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Setting up risk-based monitoring programs for mycotoxins in cereals

Setting up risk-based monitoring programs for mycotoxins in cereals

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Abstract

A monitoring program has been designed for determining the sample size (number of samples) of mycotoxin-cereal contamination based on the risk of mycotoxin for human health. The sample size calculation was developed according to the concept of 'risk-based sample size', being risk level times normal sample size. The risk level of mycotoxin-cereal contamination was assessed by estimated daily intake (EDI) values per country of origin, tolerable daily intake (TDI) values and the carcinogenic consequences of mycotoxins. The normal sample size of each contamination was determined by two factors, both stratified per country: volume of cereal and prevalence rate. Furthermore, the sampling plan of Regulation (EC) No 401/2006 was used for translating lot weights to sample size numbers. The model can be used for sample size calculation on monitoring mycotoxins in cereals both with an unlimited budget and with budget limitations.

The developed model was practically applied to calculate the risk-based sample sizes of mycotoxin-cereal contaminations in the Netherlands by using historical monitoring data. Scenario analysis (SA) and Monte Carlo simulation analysis (MCSA) were also applied to conduct the calculation. According to the results of both analysis approaches, the most risky contaminations in the Netherlands were Zearalenone-Wheat, Deoxynivalenol-Wheat, Deoxynivalenol-Maize.

Keywords: Sample size calculation, Risk-based monitoring, Mycotoxins/cereals contamination, Carcinogenic risk, Monte Carlo simulation, Scenario analysis.

Preface

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List of abbreviations

Abbreviations	Meaning
AFs	Aflatoxins
ALARA	As Low As Reasonably Achievable
CAC	Codex Alimentarius Commission
CBS	Statistics Netherlands
DON	Deoxynivalenol
EC	European Commission
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EU	European Union
$FB_1/FB_2/FB_3$	Fumonisin B_1/Fumonisin B_2/Fumonisin B_3
IARC	International Agency for Research on Cancer
LOD	Limit of Detection
MCSA	Monte Carlo Simulation Analysis
MSs	Member States
NIV	Nivalenol
NL	The Netherlands
NOAEL	No Observed Adverse Effect Level
OTA	Ochratoxin A
RASFF	Rapid Alert System for Food and Feed
RBMPs	Risk-Based Monitoring Programs
RBSS	Risk-Based Sample Size
RBSSLB	Risk-Based Sample Size with a Limited Budget
RBSSUB	Risk-Based Sample Size with an Unlimited Budget
RiBESS	Risk-Based Estimate of System Sensitivity Tool
RIKILT	Rijks Kwaliteitsinstituut voor Land-en Tuinbouwproducten (Imperial Quality Control of Agricultural and Horticultural Products)
SA	Scenario Analysis
TDI	Tolerable Daily Intake
WHO	World Health Organization
ZEA	Zearalenone

1. Introduction

1.1 Background

Mycotoxins, the secondary metabolites of several fungi, can have adverse health effects on both humans and animals. Mycotoxins can occur in a wide range of cereals like maize, barley and wheat (Boon et al., 2009). Other food products, such as nuts and seeds, also have the possibility of being infected by mycotoxins (RASFF, 2017). In order to prevent mycotoxin contaminations and protect animal and human health, the European Commission established maximum levels of mycotoxins in foodstuffs in Regulation (EC) No 1881/2006 and Regulation (EU) No 165/2010 (European Commission, 2006c, 2010). The guidance levels in feed products and ingredients were regulated in Commission Recommendation (European Commission, 2006a). In recent years, mycotoxin contamination was estimated to be more severe than before because of environmental reasons. For instance, deoxynivalenol contamination in north-western European countries was reported to increase 3 times due to global climate-changing (Van der Fels-Klerx, Olesen, et al., 2012). Furthermore, mycotoxin contamination can cause economic losses in different ways: crop production losses, disposal of contaminated products, increased health care costs, animal production losses, and extra analytical costs in laboratories (Pinotti et al., 2016).

Mycotoxins in feed and food were of concern in the Netherlands because NL is an agriculture-oriented country, in which agricultural trade accounts for almost 55% of Dutch trade surplus (Statistics Netherlands, 2016). Moreover, many agricultural commodities were used for feed and food production in the Netherlands. In such cases, establishing monitoring programs for mycotoxins is an important action to guarantee the feed and food safety. However, monitoring can be very costly in terms of time and money, so it can best be risk-based. In addition, an EC regulation indicates that the governmental controls should be risk-based (European Commission, 2004, 2017). Therefore, a monitoring program - on a risk basis - for mycotoxins in feed and food is quite suitable for the current situation. This is because such a monitoring program can minimize the costs by decreasing the normal sample size (number of samples) to risk-based sample size based on the risk levels of contaminations.

Risk-based monitoring programs are based on specific sampling plans to increase the possibility of finding specific chemical contaminants or agents in feed and food (Presi et al., 2008; Stärk et al., 2006). According to a survey, the top three reasons that why responders applying RBMPs were related to the advantages of risk-based monitoring: lower monitoring

cost, higher data quality and improved quality oversight (Shukla et al., 2016). Once a RBMP is defined, the priorities of target contaminants can be known.

Previous studies developed sampling plans (including sampling, preparation and analysis) for mycotoxins based on mathematical and statistical tools (Johansson et al., 2000; Macarthur et al., 2006), the USA governmental department also published a mycotoxin handbook for introducing an official sampling plan (United States Department of Agriculture, 2015). These sampling plans can be used for setting up risk-based monitoring programs if needed.

In Europe, in accordance with Article 9 of Regulation (EU) 2017/625 of European Union, the 'Governmental control Regulation', governmental controls of food and feed materials should be carried out based on identified risks by Member States (European Commission, 2017). For further implementation, European Food Safety Authority developed a risk-based estimate of system sensitivity tool which covers risk-based sample size calculation (European Food Safety Authority (EFSA), 2012; Verbeke & Varewyck, 2016).

Nevertheless, RiBESS is not straightforward enough for setting up such a RBMP for mycotoxins in cereals. This is because: 1) the unit of input can only be numbers, whereas the unit of volume of cereal is ton which cannot directly be an input in RiBESS; 2) there are only four distribution functions can be used for input, and the distribution cannot be truncated. Hence, a proper distribution is not easy to set up. Moreover, triangular distribution is not included in the tool, which restricted its use; 3) prevalence only can be filled with a fixed number, in this case, the variability of prevalence cannot be achieved in RiBESS; 4) since the current study is not intended to set a fixed technique for detecting. The test sensitivity of a technique, which is one of the parameters in RiBESS, is not known to be an input of the tool for further calculations; 5) the risk factors must be selected from nine fixed numbers in RiBESS, which is not convenient because users must define and classify risk levels for each factor before using the tool; 6) since the tool can only process one mycotoxin-cereal contamination at one time, following comparison and results analysis are time-consuming for users (European Food Safety Authority (EFSA), 2018).

A risk-based model for monitoring dioxins and dl-PCBs in compound feeds was reported recently. The model calculated the potential health impact scores of contaminants and was applied to rank different feed ingredients based on their health impacts on animals and humans. The results provided the Dutch government with the advice of prioritization for dioxins inspection in feed ingredients (Van der Fels-Klerx et al., 2017). The sample size

calculation is not covered in the model, but this model is highly valuable for weighing the risk levels of various mycotoxins in cereals.

Since most of the previous reports focused on setting up sampling plans for mycotoxins, and since the EC regulations recommended to set up monitoring programs based on risks for adverse contaminants (including mycotoxins), the current study aims to set up risk-based monitoring programs - at the country level - for mycotoxins in cereals. The research problem is that the variety of cereals and mycotoxins is a lot, so priority setting of mycotoxins-cereals is needed to set up such a RBMP. The question is how to take the priorities into account in risk-based sample size calculations for monitoring.

In order to develop a risk-based monitoring program - at the country level - for mycotoxins in cereals under the European Union legal frameworks. The following sub-questions will be addressed:

1. Which methods of sample size (number of samples) calculations are available from literature, not only for mycotoxins but also for other chemical contaminants?

2. Which method can be applied to the current objective and which parameters are relevant to include?

3. How to set up a risk-based monitoring program for mycotoxins in cereals, both given an unlimited budget and with budget limitations?

1.2 Outline

Chapter 2 presents a literature review on mycotoxins and the current contamination situations in both Europe and the Netherlands. It also summarizes the European regulatory frameworks for mycotoxin contaminations. Furthermore, the risk-based monitoring programs for other contaminants were discussed. The third chapter provides the methodology of the current monitoring model and the data that used in this study. Chapter 4 presents the empirical results of applying the current risk-based monitoring model in the Netherlands. The discussion and concluding remarks were given in chapter 5 and 6, respectively.

2. Literature review

Section 2.1 discusses the basic classifications and relevant characteristics of the most toxic mycotoxins. Section 2.2 provides the preventions and governmental controls for mycotoxins in EC regulations. The final section presents an overview of the current contamination

situations for mycotoxins in cereals in the Netherlands and some other European countries. The summary of the literature review is shown in section 2.4.

2.1 Mycotoxins

2.1.1 Classifications

Mycotoxins, which commonly infect different crops in the field, are toxic substances produced by a wide variety of fungi. Mycotoxins can also be produced during improper drying process or inappropriate storage. According to the classification, around 400 mycotoxins have been recognized today, but only a few of them are of worldwide concern. This is because they have serious impacts on the health of animals and humans. Economically, mycotoxin contaminations can cause substantial economic losses to producers and governments (Smith et al., 2016).

