Farm-to-fork risk assessment of aflatoxin M1 in milk under climate change scenarios – A comparative study of France and Ireland

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Farm-to-fork risk assessment of aflatoxin M1 in milk under climate change scenarios – A comparative study of France and Ireland

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ABSTRACT

Changes in temperature and precipitation patterns due to climate change (CC) may affect the growth of fungi and the subsequent release of toxic metabolites (mycotoxins). Aflatoxin B_1, a human carcinogenic mycotoxin produced by *Aspergillus flavus (A. flavus)*, can be found in animal feed and further metabolised into aflatoxin M_1 (less carcinogenic) in bovine milk. This research developed a probabilistic model in the farm-to-fork continuum to assess the potential risk from aflatoxin M_1 in milk (Irish and French consumers) under current and future CC scenarios. The effects of temperature and relative humidity changes on aflatoxin B_1 were examined. The stepwise exposure assessment model considered *A. flavus* growth during pre-harvest, aflatoxin B_1 production, carry-over rate from feed to milk (in aflatoxin M_1 form), and human consumption. Results suggest that the cancer risk from aflatoxin M_1 is relatively low under climate change scenarios as the estimated margin of exposure was greater than 10,000 (5th percentiles: 48,060 and 79,394 for males and females, respectively). Aflatoxin M_1 level in milk (95th percentiles) did not exceed the European Union's maximum permissible limits (50 ng l–1) under all scenarios. Temperatures during the plant growth period (correlation coefficient +0.78), whole milk consumption (+0.29), tillage practice (+0.25), beta coefficient (+0.18), and initial inoculation (−0.17) were found to be the most sensitive parameters to the model output. These findings help to inform farmers and policymakers to adopt mitigation strategies against CC and be climate ready. Future work may include further model development for exposure assessment of multiple mycotoxins in milk, potentially from animal feed materials produced in various geographical regions.

1. Introduction

Mycotoxins are a group of chemicals produced by toxigenic strains of fungi found in food and feedstuffs. Amongst them, aflatoxin B_1, formed by *A. flavus* and *A. parasiticus*, is a mycotoxin of serious concern as it is known to cause cancer in humans and is classified as a Group 1 carcinogen (International Agency for Research on Cancer, 2002). It is found in crops such as maize, cotton, peanuts, wheat and their by-products. Aflatoxin B_1, consumed as animal feed, is known to carry over into dairy products, such as milk, as aflatoxin M_1 (Fink-Gremmels, 2008). Dairy and dairy products are one of the most widely consumed food products worldwide, and the European Union produces around 160 million tonnes of milk per year (EUROSTAT, 2020). Its consumer base is projected to grow in the coming years (EC, 2020). Monitoring aflatoxin contamination in feed and dairy products is imperative due to its carcinogenicity.

Aflatoxins can be found in a number of animal feedstuffs, including maize. Maize is one of the most important agricultural crops in Europe since it is used for feed for animal consumption and food products for human consumption. The utilisation of maize as feed for ruminants is common in many parts of the world, including Ireland (Upton et al., 2013) and France (Richard et al., 2007). Aflatoxins may be produced in maize pre-harvest by *A. flavus*. The producing fungi *A. flavus* sclerotia and mycelium can manifest in soil and contaminate maize during the flowering period. *A. flavus* is known to survive and colonise soil from plant residue as mycelia or sclerotia, which may become the primary source of new *A. flavus* conidia. These conidia may be dispersed onto the maize plant by wind or vectored by insects, where they may colonise and produce aflatoxins if subjected to conducive growing conditions (i.e. heat stress and moisture deficit). *A. flavus* may persist and carry over

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into storage, where they may produce more mycotoxins if proper storage conditions after drying and fermentation for silage (such as anaerobic, low moisture content and a pH of around 3.5–3.8) are not met.

In the coming years, the world is expected to experience the effects of climate change, including an increase in global surface temperature, changes in precipitation patterns and changes in evapotranspiration rates which could impact the moisture in the soil (IPCC, 2021). These changes could increase maize crop susceptibility to *A. flavus* infection (Vaughan et al., 2014). Rising temperatures and changes in precipitation patterns are expected to increase the risk of aflatoxin B_1 contamination in maize in the south and central Europe (Battilani et al., 2016). French maize is also expected to be susceptible to these fungal attacks under different climate scenarios. The change in climate in Ireland is likely to be more suited to growing maize and hence resulting in higher production levels of the crop.

1.1. Agricultural systems

Global surveys conducted for mycotoxin contamination in crops include the BIOMIN survey (2020). The BIOMIN survey (2020) for mycotoxin contamination in maize showed that aflatoxin in Central Europe, comprising of countries such as Germany, France and Belgium, showed a prevalence of approximately 26% in maize with a maximum level of 15,000 ng kg^{-1}, with as much as 83% above the threshold limit of 2000 ng kg^{-1}. Compared to Southern Europe, where the prevalence of aflatoxin in maize was 24% with a maximum level of 30,000 ng kg^{-1} with 50% over the threshold of 2000 ng kg^{-1}, while Eastern Europe had the lowest prevalence of 2%, with a maximum level of 3000 ng kg^{-1} and 100% above the threshold level of 2000 ng kg^{-1}. Though the survey did not include results from Northern Europe, it is suggested that the climate of Northern Europe is currently not as conducive to aflatoxin B_1 production in maize grown in those countries, while deoxynivalenol and zearalenone are more likely to occur in cereal grains. However, maize being imported from other countries could be contaminated by aflatoxin B_1. The BIOMIN survey (2020) also suggested that aflatoxin contamination in maize kernels was very low for France, with only 5% of samples testing positive for aflatoxin B_1, with maximum contamination of 1000 ng kg^{-1}, below the feedstuff maximum permissible limit of 2000 ng kg^{-1}. In 2015, following exceptionally hot and dry climate conditions in France, maize samples from the field and maize silage tested positive for aflatoxin B_1, with maximum contamination levels reaching 66,000 ng kg^{-1} from samples tested in the field and 7200 ng kg^{-1}in maize silage (Bailly et al., 2018). The frequency of drought-like events is likely to increase in the future due to climate change, thus potentially emulating the conditions of 2015, as studied by Bailly et al. (2018). In comparison to France, the only positive occurrence of aflatoxin B_1 in maize samples in Ireland was found in 2012, with a maximum concentration value of 1000 ng kg^{-1} (FSAI, 2015).

The degree of maize cropping in both these areas differs significantly. France produced over 33,00 tonnes (EUROSTAT, 2020), while Ireland only produced 680 tonnes of forage maize (EUROSTAT, 2020; TEAGASC, 2017). However, climate change conditions are expected to increase maize crop area in Ireland (Holden & Brereton, 2003; Shrestha et al., 2015) and currently, growing maize crops is being promoted as it is a cheaper source of feed per unit of energy when compared to grass fodder (TEAGASC, 2017).

Dairy cattle in Ireland largely thrive on pasture (76 ± 8% FMB), followed by grass silage (18.6 ± 7.3% FMB), with alternative forage and concentrate making up 1.2 and 4.3% of the diet, respectively. While on a dry matter basis, pastures made up 60.2% of the diet (O'Brien et al., 2018). When fresh pasture is not available, dairy cattle in Ireland largely rely on grass silage, with up to 32% of maize silage on a dry matter basis. In comparison, in France, a mixed feeding system is employed, and feed consisting of both grazed grass and maize silage is used (Delaby et al., 2009; Richard et al., 2007). The French region of Normandy uses maize silage as feed during winter to obtain the best quality milk for cheese production (Hurtaud et al., 2009).

Ireland and France are both parts of the European Union (EU) and are expected to follow the regulations for maximum permissible limits set by the EU in feed and in dairy. Maximum allowed Aflatoxin B_1 levels in feed materials are 0.02 mg kg^{-1} (moisture content 12%) or 20,000 ng kg^{-1}, with maximum allowed content in compound feed for dairy cattle at 0.005 mg kg^{-1} (moisture content 12%) or 5000 ng kg^{-1} (DIRECTIVE 2002/32/EC) and maximum levels of Aflatoxin M_1 (EC 1881/2006) allowed in milk are 0.05 μg kg^{-1} or 50 ng kg^{-1}. Aflatoxin M_1 levels in milk did not surpass the maximum permissible levels set by the EU in France in a survey carried out by Boudra et al. (2007). However, a small percentage was found to be greater than the maximum permissible levels set by the EU for aflatoxin M_1 content in milk in the simulated exposure assessment conducted in Ireland (Coffey et al., 2009).

1.2. Quantitative assessment of aflatoxin in milk

Chhaya et al. (2021) summarise predictive models and human health risk assessment models used to assess mycotoxins in milk. Rory Coffey et al. (2009) estimated human exposure to different mycotoxins from bovine milk carried over from feed in Ireland. Mycotoxins present in the composite feed for the calculation of exposure in Coffey et al. (2009) were derived from data found in the scientific literature. The exposure assessment was a probabilistic model using Monte Carlo simulation to obtain a probability distribution of exposure to mycotoxins for the Irish population. Signorini et al. (2012) carried out a stochastic risk assessment for Argentina of different mycotoxins found in milk carried over or metabolised from the dairy feed. They included aflatoxin B_1, deoxynivalenol and fumonisin in their study. Van Der Fels-Klerx et al. (2019) carried out quantification of aflatoxin B_1 to aflatoxin M_1 in cattle milk for the Netherlands from imported maize grown in Ukraine. They included possible climate change scenarios in their model. Their model, however, did not conduct a human health risk assessment, nor did it include the potential effects of climate on storage. A limited number of other predictive models have quantified the risk of aflatoxin contamination in relation to climate change and weather variables (Battilani et al., 2013; Chauhan et al., 2008; Van Der Fels-Klerx et al., 2016). Weather variables such as temperature, relative humidity and rainfall have previously been used (Battilani et al., 2013; Chauhan et al., 2008). However, these predictive models did not extend to the consumer stage, and an exposure assessment was not conducted in any of these studies.

