these inputs were not expected to change the estimated relative risk reduction after sampling.

Developing the FDA-LmQRA App to predict risk with consideration of exceptional contamination. We developed the FDA-LmQRA App to evaluate complex contamination distributions, provide additional metrics for comparing the performance of sampling plans in reducing the risk of listeriosis in foods through sampling and testing, and compare results assuming different dose-response relationships, by using the dose-response curves from Pouillot et al. (2015) and from Pouillot et al. (2024b) (Table S2). The FDA-LmQRA App generated outputs for cases with and without sampling and testing, the percentage of lots rejected, and the relative risk reduction (calculated as [cases without test]/[cases with test]). Furthermore, the FDA-LmQRA App generated outputs for the proportion of servings with *L. monocytogenes* levels >0 or >100 CFU/serving and the proportion of lots with $>10\%$ of positive units.

The inputs for lot parameters included definitions for between-lot contamination and within-lot distribution in the FDA-LmQRA App,

where the prevalence was defined either at the unit level (e.g., 0.25 lb unit) or at the lot level (e.g., lot size = 10,000 units of 0.25 lb). For samples with a concentration >0 CFU/g, the between-lot distribution was defined either as a lognormal distribution or a beta pert distribution for *L. monocytogenes* levels ($\log_{10}$ CFU/g). The between-lot distribution represents variability in the arithmetic mean concentrations of lots in the food supply.

We used two assumptions for the within-lot contamination: (a) a lognormal distribution with a s.d. of 0.8 $\log_{10}$ CFU/g and an arithmetic mean concentration of *L. monocytogenes* in the lot drawn from the between-lot distribution, and (b) two lognormal distributions in which the lower-level distribution is the same as in assumption (a) and the higher-level distribution defines a proportion of the units that have concentrations following another, higher, lognormal distribution. For example, under the assumption (b), the within-lot contamination was defined as 99% of the samples following a lognormal distribution with a mean of −4.85 $\log_{10}$ CFU/g and s.d. of 0.8 $\log_{10}$ CFU/g, and 1% of the samples following a lognormal distribution with a mean of 2.5 $\log_{10}$ CFU/g and s.d. of 0.2 $\log_{10}$ CFU/g. We developed the FDA-LmQRA App to explore the impact of exceptional contamination that is known to occur; however, the exact distribution is usually unknown. Enumeration of many samples within a lot of naturally contaminated food is needed to derive a distribution, but remains rare (Chen et al., 2016; Jongenburger et al., 2011). Available data show that *L. monocytogenes* contamination in the RTE food supply at higher levels (e.g., above 10 or 100 CFU/g) may occur (EFSA, 2018; Gombas et al., 2003; Luchansky et al., 2017), even though the proportion of samples within a lot that have a higher-level contamination is largely unknown.

Model inputs also included the *n*, *c*, and *m* values for a 2-class plan; and the *n*, *c*, *m*, and *M* values for a 3-class sampling plan. We used the FDA-LmQRA App to evaluate a range of 2-class and 3-class mixed plans as we did in the FDA-iRISK approach, where *c* was 0 for 2-class plans, using assumption (a) for the within-lot distribution. Furthermore, using assumption (b) for the within-lot distribution, we evaluated 2-class plans (e.g., *n* = 5 or 10, *m* = 0/25 g or 0/5 g, *c* = 0 or 1), 2-class enumeration plan (e.g., *n* = 5, *c* = 0, *m* = 100 CFU/g) and 3-class mixed plan (e.g., *n* = 5, *m* = 0/25 g, *c* = 1, and *M* = 20 CFU/g; and *n* = 10, *m* = 0/5 g, *c* = 1, and *M* = 20 CFU/g) under the assumption that the within-lot distribution was a lognormal with s.d. of 0.8 $\log_{10}$ CFU/g plus a proportion of 1% or 5% of exceptional contamination at 100 CFU/g; we also evaluated 0.1%, 1%, 5%, 20% or 40% exceptional contamination at levels defined by Normal(2.5, 0.2) $\log_{10}$ CFU/g (i.e., assuming exceptional contamination with an additional peak in the within-lot distribution, for example, with 0.1% or 40% of the units in the lot expected to be contaminated at a level >20 CFU/g).

Data from sampling across many lots of RTE foods at retail (Luchansky et al., 2017; Gombas et al., 2003) show that the proportion of samples with *L. monocytogenes* level >20 CFU/g (i.e., exceptional contamination) was 0.055% for diary (total 5410 samples and 3 samples had a level >20 CFU/g, i.e., 3/5410), 0.044% (3/6749) for produce, 0.19% (4/2095) for seafood, and 0.11% (6/5232) for other commodities (Table S4). Pouillot et al. (2016) analyzed enumeration data reported by Chen et al. (2016) from testing multiple lots of ice cream products implicated in an outbreak and showed that 10% samples of a product had a level >17 CFU/g and 1% samples of two other products had a level >46 CFU/g. Pereira et al. (2023) reported enumeration data for enoki mushrooms from multiple product lots implicated in a 2016–2020 outbreak and showed that, among 30 enoki mushroom samples with enumeration results, 11 samples had *L. monocytogenes* at >100 CFU/g. Considering the enumeration data from these studies, and due to the lack of specific within-lot data for the proportion of exceptional contamination, we chose 0.1–40% to represent the possible range of proportions of exceptional contamination to evaluate the performance of sampling plans under normal operations and outbreak situations.

We used the FDA-LmQRA App to predict the potential advantage a 3-class mixed plan could have in a reduction in the number of cases per billion servings. In model simulations, enumeration (e.g., in a 3-class mixed plan) was performed using the MPN method and only on samples that tested positive in the presence/absence test. After testing, lots found to exceed the microbiological limit(s) in the model simulation were replaced by noncontaminated lots. The FDA-LmQRA App has the option to simulate other options, such as enumeration using direct plate count and replacing violative lots by lots not exceeding the microbiological limit(s).

Results and discussion

We generated risk estimates with or without testing with a sampling plan for a range of 2-class and 3-class mixed plans, quantified how different contamination levels for the between-lot distribution impact the risk estimate, and elucidated the impact of sampling in relation to contamination characteristics among product lots, as well as how the lower and upper microbiological limits of a 3-class mixed plan and exceptional contamination influence sampling performance.

Impact of sampling on risk estimate using a 2-class or 3-class mixed plan. Given the model inputs for the baseline scenario, the risk assessment model developed in FDA-iRISK predicted 0.698 cases per one billion servings with no testing for the 65+ subpopulation (Table 1). Testing and removal of positive lots from consumption resulted in a lower number of predicted cases, and the more stringent the sampling plan (e.g., larger *n* and/or smaller *m*), the lower the number of predicted cases (Table 1).