The occurrence of mycotoxins in agricultural commodities depends on environmental factors, such as temperature and moisture levels. Once the mycotoxin-contaminated feeds or foods were consumed, severe toxic effects may happen on both animals and humans. Therefore, mycotoxin contamination poses a major food safety risk. Since mycotoxins have different chemical structures, and since they have plenty of fungal producers, methods of classifications for mycotoxins are quite complicated from various perspectives. According to previous studies, major producers of mycotoxins and main crops that affected by mycotoxins (Barug, 2006; Köppen et al., 2010; Panel, 2011) were summarized in **Table 2-1**.

Table 2-1. Major producers of mycotoxins and affected commodities

Mycotoxin	**Fungi (main producers)**	**Commodities**
Aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2)	*Aspergillus flavus* *A. parasiticus*	Cereals (maize, barley, oats, wheat, rice), nuts (hazel nuts, peanuts, tree nuts), dried fruits, spices, milk and cheese, eggs, meat, and oilseeds

Deoxynivalenol	*F. graminearum* *F. culmorum*	Cereals (maize, wheat) and cereal based products
Zearalenone	*F. graminearum* *F. culmorum*	Cereals (barley, oats, maize, wheat) and soy beans
Fumonisins (FB_1, FB_2, FB_3)	*Fusarium verticillioides* *F. proliferatum*	Cereals (maize, sorghum, rice), asparagus and milk
Ochratoxin A	*A. ochraceus* *Penicillium verrucosum*	Cereals (maize, oats, wheat and barley), dried fruits, coffee, beer and wine.
T-2 and HT-2 toxin	*F. sporotrichioides* *F. poae*	Cereals and cereal based products
Patulin	*A. clavatus* *A. longivesica*	Fruits (apples, cherries, grapes, pears and bilberries) and cereals
Ergot alkaloids	*Claviceps africanana* *Claviceps purpurea*	Cereals (wheat, rye, barley, oats sorghum and triticale)

Sources: Determination of mycotoxins in foods: current state of analytical methods and limitations (Köppen et al., 2010); Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed (Panel, 2011); The mycotoxin factbook: food & feed topics (Barug, 2006).

2.1.2 Mycotoxins selected for the current study

Since some mycotoxins were not prevalent in foodstuffs, only the top-five mycotoxins that were commonly found in cereals were selected in the current study. These mycotoxins are aflatoxins, deoxynivalenol, zearalenone, fumonisin B_1 and ochratoxin A.

The following sections present a brief introduction, including the general production conditions of mycotoxins and the description of dietary exposure to each mycotoxin, for these five mycotoxins.

2.1.2.1 Aflatoxins

Aflatoxins are the most noteworthy natural contaminants in foodstuffs. They were classified as carcinogens by IARC because of their acute toxicity to humans (Moss, 2002). The most favorable production of aflatoxins is under drought and high-temperature conditions (Streit et al., 2012). Aflatoxin B_1 is mainly observed in maize, nuts, groundnuts, rice and other plants; while aflatoxin M_1 affects milk products (Boon et al., 2009). The daily intake level of aflatoxins for Dutch children was reported to cause no health risk because the toxin concentration in foods was lower than the limit of quantification (G. Bakker et al., 2009). Nevertheless, aflatoxins should be concerned by food safety authorities because of its severe carcinogenicity to humans and because of people's huge consumptions for milk-derived products and nuts.

2.1.2.2 Deoxynivalenol

Deoxynivalenol belongs to the groups of trichothecenes from a chemical perspective. It is mainly produced in temperate climate conditions with warm and wet circumstances by *Fusarium graminearum* (Kushiro, 2008). DON is so-called vomitoxin because it can be delivered into the brain and can further run dopaminergic receptors to cause serious emetic effects (Sobrova et al., 2010). DON contamination is recognized as a severe safety issue in feed and food, not only because of its acute effects on human health but also because DON affects a wide range of cereals with high prevalence rate all over the world (Rotter, 1996; Streit et al., 2012). In 1999, DON was estimated to cause significant toxic effects on one-year-old children in the Netherlands. The phenomenon was connected with high concentrations of DON in wheat samples during the twentieth century (Pieters et al., 2002). Thanks to the rapid control measures of the Dutch government, the risk level of DON intake was decreased in 2002 due to the reduced concentrations (Pieters et al., 2004). However, DON is still one of the key agents for monitoring because of its frequent occurrence in cereal grains.

2.1.2.3 Zearalenone

Zearalenone is a major pre-harvest contaminant. However, it also infests post-harvest grains with suitable conditions. The infection risks were mainly exposed to those cereals which were not dried or stored correctly. The grain straws and silages also face the risk of ZEA contamination (Aldana et al., 2014). ZEA was mostly associated with maize in the field according to the historical monitoring data, which leads to great concerns in maize-based products (European Food Safety Authority (EFSA), 2014a). The optimum temperature and water activity of ZEA production are 25°C and 0.96 respectively (Milani, 2013). The risk of ZEA intake is higher for Dutch infants and children than that for adults (Aldana et al., 2014). Therefore, prevention measures are necessary because they are the best approaches to decrease the exposure risk of adverse health effects on humans.

2.1.2.4 Fumonisin B_1

Fumonisin B_1, one of the most prevalent and the most toxic mycotoxins produced by *Fusarium moniliforme*, can cause oesophageal cancer in humans. FB_1 contamination was identified as a high-frequency risk in maize-containing foods in the NL (De Nijs et al., 1998). It also has observed synergistic interactions in the combinations with other mycotoxins (Streit et al., 2012). The most suitable water activity for FB_1 production distributed between 0.93 and 0.995, with 20-35°C of temperature (Medina et al., 2013). From the dietary exposure perspective, the intake of FB_1 has toxic effects on liver and kidney of Dutch people (Boon et al., 2009).

2.1.2.5 Ochratoxin A

Ochratoxin A is nephrotoxic, mainly produced by *Aspergillus* and *Penicillium.* It also can be produced by other fungi at different temperature conditions and within diverse regions (Larsen, 2004). It is commonly found in stored cereals, such as wheat, maize, barley and rye. The growth of OTA often happens at a localized place with raised water activity during improper storage (Streit et al., 2012). It is reported that the ingestion of OTA was contributed mainly by cereals in daily diets. Noteworthy, although OTA has no health risk for Dutch people (M. Bakker & Pieters, 2003), the Netherlands is one of the regions that have a frequent occurrence of OTA (Smith et al., 2016). Consequently, governmental controls are necessary to implement concerning the precautionary principles.

2.1.3 Tolerable daily intake of mycotoxins

Mycotoxin contaminations were attempted to be linked with some historical disease outbreaks for many times since ancient. Mycotoxins were suspected as the trigger points of plague, gastrointestinal illnesses, lesions on the skin and other diseases; they were even connected

with the death of humankind and animals (Ramos et al., 2011). However, most of these attempts were failed because there was no scientific evidence. People gradually began to fear mycotoxins because of those suspicions. The fear promotes chemical studies of mycotoxins in the 1960s. Inspired by the relevant studies, tolerable daily intake values were established to prevent animal and human health.

A TDI value is the daily tolerating intake amount of one chemical compound in human diets. This amount has been determined as safe for humans regarding the whole lifespan. 'Safe' means the chemical compound will not present noticeable risk on the long-term basis. Since the TDI values were assessed based on animal experiments and were mostly well below the NOAEL dose (Faustman & Omenn, 2001; Renwick, 1999). TDI values were consequently seen as reference tools for setting up allowable levels for undesirable substances.

Almost all the mycotoxins have their corresponding TDI values. These values were treated as favorable metrics for benchmarking human dietary exposure to mycotoxins. However, it is worth mentioning that since aflatoxins were classified as carcinogens, the tolerable exposure on humans is assessed based on the ALARA principle instead of a TDI value (G. Bakker et al., 2009). The TDI values of other selected mycotoxins of the current study were summarized in **Table 2-2**.

Table 2-2. Tolerable daily intake values of mycotoxins

Mycotoxin	Tolerable daily intake (TDI)	Reference
Deoxynivalenol	1 µg/kg body weight/day	(Gareis, 2003)
Zearalenone*	0.2 µg/kg body weight/day	(Gareis, 2003)
Fumonisins	2 µg/kg body weight/day	(Gareis, 2003)
Ochratoxin A	5 ng/kg body weight/day	(Barug, 2006; Miraglia & Brera, 2002)

* *Temporary TDI*

2.1.4 Maximum levels of mycotoxins in EC regulations

Since mycotoxins are undesirable compounds in food and feed and given their adverse health effects on animals and humans, the European Food Safety Authority published scientific opinions on the maximum tolerance levels of mycotoxins in feed and food. These tolerance

levels were used as the basis for setting up legal limits in EC regulations. The maximum levels of various contaminants were regulated in Regulation (EC) No 1881/2006 (European Commission, 2006c). In addition, guidance levels have been set in Commission Recommendation as regards DON, ZEA, OTA and FB_1&FB_2 in cereal-based feed materials (European Commission, 2006a). Having legal limits on mycotoxins in foodstuffs and animal feeds can help to protect the public health of European citizens and can prevent mycotoxin outbreaks. The maximum limits and guidance levels for mycotoxins in cereals were presented in **Table 2-3**.