The objective of this study was thus to develop a human exposure assessment model for aflatoxin in bovine milk for two different agriculture systems (Ireland and France) in Europe and to assess the potential risk of aflatoxin M_1 exposure through liquid milk in the two regions under climate change scenarios.

2. Materials and methods

2.1. Quantitative assessment framework

A quantitative exposure assessment model was developed based on the feed-to-fork processes for dairy milk production (Battilani et al., 2013; Coffey et al., 2009; Van Der Fels-Klerx et al., 2019). Fig. 1 shows the framework developed for the quantitative risk assessment model. It consists of 3 stages which are further divided into different steps. These stages include: i) the preharvest stage, where maize is infected with *A. flavus,* and aflatoxin B_1 is produced; ii) the livestock stage, where aflatoxin B_1 in maize is consumed as part of feed (composition dependent on the country) by the dairy cows and metabolised into aflatoxin M_1 and transferred into milk, and iii) the consumer stage, where the amount of aflatoxin M_1 consumed through milk is calculated based on consumption patterns in both countries, and thus the risk is calculated using a hazard index. A sensitivity analysis is then conducted to account for uncertainty and variability in the model using Spearman's rank order of correlation coefficients.

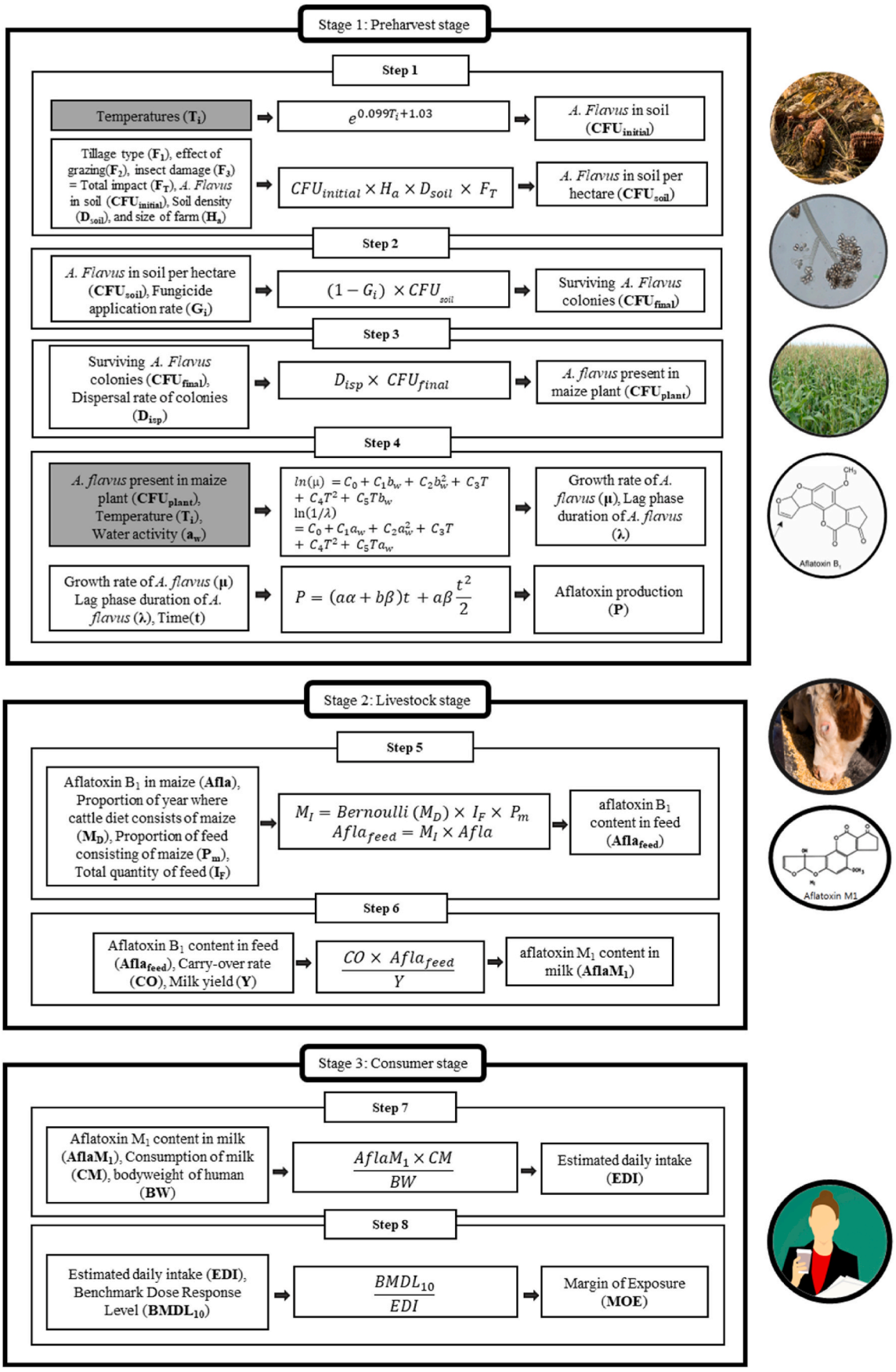

Fig. 1. Feed-to-fork risk assessment model framework. Greyed-out boxes represent climate change influences.

Based on the risk assessment framework, a search was carried out using the Web of Knowledge and Scopus to find data appropriate for each stage. Step 1 involves the calculation of *A. flavus* colonies found in soil as a result of previous crop debris. Step 2 involves the application of fungicide and the reduction of viable *A. flavus* colonies. Step 3 involves the dispersal of these spores onto nearby maize plants. Step 4 calculates the lag time, growth and diameter of *A. flavus* colonies based on temperature and water activity. Step 5 calculates the production of aflatoxin B_1 based on *A. flavus* diameter. Steps 1 to 5 are included in the pre-harvest stage. Step 6 calculates the amount of aflatoxin B_1 consumed by bovines. Step 7 calculates the bio-transfer of aflatoxin B_1 to aflatoxin M_1 from feed to milk. Steps 6 and 7 are part of the livestock stage. Step 8 calculates the estimated daily intake of aflatoxin M_1 by human consumers. Finally, step 9 characterises the risk from the consumption of aflatoxin M_1 for the population of Ireland and France. A comparison was carried out between aflatoxin M_1 consumption from milk in Ireland and France. Both Irish and French scenarios were considered under 2 different climate change scenarios, and a baseline scenario was used by taking weather data from a 30-year average (1980–2010) for both countries. The effect of change in climate was considered by taking the potential changes in temperature and relative humidity, which could impact the growth of *A. flavus*.

2.2. Model development

2.2.1. Pre-harvest

The pre-harvest stage consists of three steps. These include the formation of *A. flavus* colonies (in the form of CFU g^{-1}) based on previous crop types and temperature variations (Jaime-Garcia & Cotty, 2010). The previous crop can have a significant effect on the *A. flavus* population found in the soil. Maize, as a previous crop, is reported to have significantly greater quantities of *A. flavus* population in soil than cotton and sorghum (Jaime-Garcia & Cotty, 2010). Soil surface temperature also significantly influenced the quantity of *A. flavus* in the soil, with temperatures below 18 °C and above 30 °C having a negative effect on the population. In Table 1, *A. flavus* presence in soil is denoted by $\boldsymbol{CFU_{initial}}$. Temperatures for April (T_i in Table 1) are used to quantify the *A. flavus* propagules in soil, as mid-April is the time for planting maize in France and Ireland. The equation used to calculate the quantity of *A. flavus* propagules in soil was derived from the relationship reported by Jaime-Garcia and Cotty (2010) and is given as Eq (1). As the study was conducted outside Europe, the equation was classified as having uncertainty.

$$CFU_{initial} = e^{0.099AT_1+1.03} \quad (1)$$

Where $CFU_{initial}$ = *A. flavus* propagules in soil (CFU g^{-1}).

AT_1 = Temperatures in April (°C)

The amount of *A. flavus* remaining in the soil post-harvest of a crop depends on several factors, including tillage practice and grazing practice. Differences in tilling practices influence the amount of *A. flavus* in the soil. Grazing facilitates the distribution of *A. flavus* propagules in soil (Nesci et al., 2006). Table 2 displays the percentage of *A. flavus* remaining available in soil under different conditions (F_T) based on data from Nesci et al. (2006).

After obtaining the levels of *A. flavus* propagules in soil (CFU g^{-1}), a spatial unit of 1 ha is assumed with the presence of *A. flavus* in 1 inch of soil and the density of the soil is assumed to be a distribution between 0.9 and 1.39 g cm^{-3}. To estimate the *A. flavus* population present in the soil, $\mathbf{CFU_{initial}}$ is multiplied by the density of soil and the volume of soil present in 1 ha (Eq. (2)).

$$CFU_{soil} = CFU_{initial} \times H_a \times D_{soil} \times F_T \quad (2)$$

Where $CFU_{initial}$ = *A. flavus* propagules in soil (CFU g^{-1}).