Given that all lots were subject to sampling and testing, implementing a 2-class plan of *n* = 10 or 5, *m* = 0 CFU/25 g, and *c* = 0 was predicted to reduce the risk to 0.0198 and 0.0464 cases per billion servings, respectively. If *m* = 0 CFU/5 g, a 2-class plan with *n* = 10 and *c* = 0 would reduce the risk to 0.0698 cases, while a 2-class plan with *m* = 100 CFU/g, *n* = 5, and *c* = 0 would result in predicted 0.688 cases (Table 1A). Of note, the 2-class plan of *n* = 10, *m* = 0 CFU/5 g, and *c* = 0 is equivalent to the composite sample analysis in the FDA BAM method for *L. monocytogenes* (Hitchins et al., 2022), where each of two composites (25 g each) is prepared from a composite mixture consisting of 5 independent subsamples (i.e., samples) taken from a lot, according to our earlier analysis (Pouillot et al., 2024a). For a 2-class plan of *n* = 2, *m* = 0 CFU/25 g, and *c* = 0, which is sometimes used for lot acceptance testing (Farber et al., 2021), i.e., two samples taken from the lot and 25 g of each sample is tested, the predicted risk reduction would be 0.126 cases (Table 1A) with a relative risk reduction lower than the FDA BAM equivalent sampling plan, 5.5 vs. 10.0 (Table 1C).

The predicted risk was 0.115, 0.204, and 0.318 cases, respectively, after implementing a 3-class mixed plan of *n* = 5, *m* = 0 CFU/25 g, *M* = 100 CFU/g, and *c* = 1, 2 or 3. Changing *M* to *M* = 500 CFU/g did not change the predicted risk for the 3-class mixed plan (Table 1B). Similarly, no change or little change in the predicted risk was observed when changing *M* to *M* = 20 CFU/g or *M* = 10 CFU/g (Table 1B). These results are expected because for the 3-class mixed plans evaluated, the value *c* has substantial influence (Fig. S2A and C) while *M* has little influence (Fig. S2B) on the OC curves, which confirmed observations reported by Farber et al. (2021) and Pouillot et al. (2024a). The combined changes in the values of *c* and *n* have a substantial effect on the OC curves (Fig. S2C). For the 3-class mixed plans, after testing the predicted relative risk reduction varied from 2.2 (*c* = 3) to 6.1 (*c* = 1), depending on the acceptable number of samples exceeding the microbiological limits (Table 1C); the relative risk reduction was 1.01, 10.0, 15.0, and 35.3 for the 2-class plans evaluated. The degree of relative risk reduction has a trend similar to that

Table 1
Predicted cases per billion servings of no-growth RTE foods for the 65+ subpopulation with no test or test using FDA-iRISK and OC curves[a]

(A) Test using a 2-class plan

Test scenario and sampling plan	Predicted cases
No Test	0.698
$n = 5$, $m = 100$ CFU/g, $c = 0$	0.688
$n = 10$, $m = 0$ CFU/5 g, $c = 0$	0.0698
$n = 2$, $m = 0$ CFU/25 g, $c = 0$	0.126
$n = 5$, $m = 0$ CFU/25 g, $c = 0$	0.0464
$n = 10$, $m = 0$ CFU/25 g, $c = 0$	0.0198

(B) Test using a 3-class mixed plan

Test scenario and sampling plan	Predicted cases with test		
	$c = 3$	$c = 2$	$c = 1$
$M = 500$ CFU/g, $m = 0$ CFU/25 g, $n = 5$	0.318	0.204	0.115
$M = 100$ CFU/g, $m = 0$ CFU/25 g, $n = 5$	0.318	0.204	0.115
$M = 20$ CFU/g, $m = 0$ CFU/25 g, $n = 5$	0.317	0.204	0.115
$M = 10$ CFU/g, $m = 0$ CFU/25 g, $n = 5$	0.315	0.203	0.115

(C) Relative risk reduction after testing with a 2-class or 3-class mixed plan

Test scenario and sampling plan	Relative Risk Reduction [b]
2-class: $n = 5$, $m = 100$ CFU/g, $c = 0$	1.01
2-class: $n = 10$, $m = 0$ CFU/5 g, $c = 0$	10.0
2-class: $n = 2$, $m = 0$ CFU/25 g, $c = 0$	5.5
2-class: $n = 5$, $m = 0$ CFU/25 g, $c = 0$	15.0
2-class: $n = 10$, $m = 0$ CFU/25 g, $c = 0$	35.3
3-class: $M = 20$ CFU/g, $m = 0$ CFU/25 g, $n = 5$, $c = 3$	2.2
3-class: $M = 20$ CFU/g, $m = 0$ CFU/25 g, $n = 5$, $c = 2$	3.4
3-class: $M = 20$ CFU/g, $m = 0$ CFU/25 g, $n = 5$, $c = 1$	6.1

[a]Between-lot concentration distribution Normal (−2.5, 1.0) $\log_{10}$ CFU/g; prevalence = 100% of lots (lot size = 1,135 kg). Sampling and testing all lots, where the probability of rejection was estimated by the OC curve, resulting in a posttest adjustment in the between-lot concentration distribution in the RTE foods. Serving size 113.4 g (0.25 lb). Not considering growth between retail and consumption.
[b]Calculated as [(Cases_No Test)]/[Cases_Test].

for the OC curves (Fig. S2, and –C), and the sampling plan performance is measured as the arithmetic mean concentration (Table S3).

Many studies have been published on the application of the ICMSF methodology to inform sampling strategies by assessing whether a food lot is acceptable according to predetermined microbiological criteria (ICMSF, 2002, 2011, 2020), and to relate microbiological criteria to food safety objectives and food safety management (e.g., van Schothorst et al., 2009; Scott et al., 2015; Zwietering et al., 2015; Zwietering & den Besten, 2016; Farber et al., 2021). In a typical application, these studies focused on the acceptability of individual lots of food, where a key assumption used to assess sampling plan performance was a lognormal distribution of microorganisms in the lot (Legan et al., 2001; Dahms, 2004; ICMSF, 2020). Scott et al. (2015) suggested that within-lot s.d. is usually uncertain for a food lot and may vary among lots. In their study, Scott et al. (2015) used a range of within-lot s.d. values (0.25, 0.50, 0.80, and 1.2 $\log_{10}$ CFU/g) to evaluate the performance of potential sampling plans designed to test for *Salmonella* spp. in milk powder. Similarly, Farber et al. (2021) used a range of within-lot s.d. values (0.25, 0.40, 0.80, and 1.2 $\log_{10}$ CFU/g) to evaluate the performance of sampling plans codified in regulations, recommended in guidance, or as potential options designed to test for *L. monocytogenes* in RTE foods. As the influence of the within lot s.d. has been extensively evaluated (e.g., Scott et al., 2015; Farber et al., 2021) and expert judgment based on limited available data is usually needed to determine the relevance of a given distribution and s.d. for a given type of product, in this study, we chose s.d. of 0.8 $\log_{10}$ CFU/g as an assumption for within-lot contamination to evaluate sampling performance measured as risk estimate. Furthermore, we evaluated sampling performance given different between-lot distributions, as well as considering the assumption of heterogeneous within-lot contamination that is a lognormal distribution plus exceptional contamination to predict risk to the consumer.

Impact of sampling given different between-lot distributions. We evaluated the relative risk reduction in alternative scenarios with different values for the mean of the between-lot distribution to represent different potential contamination levels in the food supply. Keeping the same s.d. and using a mean of −0.5, −1.5, and −3.5 $\log_{10}$ resulted in a relative risk reduction of 361.6, 33.2, and 2.1, respectively, compared to 6.1 when using Normal(−2.5, 1.0) $\log_{10}$ CFU/g as the between-lot distribution (Table 2). Although the predicted cases before testing differed by an order of magnitude proportional to the difference in the mean value, e.g., 24.7 and 2.47 cases for the scenarios with the mean of −0.5 and −1.5 $\log_{10}$, respectively, the cases after testing were 0.0683 and 0.0743, respectively. The contaminated product lots with a lower mean for the between-lot distribution were less likely to be detected by using the same sampling plan. As the mean value was lowered further, to −2.5 and −3.5 $\log_{10}$, contaminated lots were even less likely to be detected by the same sampling plan, resulting in a lower relative risk reduction (Table 2).