Table 2-3. Maximum and guidance levels of mycotoxins in cereals

Mycotoxin	**Cereal**	**Maximum levels (µg/kg)**			**Guidance levels* (mg/kg)**
Aflatoxins	All cereals and cereal derived products	B_1	Sum of B_1, B_2, G_1 and G_2	M_1	-
		2.0	4.0	-	-
Fumonisins B_1 & B_2	Unprocessed maize		4000		-
	Maize and maize products		-		60
Deoxynivalenol	Unprocessed cereals other than durum wheat, oats and maize		1250		-
	Unprocessed durum wheat and oats		1750		-
	Unprocessed maize		1750		-
	Cereals and cereal products other than maize by-products		-		8

	Maize by-products	-	12
Zearalenone	Unprocessed cereals other than maize	100	-
	Unprocessed maize	350	-
	Cereals and cereal products other than maize by-products	-	2
	Maize by-products	-	3
Ochratoxin A	Unprocessed cereals	5.0	-
	Cereals and cereal products	-	0.25

** Guidance values are relative to feed materials with a moisture content of 12%*

Sources: Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (European Commission, 2006a); Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins (European Commission, 2010); Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (European Commission, 2006c).

2.1.5 Carcinogenicity of selected five mycotoxins

The main reason why people paid much attention to chemical contaminants is that contaminants have acute effects on humans regarding the whole lifetime. In order to identify the carcinogenicity levels of chemical compounds, the international agency for research on cancer of WHO defined five classes and classified every undesirable compound to its matching class based on proper assessments. The descriptions for each group and the classifications for five selected mycotoxins were shown in **Table 2-4**.

Table 2-4. Classifications of carcinogenicity of mycotoxins

Class	Description	Mycotoxin
Group 1	Carcinogenic to humans	Aflatoxins
Group 2A	Probably carcinogenic to humans	-
Group 2B	Possibly carcinogenic to humans	Ochratoxin A Fumonisin B_1
Group 3	Not classifiable as to its carcinogenicity to humans	Zearalenone Deoxynivalenol
Group 4	Probably not carcinogenic to humans	-

Source: International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans (IARC, 2017).

2.1.6 RASFF notifications on mycotoxins

In order to provide an information exchange platform concerning food safety hazards between European Member States, the Rapid Alert System for Food and Feed was created in 1979 (European Commission, 2018a, 2018b). RASFF is responsible for receiving food safety issues from each Member State, classifying the severities and publishing the notifications to all MSs and the public.

According to previous annual reports of RASFF, three original notifications with different severities can be notified by RASFF. The most urgent notification is an alert; this notification is a trigger point for initiating rapid control measures in all Member States. The second notification is an information notification, which provides the public with information of identified hazards in food and feed, rather than activates rapid actions. The third one is a

border rejection notification. This is a notification for rejecting the entry of some unsafe ingredients in foods into European countries based on reasonable concerns (RASFF, 2017).

Compared with the three notifications mentioned above, a follow-up notification is transmitted by the Member States rather than RASFF. This notification is proposed when a Member State has valuable information that must be added to the original notification. Follow-up notifications can influence further reactions of other Member States since the added information may re-classify the severities of original notifications.

The number of each notification regarding mycotoxins in past five years was summarized in **Table 2-5**.

Table 2-5. Summary of the number of RASFF notifications concerning mycotoxins during the period 2012-2016

Year	Alert	Border rejection	Information for attention	Information for follow-up	Total
2016	82	418	49	2	551
2015	74	388	29	4	495
2014	54	280	44	5	383
2013	78	269	55	3	405
2012	38	425	53	9	525

Sources: The Rapid Alert System for Food and Feed - annual report of 2012, 2013, 2014, 2015, 2016 (RASFF, 2013, 2014, 2015, 2016, 2017).

In 2016, most of the notifications were classified as 'serious': 18 out of 19 in feed products, 527 out of 532 in foodstuffs respectively, only two notifications were classified as 'not serious' (RASFF, 2017). Aflatoxins-nuts contaminations were the top notifications according to all the previous reports because of the confirmed carcinogenicity of aflatoxins and the frequent occurrence. Apart from aflatoxins, ochratoxin A was the second critical mycotoxin although its genotoxic carcinogenic is still not confirmed yet. OTA was mainly found in various dried fruit samples. In the Netherlands, the number of notifications for mycotoxins was 84 in 2016, which increased by 31 compared with that in 2015 (RASFF, 2016).

2.2 Control framework for mycotoxins

2.2.1 Preventions of mycotoxin contaminations

Concerning the negative consequences of mycotoxin contaminations, preventions and control measures were therefore taken to prevent significant economic damages and health effects. In order to have the basic guidance for preventing contaminations, the Codex Alimentarius Commission developed Good Agricultural Practices, Good Manufacturing Practices, and Hazard Analysis Critical Control Point (Magan, 2006). National authorities of each country modified the suggested codes of practices by taking into account the local agricultural products, climate and other environmental factors that may influence the occurrence of chemical contaminants (Kabak et al., 2006).

The core prevention measures were divided into two phases: the first phase is precautionary, which aims to avoid or prevent contaminations. The second phase is controlling, which is performed on contaminated products to protect other products away from the contaminations.

2.2.1.1 First phase of prevention

The first phase which aims to avoid mycotoxin formation focuses on management. Proper soil cultivation, appropriate irrigation method and correct fertilization approaches are the major field management practices. These methods can reduce the probability of fungal infections before harvest. Post-harvest management measures emphasize the correct drying procedure and proper storage process. Rapid drying facilities, moisture control system of hygienic storage space and unspoiled carriage conditions are the critical safeguards of contaminations (Kabak et al., 2006).

2.2.1.2 Second phase of control

Concerning the contaminated products, control is focused on toxic substances elimination. There are three different methods to remove mycotoxins from products: physical, chemical and biological methods (Kabak et al., 2006; Magan, 2006). The detailed treatments and characteristics of each method were summarized in **Table 2-6**.

Table 2-6. Treatments and characteristics of three different removal methods for mycotoxins

Method	Treatments	Characteristics
Physical method	- Physical separation of contaminated products - Heat treatment - Ionizing radiation (including UV rays of sunlight for aflatoxins)	Physical methods are the cheapest and the simplest acceptable approaches. The treatments are well developed but cannot achieve desired objectives by a single treatment.
Chemical method	- Acid or bases treatment - Oxidizing agents treatment - Reducing agents treatment - Chlorinating agents treatment	Chemical methods can significantly decrease the nutritional value of feeds and foods. The treatments can cause undesirable sensory properties. Chemical treatments are restricted to human consumption in some countries.
Biological method	- Biological agents (yeast, fungi and bacteria) treatment based on the method of molecular biology, genetic engineering and microbial genomics	Biological methods have been undertaken only in laboratories. Organisms that used in treatments may produce pigment during fermentation, which restricts its use.

Sources: Mycotoxin contamination of food in Europe: early detection and prevention strategies (Magan, 2006); Strategies to prevent mycotoxin contamination of food and animal feed: a review (Kabak et al., 2006; Magan, 2006).

Since the selection of the removal methods for mycotoxins in foods or animal feeds depends on the environmental conditions, the features of the products, the expected objectives and the

natures of the mycotoxins, no single method is suitable for all kinds of foods and feeds. Therefore, the best prevention measure regarding all foodstuffs and animal feeds does not exist.

2.2.2 Governmental control regulation

Governmental controls were fulfilled by national food safety authorities with the aim of ensuring that feed and food products comply with EC regulations and protecting public health.

According to Article 3 (12) of Regulation (EU) 2017/625, the definition of 'food' refers to Article 2 of the General Food Law. Cereals were defined as 'food' or 'foodstuffs' since they are expected to be ingested by humans whether processed or not (European Commission, 2002). Mycotoxins in cereals, which may have toxic effects on humans, were treated as risks in accordance with the definition of 'risk' in the governmental control regulation (European Commission, 2017). As a consequence, governmental controls and other official activities for mycotoxins in cereals shall be performed by competent authorities of each Member State based on the rules in Regulation (EU) 2017/625 and shall be applied to the field of food safety at every phase of production.

2.2.3 Risk-based monitoring programs

In accordance with Article 9 of the governmental control regulation, governmental controls for foods shall be regularly performed on a risk basis before placing foods on the market (European Commission, 2017). Furthermore, sampling and analysis were seen as control measures according to Article 14 (European Commission, 2017). Therefore, governmental controls for chemical contaminants should best be done with a risk-based monitoring plan (RBMP). Applying risk-based monitoring programs will not only protect human health since it focuses on the most risky contaminations but also can save money.

Risk-based monitoring programs can enhance the effectivity of finding undesirable contaminants in target groups based on pre-defined risk-based sampling. The monitoring goals of each program varies with the defined program. For example, the most common objectives of risk-based monitoring programs for chemical contaminants in feed and food were: 1) classifying risk level of each chemical compound in products based on the prevalence rates and the potential adverse consequences; 2) determining how many samples should be taken for detecting the residue levels of a specific contaminant in foodstuffs. But the final purpose of such risk-based monitoring programs is to protect public health.