CFU_{soil} = Amount of *A. flavus* propagules in soil per hectare (CFU $hectare^{-1}$)
H_a = Hectare of 1 inch soil (cm^3 $hectare^{-1}$)
D_{soil} = Density of soil (kg cm^{-3})
F_T = Effect of grazing and tillage

The second step involves the application of the fungicide before flowering, which could potentially reduce the *A. flavus* population. For this step, *in-vitro* and in-field experiments conducted by Masiello et al. (2019) were taken into consideration. Masiello et al. (2019) evaluated the response of 4 fungi, including *A. flavus,* to 11 fungicides. They found the fungicides had a range of effectiveness in inhibiting the mycelial growth of *A. flavus* depending on the main chemical compound and concentration of the fungicide chemical compound, with Folpet giving as low as 7% mycelial growth inhibition to several fungi and fungicides, including Boscalid (Prothioconazole as the active ingredient) inhibiting the mycelial growth of *A. flavus* up to 100%. A distribution was taken by fitting the mycelial growth inhibition data as a response to different fungicides from Masiello et al. (2019). A best-fit distribution in the form of a Triangular distribution was used with the values given in Table 1 (minimum = 0.016, most likely = 1 and maximum = 1) used to define the growth inhibition due to the application of fungicides (G_i). The amount of colonies of *A. flavus* leftover is given in Eq. (3).

$$CFU_{final} = (1 - G_i) \times CFU_{soil} \quad (3)$$

Where G_i = growth inhibition (fraction %)

CFU_{final} = *A. flavus* colonies which remain after application of fungicide (CFU $hectare^{-1}$)
CFU_{soil} = Amount of *A. flavus* propagules in soil per hectare (CFU $hectare^{-1}$)

Due to differences in practices and fungicides used in farms across Europe, this step was marked to have both uncertainty and variability while running the exposure assessment. The third step assumes spores are dispersed across the field from sites of waste debris (Olanya et al., 1997). As high as 50% of *A. flavus* conidia were found on leaf pieces near a waste corn site (0 m, 2 m and 1.7 m), with conidia spreading as far as 14 m from the waste corn disposal site. Since it is assumed in this study that *A. flavus* conidia are potentially available throughout the field, a uniform distribution of 0–50% was taken to account for the amount dispersed onto the plants in the field (Olanya et al., 1997). This is denoted by $\boldsymbol{D_{isp}}$ in Table 1. The final CFU on each maize plant is calculated according to Eq. (4).

$$CFU_{plant} = \frac{D_{isp} \times CFU_{final}}{P} \quad (4)$$

Where CFU_{plant} = CFU present on maize stalks (CFU maize $stalk^{-1}$).

D_{isp} = rate of dispersal (%)
P = Number of maize plants present in 1 ha (maize stalk $hectare^{-1}$) (Uniform (minimum = 6000, maximum = 12,000))

This is denoted by $\boldsymbol{CFU_{plant}}$ in Table 1, and it is obtained by dividing the CFU dispersed in the air by the probable amount of maize stalks present in the field. The level on each plant is thus calculated as a probability distribution to account for different scenarios, including differences in air temperature. The fourth step involves the growth rate of *A. flavus* colonies established as a function of air temperature and water activity (Samapundo et al., 2007). The equation from Samapundo et al. (2007) was used, which was based on *in-vitro* experiments on the growth rate (denoted by μ in Table 1), and lag phase (denoted by λ in Table 1) of *A. flavus* strains on maize. They derived an equation after studying the growth under different temperatures and water activity conditions. Therefore, the equation with the best fit as per the R^2 value

Table 1
Inputs and equations used in the exposure model.

Variable	Symbol	Equation/Distribution	Unit	Uncertainty/ Variability	Source
Temperature	T_i	Pert (min, mode, max)	°C	Variability	(IPCC, 2021; Met Éireann, 2020; Meteo France, 2020)
Relative humidity	Rh			Variability	
Saturated vapour pressure	ES			Variability	
Actual vapour pressure	EA	RH × ES		Derived	
Vapour pressure deficit	VPD	ES - EA		Derived	Table 6
Moisture content	Mc	77.5 - (1.1 × ΣVPD)		Derived	Manstretta and Rossi (2015)
Water activity	a_w	$e^{-\left(\frac{0.68}{25^{-.41}}\right)e^{\frac{18.69\times T^{-0.05}\times Mc}{100}}}$		Derived	
	b_w	$\sqrt{1-a_w}$		Derived	
Soil inoculum of mycelium	$CFU_{initial}$	$e^{0.099T1+1.03}$	CFU g^{-1} soil	Uncertainty	Jaime-Garcia and Cotty (2010)
Type of tillage	F_1	Integer {Uniform (min = 1, max = 3) } (Table 2)		Uncertainty	
Grazing	F_2	Integer {Uniform (min = 1, max = 2) } (Table 2)		Uncertainty	
Effect of grazing and tillage	F_T	f (F_1,F_2), (Table 2)		Derived	
Hectare of 1-inch soil	H_a	100 × 100 × 10^4 × 2.54	cm^3 $hectare^{-1}$	Fixed	
Density of soil	D_{soil}	Uniform (min = 1.2, max = 1.4)	g cm^{-3}	Variability	
CFU in soil	CFU_{soil}	$CFU_{initial} \times F_T \times H_a \times D_{soil}$	CFU $hectare^{-1}$	Derived	
Growth inhibition due to fungicide	G_i	Triangular (min = 0.016, mode = 1, max = 1)		Variability	Masiello et al. (2019)
Viable mycelium in the soil	CFU_{final}	$CFU_{soil} \times (1\text{-}G_i)$	CFU $hectare^{-1}$	Variability	
Maize plants density	D_{plants}	Uniform (6, 12)	plant m^{-2}	Variability	
Maize plants/hectare	P	$D_{plants} \times 100 \times 100$	plants $hectare^{-1}$	Derived	
Dispersal rate	D_{isp}	Uniform (0,50)	%	Uncertainty	Olanya et al. (1997)
CFU/plant	CFU_{plant}	$D_{isp} \times CFU_{final}/P$	CFU $plant^{-1}$	Derived	
CFU diameter (initial)	$X_{initial}$	Uniform (0, 300)	μm	Uncertainty	
Growth rate	M	$\ln(\mu) = C_0 + C_1 b_w + C_2 b_w^2 + C_3 T + C_4 T^2 + C_5 T b_w$	mm day^{-1}	Uncertainty	Samapundo et al. (2007)
Lag phase	Λ	$\ln\left(\frac{1}{\lambda}\right) = D_0 + D_1 a_w + D_2 a_w^2 + D_3 T + D_4 T^2 + D_5 T a_w$	day^{-1}	Uncertainty	Samapundo et al. (2007)
Days of growth	D_g	Uniform (85–125)	days	Variability	
Time	T	$D_g - \lambda$		Derived	
Maximum time	t_{max}	125		Fixed	
Maximum radius at maximum	X_{max}	(μ × t_{max})/2		Derived	
Radius at time t	X	$X_{initial}$ + (μ × t)/2		Derived	
Coefficient a	A	~ μ at t_{max}		Approximation	
Coefficient b	B	X_{max} = a + bt_{max}		Derived	
Aflatoxin production	P_a	$(a\alpha + b\beta) \times (t) + a\beta\frac{t^2}{2}$	ng g^{-1}	Uncertainty	Garcia et al. (2013)
Increase in aflatoxin calculated due to insect damage	I_D	Integer {Uniform (min = 1, max = 6)} (Table 4)		Uncertainty	
Total aflatoxin produced in the plant	A	$\frac{P_a \times CFU_{plant}}{M_{weight}}$	ng kg^{-1}	Derived	
Aflatoxin increase due to insect damage	I_D	Table 4	%	Uncertainty	McMillian et al. (1978)
Total aflatoxin in feed	T_{afla}	A × (1 + I_D)	ng kg^{-1}	Derived	
Feed intake	I_F	Uniform (min = 16, max = 21)	kg day^{-1}	Variability	Teagasc
The proportion of feed (maize)	P_m	24% (Ireland) 50% (France)		Fixed	
Quantity of maize intake	M_I	$P_m \times I_F$	kg day^{-1}	Derived	
Aflatoxin in feed	$Afla_{feed}$	$M_I \times T_{Afla}$	ng day^{-1}	Derived	
Milk yield	Y	Pert (10, 13,15) + Pert (10, 13, 15)	l day^{-1}	Variability	Teagasc
Carry-over rate	CO	0.0056 × $e^{0.0519\times Y}$	decimal	Uncertainty	
Aflatoxin in milk	$AflaM_1$	CO × $Afla_{feed}$/Y	ng l^{-1}	Derived	
Daily intake of milk	CM	Lognormal (mean, SD)	ml day^{-1}	Variability	(Agence nationale de sécurité sanitaire de l'alimentation del'environnement et du travail, 2017; IUNA, 2011)
Body weight	BW	Normal (mean, SD)	kg	Variability	(Agence nationale de sécurité sanitaire de l'alimentation del'environnement et du travail, 2017; IUNA, 2011)
Exposure to aflatoxin M_1	EDI	$AflaM_1$ × CM/BW	ng kg^{-1} bw day^{-1}	Derived	
Benchmark dose level (10)	$BMDL_{10}$	400	ng kg^{-1} bw day^{-1}	Fixed	EFSA (2020)
Margin of exposure	MOE	$BMDL_{10}$/EDI			

Table 2
Effect of tillage practices and grazing on the percentage of A. flavus available in soil (F_T)(Nesci et al., 2006).