We used two different inputs for dose response to illustrate that this model input does not affect the predicted relative risk reduction. Using Normal(−2.5, 1.0) $\log_{10}$ CFU/g as the between-lot distribution, the predicted risk for the whole population was 0.247 and 0.0407 cases per billion servings before and after testing (Table 2), compared to 0.698 and 0.115 cases for the 65+ subpopulation (Table 1B). The predicted cases differ between the whole population and the 65+ subpopulation because of the different model inputs for consumption and dose-response; however, as expected, the relative risk reduction was the same (i.e., 6.1) for the whole population (Table 2) and for the 65+ subpopulation (Table 1C) after testing with the sampling plan, e.g., a 3-class mixed plan of $n = 5$, $c = 1$, $m = 0$ CFU/25 g, and $M = 20$ CFU/g. Similarly, the predicted relative risk reduction was the same for the whole population and the 65+ subpopulation scenarios after testing using other 3-class mixed plans (Fig. 1A) or 2-class plans (Fig. 1B), assuming that the within-lot lognormal distribution had a s.d. of 0.8 log10 CFU/g. As expected, the s.d. of the within-lot distribution had a substantial impact on sampling plan performance (Fig. 1C): if the s.d. was 0.4 the predicted relative risk reduction was greater, e.g., 12.7 compared to 6.1 from sampling with the 3-class mixed plan $n = 5$, $c = 1$, $m = 0$ CFU/25 g, $M = 20$ CFU/g; while if the s.d. was 1.2, the predicted relative risk reduction was lower, e.g., 2.6 compared to 6.1 for the same sampling plan. These risk estimates are expected because of the changes in the arithmetic mean of a lognormal distribution that is detected with a 95% probability from changing s.d. values (e.g., a lower s.d. resulting in a lower arithmetic mean), as reported in previous studies (Farber et al., 2021).

Both FDA-iRISK (a general tool for risk assessment of any microbial and chemical hazards) and the FDA-LmQRA App (a specific risk assess-

Table 2
Predicted cases per billion servings of no-growth RTE foods for the whole population using FDA-iRISK and OC Curve approach[a]

Between-lot distribution of *L. monocytogenes* ($\log_{10}$ CFU/g)	Cases_No Test	Cases_Test	Relative Risk Reduction
Normal (−0.5, 1.0)	24.7	0.0683	361.6
Normal (−1.5, 1.0)	2.47	0.0743	33.2
Normal (−2.5, 1.0)	0.247	0.0407	6.1
Normal (−3.5, 1.0)	0.0244	0.0117	2.1

[a] Sampling and testing all lots with 3-class mixed plan $n = 5$, $c = 1$, $m = 0$/25 g, $M = 100$ CFU/g. Between-lot concentration distribution Normal(−2.5, 1.0) $\log_{10}$ CFU/g, with alternative means indicated; prevalence = 100% of lots (lot size = 1,135 kg). Dose−response model: Pouillot et al, 2015, marginal across all subpopulations; serving size 50 g (0.11 lb). Not considering growth between retail and consumption.

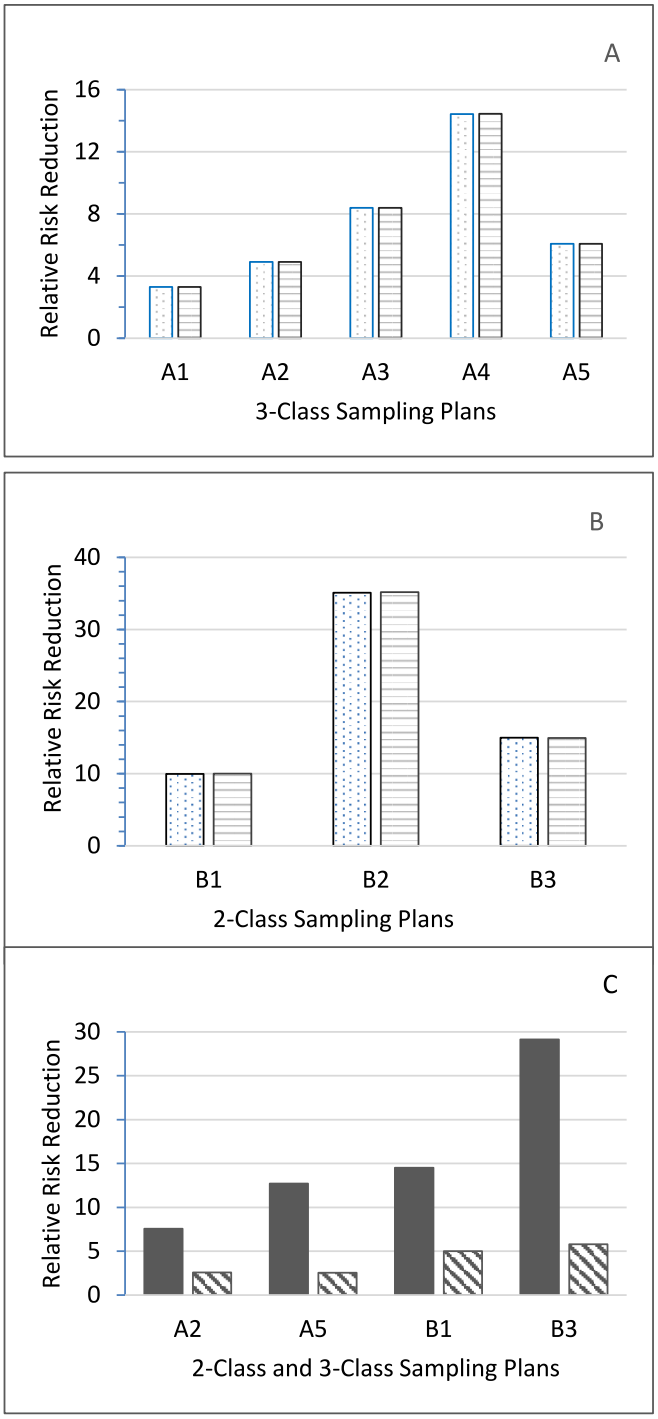

Figure 1. Predicted relative risk reduction for no-growth RTE foods after testing with various sampling plans for the 65+ subpopulation (dotted bars) and the whole population (horizontal stripes bars) using FDA-iRISK given the within-lot lognormal distribution with a s.d. of 0.8 (panel A and panel B), and with a s.d. of 0.4 or 1.2 $\log_{10}$ CFU/g (panel C). Panel A: 3-class mixed plans with $M = 20$ CFU/g and $n = 10$, $c = 2$, $m = 0$ CFU/5 g (A1); $n = 10$, $c = 1$, $m = 0$ CFU/5 g (A2); $n = 10$, $c = 2$, $m = 0$ CFU/25 g (A3); $n = 10$, $c = 1$, $m = 0$ CFU/25 g (A4); or $n = 5$, $c = 1$, $m = 0$ CFU/25 g (A5). Panel B: 2-class plans $n = 10$, $c = 0$, $m = 0$ CFU/5 g (B1); $n = 10$, $c = 0$, $m = 0$ CFU/25 g (B2); or $n = 5$, $c = 0$, $m = 0$ CFU/25 g (B3). Panel C: 3-class mixed plans A2 and A5, and 2-class plans B1 and B3 with a s.d. of 0.4 (solid bars) and a s.d. of 1.2 (diagonal bars) for the 65+ subpopulation. Relative risk reduction was (Cases_No Test)/(Cases_Test); cases per billion servings of 50 g using corresponding dose-response model from Pouillot et al. (2015). Not considering growth between retail and consumption. Sampling and testing all lots. L. monocytogenes concentration distribution Normal (−2.5, 1.0) $\log_{10}$; prevalence = 100% at the lot level, lot size = 1,135 kg.