The typical procedure of setting up risk-based monitoring programs is separated into two steps. The first step is to develop a calculation method which can further determine the priorities for target contaminants based on defined risks. The developed model is then applied to a specific practical case for further demonstrations in the second step. According to previous studies, risk-based monitoring programs were used in various fields: Eide et al. developed an environmental risk model for oil spill monitoring to determine the prioritization rankings of individual crude oil tankers (Eide et al., 2007). The hazard-based models, developed by Khan and Sadiq, provide the prioritization of different locations for monitoring air pollutants based on risk levels of adverse hazards (Khan & Sadiq, 2005).

Khadam & Kaluarachchi developed a calculation method to assess the risk levels for contaminated groundwater. The methodology provides an approach for determining risk levels and risk-based priorities. The single risk level was determined by multiplying daily exposure with a consequence factor. All single risk levels were then added up to get the overall risk level for specific contaminants. According to the calculation results, a risk-based priority ranking can be obtained by sorting the results from largest to smallest. (Khadam & Kaluarachchi, 2003). The method presented calculation approach that can be useful for the current study: a single hazard level equals the probability of the hazard happening times the consequence factor which is caused by the hazard.

In the field of feed safety, Van der Fels-Klerx et al. established a model for dioxins and dl-PCBs in compound feed. The model estimates the monitoring priorities for chemical contaminants in feed ingredients that used in compound feed production. This monitoring model provided competent authorities with scientific recommendations for monitoring toxic contaminants in feed ingredients based on the concern of human health, animal health, and the overall health respectively (Van der Fels-Klerx et al., 2017). Since the objective of this model and that of the current study are similar: setting up monitoring priorities for chemical contaminants in feedstuffs as regards the animal and human health based on defined risks, the method of priority determination can be a valuable reference of the current study, especially for the development of taking priorities into account in risked-based sample size calculations.

2.2.4 Existing methods of sampling and analysis for mycotoxins

Almost every study of risk-based monitoring model includes one suitable existing sampling plan which matches its study objectives. This is because the essential contents that were outlined in a sampling plan, e.g., target samples, sampling units, sampling procedure and other relevant parts, may be applied to determine the calculations of a risk-based monitoring

program. Therefore, selecting a proper sampling method is the basis for setting up the current risk-based monitoring programs.

The European Regulation (EC) No 401/2006 regulated the methods of sampling and analysis for contaminants to support the implementation of governmental controls (European Commission, 2006b). This regulation covers the general methodology for sampling mycotoxins in foodstuffs in European countries, which was shown in **Figure 2-1**.

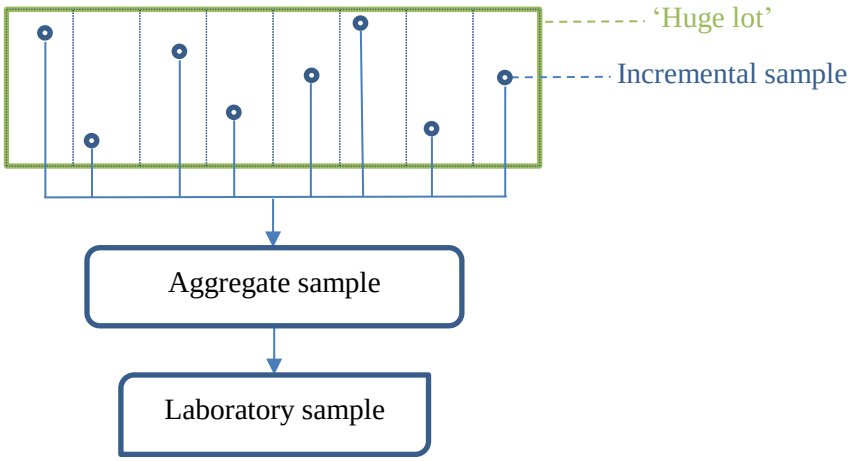


Figure 2-1. Legal sampling methodology for mycotoxins in foodstuffs

According to the descriptions of the sampling methodology for mycotoxins in cereals, a 'huge lot' is first divided into several sublots (based on weights). The incremental samples are then collected from each sublot. All the incremental samples of the 'huge lot' are mixed as an aggregate sample which is further processed into a laboratory sample. The sampling methodology enhances the accuracy of the sampling and analysis since the laboratory sample is a small representative mixture that evenly collected from a 'huge lot' (Whitaker et al., 2010).

The number of incremental samples per sublot, which was presented in **Table 2-7**, was well-defined in 'Method of Sampling for Cereals and Cereal Products', Part B of Annex I in Regulation (EC) No 401/2006 (European Commission, 2006b).

Table 2-7. Classification of the number of incremental samples

Weight of the huge lot (tonnes)	Weight or number of sublots	Number of incremental samples
≥ 1500	500 tonnes	100
> 300 and < 1500	3 sublots	100
> 50 and ≤ 300	100 tonnes	100
< 50	-	3-100 (depends on the specific weight of sublots)

Source: Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the governmental control of the levels of mycotoxins in foodstuffs (European Commission, 2006b).

2.2.5 Sample size calculation

The sample size calculations in previous studies were based on mathematical principles regarding these parameters: type I and type II error of a test, standard error, confidence level, volume of cereals and prevalence rate of mycotoxins (Dillon et al., 2005). From the results of these calculations, the question - how many grams should be taken per lot to represent the situation of the whole huge lot - can be answered (Tittlemier et al., 2011). But the number of samples cannot be given because these parameters cannot convert the weights of cereals to the numbers of samples.

However, since different sample sizes have different objectives and different input, sample size determination methods and the parameters are not fixed (Lenth, 2001). There are some sample size studies that focus on the conversion between the weights and the numbers, which provides some ideas for developing the current risk-based monitoring program. The number of samples was well-classified based on the weight values of the lots in these sampling plans (Canadian Food Inspection Agency, 2017; De Saeger, 2011; Freese et al., 2015). These ideas of specifying a correspondence between lot sizes and sample sizes can be an approach to determine the sample size for monitoring mycotoxins in cereals. Once ‘how many samples should be taken from a fixed weight of cereals’ is known, the total sample size can be calculated as the sum of single sample sizes.

2.3 Contamination situation of mycotoxins in cereals

2.3.1 In Europe

The major mycotoxins in cereals in European countries, especially in wheat in the field, are ZEA, DON, T-2 toxin and nivalenol (Kabak et al., 2006). However, since the occurrence rates of mycotoxins vary greatly with geographical characteristics, the situations of mycotoxin contaminations were different in different countries. In order to illustrate the differences, the mycotoxin contaminations of some European countries were described in **Table 2-8**.

Table 2-8. Mycotoxin contaminations in European countries

Country	Description of mycotoxin contaminations
Austrian	The top-two frequent contaminations are DON in maize, ZEA in unprocessed maize kernel. DON concentration was detected higher than the corresponding guidance level in one maize sample in 2009, 2010 respectively.
German	Aflatoxin B_1 was found to exceed the maximum level in 7 oilseed samples in 2010. DON levels of two contaminated samples exceeded the guidance level. OTA levels of three samples were found higher than the guidance level.
Greece	There was no significant mycotoxin hazard according to the test results of 71 samples in 2010. Almost all mycotoxin levels compliant with the regulations.
Sweden	In 2006, aflatoxin M_1 contaminated milk products, which further led to the contamination in rice. Since 2006, mycotoxin concentrations of all analyzed samples compliant with the guidance values.

Source: Current situation of mycotoxin contamination and co-occurrence in animal feed - Focus on Europe (Streit et al., 2012).

According to the table, it is obvious that different mycotoxin contaminations were of concern in different countries. However, it is noteworthy that mycotoxin contamination levels were controlled well below the guidance levels after the aflatoxin hazard in Sweden in 2006, which means that effective governmental controls can prevent contaminations significantly to some extent.

2.3.2 In the Netherlands

Since Dutch people consume lots of cereal-derived foods such as bread, beer, biscuits and cookies, the cereals safety is highly concerned. Besides the occurrence rates of mycotoxins, the human exposure levels to each mycotoxin as regards different age groups were also the core research topics for scientists in determining the risk levels of mycotoxins. By reviewing these studies, the mycotoxin contamination situations of the Netherlands were described below.

RIKILT published a report regarding the governmental controls for chemical contaminants between 2001 and 2009 in the Netherlands. There are some noteworthy contents about mycotoxins in the report: ZEA concentrations in soya bean samples were found relatively high, but most of the soya bean products originated from Argentina; DON levels in several liquid pig feed samples exceeded ten percent of the guidance value; aflatoxin B_1 levels in nuts and oilseeds were described as infrequently higher than the guidance level (Streit et al., 2012). However, since these mycotoxin levels were mostly well below the maximum level of EC regulations, the toxicities of these mycotoxins in the Netherlands were considered as not very critical (Streit et al., 2012).

The results of a risk assessment regarding dietary exposure to mycotoxins for Dutch children illustrated that the possibility of appearing the toxic effects is limited (Boon et al., 2009). The toxic effects of mycotoxins were therefore considered as ‘not severe’ for young children. However, the assessment did not include aflatoxin B_1 and OTA.

Another study reported that the human exposure levels to OTA, trichothecenes and fumonisins were well below the corresponding tolerable daily intake values. The study also assessed aflatoxins based on ALARA principle. The result presented a quite low level of human exposure. Consequently, there was no significant health risk for Dutch children according to the limited data that used in the study (G. Bakker et al., 2009; M. Bakker & Pieters, 2003).