Tillage Type	Grazing (F_2)	
(F_1)	1) Yes	2) No
1) Conventional	10	5
2) Reduced Tillage	40	20
3) No-Tillage	100	60

was taken for the growth rate and lag phase of *A. flavus* on maize plants (Eq.s 5 and 6). The constants used in Eq.s 5 and 6 are given in Table 3.

$$\ln(\mu) = C_0 + C_1 b_w + C_2 b_w^2 + C_3 T + C_4 T^2 + C_5 T b_w \quad (5)$$

$$\ln\left(\frac{1}{\lambda}\right) = D_0 + D_1 a_w + D_2 a_w^2 + D_3 T + D_4 T^2 + D_5 T a_w \quad (6)$$

Where μ = growth rate of *A. flavus* (mm day^{-1}).

C_i = constants for growth equation where *i* indicates numbers from 0 to 5 (Table 3)
D_i = constants for a lag equation where *i* indicates numbers from 0 to 5 (Table 3)
λ duration of lag phase (days)
T = Temperature (°C)
a_w = water activity
b_w = derivative of a_w given as ($\sqrt{1\text{-}a_w}$)

The temperatures for this step were taken for the month of July (denoted by $\boldsymbol{T_i}$ in Table 1), where *i* represents different countries and climate scenarios), and water activity (denoted by $\boldsymbol{a_w}$ in Table 1) was derived from relative humidity (denoted by **RH** in Table 1) using the formula described in Table 1 (Manstretta & Rossi, 2015). Equations (7) and (8) were used to derive the aflatoxin production equations (Eqs 9 and 10) as given in Baranyi and Roberts (1994) and Garcia et al. (2013) and are denoted by $\mathbf{P_a}$ in Table 1. The experiment predicted the production of aflatoxin based on the diameter of *A. flavus* and time (Eqs 9 and 10) for 1 to 5 × 10^4 spores of *A. flavus*. Coefficients in each of the equations were used as per Garcia et al. (2013), and a normal distribution was used to represent the variation in the coefficient (coefficients represented by **α** and **β** in Table 1). Variables $\boldsymbol{a}$ and $\boldsymbol{b}$ were calculated using maximum diameter based on growth rate and maximum growing days.

$$A = t + \left(\frac{1}{\mu_r}\right)\ln\left[exp^{-\mu_r t} + exp^{-\mu_r \lambda} - exp^{-\mu_r t - \mu_r \lambda}\right] \quad (7)$$

$$X = t + \left(\frac{1}{\mu_r}\right)\ln\left[1 + \frac{exp^{-\mu_r A} - 1}{exp^{r_{max}}}\right] \quad (8)$$

$$\text{If } t < \lambda X = 0; P_a = 0 \quad (9)$$

$$\text{If } \lambda < t < t_{xmax} X = at + bP_a = (a\alpha + b\beta)t + a\beta\frac{t^2}{2} \quad (10)$$

Where t = time (days).

Table 3
Values of constants for Equations (5) and (6) taken from Samapundo et al. (2007).

Constant	*I*					
	0	1	2	3	4	5
C	−12.01	n.s[1]	−26.82	1.02	−0.02	0.18
D	−37.4	32	n.s[1]	1	−0.009	−0.546

[1] n.s Not significant.

Table 4
Increase in aflatoxin contamination due to insect damage (I_D) (McMillian et al., 1978).

Insect damage severity	Aflatoxin increase (%) (I_D)
No damage (1)	Uniform (min = 0, max = 5)
Very Low (2)	Uniform (min = 0, max = 4.2)
Low (3)	Uniform (min = 0, max = 6.5)
Medium (4)	Uniform (min = 0, max = 40.8)
High (5)	Uniform (min = 5.5, max = 46.4)
Very High (6)	Uniform (min = 4.1, max = 100)

λ = lag
μ_r = maximum growth rate
X = radius
X_{max} = maximum radius at time t_{xmax}
P_a = aflatoxin produced in maize (ng g^{-1})
α = 39.15 ± 7.54 ng g^{-1} mm
β = 8.87 ± 3.60 ng g^{-1} mm d

When t reaches t_{xmax}, a ~ μ_r, thus, a and b were calculated using inputs from Samapundo et al. (2007) for lag and growth rate, and aflatoxin production in ng g^{-1} was obtained. Aflatoxin produced in ng kg^{-1} of maize was calculated according to Eq. (11).

$$Afla = \frac{P_a \times CFU_{plant}}{M_{weight}} \quad (11)$$

Where Afla = aflatoxin produced in ng kg^{-1} plant.

P_a = aflatoxin produced in maize (ng g^{-1})
M_{weight} = weight of maize plant (kg)

To account for insect damage, aflatoxin content is increased based on insect damage severity (Eq. (12)):

$$T_{Afla} = Afla \times (1 + I_D) \quad (12)$$

Where T_{Afla} = Total aflatoxin in ng kg^{-1} maize.

Afla = aflatoxin produced in ng kg^{-1} plant
I_D = aflatoxin contamination increase due to insect damage severity (%) (Table 4)

Here, insect damage was calculated based on data from McMillian et al. (1978). Severity and % increase in aflatoxin was grouped based on 6 bins created from the insect damage severity and aflatoxin contamination data in McMillian et al. (1978). Aflatoxin increase due to insect damage (I_D) used in the calculations is given in Table 4.

Maize sowing in Ireland is recommended between late March and early May and when the soil temperatures are greater than 10 °C. Similarly, for France, the recommended maize sowing dates are mid to late April, with air temperatures between 8 and 10 °C, depending on the soil type. Maize grows from April to September. Hence, temperatures for April were considered for spore formation in the ground. Temperatures for July were used for fungi growth and mycotoxin production equations. Maize is typically grown in the southeast of France. Therefore, temperatures for the preharvest stage were taken for the region of Auvergne-Rhône-Alps in France. The normal minimum and maximum air temperatures ($\mathbf{T_i}$ in Table 1) for this region between 1980 and 2010 were 6.5 and 16.3 °C for April ($\mathbf{AT_f}$) and 16.6 and 27.7 °C for July ($\mathbf{JT_f}$). The normal minimum and maximum air temperatures ($\mathbf{T_i}$ in Table 1) for Ireland between 1980 and 2010 were −4 and 20.5 °C for April ($\mathbf{AT_{IRE}}$) and 4.6 and 27.6 °C for July ($\mathbf{JT_{IRE}}$).

2.2.2. Livestock stage

It is assumed that aflatoxin B_1 from the pre-harvest stage completely carried over into the storage stage and was present in animal feed. The

amount of aflatoxin B_1 present in maize in feed consumed is calculated by using the quantity of maize intake as given in Eq. (13):

$$M_I = P_M \times I_F \quad (13)$$

Where M_I = Quantity of maize intake (kg cow^{-1} day^{-1}).

P_M = Composition of maize in feed (%)
I_F = Quantity of feed intake by a dairy cow (kg cow^{-1} day^{-1})

Aflatoxin B_1 in feed is given in Eq. (14):

$$Afla_{feed} = M_I \times T_{Afla} \quad (14)$$

Where $Afla_{feed}$ = aflatoxin B_1 content in feed (ng kg^{-1} cow day^{-1}).

T_{Afla} = Total aflatoxin in ng kg^{-1} maize
M_I = Quantity of maize intake (kg cow^{-1} day^{-1})

The quantity of maize in the bovine diet was dependent on agricultural practices; for example, France has a high percentage of maize in the diet of dairy cattle, whereas bovines in Ireland are mostly fed a grass diet all year round. Even if maize is in the diet, it is a small percentage of about 32% fed as silage in Ireland. Carry-over of aflatoxin B_1 to aflatoxin M_1 is denoted as ***CO*** in Table 1, and the values were expressed in ng l^{-1} of milk (Britzi et al., 2013; Masoero et al., 2007; Veldman et al., 1992). The carry-over was taken as reported by Britzi et al. (2013); Masoero et al. (2007); Veldman et al. (1992) as influenced by the yield of milk taken in the study, combining the three studies to obtain a function for carry-over dependent on milk yield. This accounted for late lactation, and mid-lactation cycles as the three studies had undertaken experiments to check aflatoxin M_1 carry-over into milk during the two lactation stages. Fig. 2 shows the carry-over and milk yield data plotted to obtain the line of best fit for Eq. (15).

$$CO = 0.0056 \times e^{0.0519 \times Y} \quad (15)$$

Where CO = Carry-over rate.

Y = milk yield (L)

Therefore, the amount of aflatoxin M_1 in milk was calculated as follows (Eq. (16))

$$AflaM_1 = \frac{CO \times Afla_{Feed}}{Y} \quad (16)$$

Where $AflaM_1$ = amount of aflatoxin M_1 in milk (ng l^{-1}).

CO = Carry-over rate
Y = milk yield (L)

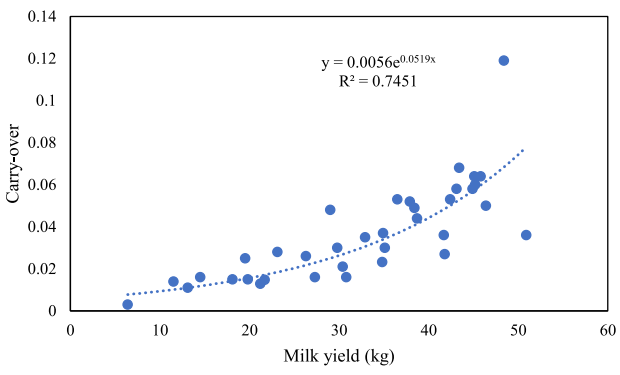

Fig. 2. Carry-over estimated from milk yield vs carry-over %.