ment tool for *L. monocytogenes*) have the capacity to define prevalence at the lot level for contamination in the food supply and to define an associated between-lot distribution for evaluating the impact of sampling on the risk of illness, while the FDA-LmQRA App provides an additional option to define prevalence at the unit (sample unit) level to predict risk. Furthermore, the FDA-LmQRA App has new capacities that enable specific definitions of both the between-lot distribution and the within-lot distribution for the food supply, consideration of exceptional contamination, consideration of bacterial growth, and the choice of different enumeration methods associated with implementing a sampling plan. In addition to risk estimates, the FDA-LmQRA App generates intermediate results such as the characteristics of contamination among lots, and the percentage of lots rejected.

Impact of sampling in relation to contamination characteristics among lots. To elucidate how the characteristics of contamination among lots influences sampling plan performance, we used the FDA-LmQRA App to evaluate two hypothetical while plausible scenarios (Table 3), where *L. monocytogenes* contamination for between-lot concentration distribution was Normal(−4.85, 1.0) $\log_{10}$ CFU/g and prevalence was 100% at the lot level for Scenario A (representing low, sporadic contamination in the food supply); and Pert(−1.4, −1.4, 3.1, shape = 1) (equivalent to Triangular(−1.4, −1.4, 3.1)) $\log_{10}$ CFU/g and prevalence was 1.1% at the unit level for Scenario B (representing moderate contamination in the food supply). The between-lot distribution for scenario A was within the range of *L. monocytogenes* levels in RTE foods reported in the FDA/FSIS 2003 risk assessment (FDA/FSIS, 2003), and for scenario B was based on the FDA-ARS-FSIS Survey data for produce that was approximated using a triangular distribution. Given these inputs, the FDA-LmQRA App predicted the prevalence of contaminated servings of 0.581% and 0.894% for Scenario A and Scenario B, respectively (Table 3A), along with the predicted prevalence of servings with levels >100 CFU/serving, and the proportion of lots with prevalence at above 10%.

For Scenario A, implementing a 2-class plan of $n = 5$ or 10, $m = 0$ CFU/25 g, and $c = 0$, on all lots, when positive lots were replaced by uncontaminated lots, was predicted to reduce the risk (1.17×10^{-3} cases per billion servings with no testing) to 0.798×10^{-3} and 0.648×10^{-3} cases, respectively (Table 3B). If $m = 0$ CFU/5 g, a 2-class plan with $n = 10$ and $c = 0$ would reduce the risk to 0.908×10^{-3} cases. The corresponding predicted percentage of lots rejected was 0.722–2.63% and relative risk reduction was 1.29–1.81 (Table 3B). After implementing a 3-class mixed plan of $M = 20$ CFU/g, $m = 0$ CFU/25 g, $n = 5$, and $c = 1$, the FDA-LmQRA App predicted that 0.138% lots would be rejected and a relative risk reduction of 1.14. No change was observed in the results when changing M to 50 or 100 CFU/g. For a 3-class mixed plan of $M = 20$ CFU/g, $m = 0$ CFU/5 g, $n = 10$, and $c = 1$, 0.0603% of the lots would be rejected and the relative risk reduction was 1.08 (Table 3B). Of note, little risk reduction was predicted from implementing the sampling plans if positive lots were replaced by lots that tested negative (that could be contaminated, i.e. false negative) when compared to situations where positive lots are replaced by noncontaminated lots.

For Scenario B, the predicted number of cases was 5.13 per billion servings with no testing. This was due to the between-lot distribution that gave 0.265% prevalence of servings with level >100 CFU/serving, compared to 0.000214% for Scenario A. For Scenario B, the predicted percentage of rejected lots for the 2-class plans (resp. 3-class mixed plans) ranged from 4.81-9.39% (resp. 0.618–2.46%) and the relative risk reduction ranged from 1.06 to 1.12 (resp. 1.04–1.10). Given the same sampling plan, the smaller degree of predicted relative risk reduction for Scenario B than Scenario A reflects the influence of the proportion of lots with high prevalence, e.g., above 10% (Table 3A). This contamination characteristic has a substantial influence on the proportion of the higher-contaminated lots in the food supply being detected in Scenario A and removed from the food supply,

Table 3
Predicted cases per billion servings of no-growth RTE foods for the whole population and relative risk reduction using the FDA-LmQRA App[a]

(A) Characteristics of contamination

Contamination Characteristics	Scenario A		Scenario B	
Prevalence contaminated servings	0.581%	1 positive every 172 servings	0.894%	1 positive every 112 servings
Prevalence servings with *L. monocytogenes* levels >100 CFU/serving	0.000214%	1 serving every 467,300 servings.	0.265%	1 serving every 377 servings.
Proportion of lots with prevalence >10%	0.852%	1 lot every 117 lots	0.000%	No lot with prevalence >10%

(B) Results (cases per billion servings) after sampling compared to predicted cases without testing was 1.17×10^{-3} (Scenario A) and 5.13 (Scenario B) cases per billion servings.

Test scenario and sampling plan	Scenario A			Scenario B		
	Cases_Test	Percentage of Lots Rejected[b]	Relative Risk Reduction	Cases_Test	Percentage of Lots Rejected	Relative Risk Reduction
2-class: $n = 5$, $m = 0$ CFU/25 g, $c = 0$	0.798×10^{-3}	1.45%	1.47	4.85	4.81%	1.06
2-class: $n = 10$, $m = 0$ CFU/25 g, $c = 0$	0.648×10^{-3}	2.63%	1.81	4.59	9.39%	1.12
2-class: $n = 10$, $m = 0$ CFU/5 g, $c = 0$	0.908×10^{-3}	0.722%	1.29	4.59	7.93%	1.12
3-class: $M = 20$ CFU/g, $m = 0$ CFU/25 g, $n = 5$, $c = 1$	1.03×10^{-3}	0.138%	1.14	4.90	1.22%	1.05
3-class: $M = 50$ CFU/g, $m = 0$ CFU/25 g, $n = 5$, $c = 1$	1.03×10^{-3}	0.138%	1.14	4.95	0.681%	1.04
3-class: $M = 100$ CFU/g, $m = 0$ CFU/25 g, $n = 5$, $c = 1$	1.03×10^{-3}	0.138%	1.14	4.95	0.681%	1.04
3-class: $M = 20$ CFU/g, $m = 0$ CFU/5 g, $n = 10$, $c = 1$	1.09×10^{-3}	0.0603%	1.08	4.68	2.46%	1.10