2.4 Summary

By reviewing the previous studies and the current contamination situations, setting up a risk-based monitoring program which contains a sample size calculation can provide not only the priorities for monitoring mycotoxins but also the risk-based sample sizes. This monitoring model can directly support decision-making process since the priorities and the risk-based sample sizes are necessary indicators of a governmental monitoring plan.

3. Methodology

The current risk-based monitoring model is designed to determine the sample sizes based on defined risks. The conceptual framework of the model was presented in the first section of methodology. Whereas the second and third section stated the introduction of two calculation approaches, being scenario analysis and Monte Carlo simulation analysis. These analyses were used to conduct the calculation to further obtain the risk-based sample sizes without budget limitations. Moreover, the calculation method of risk-based sample sizes with budget limitations was stated in section 4.

In order to have a practical application, the current model was used for determining sample sizes for monitoring mycotoxins in cereals in the Netherlands. The descriptive statistics to determine input values were presented in Appendix 1, 2, 3 and 4 respectively.

3.1 Conceptual framework of monitoring model

The current monitoring model was designed based on the definition of ‘risk-based sample size’, being the normal sample size times the carcinogenic risk of mycotoxins on human health. The detailed risk-based sample size calculation determination was shown by four steps with different colors in **Figure 3-1**.

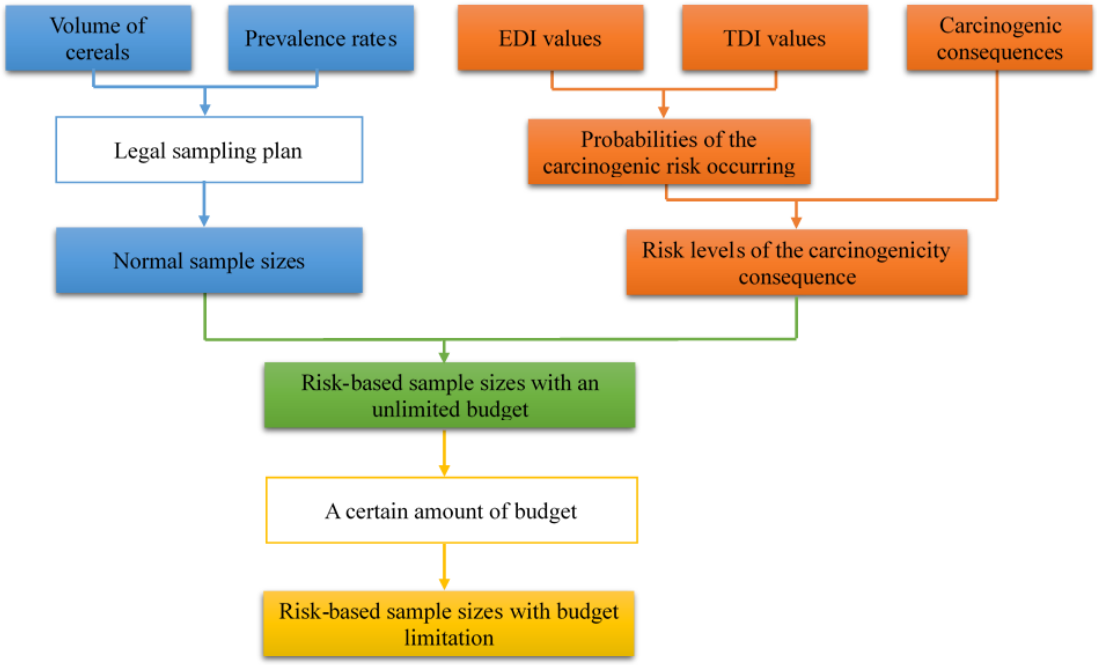


Figure 3-1. Flowchart for calculating risk-based sample sizes

The normal sample size was calculated based on the total volume of cereals, the prevalence rate of mycotoxins in cereals, and a legal sampling plan which was included in European Regulation (EC) No 401/2006. In the current study, the defined risk only considered the carcinogenic risk that occurs through daily dietary ingestions. The level of the carcinogenic risk is calculated by using the definition of 'risk' from the risk assessment perspective, being the probability of a hazard occurring times its possible health impacts.

Because of difference of climate and that of geographical characteristics, volume of cereals and prevalence rates vary from country to country. These two parameters also vary within a single country from one year to the next year. EDI values are also variable values because of the diverse diets and eating habits in different countries. However, other factors are constants regardless environmental and dietary differences: 1) the number of incremental samples that should be taken from each lot is determined by the existing sampling plan in EC regulations (European Commission, 2006b); 2) the TDI values which were shown in **Table 2-2** are constants in all European countries for years; 3) the fixed carcinogenic consequences of mycotoxins on human health were classified by IARC (IARC, 2017).

Therefore, the risk-based sample size with an unlimited budget can be calculated by:

$$RBSS_{ij} = NSS_{ij} * c_{ij}$$

Where:

$$NSS_{ij} = \left[\left(\frac{a_i * 1000}{500}\right) * 100\right] * b_{ij} = 200a_i * b_{ij}$$

$$c_{ij} = \frac{d_{ij}}{e_j} * f_j$$

With:

$RBSS_{ij}$ = Risk-based sample size of mycotoxin *j* in cereal *i*, which includes the normal sample size of a specific mycotoxin-cereal combination (*ij*) in a country (NSS_{ij}) and the level of carcinogenic risk on human health (c_{ij});

a_i = Total volume of cereal *i* (1000 t) of a specific country, equals the sum of imported volume and domestic production;

b_{ij} = Prevalence rate (%) of a mycotoxin-cereal combination *ij* in a specific country;

c_{ij} = The carcinogenic risk of a mycotoxin-cereal contamination *ij* on human health in a specific country (classes, values of 1 (low), 2 (medium), 3 (high), 4 (severe));

$\frac{d_{ij}}{e_j}$ = Probability of the carcinogenic risk (classes, values of 0.01 (low), 0.1 (medium), 1 (high), 10 (severe)) is classified by the ratio of the EDI value of a mycotoxin-cereal combination *ij* (d_{ij}) to the TDI value of the corresponding mycotoxin *j* (e_j);

f_j = Carcinogenic consequence of a mycotoxin *j* on human health (classes, values of 0.01 (low), 0.1 (medium), 1 (high), 10 (severe)).

Factor NSS_{ij} is the normal sample size of a specific mycotoxin-cereal contamination *ij*. It involves the total volume of cereal and the prevalence rate. The total volume of cereal (a_i) was assumed as the weight of the 'huge lot' of the corresponding cereal. According to the sampling plan of Regulation (EC) No 401/2006 (European Commission, 2006b), which has been described in section 2.2.4 of this report, each 'huge lot' was divided into numbers of

sublots. The sample size of a 'huge lot' that only considers the volume was therefore the total number of incremental samples that is taken from all the sublots.

Regarding the statement "*100 incremental samples should take as a minimum level from every sublot regarding that the weight of whole lot is not always an exact multiple of the weight of the sublots*" (European Commission, 2006b) of the legal sampling plan for cereals and the idea of specifying a correspondence between sample size and lot size which mentioned in section 2.2.5. The sampling plan of the current monitoring program was defined as taking 100 incremental samples from every 500 tonnes cereal without considering the sublot division. Therefore, the number of samples which only consider the volume of cereal equals $\left[\left(\frac{a_i*1000}{500}\right)*100\right]$. Taking into account the prevalence rate, the normal sample size was calculated as $\left\{\left[\left(\frac{a_i*1000}{500}\right)*100\right]*b_{ij}\right\}$.

Factor c_{ij} is the defined carcinogenic risk, it was calculated based on the definition: risk equals the probability of a risk occurring multiplies the expected consequence that caused by the risk. The classification of carcinogenic risk levels was presented in **Table 3-1**.

Table 3-1. Classification of the carcinogenic risk levels

RISK	**Probability of the consequence occurring**				
Expected carcinogenic consequence		Low (0.01)	Medium (0.1)	High (1)	Severe (10)
	Low (0.01)	*Low 1*	*Low 1*	*Low 1*	Medium 2
	Medium (0.1)	*Low 1*	*Low 1*	Medium 2	High 3
	High (1)	*Low 1*	Medium 2	High 3	**Severe 4**
	Severe (10)	Medium 2	High 3	**Severe 4**	**Severe 4**

Source: A risk-based strategy for controlling chemical contaminants as relevant hazards in food ingredients (Hanlon et al., 2015).

In the current study, the probability of the carcinogenic consequence was classified by the ratio of the EDI value of a specific mycotoxin-cereal combination to the TDI value of the corresponding mycotoxin ($\frac{d_{ij}}{e_j}$). The carcinogenic consequence (f_j) on human health, which was caused by the presence of mycotoxins in daily cereal-derived diets, was expressed by

four classes (values of 0.01 (low), 0.1 (medium), 1 (high), 10 (severe)). The classifications for both probabilities and carcinogenic consequences were listed in **Table 3-2**.