2.2.3. Consumer stage

Consumption data for France and Ireland were taken from their respective national dietary surveys. For Irish data, the Irish Universities Nutrition Alliance's (IUNA) National Adult Nutrition Survey (NANS) data report from 2008 to 2010 was used (denoted as *Intake* in Table 1) (Irish Universities Nutrition Alliance, 2011). The mean consumption of liquid whole milk in Ireland for adult men was 215 g day^{-1}, and the standard deviation was 214 g day^{-1}. For adult women, the mean consumption of liquid whole milk was 116 g day^{-1}, and the standard deviation was 139 g day^{-1}. The mean body weight (bw) for Irish men and women was 86.2 and 70 kg, respectively, and the standard deviation was 15 and 13.7 kg, respectively.

France's liquid whole milk consumption data was obtained from the Third Individual and National Survey on Food Consumption (INCA3) (Agence nationale de sécurité sanitaire de l'alimentation del'environnement et du travail, 2017). Mean consumption of liquid whole milk for men and women were 79.7 and 80.4 g day$^{-1,}$ respectively, and standard deviations were 147.9 and 161 g day^{-1}, respectively. Estimated Daily Intake (EDI in Table 1) for France was calculated using body weight (bw) given by a normal distribution, where mean body weight for men and women were 80.3 and 67.3 kg, respectively, and the standard deviation was 16.2 and 14.7 kg, respectively. To calculate EDI, Eq. (17) was used.

$$EDI = \frac{AflaM_1 \times CM}{BW} \quad (17)$$

Where EDI = Estimated daily intake (ng of aflatoxin M_1 kg^{-1} bw day^{-1}).

$AflaM_1$ = amount of aflatoxin M_1 in milk (ng l^{-1})
CM = Consumption of milk (g day^{-1})
BW = body weight of human (kg)

The carcinogenic risk associated with consumption of aflatoxin M_1 was calculated using the margin of exposure method (Eq. (18)). The margin of exposure method (**MOE** in Table 1) is calculated using the benchmark dose level for 10% ($BMDL_{10}$) extra risk to hepatocellular carcinoma (HCC) compared to background levels for aflatoxin B_1 (400 ng kg^{-1} bw day^{-1}) for male Fischer rats at one-tenth its potency for aflatoxin M_1 (4000 ng kg^{-1} bw day^{-1}) as aflatoxin M_1 is ten times less potent than aflatoxin B_1 (EFSA, 2020).

$$MOE = \frac{BMDL_{10}}{EDI} \quad (18)$$

Where MOE = margin of exposure.

EDI = estimated daily intake (ng kg^{-1} bw day^{-1})
$BMDL_{10}$ = Benchmark dose level (ng kg^{-1} bw day^{-1})

A MOE below 10,000 indicated (EFSA, 2020) that people are at risk from exposure to aflatoxin M_1 through the milk and if greater than 10, 000, it indicates reduced consumer risk.

2.2.4. Climate data

Climate data for both France and Ireland were taken from their respective meteorological websites, Met Éireann (Met Éireann, 2020) and Meteo France (Meteo France, 2020). The temperature (**T**) distributions for each of these countries were used based on historical data of 30 years. Hourly relative humidity data and saturated vapour pressure were collected from Met Éireann (2020) for the years 2011–2022. This was used to obtain vapour pressure deficit for 2 different scenarios based on the months March, April and May (MAM) and June, July and August (JJA). Two future potential temperature scenarios were considered in the model, scenario 2–4.5 and scenario 5–8.5, based on the shared socioeconomic pathways published in the sixth Intergovernmental Panel on Climate Change (2021) report. Scenario 2–4.5 was a

middle-of-the-road pathway from the baseline scenario, where there was a moderate increase in greenhouse gas emissions and carbon dioxide emissions. Scenario 5–8.5 is a worst-case scenario where greenhouse gas and carbon dioxide emissions have doubled by the 2100 (IPCC, 2021). The current (normal) temperatures based on the IPCC report have changed by 0.8–1.3 °C relative to 1850–1900. By 2041–2060, the IPCC forecasts temperature changes of 1.9–3.0 °C for SSP 5–8.5 and 1.6–2.5 °C for SSP 2–4.5. For future climate change scenarios, temperature distributions were increased by 0.4–1.8 for scenario 2–4.5 for the year 2041–2060 and 1.2 to 2.2 for scenario 5–8.5 (year 2041–2060) relative to current normal temperatures (baseline).

2.3. Running the model

The inputs and model equations for both case studies were combined into a quantitative exposure assessment using @Risk 7.5 software (Palisade inc.), a Microsoft Excel add-in. The model was designed to be a probabilistic model and run for 100,000 iterations.

2.4. Sensitivity analysis

A sensitivity analysis was performed to account for uncertainty and variability in the main exposure assessment model output using Spearman's rank correlation coefficient in @Risk 7.5. The stages where uncertainty or variability was applicable were identified in the model. This is shown in Table 1.

2.5. Scenario analysis

Scenario analysis included different temperature distributions incorporating temperature fluctuations expected due to climate change under 2 Shared Socioeconomic pathway scenarios as per Intergovernmental Panel on Climate Change (IPCC) sixth assessment report (AR6) 2–4.5 and 5–8.5, on minimum, mean and maximum temperatures. Scenario 2–4.5 estimated global surface temperatures to most likely increase by 2.1–3.5 °C (years 2081–2100) and by 3.3–5.7 °C (years 2081–2100) for scenario 5–8.5 relative to average global surface temperatures in the period 1850–1900. Each climate scenario for the two countries had additional scenarios for relative humidity and agricultural practices decided by a uniform distribution at different steps, with numbers assigned based on Table 5. Two scenarios were created to account for the influence of changing relative humidity using distribution data available from the Met Éireann database. The relative humidity was then converted to vapour pressure deficit (**VPD** in Table 1, distributions for VPD based on hourly data in Table 6) (correlation with temperature 0.654) to be used to obtain water activity values.

Table 5
Possible scenarios based on the random effect of factors while performing iterations.

Factors	Possible scenarios based on the random effect of iterations (Integer Uniform distribution for selection)
Tillage (F_1) (Table 2)	1. Conventional (1) 2. Reduced tillage (2) 3. No-tillage (3)
Grazing (F_2) (Table 2)	1. Yes (1) 2. No (2)
Vapour pressure deficit (VPD) (Table 6)	1. March, April, and May (1) 2. June, July, and August (2)
Insect damage (I_D) (Table 4)	1. No damage (1) 2. Very low (2) 3. Low (3) 4. Medium (4) 5. High (5) 6. Very High (6)

Table 6
Adopted distributions for vapour pressure deficits (VPD) for two scenarios.

Time (24-h format)	March, April, and May (MAM)	June, July, and August (JJA)
0	Uniform (0,5.0926)	Triangular (0,0,5.3676)
1	Uniform (0,4.7473)	Triangular (0,0,4.7842)
2	Uniform (0,4.374)	Triangular (0,0,5.0578)
3	Uniform (0,4.1397)	Triangular (0,0,5.1502)
4	Uniform (0,3.7754)	Pert (0,0.81534,5.0701)
5	Pert (0,0.86638,3.7485)	Pert (0,0.89931,4.6989)
6	BetaGeneral (2.4941,7.9233,0,5.0551)	Pert (0,1.1432,4.8581)
7	Uniform (0,4.9064)	Pert (0,1.5063,5.5096)
8	Uniform (0,5.505)	Pert (0,1.7478,7.0129)
9	Triangular (0,0,5.8735)	Pert (0,2.1018,7.4209)
10	Triangular (0,0,6.2609)	Triangular (0,0,8.2249)
11	Triangular (0,0,7.0815)	Triangular (0,0,8.8389)
12	Triangular (0,0,7.3839)	Triangular (0,0,9.2322)
13	Pert (0,2.2096,8.2072)	Triangular (0,0,9.6593)
14	Triangular (0,0,7.9137)	Uniform (0,9.6613)
15	Triangular (0,0,8.1314)	Triangular (0,0,9.6132)
16	Triangular (0,0,8.3305)	Triangular (0,0,9.1228)
17	Triangular (0,0,8.0824)	Triangular (0,0,9.1887)
18	Triangular (0,0,7.616)	Triangular (0,0,9.4702)
19	Pert (0,1.6906,7.3508)	Triangular (0,0,8.8582)
20	Pert (0,1.4888,6.5373)	Triangular (0,0,7.7456)
21	Uniform (0,5.8133)	Triangular (0,0,7.2063)
22	Uniform (0,5.3048)	Triangular (0,0,6.237)
23	Uniform (0,5.1527)	Triangular (0,0,5.547)

2.6. Assumptions

1. It is assumed that the field has previous plant debris scattered randomly on the entire field to act as the initial source of contamination of *A. flavus*.
2. Aflatoxin B_1 in maize is assumed to be evenly distributed in cattle feed.
3. Consumption of milk by humans and feed composition for cattle is assumed not to change in the different scenarios.
4. The bioconversion of aflatoxin B_1 to aflatoxin M_1 is assumed not to be influenced by climate-related influences; the gut biome remains the same.
5. Post-harvest storage impacts on aflatoxin B_1 production are not considered
6. Processing steps during milk production are assumed not to influence aflatoxin M_1 contamination of milk.

3. Results

The results for the output of the model for the baseline scenario are provided in Fig. 3. The amount of aflatoxin M_1 in milk (**AflaM_1**) as a result of maize included in feed is given in Fig. 3a. Exposure to aflatoxin M_1 through consumption of milk for France and Ireland is displayed in Fig. 3b and **c** for males and females, respectively, while Fig. 3d and **e** displays the results for margin of exposure for the baseline model for males and females, respectively. Simulated mean aflatoxin M_1 content in milk, human exposure, and MOE are provided in Table 7, along with the confidence intervals for all the scenarios for both countries.