[a]*L. monocytogenes* contamination for between-lot concentration distribution of the arithmetic mean: (Scenario A) Normal(−4.85, 1.0) $\log_{10}$ CFU/g; prevalence = 100% of lots (lot size = 10,000 units); (Scenario B) Triangular(−1.4, −1.4, 3.1) $\log_{10}$ CFU/g; prevalence = 1.1% of units (unit level). For both scenarios, s.d. = 0.8 for within-lot distributions. Dose−response model (Pouillot et al., 2015): marginal across all subpopulations; serving size 50 g (0.11 lb). Not considering growth between retail and consumption. Sampling and testing all lots, and if positive replace the lot with a noncontaminated lot. Enumeration (when performed) is done using MPN (3 tubes of 10, 1, 0.1, and 0.01 g) only on samples tested positive in detection. Model simulation 10 K iterations.
[b]The percentage of lots detected to exceed the microbiological limit(s).

resulting in a proportionally larger relative risk reduction for Scenario A than that for Scenario B.

The influence of the lower and the upper microbiological limits of a 3-class mixed plan and exceptional contamination on sampling performance. We used the FDA-LmQRA App to examine how the lower and the upper microbiological limits of a 3-class mixed plan influence the percentage of rejected lots, i.e., lots detected to be exceeding the microbiological limit(s). For the 3-class mixed plan $n = 10$, $c = 1$, $m = 0$ CFU/25 g, and $M = 20$ CFU/g, our parallel study (Pouillot et al., 2024a) found that nearly all rejected lots were based on detecting >1 sample exceeding the lower limit ($m = 0$ CFU/25 g, related to detection of the pathogen in a sample), rather than detecting any sample exceeding the upper limit ($M = 20$ CFU/g, related to enumeration of the pathogen in a sample). Given a Normal(−4.85, 1.0) $\log_{10}$ CFU/g between-lot distribution and assuming a single lognormal distribution with s.d. of 0.8 $\log_{10}$ for the within-lot distribution (the inputs for Scenario A in Table 3), the FDA-LmQRA App predicted a relative risk reduction of 1.34 after testing with the 2-class plan $n = 10$, $c = 1$, $m = 0$ CFU/25 g, essentially the same as that after testing with the 3-class mixed plan $n = 10$, $c = 1$, $m = 0$ CFU/25 g, and $M = 20$ CFU/g (data not shown). This finding, i.e., little influence on the risk estimate from adding the upper microbiological limit in the 3-class mixed plan, is expected because of the finding from our parallel study (Pouillot et al., 2024a), where these two sampling plans have essentially overlapped OC curves when the underlying within-lot distribution is assumed to be a single lognormal distribution.

However, the within-lot contamination may be more complex than a single lognormal distribution. We found that the upper microbiological limit would have a substantial influence, if the within-lot distribution included exceptional contamination. When the between-lot distribution was Normal(−4.85, 1.0) $\log_{10}$ CFU/g and the within-lot distribution included a lognormal plus exceptional contamination at 100 CFU/g of 1% (Table 4A, Scenario A1) or 5% (Table 4A, Scenario A2), the FDA-LmQRA App predicted risk of 5.58 and 27.9 cases per billion servings without testing, respectively. For Scenarios A1 and A2, after testing with the 2-class plan $n = 5$, $c = 1$, $m = 0$ CFU/25 g, the predicted percentage of lots rejected was 0.29% and 2.61%, respectively, and the predicted relative risk reduction was 1.00 and 1.03, respectively. Including an upper limit in the sampling plan, i.e., using the 3-class mixed plan $n = 5$, $c = 1$, $m = 0$ CFU/25 g, $M = 20$ CFU/g, the predicted percentage of lots rejected was 5.03% and 22.7%, respectively, and the predicted relative risk reduction was 1.05 and 1.29, respectively, for Scenarios A1 and A2 (Table 4A), which is comparable to the relative risk reduction from using the 2-class plan $n = 5$, $m = 0$ CFU/25 g, and $c = 0$ (Table 4A). The driver for the performance of the 3-class mixed plan when the within-lot distribution included exceptional contamination is its preferential rejection of the more highly contaminated lots in the food supply, despite that lots contaminated at low level would be released. For example, for Scenario A1 (1% exceptional contamination), the percentage of rejected lots was 5.03% from the 3-class mixed plan $n = 5$, $c = 1$, $m = 0$ CFU/25 g, $M = 20$ CFU/g, compared to 6.28% from the 2-class plan $n = 5$, $c = 0$, $m = 0$ CFU/25 g. Notably, 4.74% of lots rejected (the difference between scenarios A1.4 and A1.3) from the 3-class mixed plan (94.2% of the lots rejected) were lots with a level $>M$ in the tested samples, compared to 4.90% of lots rejected (scenario A1.2) from the 2-class plan (78.0% of the lots rejected) that had a level $>M$. The 3-class mixed plan preferentially rejected a greater proportion of the more highly contaminated lots, which translated into an advantage in that it could achieve comparable risk reduction without having to reject nearly as large a proportion of the lots in the food supply as that by the 2-class plan. After testing, the predicted cases per billion servings was 5.30 from the 3-class mixed plan compared to 5.23 from the 2-class plan, corresponding to a relative risk reduction of 1.05 vs. 1.07 (Table 4A). If the exceptional contamination increased, the performance of the 3-class mixed plan with $c = 1$, relative to that of the 2-class plan with $c = 0$, would further improve. When the exceptional contamination was 5%, the predicted percentage of lots rejected was 22.7% from the 3-class mixed plan (vs. 23.7% from the 2-class plan), and furthermore, 88.5% of the lots rejected were lots with a level $>M$ in the tested samples, which translated into a relative risk reduction of 1.295 (vs. 1.312 from the 2-class plan).

To further evaluate the impact of exceptional contamination on sampling plan performance, we used a wide range of exceptional contamination proportions, from 0.1% to 40% of the units within the lot that have higher levels sampled from the distribution Normal(2.5, 0.2) $\log_{10}$CFU/g, building on the results of the percentage of lots rejected we obtained from our parallel study (Pouillot et al., 2024a). As the proportion of exceptional contamination increased from 1% to 40%, the

Table 4
Predicted cases per billion servings of no-growth RTE foods as influenced by exceptional contamination using the FDA-LmQRA App[a]

(A) Results with exceptional contamination of 1% (Scenario A1) or 5% (Scenario A2) of within-lot samples at 100 CFU/g[b]

Test scenario and sampling plan	Cases_No Test	Cases_Test	Percentage of lots rejected	Relative risk reduction
A1.1: 2-class $n = 5$, $m = 0$ CFU/25 g, $c = 0$	5.58	5.23	6.28%	1.07
A1.2: 2-class $n = 5$, $m = 20$ CFU/g, $c = 0$	5.58	5.31	4.90%	1.05
A1.3: 2-class $n = 5$, $m = 0$ CFU/25 g, $c = 1$	5.58	5.57	0.29%	1.00
A1.4: 3-class $n = 5$, $m = 0$ CFU/25 g, $M = 20$ CFU/g, $c = 1$	5.58	5.30	5.03%	1.05
A2.1: 2-class $n = 5$, $m = 0$ CFU/25 g, $c = 0$	27.9	21.3	23.7% [b]	1.31
A2.2: 2-class $n = 5$, $m = 0$ CFU/25 g, $c = 1$	27.9	27.1	2.61%	1.03
A2.3: 3-class $n = 5$, $m = 0$ CFU/25 g, $M = 20$ CFU/g, $c = 1$	27.9	21.5	22.7%	1.29