Table 3-2. Classifications of probabilities and that of carcinogenic consequences

Classification of probabilities		**Classification of carcinogenic consequences***	
Class of the probability	Ratio results range description	Class of the carcinogenic consequence	Carcinogenicity description
Low (0.01)	$\frac{e_{ij}}{f_j} < \frac{1}{1000}$	Low (0.01)	Probably not carcinogenic to humans
Medium (0.1)	$\frac{1}{1000} \leq \frac{e_{ij}}{f_j} < \frac{1}{100}$	Medium (0.1)	Not classifiable as to its carcinogenicity to humans
High (1)	$\frac{1}{100} \leq \frac{e_{ij}}{f_j} < \frac{1}{10}$	High (1)	Probably /Possibly carcinogenic to humans
Severe (10)	$\frac{1}{10} \leq \frac{e_{ij}}{f_j}$	Severe (10)	Carcinogenic to humans

**Source: International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans (IARC, 2017).*

By using the calculation mentioned above, the risk-based sample sizes with unlimited budget can be obtained, the risk-based sample sizes with limited budget can be calculated by scaling the results of RBSSUBs based on a specific amount of budget and the cost of each sample.

Considering the priorities of mycotoxin-cereal contaminations, the mycotoxin-cereal combination which has the largest RBSS value is the most risky contamination; the corresponding priority is therefore the highest, and vice versa. In other words, the priority rankings are positively correlated with the numerical orders, from large to small, of RBSS values. In order to have a better understanding of this, the relationship of RBSS values and priorities were shown in **Figure 3-2**.

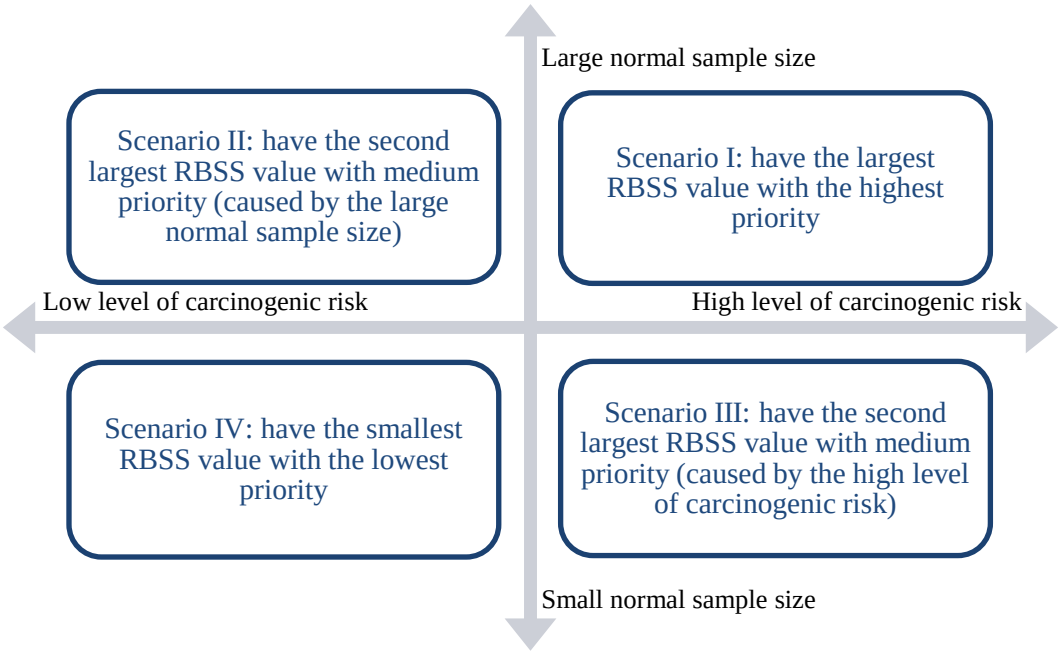


Figure 3-2. Relationship of RBSS values and priorities

3.2 Risk-based monitoring programs: scenario analysis approach

Since the risk level and risk-based sample size of each mycotoxin-cereal combination may vary with variable factors, the risk-based sample size was discussed in various scenarios to have a comprehensive overview.

Considering the variable inputs of the calculation, the estimated daily intake values were assumed as constants in the current model because the available data was limited. However, the other yearly variable inputs that have enough available data, being the volume of cereals and the prevalence rates of mycotoxins, were considered in the scenario settings since they affect the risk level and risk-based sample size of each mycotoxin-cereal combination. Therefore, different scenarios were developed to obtain insights into the results from the variability in the inputs. As a first step, the variability of the volume of cereals was determined by considering risk-based sample sizes in each different year since the volume of cereals was changing every year. As a second step, the minimum, medium and maximum prevalence rates were used in 3 scenarios of each year to reflect the variable prevalence rate of mycotoxins. As a result, the representative minimum, medium and maximum prevalence rate were used in scenario 1, 2 and 3 of each year to obtain minimum, medium and maximum risk-based sample sizes of each year.

The risk-based sample sizes of all the studied mycotoxin-cereal contaminations, within each scenario, were calculated in a spreadsheet in Microsoft Excel 2016. Previous monitoring data was used in the calculation to obtain the results. The final results would be risk-based sample size tables of all studied years, with 3 scenarios per year.

3.3 Risk-based monitoring programs: Monte Carlo simulation analysis approach

Because of the variable inputs, e.g., volume of cereals, prevalence rates, EDI values, the number of outputs will be countless. Therefore, using random numbers as inputs to simulate the results can be the best way to solve the problem. As long as the range of each random number is well-defined based on the reality, the results of calculation would be comprehensive since all the possible situations of all variable inputs were included. In order to do so, Monte Carlo simulation analysis was used.

The Monte Carlo simulation method can mathematically repeat the sampling process in a spreadsheet to simulate the possible results. Since the variabilities of the variable factors were determined by using distribution functions in MCSA, the outcomes of the method would also be distributions. In this approach, distribution selections and parameter determinations of each distribution were the core steps. This is because the results would be useful only in the case of using proper distributions with reasonable ranges.

A normal distribution is commonly used to present continuous numbers in Monte Carlo simulation analysis by using the mean value and the variance of previously recorded data as the basic parameters. Since the volume of cereals is a continuous variable factor, and since the volume would never be a negative number. A truncated normal distribution was therefore selected to express the volume of cereal, being [Truncated Normal (mean value, variance value), Truncate (0,)].

Considering that only limited historical prevalence rates (expressed by percentages) can be collected, which because it was not possible to sample all the cereals for every mycotoxin-cereal combination. A distribution that only need limited data as the basic parameters is suitable to determine the prevalence rate of mycotoxins in cereals. Since the values of minimum and maximum prevalence rate, and the medium value can be obtained from the collected data, a triangular distribution with three values being minimum(x), medium(y), maximum(z) was applied to represent the prevalence rate. As the outcomes of triangular distributions would not be smaller than the minimum value which would not be negative values, it is not necessary to truncate the distributions.

Another variable factor is EDI value. However, since EDI values were used to determine the probability of the carcinogenic risk, rather than used directly in the calculation, the variability of the probability of the carcinogenic risk instead of EDI values was presented by a distribution. A discrete function was used to express the probability of the carcinogenic risk [Discrete, values of 0.01 (low), 0.1 (medium), 1 (high), 10 (severe)] based on the discrete classes. According to the calculation $c_{ij} = \frac{d_{ij}}{e_j} * f_j$, this probability distribution was then multiplied with the carcinogenic consequence factors which are numbers. Another discrete distribution regarding the carcinogenic risk level was further developed as [Discrete, values of 1 (low), 2 (medium), 3 (high), 4 (severe)] according to the classifications. The reason why the carcinogenic risk level was also a distribution is that the results of multiplying distributions with numbers are still distributions.

In order to accurately simulate the risk-based sample sizes, the iterations of all the distributions were set as 100,000. All simulations were performed by using the @RISK 7.0 software as an add-in function in Microsoft Excel 2016.

3.4 Risk-based sample sizes with budget limitations

Risk-based sample sizes with an unlimited budget were directly calculated by using the collected data in the calculation formula. For the RBSSLBs calculation, assumptions were made for scaling: the total fixed budget for monitoring mycotoxins in cereals per year is €10,000; the cost of each sample is €20 (Van der Fels-Klerx, 2017). In this case, the limited budget allows taking 500 samples in total for all kinds of mycotoxin-cereal combinations per year in the current monitoring programs. Multiplying the ratio of each RBSSUB to the total RBSSUBs with the total RBSSLBs (500), each RBSSLB could be obtained.

4. Results

4.1 Results of the scenario analysis approach

In order to have an overview of the results of the scenario analysis, the RBSSUBs and RBSSLBs in the NL in 2015 and 2016 were calculated by scenario analysis approach and shown in **Table 4-1** and **Table 4-2** respectively.