3.1. Aflatoxin in milk

3.1.1. Scenario for France

Table 7 displays the results from the risk assessment model for France and Ireland. Predicted aflatoxin M_1 concentration levels in milk for France ranged from 0.33 to 37.8 ng l^{-1} (95th percentile). Under all the scenarios, aflatoxin M_1 concentration levels did not exceed the maximum permissible limits of aflatoxin M_1 in milk (50 ng l^{-1}). Exposure to aflatoxin from milk was greatly affected by the variations in milk consumption for the French scenario. Model predictions indicated that

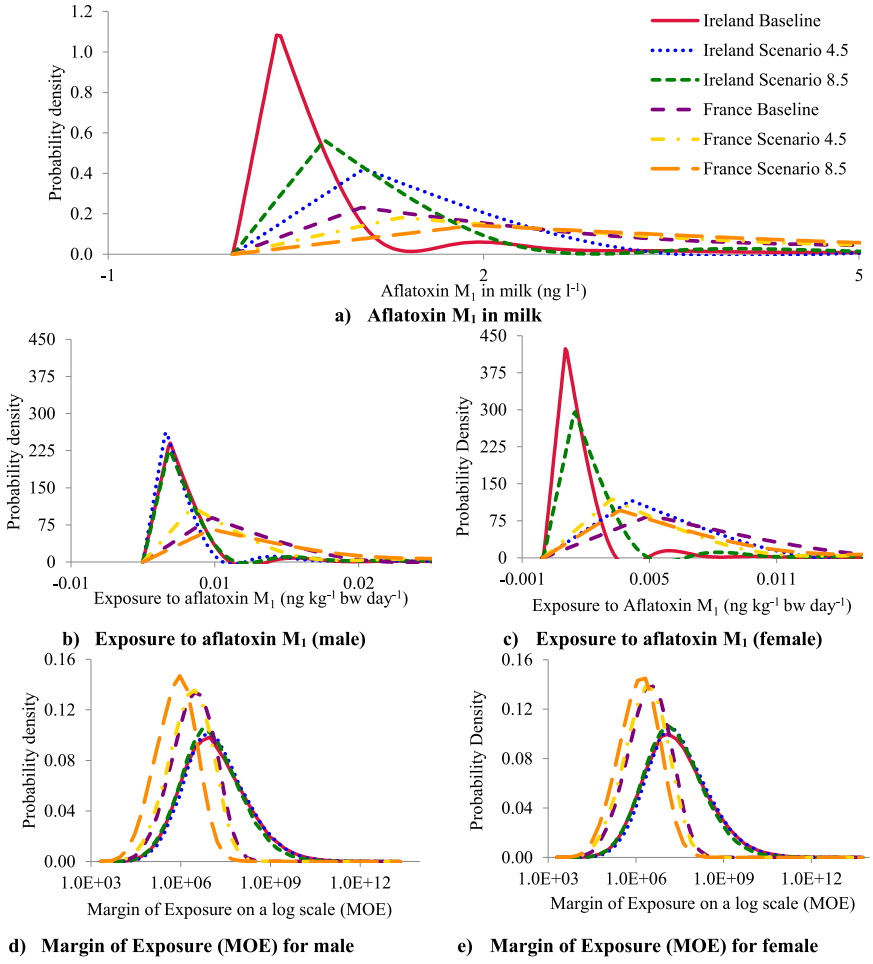

Fig. 3. Comparative results of the probability distributions for baseline model outcomes.

females in France are potentially exposed to greater levels of aflatoxin M_1 from milk in each of the scenarios compared to males (Table 7). This could be due to higher consumption rates of milk per day compared to males and also lower body weights, resulting in higher exposure kg^{-1} body weight. Under all three scenarios, the margin of exposure was greater than 10,000 (lowest 5th percentile in log values 4.68 and 4.90 for male and female respectively in scenario 8.5, which is greater than the log value of 10,000, i.e. 4) (Table 7). This suggested that a very small number in the population were at risk from aflatoxin M_1 consumption through milk under the baseline (i.e. the current scenario) and the different climate change scenarios.

3.1.2. Scenario for Ireland

For Ireland, the level of aflatoxin M_1 in milk ranged from 0.00087 to 5.72 ng l^{-1} (95th percentile) in the different scenarios, as given in Table 7. Similar to France, aflatoxin M_1 levels in milk did not exceed the EU maximum permissible limit of 50 ng l^{-1} for two scenarios, i.e. scenario 4.5 and scenario 8.5. Adult males consumed higher quantities of milk per day when compared to females, which is also reflected in the exposure in each of the scenarios (Table 7). Under all the scenarios considered, there were low levels of risk for the Irish population from levels of aflatoxin M_1 in milk as the MOE was greater than 10,000 (lowest 5th percentile in log values 5.47 and 5.71 for male and female respectively in scenario 8.5, which is greater than the log value of 10,000, i.e. 4) (Table 7).

3.2. Sensitivity analysis

The sensitivity analysis in Fig. 4 displays the most sensitive parameters for the exposure output for France and Ireland (both male and female). For France, the most sensitive parameters were whole milk consumption (CM; correlation coefficient 0.55 and 0.58 for male and female exposure models, respectively), tillage practice (F_1; correlation coefficient 0.43 and 0.42 for male and female exposure models, respectively), growth inhibition of mycelium due to fungicide (G_i; correlation coefficient 0.29 and 0.29 for male and female exposure models, respectively), initial inoculation used in Garcia et al. (2013) (correlation coefficient −0.25 and −0.25 for male and female exposure models, respectively), and June temperature (JT; correlation coefficient 0.22 and 0.22 for male and female exposure models, respectively). For Ireland, the most sensitive parameters were June temperature (JT; correlation coefficient 0.78 and 0.77 for male and female exposure models, respectively), whole milk consumption (CM; correlation coefficient 0.29 and 0.33 for male and female exposure models, respectively), tillage practice (F_1; correlation coefficient 0.25 and 0.24 for male and female exposure models, respectively), beta coefficient (β; correlation coefficient 0.18 and 0.17 for male and female exposure models, respectively), and growth inhibition of mycelium due to fungi (G_i; correlation coefficient 0.17 and 0.16 for male and female exposure models, respectively).

Input parameters taken in this study are either driven by uncertainty or variability. There is a large variability influence on the model output due to dispersal rate, JT, and CM in France and Ireland. Risk managers may need to consider the influence of this variability while

Table 7
Mean aflatoxin M_1 content in milk, human exposure and risk to aflatoxin M_1 from milk consumption estimated for France and Ireland under different climate change scenarios (5th,95th Percentile).

Parameters	Scenario (Ireland)			Scenario (France)		
	Baseline	4.5	8.5	Baseline	4.5	8.5
Aflatoxin M_1 content in milk (ng l^{-1})	0.78 (8.74 × 10^{-4}, 3.35)	1.13 (2.11 × 10^{-3}, 4.93)	1.30 (4.27 × 10^{-3}, 5.72)	6.52 (0.33, 26.4)	8.32 (0.42, 34.2)	9.22 (0.49, 37.8)
Male exposure to aflatoxin M_1 through milk (ng kg^{-1} bw day^{-1})	1.69 × 10^{-3} (1.19 × 10^{-6}, 6.97 × 10^{-3})	2.46 × 10^{-3} (2.87 × 10^{-6}, 1.02 × 10^{-2})	3.23 × 10^{-3} (6.22 × 10^{-6}, 1.35 × 10^{-2})	6.66 × 10^{-3} (1.19 × 10^{-4}, 2.70 × 10^{-2})	8.40 × 10^{-3} (1.47 × 10^{-4}, 3.46 × 10^{-2})	2.00 × 10^{-2} (5.01 × 10^{-4}, 8.29 × 10^{-2})
Risk to males from aflatoxin M_1 exposure (log MOE)	9.36 (5.75, 9.28)	9.16 (5.59, 9.02)	8.54 (5.47, 8.75)	6.94 (5.17, 7.51)	6.88 (5.06, 7.41)	6.33 (4.68, 6.88)
Female exposure to aflatoxin M_1 through milk (ng kg^{-1} bw day^{-1})	1.02 × 10^{-3} (6.30 × 10^{-7}, 4.08 × 10^{-3})	1.48 × 10^{-3} (1.49 × 10^{-6}, 5.93 × 10^{-3})	1.95 × 10^{-3} (3.10 × 10^{-6}, 7.81 × 10^{-3})	6.33 × 10^{-3} (1.02 × 10^{-4}, 2.64 × 10^{-2})	8.00 × 10^{-3} (1.26 × 10^{-4}, 3.39 × 10^{-2})	1.18 × 10^{-2} (2.44 × 10^{-4}, 5.03 × 10^{-2})
Risk to females from aflatoxin M_1 exposure (log MOE)	9.71 (5.99, 9.56)	9.48 (5.83, 9.30)	8.99 (5.71, 9.05)	7.01 (5.81, 7.57)	6.93 (5.07, 7.48)	6.66 (4.90, 7.19)

implementing mitigation strategies, as the significant influence of variability might make it difficult to employ a standard mitigation strategy in both countries. At the same time, the influence of the tillage practice ($\mathbf{F_1}$), inhibition of viable mycelium due to fungicide ($\mathbf{G_i}$), and dispersal rate of propagules on the plant ($\mathbf{D_{isp}}$) caused uncertainty in the model. More knowledge regarding the input parameters driven by uncertainty, such as $\mathbf{D_{isp}}$, would help refine the model and make it more precise. For example, the dispersal rate in this study was extracted from a single study. Therefore, it is not widely studied, and information available in the literature about the locations chosen in the study was limited.