4B. Results with exceptional contamination: within-lot varying proportion of samples at levels defined by Normal (2.5, 0.2) $\log_{10}$ CFU/g[c]

Test scenario and sampling plan	Predicted cases Proportion of highly contaminated samples						Relative risk reduction Proportion of highly contaminated samples					
	0%	0.10%	1%	5%	20%	40%	0%	0.10%	1%	5%	20%	40%
No Test	0.00117	1.81	18.1	90.5	362	724	1.00	1.00	1.00	1.00	1.00	1.00
2-class $n = 5$, $m = 0/25$ g, $c = 0$[d]	0.000798	1.77	17.0	69.0	117	55.4	1.47	1.02	1.07	1.31	3.10	13.1
2-class $n = 10$, $m = 0/5$ g, $c = 0$[e]	0.000908	1.78	16.2	53.7	38.5	4.32	1.29	1.02	1.11	1.68	9.39	167
2-class $n = 10$, $m = 0/5$ g, $c = 1$	0.00109	1.81	18.0	82.4	135	33.4	1.08	1.00	1.01	1.10	2.67	21.7
3-class mixed $n = 5$, $m = 0/25$ g, $c = 1$, $M = 20$ CFU/g	0.00103	1.80	17.2	69.9	118	56.2	1.14	1.01	1.05	1.29	3.06	12.9
3-class mixed $n = 5$, $m = 0/25$ g, $c = 1$, $M = 100$ CFU/g	0.00103	1.80	17.2	70.4	122	61.2	1.14	1.01	1.05	1.29	2.96	11.8
3-class mixed $n = 10$, $m = 0/5$ g, $c = 1$, $M = 20$ CFU/g	0.00109	1.79	16.3	54.1	38.8	4.35	1.08	1.01	1.11	1.67	9.33	166
3-class mixed $n = 10$, $m = 0/5$ g, $c = 1$, $M = 100$ CFU/g	0.00109	1.79	16.4	54.8	41.4	5.13	1.08	1.01	1.10	1.65	8.75	141
2-class enumeration, $n = 5$, $m = 100$ CFU/g, $c = 0$	0.00117	1.80	17.2	70.5	123	61.5	1.00	1.01	1.05	1.28	2.95	11.8
2-class $n = 10$, $m = 0/25$ g, $c = 0$[g]	0.000648	1.74	15.9	52.7	37.8	4.24	1.81	1.04	1.14	1.72	9.57	171

[a]*L. monocytogenes* contamination for between-lot concentration distribution of the arithmetic mean for Scenario A: Normal(−4.85, 1.0) $\log_{10}$ CFU/g; prevalence = 100% of lots (lot size = 10,000 units). Dose response model (Pouillot et al., 2015): marginal across all subpopulations; serving size 50 g (0.11 lb). Not considering growth between retail and consumption. Sampling and testing all lots, and if positive replace the lot with a noncontaminated lot. Enumeration (when performed) is done using MPN (3 tubes of 10, 1, 0.1, and 0.01 g) only on samples tested positive in detection. Model simulation 10 K iterations.

[b]Predicted cases per billion servings with no test and with test shown. Between-lot distribution and model simulation as that in Scenario A. Within-lot contamination defined by two lognormal distributions: a higher-level distribution that defines the proportion of exceptional contamination at 100 CFU/g of 1% for alternative Scenario A1.1 to A1.3, and 5% for alternative Scenario A2.1 to A2.3; and a low-level lognormal distribution with an arithmetic mean sampled from Normal (−4.85, 1.0) log and s.d. of 0.8 $\log_{10}$ CFU/g that defines contamination in the remaining samples within the lot. On a practical note, some of the scenarios here were used to investigate sampling performance, but may not be considered realistic, e.g., 23.7% lots rejected in scenario A2.1. If such a scenario occurred, producers would have cost-benefit incentive to improve preventive controls to reduce the frequency of rejections and avoid destruction of large amounts of product.

[c]Predicted cases per billion servings shown. Between-lot distribution and model simulation as that in Scenario A. Within-lot contamination defined by two lognormal distributions: a higher-level distribution that defines the proportion of exceptional contamination at Normal(2.5, 0.2) $\log_{10}$ CFU/g of 0.1–40% (i.e., 0.1–40% of the units in the lot expected to be contaminated at a level >20 CFU/g), compared to 0% (no exceptional contamination); and a low-level lognormal distribution with an arithmetic mean sampled from Normal(−4.85, 1.0) log and s.d. of 0.8 $\log_{10}$ CFU/g that defines contamination in the remaining samples within the lot.

[d]EU/Canada foods supporting *L. monocytogenes* growth and Canada foods for vulnerable populations (EU, 2005; Health Canada, 2023).

[e]Equivalent of 2-composite sample analysis for foods in the FDA BAM chapter 10 (Hitchins et al., 2022; Pouillot et al., 2024a).

[f]EU/Canada foods not supporting *L. monocytogenes* growth (EU, 2005; Health Canada, 2023).

[g]EU foods for infants and special medical purposes (EU, 2005).

relative risk reduction for the 2-class plan $n = 10$, $c = 0$, $m = 0$ CFU/5 g (resp. 3-class mixed plan $n = 10$, $c = 1$, $m = 0$ CFU/5 g, $M = 20$ CFU/g or $M = 100$ CFU/g) improves from 1.11 to 167 (resp. 1.11–166 or 1.10–141) (Table 4B). Notably, given the model inputs and assumptions, the 2-class plan $n = 10$, $c = 0$, $m = 0$ CFU/5 g was predicted to perform better than the 2-class plan $n = 5$, $c = 0$, $m = 0$ CFU/25 g when the exceptional contamination was 1 –40%, e.g., with 5% exceptional contamination, the predicted risk was 53.7 vs. 69.0 cases after testing (relative risk reduction 1.68 vs. 1.31). Similar patterns of risk estimate and relative risk reduction were predicted for 3-class mixed plans, where the 3-class mixed plan $n = 10$, $c = 1$, $m = 0$ CFU/5 g, $M = 20$ CFU/g was predicted to perform better than the 3-class mixed plan $n = 5$, $c = 1$, $m = 0$ CFU/25 g, $M = 20$ CFU/g (Table 4B). If the exceptional contamination was 0.10%, the two 2-class plans with $c = 0$ were predicted to have comparable performance (Table 4B), as were the two 3-class mixed plans with $c = 1$. The 3-class mixed plan $n = 10$, $c = 1$, $m = 0$ CFU/5 g, $M = 20$ CFU/g or $M = 100$ CFU/g was predicted to perform consistently better than the 2-class plan $n = 5$, $c = 0$, $m = 0$ CFU/25 g, and to have performance comparable to (but slightly less than) that for the 2-class plan $n = 10$, $c = 0$, $m = 0$ CFU/5 g for the range of 1 –40% exceptional contamination. As described in Pouillot et al. (2024a), the 2-class plan $n = 10$, $m = 0/5$ g, $c = 0$ is equivalent to a 2-composite sample analysis option in the FDA BAM chapter 10 (Hitchins et al., 2022). We chose to evaluate this sampling plan in comparison to several sampling plans for RTE foods in EU regulation (EU, 2005) and Canadian policy (Health Canada, 2023), as well as potential 3-class mixed plans. The risk estimates from this study complement those in our parallel study (Pouillot et al., 2024a).