Table 4-1. Risk-based sample size with an unlimited budget (SA)

	Scenario 1									
	2015					2016				
Cereal	Risk-based sample size					Risk-based sample size				
	AF	DON	ZEA	FB1	OTA	AF	DON	ZEA	FB1	OTA
Wheat	0	1360605	377785	14475	752675	0	1146540	318348	12197	634256
Maize	0	963505	722628	0	0	0	829797	622348	0	0
Barley	0	180553	23264	0	20833	0	232482	29954	0	26825
Oats	0	0	0	0	0	0	0	0	0	0
Rye	2053	1027	0	257	8725	1459	729	0	182	6201
	Scenario 2									
	2015					2016				
Cereal	Risk-based sample size					Risk-based sample size				
	AF	DON	ZEA	FB1	OTA	AF	DON	ZEA	FB1	OTA
Wheat	627471	1884583	2073041	868471	1592198	528750	1588079	1746887	731834	1341695
Maize	563675	3691004	1810763	1016835	0	474991	3110294	1525873	856855	0
Barley	1929937	1077870	342684	21712	1157962	1626297	908287	288769	18296	975778
Oats	0	395637	0	0	0	0	333391	0	0	0
Rye	115796	101322	21712	14475	723726	97578	85381	18296	12197	609862
	Scenario 3									
	2015					2016				
Cereal	Risk-based sample size					Risk-based sample size				
	AF	DON	ZEA	FB1	OTA	AF	DON	ZEA	FB1	OTA
Wheat	1259284	2066962	3994969	1447452	2431720	1061159	1741764	3366435	1219723	2049135
Maize	998742	3799563	1810763	2113281	0	841609	3201773	1525873	1780796	0
Barley	2894905	1186911	342684	43424	1852739	2439446	1000173	288769	36592	1561245
Oats	0	1186911	0	0	0	0	1000173	0	0	0
Rye	115796	144745	43424	14475	955319	97578	121972	36592	12197	805017

Table 4-2. Risk-based sample size with a limited budget (SA)

Scenario 1

Cereal	2015 Risk-based sample size					2016 Risk-based sample size				
	AF	DON	ZEA	FB1	OTA	AF	DON	ZEA	FB1	OTA
Wheat	0	154	43	2	85	0	148	41	2	82
Maize	0	109	82	0	0	0	107	81	0	0
Barley	0	20	3	0	2	0	30	4	0	3
Oats	0	0	0	0	0	0	0	0	0	0
Rye	0	0	0	0	1	0	0	0	0	1

Scenario 2

Cereal	2015 Risk-based sample size					2016 Risk-based sample size				
	AF	DON	ZEA	FB1	OTA	AF	DON	ZEA	FB1	OTA
Wheat	16	47	52	22	40	16	47	52	22	40
Maize	14	92	45	25	0	14	92	45	25	0
Barley	48	27	9	1	29	48	27	9	1	29
Oats	0	10	0	0	0	0	10	0	0	0
Rye	3	3	1	0	18	3	3	1	0	18

Scenario 3

Cereal	2015 Risk-based sample size					2016 Risk-based sample size				
	AF	DON	ZEA	FB1	OTA	AF	DON	ZEA	FB1	OTA
Wheat	22	36	70	25	42	22	36	70	25	42
Maize	17	66	32	37	0	17	66	32	37	0
Barley	50	21	6	1	32	50	21	6	1	32
Oats	0	21	0	0	0	0	21	0	0	0
Rye	2	3	1	0	17	2	3	1	0	17

In both tables, the mycotoxin-cereal combinations with red and dark orange color were the top two risky contaminations. The risk level of contaminations that with dark green color was the lowest. Since the different prevalence rates were used in different scenarios, the results of final RBSSs and the risk rankings in three scenarios vary with numbers and colors. Furthermore, it is noteworthy that the priorities were sorted with the risk-based sample sizes from large to small or based on the colors from dark red to dark green. Some RBSSs were zero in all scenarios, this is caused by the collected prevalence rate which equals zero. By comparing RBSSLBs in scenario 2&3 in 2015 and 2016, the results were the same. The reason is that the minor differences between large numbers of RBSSUBs were mathematically ignored during scaling.

According to the results of risk-based sample size, the most risky mycotoxin-cereal combinations in the Netherlands in both 2015 and 2016 were: DON-Wheat and DON-Maize in scenario 1; DON-Maize in scenario 2; ZEA-Wheat and DON-Maize in scenario 3. Concluding these combinations, four-fifths of mycotoxins is DON, which is caused by the highest prevalence rates. Furthermore, maize and wheat were the main affected crops among the most risky mycotoxin-cereal contaminations because the volumes were bigger than the volume of other cereal grains.

Summarizing the tables, RBSSUBs were quite substantial for practical monitoring since it cost too much: €337,587,829.86 per year on average, with a cost €20 per sample. Moreover, taking too many samples is time-consuming. On the contrary, RBSSLBs which were scaled by a given budget is more practical for real national monitoring programs. This is because: 1) RBSSLBs can be adjusted based on the budget, therefore, both the budget and the total number of samples is controllable; 2) the total number of samples is only 500, which is feasible for people to take samples.

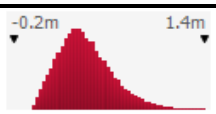
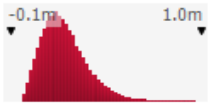
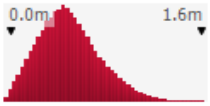
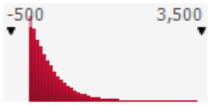
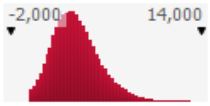
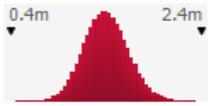
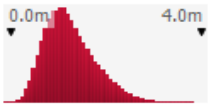
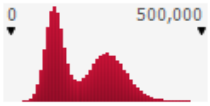
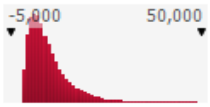
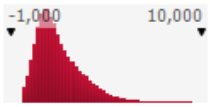
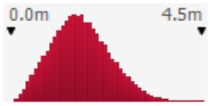
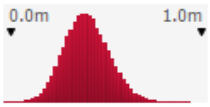
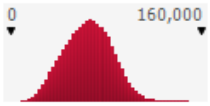
From the results of **Table 4-2**, the RBSSLBs may differ between years because of the variable volumes. However, the risk-based priorities present the same rankings in each scenario although the prevalence rates vary with years. This is because the sample sizes are risk-based, the relative risk levels are fixed once the risk is defined. Moreover, from the color distributions, a conclusion was obtained: scaling process did not change the priority orders.

4.2 Results of the Monte Carlo simulation approach

The results of MCSA were listed in **Table 4-3** and **Table 4-4** to give an overview of RBSSs, both given an unlimited budget and with budget limitations. Simulation output figures,

minimum, mean, maximum, 5th and 95th percentile values were presented for demonstrations in each table.

Table 4-3. Risk-based sample size with an unlimited budget (MCSA)

Contamination	Simulation output	Min value	Mean	Max value	5th percentile	95th percentile
AF-Wheat		1313	418115	1367716	121085	792180
AF-Maize		349	229350	942435	62127	450604
AF-Barley		1713	513407	1583652	153249	939834
AF-Oats		0	393	3338	21	1120
AF-Rye		0	3721	12753	1049	6989
DON-Wheat		511917	1412549	2307418	1071019	1770278
DON-Maize		14854	1304432	3771183	619179	2205453
DON-Barley		46853	192633	462915	99568	315501
DON-Oats		2	6874	47728	1142	17065
DON-Rye		0	1954	9276	467	4228
ZEA-Wheat		237655	1714152	4457649	719968	2815472
ZEA-Maize		3821	410897	922204	238303	597074
ZEA-Barley		13632	66597	146414	36561	95662

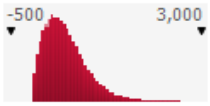
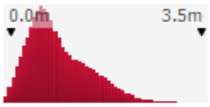
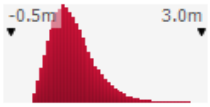
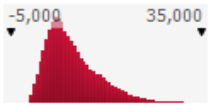
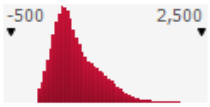
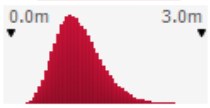
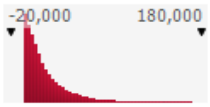
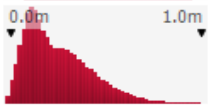
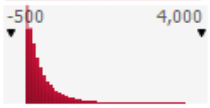
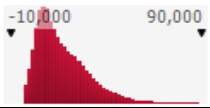
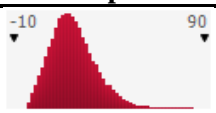
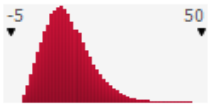
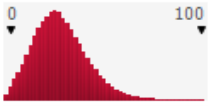
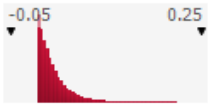
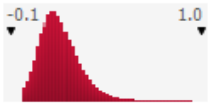
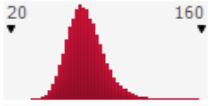
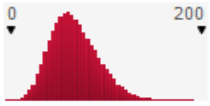
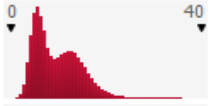
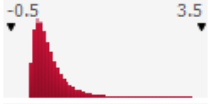
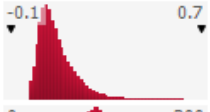
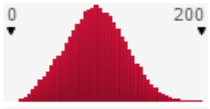
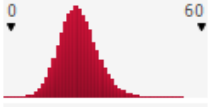
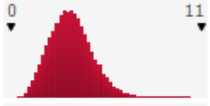
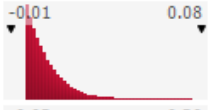
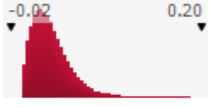
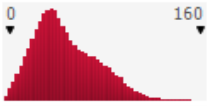
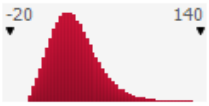
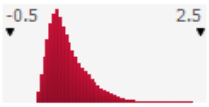
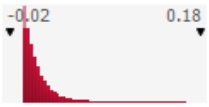
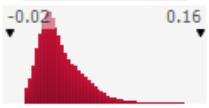
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ZEA-Rye		0	559	2569	98	1211
FB_1-Wheat		11534	929839	3006051	256506	1886076
FB_1-Maize		817	688757	2745608	183752	1378051
FB_1-Barley		21	8319	30611	2146	17613
FB_1-Oats		0	235	2768	12	711
FB_1-Rye		0	559	2125	139	1169
OTA-Wheat		329087	1111204	2554780	637387	1697888
OTA-Maize		0	19103	165913	1064	56565
OTA-Barley		9986	263641	972107	69932	556966
OTA-Oats		0	315	3549	14	981
OTA-Rye		1	18625	86502	3965	41683