4. Discussion

This risk assessment model accounts for the impact of climate change influences by considering changes in temperature and relative humidity on human exposure to aflatoxin M_1 through dairy milk consumption. It considers different climate change scenarios to obtain information on potential human exposure to aflatoxin M_1 through milk in the future. The results of the exposure model suggested that the risk of aflatoxin M_1 through consumption of liquid milk was not high for 95% of the population under all scenarios, as the amount of aflatoxin M_1 content in milk did not exceed the European Union's maximum permissible limits. The comparison of Ireland and France was made to account for differences in agricultural practices and the impact of climate change on the temperature in the two countries. The findings of this study suggested that the risk to human health from aflatoxin M_1 consumption through milk was marginally different between consumer groups (the 5th percentile for MOE was greater than 10,000). The margin of exposure under all scenarios for Ireland and France was greater than 10,000 (lowest 5th percentile 48,060). There was a relative increase in the aflatoxin levels in milk for France and Ireland in the different scenarios depending on the severity of climate change.

Previous studies in Europe assessed human exposure and risk to aflatoxin M_1 and other mycotoxins through milk varied in their findings according to the country and climate type. For example, Udovicki et al. (2019) undertook an exposure assessment to aflatoxin M_1 through the consumption of milk and yoghurt for students (18–27 years old) in Greece and Serbia, which have two different climate types. The exposure results estimated the range of student exposure to aflatoxin M_1 in Serbia to be between 1.238 and 2.674 ng kg^{-1} bw day^{-1} and 0.350–0.499 ng

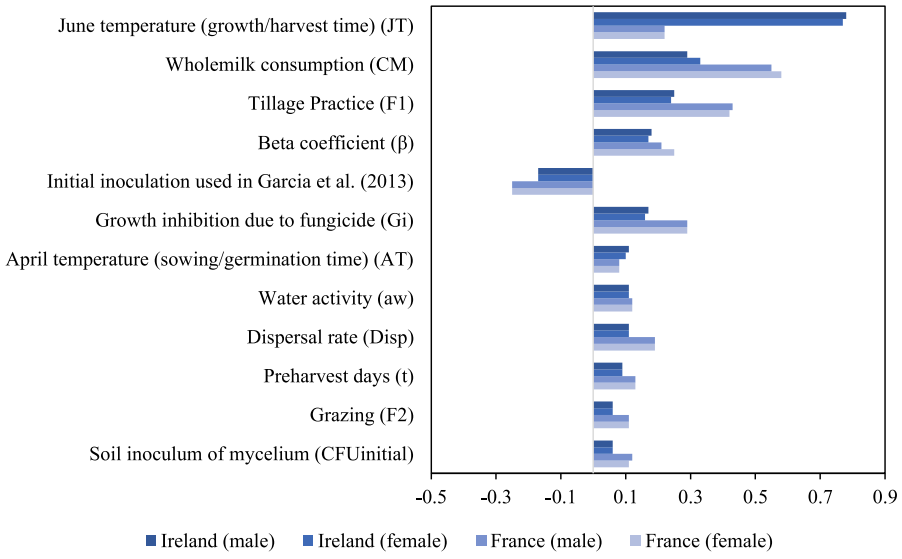

Fig. 4. Sensitivity Analysis (Spearman's rank correlation coefficient) of model inputs to simulated human exposure level to Aflatoxin M_1 in milk.

kg^{-1} bw day^{-1} in Greece. Whereas the margin of exposure score calculated had mean values of 1887.4 and 3958.3 for Serbia and 7307.7 and 7125 for Greece based on 1-day recall and 7-day recall, respectively. These values were considerably higher than the mean margin of exposure measured in this current study, suggesting that the population are at a relatively higher risk from aflatoxin M_1 consumption of milk. However, while the current study focussed only on milk consumption, Udovicki et al. (2019) considered cumulative exposure to aflatoxin M_1 through milk and yoghurt and associated risks. Udovicki et al. (2019) also used $BMDL_{10}$ values of 570 ng kg^{-1} bw day^{-1} for the MOE calculations, which is lower than the $BMDL_{10}$ value used in this study. Drought-like conditions in Serbia in 2012 impacted aflatoxin contamination in maize and, therefore, would have likely impacted the amount found in milk and dairy products produced in Serbia. Also, since Greece is a member of the European Union, it is required to follow the maximum permissible limits of aflatoxin M_1 in milk and the regulated amount of aflatoxin B_1 present in feed for dairy cows. Therefore, Greece would have lower exposure values when compared to Serbia. However, since Greece has conditions suitable for aflatoxin production, it is possible for it to have relatively higher values than Ireland and France. Milićević et al. (2021) characterised the risk of aflatoxin M_1 through the consumption of multiple food products including milk beverages, fermented milk, and pasteurised and UHT milk for children (age groups 1–3 and 3–9 years). The highest dietary exposure to aflatoxin M_1 estimated was for fermented milk, and for pasteurised and UHT milk at 95th percentile for the younger age group (1–3 years) with values of 0.508 and 0.457 ng kg^{-1} bw day^{-1} and the MOE associated with that population was 7881 and 8756 respectively suggested that there was a small population at risk from consuming those beverages.

A study conducted in Italy (Serraino et al., 2019) also calculated human exposure to aflatoxin M_1 through milk and hazard index for different age groups. The exposure values ranged from 0.02 to 0.13 ng kg^{-1} bw day^{-1} for adults, 0.19–1.62 ng kg^{-1} bw day^{-1} for infants and 0.16–1.16 ng kg^{-1} bw day^{-1} for toddlers. The exposure values in Serraino et al. (2019) were higher than those calculated in the current study. Upon calculation of the hazard index, it was observed that the groups of infants and toddlers were at increased risk since the hazard indices exceeded 1, whereas, for adults, the hazard indices were below 0.50. Roila et al. (2021) studied human exposure to aflatoxin M_1 through milk and dairy products (cow and ewe dairy and dairy products) in Italy using a deterministic approach for different age groups from 2014 to 2020. The average estimated exposure ranged from 0.00049 g kg^{-1} bw day^{-1} (50th percentile) to 0.00195 g kg^{-1} bw day^{-1} (95th percentile) for toddlers, 0.00015 g kg^{-1} bw day^{-1} (50th percentile) to 0.00045 g kg^{-1} bw day^{-1} (95th percentile) for children, 0.00007 g kg^{-1} bw day^{-1} (50th percentile) to 0.00017 g kg^{-1} bw day^{-1} (95th percentile) for adolescents, 0.00005 g kg^{-1} bw day^{-1} (50th percentile) to 0.00013 g kg^{-1} bw day^{-1} (95th percentile) for adults, and 0.00005 g kg^{-1} bw day^{-1} (50th percentile) to 0.00012 g kg^{-1} bw day^{-1} (95th percentile) for the elderly. Cow-drinking milk was the majority contributor to human exposure to aflatoxin M_1 in Italy for all age groups, followed by soft cow cheese, semi-soft cow cheese, hard cheese and ewe cheese. The calculated margin of exposure values, using a $BMDL_{10}$ value of 0.4 with a potency of 0.1 as per EFSA (2020), for risk characterisation, suggested the consumption of milk was safe for all age groups barring toddlers and children, for whom the average MOE was below 10,000 (for both 50th and 99th percentile). Italy lies in an area more prone to aflatoxin-producing conditions such as high temperatures and drought-like conditions and often faces such weather conditions, leading to high aflatoxin B_1 levels in maize. However, as Italy is part of the European Union, it has to maintain the maximum permissible levels in milk and feed. The margin of exposure for different age groups calculated by EFSA (2020) for European countries was between 100,000 and 2564 for the mean lower bound exposure (0.04–1.98 ng kg^{-1} bw day^{-1} mean exposure to aflatoxin M_1), and between 33,333 and 642 for the mean upper bound exposure (0.12–7.88 ng kg^{-1} bw day^{-1} 95th percentile dietary exposure to aflatoxin M_1) suggesting that certain populations in Europe might be at risk to hepatocellular cancer by dietary exposure to aflatoxin M_1. Farkas et al. (2022) used a probabilistic approach to characterise the risk of aflatoxin M_1 exposure through milk and dairy products for different age groups in Hungary. The study used aflatoxin M_1 concentrations and dairy product consumption data from a survey conducted between 2018 and 2020. Due to the absence of sufficient contamination data for dairy products, a concentration factor was used to estimate the presence of aflatoxin M_1 taken from literature. The study (Farkas et al., 2022) followed the calculation method for MOE used in EFSA (2020) and also in this study, by taking $BMDL_{10}$ of 400 ng kg^{-1} bw day^{-1} with a tenth of the potency, i.e. a value of 4000 ng kg^{-1} bw day^{-1}.