Impact of sampling for commodity groups based on contamination levels observed in the food supply. As expected, before testing, the predicted cases per billion servings varied for the four groups of commodities because of the differences in the initial prevalence and levels of *L. monocytogenes* in the RTE food supply. Among several sampling plans evaluated, the predicted relative risk reduction was the lowest after testing with the 2-class plan $n = 5$, $m = 0$ CFU/25 g, and $c = 0$, ranging from 1.01 to 1.03 for the four commodity groups (Table 5). We estimated risk using two different dose-response models, which predicted as an example for the dairy commodities 29.2 and 6.66 cases per billion servings when no sampling and testing of lots was applied because of the difference in their exponential r values, i.e., 1.25×10^{-11} (EFSA model evaluated from Pouillot et al., 2024b)

vs. 1.19×10^{-10} (Pouillot et al., 2015, a purposely conservative dose-response model that may overestimate the number of cases); however, the predicted relative risk reduction is the same regardless of the dose-response model used (Table 5A and Table 5B). Notably, for all four commodity groups, the predicted relative risk reduction was the same after testing with the 2-class plan of $n = 10$, $m = 0$ CFU/5 g, and $c = 0$ and the 3-class mixed plan of $n = 10$, $c = 1$, $m = 0$ CFU/5 g, and $M = 20$ CFU/g: 1.02 for dairy commodities, 1.04 for produce commodities, and 1.06 for seafood commodities and for other commodities (Table 5). In general, assuming every lot of the food supply was tested, the percentage of lots rejected was from <1% to 4.46% depending on the commodity group and the sampling plan (Table 5). Logically, when the proportion of lots subject to sampling and testing was 10%, 5%, and 1%, the percentage of lots rejected was 10%, 5%, and 1% of the results shown in Table 5, and furthermore, the FDA-LmQRA App predicted a proportionally higher risk (a higher number of cases per billion servings) after testing a fraction of the lots compared to when all lots were tested (data not shown).

In an alternative scenario to model inputs in Table 5A, we evaluated the same sampling plans assuming 10% contaminated lots and within-lot prevalence at the unit level 10 times the values used for Table 5. As expected, the predicted relative risk reduction was higher for each of the sampling plans (Table S5) than that in Table 5. For example, for dairy commodities after implementing the 2-class plan $n = 5$, $m = 0$ CFU/25 g, and $c = 0$, the predicted relative risk reduction was 1.08 (0.252% lots rejected) (Table S5) compared to 1.01 (0.258% lots rejected) (Table 5A). The sampling plans are more efficient to reduce risk under the alternative assumption preserving the overall prevalence of contaminated units (Table S5). This result is expected since, when a lot is found contaminated and rejected, more contaminated units are discarded; this results in a greater relative risk reduction, even though the % lots rejected for a given sampling plan is comparable to that in Table 5A. However, under this assumption, the comparative performances of the various sampling plans are similar (Table S5).

Considerations for sampling strategy. For RTE foods that do not support *L. monocytogenes* growth, results from this study show that, under most conditions, the predicted risk reduction from testing with the sampling plans evaluated is predicted to be small, even when all lots were subject to sampling and testing and when positive lots were replaced with uncontaminated lots. This overall finding reaffirms that finished product testing is most suitable as, among other things, a verification of preventive controls, rather than a control measure in and of itself. We conducted a comprehensive evaluation of a range of initial contamination levels for lots subject to testing with 2-class and 3-class mixed sampling plans, and found that testing product lots, whatever the sampling plan, does not work well as a control measure in reducing risk, except under certain obvious situations, such as if most samples are contaminated. Notwithstanding the limited direct impact of sampling *per se* on risk reduction, sampling and testing finished product lots over time may have an indirect impact that is often considered more important than the direct effect on lot rejection. An example of indirect impact is well illustrated in a risk assessment of *Enterobacter sakazakii* (i.e., *Cronobacter sakazakii*) in powdered infant formula (FAO/WHO, 2016): if a relatively high proportion of product lots was rejected, a producer would have an economic incentive to conduct root cause investigations, implement corrective actions, and improve preventive controls to reduce the frequency of lots rejected from sampling. Detections of *L. monocytogenes* in RTE foods would trigger root cause investigations, intensified environmental testing, and corrective actions. The knowledge gained from these actions may be used to enhance the overall *Listeria* control program in production. Implemented across industry, such indirect impact, if realized, could collectively shift the between-lot distribution of *L. monocytogenes* in RTE food categories to lower levels (e.g., a lower mean and smaller s.d.); this in turn could result in greater risk reduction than that from rejected lots when the pathogen is detected by sampling.

Although the focus of this study was on the impact of sampling plans on the risk associated with RTE foods that do not support *L. monocytogenes* growth, the FDA-LmQRA App includes a component

Table 5
Predicted cases per one billion servings of dairy, produce, seafood and other commodity groups and relative risk reduction (RRR) for the whole population[a]

(A) Based on dose response model by Pouillot et al. (2015)

Sampling Plan				Dairy Commodities			Produce Commodities			Seafood Commodities			Other Commodities		
n	*c*	*m*	*M*	Cases_ Test	% Lots Rejected	RRR	Cases_ Test	% Lots Rejected	RRR	Cases_ Test	% Lots Rejected	RRR	Cases_ Test	% Lots Rejected	RRR
10	1	0 CFU/5 g	20 CFU/g	28.7	0.189%	1.02	5.98	0.597%	1.04	16.1	1.89%	1.06	8.20	0.825%	1.06
10	1	0 CFU/5 g	100 CFU/g	28.7	0.141%	1.02	6.00	0.347%	1.04	16.1	1.17%	1.05	8.23	0.489%	1.06
5	0	0 CFU/25 g	NA*	28.9	0.258%	1.01	6.11	1. 35%	1.02	16.5	2.53%	1.03	8.45	1.85%	1.03
10	0	0 CFU/5 g	NA*	28.7	0.417%	1.02	5.98	2.09%	1.05	16.0	4.46%	1.06	8.19	2.86%	1.06

(B) Based on dose response model by Pouillot et al. (2024b)

Sampling Plan				Dairy Commodities			Produce Commodities			Seafood Commodities			Other Commodities		
n	*c*	*m*	*M*	Cases_ Test	% Lots Rejected	RRR	Cases_ Test	% Lots Rejected	RRR	Cases_ Test	% Lots Rejected	RRR	Cases_ Test	% Lots Rejected	RRR
10	1	0 CFU/5 g	20 CFU/g	6.56	0.189%	1.02	1.09	0.597%	1.05	2.76	1.89%	1.06	1.50	0.825%	1.06
10	1	0 CFU/5 g	100 CFU/g	6.56	0.141%	1.02	1.09	0.347%	1.04	2.76	1.17%	1.05	1.50	0.489%	1.06
5	0	0 CFU/25 g	NA*	6.61	0.258%	1.01	1.11	1.35%	1.02	2.83	2.53%	1.03	1.54	1.85%	1.03
10	0	0 CFU/5 g	NA*	6.56	0.417%	1.02	1.09	2.09%	1.05	2.75	4.46%	1.06	1.49	2.86%	1.06