Recent studies across the globe that have conducted a risk assessment of aflatoxin M_1 include Conteçotto et al. (2021), Kortei et al. (2022), Hasninia et al. (2022) and Costamagna et al. (2021). Conteçotto et al. (2021) studied the occurrence of aflatoxin M_1 in dairy products intended for consumption by children in Brazil and conducted a deterministic exposure assessment based on the average aflatoxin M_1 contamination in dairy products, daily consumption, and body weight. Risk characterisation was calculated using MOE (reference dose value of 0.9124 μg kg^{-1} bw day^{-1} or 912.4 ng kg^{-1} bw day^{-1}, less than the current study) and cancer potential. The range of average results for the MOE for children aged 0–6 years was 418–459, which suggested that the children in Brazil were at potential risk of cancer from aflatoxin M_1 consumption. Kortei et al., 2022 conducted a deterministic exposure assessment and risk characterisation following the quantification of aflatoxin M_1 in raw milk samples from southern Ghana. The reference dose used for MOE calculations was 400 ng kg^{-1} bw day^{-1}. The calculated MOE for all age groups was below 10,000, suggesting the population in Ghana was at risk of liver cancer through exposure to aflatoxin M_1. Hasninia et al. (2022) analysed the aflatoxin M_1 content in raw and pasteurised milk from Iran to use as input for a probabilistic exposure assessment and risk characterisation. The reference dose used for the MOE calculations was 570 ng kg^{-1} bw day^{-1}, with estimated MOE for all the age groups falling below 10,000 during the summer season for raw milk. Pasteurised milk was safer to consume as per the MOE calculations for all age groups except age groups of 7–16 in summer and winter. Costamagna et al. (2021) built a probabilistic human health risk assessment model that captured contamination at feed of aflatoxin B_1 and its subsequent carry-over as aflatoxin M_1 in milk and dairy products for Argentina. The estimated MOE, calculated using a reference dose of 570 ng kg^{-1} bw day^{-1}, did fall below 10,000 for some of the age groups (infants, toddlers, children and adolescents).

The MOE values for the studies are displayed in Fig. 5, with the range

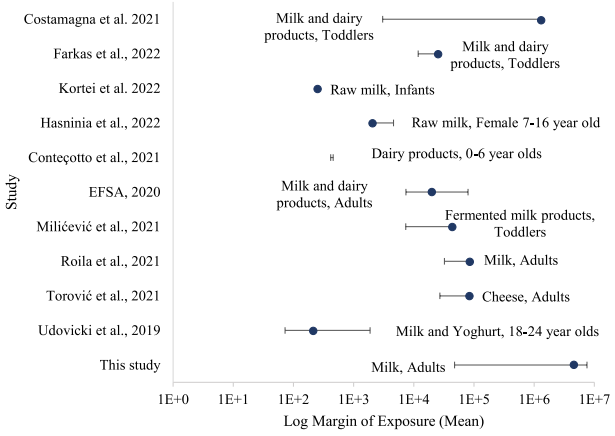

Fig. 5. Log mean margin of exposure as per literature for different dairy products (lowest MOE values indicated here in case of multiple age groups/ dairy products).

shown where possible, or in the case of multiple age groups and/or multiple dairy products, the lowest MOE ranges were selected, including for the current study for which the lowest MOE values were from the France 8.5 scenario.

An Irish study conducted by Rory Coffey et al. (2009) suggested that human exposure to aflatoxin M_1 was between 0.356 and 212 pg kg^{-1} bw day^{-1} through milk for Irish males and 0.381–238 pg kg^{-1} bw day^{-1} for Irish females. The current study's exposure levels slightly exceeded the exposure values provided by Rory Coffey et al. (2009). Rory Coffey et al. (2009), however, did not quantify the risk associated with the consumption of aflatoxin M_1 through milk. The sensitivity analysis conducted, however, pointed out that the highest impact on the output of the model was caused by the concentrations of aflatoxin B_1 in maize, followed by the carry-over rate of aflatoxin B_1 consumed in feed by cattle to aflatoxin M_1 produced in milk. The difference between the current study and the study conducted by Rory Coffey et al. (2009) was that Rory Coffey et al. (2009) took the initial concentration of aflatoxin in maize from imported maize feed data, whereas this model attempted to calculate aflatoxin B_1 presence in maize grown in Ireland.

Table 8 summarises the differences between previous studies and the current study. The current study modelled climate change influences (relative humidity and temperature) on *A. flavus* growth and aflatoxin B_1 production, followed by its presence in feed consumed by cattle and carry-over as aflatoxin M_1 in milk. Previous studies have either considered only contamination in milk without carry-over or not considered influences of climate change on feed contamination. The current study has also taken a probabilistic approach to determine the risk and exposure.

Boudra et al. (2007) found that no milk sample produced in France exceeded the European maximum permissible limit for aflatoxin M_1 in milk. In the case of this study, less than 1% of milk produced in France was predicted to exceed the maximum permissible limit for aflatoxin M_1 in milk. Though drought-like conditions in France in 2015 did increase mycotoxin contamination in maize silage in France (Bailly et al., 2018), which is likely to occur more frequently in the future.

The exposure assessment highlighted that neither of the two countries had high levels of aflatoxin contamination in milk. Under the conditions considered, the simulation model indicated that around 5% of the French milk could potentially exceed the European maximum permissible limits for aflatoxins in all the scenarios, whereas 1% of Irish milk could exceed the maximum permissible limits for aflatoxins in milk. A significant difference between the two countries is the amount of maize present in the diets of dairy bovines. In Ireland, the bovine diet consists primarily of pasture or grass silage, with around 24% of maize being fed to bovines during winter. Whereas, in France, the bovine diet can have a high composition of maize, as high as 50%. Another difference between the two countries is the temperature during the growing months. The maximum air temperature in Ireland during the maize growing months was 24.5 °C, which is less than the optimum temperature for aflatoxin production of 28 °C. Whereas, in the maize-growing regions of France, temperatures are predicted to reach 30 °C.

Maize in Ireland is grown for forage purposes and only in recent years has Ireland begun producing greater quantities. It is expected that under the climate change scenario, the amount of maize to be grown in Ireland will increase as the weather conditions become more conducive for maize production. Irish maize may likely be less susceptible to *A. flavus* due to comparatively cooler environments than in Eastern Europe. The risk of aflatoxin B_1 contamination in maize is also expected to increase in south and central Europe, including France, under different climate scenarios (Battilani et al., 2016).

This model was developed for maize used as feed. Maize, however, is not the only grain fed to bovines, and there can be multiple sources of aflatoxin in the bovine diet, including other cereal grains like wheat. Other than aflatoxin M_1, there is evidence of multiple mycotoxins being present in bovine milk, such as ochratoxin a, deoxynivalenol, enniatin and beauvercin (Akinyemi et al., 2022; Rocchetti et al., 2021). The development of mycotoxins present in the grass, which constitutes a major part of the bovine diet, also needs to be studied for carry-over as they were found to be present in bovine milk (Akinyemi et al., 2022; Rocchetti et al., 2021). Future work could include models developed for exposure assessment of multiple mycotoxins in milk from feed to fork with the analysis done for multiple countries. The current model can be adapted to suit different regions or countries. Parameters such as temperature and relative humidity, which are readily available, can be

Table 8
Comparison of risk assessment studies.

Parameter	CC influences	Feed stage	Dairy Product	Human Health Risk Assessment	Margin of Exposure reference dose (ng kg^{-1} bw day^{-1})	Age group	Deterministic, Probabilistic or mix	Country/ Region
Study								
Coffey et al. (2009)	×	✓	Liquid milk (various)	✓	n/a	Adults	Probabilistic	Ireland
Conteçotto et al. (2021)	×	×	Various	✓	912.4	0–6 years	Deterministic	Brazil
Kortei et al. (2022)	×	×	Raw cow milk	✓	400	Various	Deterministic	Ghana
Farkas et al. (2022)	×	×	Various	✓	4000	Various	Probabilistic	Hungary
Hasninia et al. (2022)	×	×	Raw and pasteurised milk	✓	570	Various	Probabilistic	Iran
Udovicki et al. (2019)	×	×	Milk and yoghurt	✓	570	18–27 years	Probabilistic	Serbia and Greece
Torović et al. (2021)	×	×	Cheese	✓	4000	Various	Deterministic	Serbia
Roila et al. (2021)	×	×	Various	✓	4000	Various	Deterministic	Italy
EFSA (2020)	×	×	Various	✓	4000	Various	Deterministic	Europe
Serraino et al. (2019)	×	×	Milk	✓	870	Various	Deterministic	Italy
Costamagna et al. (2021)	×	✓	Various	✓	570	Various	Probabilistic	Argentina
Milićević et al. (2021)	×	×	Various	✓	4000	1-3 and 3–9 years	Deterministic	Serbia
Battilani et al. (2016)	✓	✓	×	×	n/a	n/a	n/a	Europe
Van Der Fels-Klerx et al. (2019)	✓	✓	Milk	×	n/a	n/a	Probabilistic	Europe
This study	✓	✓	Milk	✓	4000	Adults	Probabilistic	France and Ireland

changed to suit the country. While influences of agricultural practices are standard, for example, the variations between not applying fungicide/differences in tillage practices are considered in the current model. Due to its toxicity, the current model considered a combination of aflatoxin B1 and aflatoxin M1. However, it can be adapted to suit fungi-mycotoxin combinations to predict exposure to different mycotoxins from milk and dairy products, depending on the availability of growth models in the literature.

5. Conclusions

This study aimed to conduct a human health risk assessment of aflatoxin M_1 in milk. Based on the risk assessment and the margin of exposure values, for France, both males and females were at risk from aflatoxin M_1 consumption under climate change scenarios 4.5 and scenario 8.5 (5th percentile was under 10,000). While, for Ireland, under none of the scenarios, the margin of exposure went below 10,000 (5th percentile). The most sensitive parameters for France and Ireland were different. Whole milk consumption was the most sensitive parameter for France, whereas, for Ireland, the most sensitive parameter was temperature during the maize growth period. This risk assessment model may be adapted to different countries to assess the potential risk of exposure to aflatoxin M_1 in milk under climate change scenarios and, through a sensitivity analysis, enable risk managers to identify possible risk reduction measures.

CRediT authorship contribution statement

Rhea Sanjiv Chhaya: Conceptualization, Methodology, Software, Data curation, Visualization, Investigation, Writing – original draft. **Jeanne-Marie Membré:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Rajat Nag:** Conceptualization, Supervision, Visualization, Writing – review & editing. **Enda Cummins:** Project administration, Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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