[a]Not considering growth between retail and consumption. Using the dose response mode by Pouillot et al., 2015 (resp. by Pouillot et al., 2024b), without testing the predicted number of cases per billion servings was 29.2 (6.66) for Dairy Commodities, 6.25 (1.14) for Produce Commodities, 17.0 (2.92) for Seafood commodities, and 8.72 (1.59) for Other Commodities; with testing the results are shown in 5A (5B). Dose-response model marginal across all subpopulations; serving size 50 g (0.11 lb). *L. monocytogenes* contamination: between-lot concentration distributions as in Table S1; prevalence as in Table S1 and defined at the unit level; lot size = 10,000 units; s.d. = 0.8 $\log_{10}$ CFU/g for within-lot distributions. Sampling and testing all lots, and if positive replace the lot with a noncontaminated lot. Enumeration (when performed) is done using MPN (3 tubes of 10, 1, 0.1, and 0.01 g) and is only on samples tested positive in detection. Model simulation 10 K iterations.
*M is not applicable in a 2-class sampling plan.

for considering growth in the risk assessment. For example, the model predicted 1.18 cases per billion servings associated with a no-growth RTE food (exceptional contamination at 1% within-lot samples at 20 CFU/g) with no sampling and testing, and 1.06 and 1.10 cases per billion servings after testing with a 2-class and a 3-class mixed plan, respectively. However, the risk would be significantly higher if the RTE food supported the growth of *L. monocytogenes*. If, after sampling and testing with the 2-class plan, growth occurred between retail and consumption, the predicted risk would be 9.67 and 80.2 cases, respectively, if the growth was 1 and 2 $\log_{10}$ (Table S6A). A similar trend was predicted given exceptional contamination of 1% within-lot samples at 100 CFU/g (Table S6B), where the predicted risk after sampling and testing was orders-of-magnitude higher for RTE foods that support *L. monocytogenes* growth than for those that do not support growth. Even larger amounts of growth (>2 $\log_{10}$) can occur given long storage at refrigeration temperature or higher temperatures. For example, if postsampling growth reached a maximum population density of 5 or 8 $\log_{10}$ CFU/g given sufficient time at storage temperature below 5 °C or above 7 °C, respectively (FDA/FSIS, 2003), the predicted risk would be several additional orders-of-magnitude higher than the risk estimates for 2 $\log_{10}$ growth shown in Table S6. Our results regarding the impact of growth, evaluated with or without sampling, confirm well-known findings from published risk assessments (FDA/FSIS, 2003; EFSA, 2018) regarding the relative risk between RTE foods that do and those that do not support the growth of *L. monocytogenes*, in terms of the predicted risk being very sensitive to growth and the maximum population density and that preventing *L. monocytogenes* growth in RTE foods dramatically reduces the risk of listeriosis.

The FDA-LmQRA App provides a tool to specifically model both between-lot variability and within-lot variability in contamination in estimating risk before and after sampling and testing in a risk assessment. We found that a 3-class sampling plan may provide an advantage if the within-lot contamination is heterogeneous, and a proportion of exceptional contamination exists in addition to the variability represented by a single lognormal distribution, which is expected in the food supply. Under contamination conditions (including prevalence and between-lot contamination derived from RTE food survey data) relevant to the U.S. food supply, using a 3-class mixed sampling plan with $c = 1$ may provide an advantageous sampling strategy, in that, it can result in comparable relative risk reduction but a lower percentage of lots rejected than using a corresponding 2-class sampling plan with the same n and m but $c = 0$, because the 3-class mixed plan can detect the more highly contaminated lots in the food supply. This study elucidates the influence of the lower and upper microbiological limits of a 3-class sampling plan in the context of quantitative risk assessment. While the impact on risk was mostly inferred in previous studies that focused on the OC curve and within-lot pathogen contamination distribution (Farber et al., 2021; Pouillot et al., 2024a), this study advances our understanding and affirms the utility of a 3-class sampling plan under certain conditions based on risk estimates.

A default model input in the risk assessment was that all lots in the food supply were subject to testing. In reality, not all lots, or in some instances only a small fraction of the lots, might be tested. We used the FDA-LmQRA App to compare different sampling strategies where a greater fraction of the lots would be tested by using a 3-class mixed plan with $c = 1$ compared to a 2-class plan with $c = 0$. We found comparable risk reduction could be achieved by using a 3-class mixed plan when exceptional contamination was present (Fig. S3). Increasing the fraction of lots tested reduces predicted cases and increases relative risk reduction. Indeed, if exceptional contamination was 1% or 5%, using the 3-class mixed plan $n = 10$, $m = 0/5$ g, $c = 1$, and $M = 20$ CFU/g to test 2% or more of lots would achieve slightly better risk reduction than using the 2-class plan $n = 10$, $m = 0/5$ g, and $c = 0$ to test 1% of lots. Similarly, using the 3-class mixed plan to test 0.5% of lots would achieve better risk reduction than using the 2-class plan test 0.1% of lots.

Conclusions

Results from the risk assessments developed in both FDA-iRISK and the FDA-LmQRA App show that testing for *L. monocytogenes* in RTE foods that do not support growth of the pathogen according to 3-class mixed or 2-class sampling plans can result in quantifiable, but low, risk reduction if positive lots are replaced with noncontaminated ones. This overall finding was substantiated through parallel risk scenarios we created in both tools using the same model inputs, data, and assumptions. We used the FDA-iRISK tool to evaluate the potential public health impact associated with 2-class or 3-class mixed plans with different stringency, by integrating OC curve data into a risk assessment that includes between-lot contamination distribution, food consumption, and dose-response relationship. We also created the FDA-LmQRA App that enables a more specific representation of contamination of food units within a lot and contamination across lots and provides new capacity to explore the impact of various assumptions on the within-lot contamination pattern and the between-lot distribution of *L. monocytogenes* in RTE foods. Collecting more data on concentration distribution for *L. monocytogenes* in RTE foods will help refine risk assessment on the impact of sampling because data on many samples in a lot are necessary to have a robust definition of the within-lot distribution, and data on many more samples in each of many lots are necessary to estimate the distribution of arithmetic means across lots in the food supply. The risk assessments developed in this study show the impact of a range of 2-class or 3-class mixed sampling plans, considering both between-lot and within-lot distributions of *L. monocytogenes*, on the estimated number of listeriosis cases based on contamination data relevant to the U.S. food supply.

Data availability statement

Risk scenarios reported herein and associated data in FDA-iRISK are available upon request.

CRediT authorship contribution statement

Yuhuan Chen: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Régis Pouillot:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Jane M. Van Doren:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

Funding

This work was carried out under official FDA duties and contract (HHFS223201710033I with Goldbelt C6, LLC (RP)).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Dr. Sherri Dennis and Dr. Benjamin Warren, FDA Human Foods Program, for helpful discussions on how to frame this research and for careful reviews of the manuscript.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.jfp.2025.100478.

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