Table 4-4. Risk-based sample size with a limited budget (MCSA)

Contamination	Simulation output	Min value	Mean	Max value	5th percentile	95th percentile
AF-Wheat		0	23	82	7	42
AF-Maize		0	12	46	3	24
AF-Barley		0	28	99	8	51
AF-Oats		0	0	0	0	0
AF-Rye		0	0	1	0	0
DON-Wheat		39	77	155	58	98
DON-Maize		1	70	188	36	110
DON-Barley		2	11	35	5	18
DON-Oats		0	0	3	0	1
DON-Rye		0	0	1	0	0
ZEA-Wheat		16	91	197	43	138
ZEA-Maize		0	22	53	14	32
ZEA-Barley		1	4	10	2	6
ZEA-Oats		0	0	0	0	0
ZEA-Rye		0	0	0	0	0

FB_1-Wheat		1	49	150	15	93
FB_1-Maize		0	37	130	11	69
FB_1-Barley		0	0	2	0	1
FB_1-Oats		0	0	0	0	0
FB_1-Rye		0	0	0	0	0
OTA-Wheat		18	60	145	36	89
OTA-Maize		0	1	8	0	3
OTA-Barley		1	14	60	4	30
OTA-Oats		0	0	0	0	0
OTA-Rye		0	1	6	0	2

The expression of results is different with that of the results obtained in scenario analysis. There are no specific samples sizes for each contamination in each year in this approach. However, the simulation output with essential characteristics was given for each mycotoxin-cereal contamination. These key characteristics, especially the mean, 5th and 95th percentile values, can be the supportive evidence for decision-makers to determine the specific risk-based sample sizes. The approach also provides priorities based on the numerical orders of mean values from large to small. Analyzing the **Table 4-4**, it is obvious that the sum of mean values is 500 which equals the total sample size with the limited budget mentioned in section 3.4. Therefore, the total cost would equal the total budget €10,000 if using the mean values as the final applied risk-based sample sizes.

Comparing the mean value of each mycotoxin-cereal combination, the top five risky combinations in the Netherlands throughout 2004 to 2016 were ZEA-Wheat, DON-Wheat, DON-Maize, OTA-Wheat and FB_1-Wheat.

4.3 Comparison of two analysis approaches

By comparing the two results of different approaches, the conclusion can be obtained: 1) the results of SA are specific situations, which are included in the range of the results of MCSA. This is because MCSA contains all possible scenarios in all studied years from 2004 to 2016, whereas the SA only analyzed three scenarios for two years in the current study. It is noteworthy that the results can be exactly the same within a specific situation by using these two different analysis approaches. Consequently, there is no difference between two methods, only the expressions of the results are different.

In order to have an intuitive view of MCSA, some possible results were screen-printed and shown in **Appendix 5**.

5. Discussion

5.1 Designation of risk-based monitoring programs

The current study presents a calculation method that can be used by decision-makers to set up risk-based sample sizes for monitoring mycotoxins in cereals and to decide the priorities in governmental controls at a national level. Two approaches were used to express the inputs, which gives comparable results but with two different kinds of expressions. The scenario analysis approach provided specific risk-based sample size values in each year with three scenarios. Whereas the Monte Carlo simulation analysis presented a range of risk-based sample sizes for each contamination throughout past years, the results are simulations rather than specific numbers. However, the determinations of the priority ranking were the same in both approaches, being sorting the numerical orders of sample sizes from large to small.

The total volume of cereal taken into account both the domestic production volume and the imported volume. Setting up this target group can efficiently include all cereals that have possibilities of being consumed in a specific country. Factor prevalence rate was based on the real historical monitoring data, which is the strong supportive evidence for the model application. The level of the carcinogenic risk on human health was the defined risk in the current risk-based monitoring model. The EDI values were included in this factor to make the risk levels vary with countries and make the model can be applicable for other European countries rather than only for the Netherlands.

In a previous study, it was reported that multiple mycotoxin contaminations were found in most of the tested milk thistle samples (Veprikova et al., 2015). These phenomena are so-called co-occurrence which means more than one mycotoxin was found in one sample. The co-occurrence was highlighted by scientists because the toxic effects of individual mycotoxins may have synergistic interactions during co-occurrence (Veprikova et al., 2015). The most common natural co-occurring mycotoxins found in food and feed in Europe were AFs & OTA (24%), ZEA & DON (15%), DON & NIV (13%), and DON & T-2 toxin (12%) (Smith et al., 2016). DON and ZEA co-contaminations were found in almost fifty percent of analyzed feed samples for dairy cows in the Netherlands, which the samples were mainly compound feed and grass silage (Driehuis et al., 2008). The synergistic toxicities of mycotoxins co-occurring in human diets are not known, but the issue is indeed a potential risk. Although several scientific reports were concerning the co-occurrence (European Food Safety Authority (EFSA), 2013a, 2013b) and the modified (masked) mycotoxins (European Food Safety Authority (EFSA), 2014b) have been published by EFSA, the question of 'how risky the co-occurrence is' cannot be surely answered because of the lack of supportive evidence: the prevalence rate of co-occurrence was rarely recorded, and the carcinogenic consequence of co-occurrence mycotoxins was not classified by IARC.

Due to the data shortage, the calculation cannot be processed for co-occurrence. The co-occurrence of mycotoxins is therefore not included in the current study, which is the first shortcoming of the model. However, the calculation formula is also suitable for co-occurrence contaminations if the inputs were available.

Another drawback of the current model is about scenario analysis approach. Since analyzing all various variables by SA is infeasible, the current scenario analysis approach only discussed three different level of prevalence rates for each mycotoxin-cereal contamination per year in the corresponding three scenarios. The variability of carcinogenic risk levels which is caused by variable EDI values was not considered in the current SA. This approach could be theoretically improved easily by including all variable factors with plenty of scenarios, while the number of scenarios is enormous in that case. Nevertheless, the Monte Carlo simulation approach considered all possible scenarios by proper distributions. Therefore, from the perspective of the entire model, this drawback was weakened by MCSA.

Comparing both results of SA and MCSA, SA presented specific numbers of samples in each year with different scenarios. MCSA provided a range of risk-based sample size values considering all variabilities of the volume of cereal, the prevalence rate and the carcinogenic

risk levels regarding all the analyzed years. It is obvious that the results of MCSA are more accessible to evaluate. This is because only several parameters of simulation results need to be analyzed, while the results of SA must be analyzed from year to year, and from scenario to scenario in every single year.

Moreover, it is noteworthy that more risk factors are existing in reality in different countries. Whereas the current study only considered prevalence rate, dietary exposure to mycotoxins (EDI values), and the carcinogenic consequences. The other adverse effects on animal and human health were ignored, e.g., acute effects, chronic diseases, neurologic toxicity and so on. However, the current model allows being improved by involving more risk factors with proper definitions and classifications in the current calculation. The determination of overall risk levels, when involving more risk factors in the model, can refer to the methodology of Khadam & Kaluarachchi (Khadam & Kaluarachchi, 2003) that mentioned in section 2.2.3.

5.2 Application in the NL

The shortcoming of the practical application is that only five mycotoxins and five types of cereals in the NL were included in the study. In reality, there are more affected crops such as seeds, nuts and dried fruits. The natural mycotoxins, such as NIV, T-2, HT-2 toxin, were also neglected in the application. Therefore, the current priority ranking is not fully comprehensive for national monitoring programs. However, regarding the budget limitations, the final RBSSLBs of those less risky mycotoxin-cereal contaminations might be zero according to the results in Chapter 4. In this case, the priority settings may not be affected.

5.3 Further study

To improve the current study, more mycotoxin-cereal combinations can be involved to have a more comprehensive overview for monitoring mycotoxins in cereals. For the calculation formula, various identified risks on both human and animal health can be defined and included in the calculations by using the same methodology that used in the current model.

Furthermore, if enough data of co-occurrence is available, the model can be improved to monitor both single-occurring mycotoxins and co-occurring mycotoxins in cereals in the follow-up study.

Another idea for a follow-up study is that modifying the sampling plan based on other countries' regulations rather than only based on EC regulations. In this way, different calculation formulas can be used under different regulation frameworks for different governmental monitoring.

6. Conclusions

Based on the results of model application in the Netherlands, it is obvious that some mycotoxin-cereal combinations were more risky than others, e.g., Zearalenone-Wheat, Deoxynivalenol-Wheat, Deoxynivalenol-Maize. This is because the volume of wheat and that of maize were larger than the volume of other three kinds of cereals (barley, oats and rye) in the Netherlands. Considering the risk-based priority of all studied mycotoxin-cereal contaminations, the priority rankings that were obtained from Monte Carlo simulation analysis (MCSA) and the scenario analysis (SA) were different. This is due to MCSA analyzed all the possible scenarios by simulating, while SA only analyzed risk-based sample sizes with three scenarios per year, in other words, the results of SA is only three scenarios from all scenarios that analyzed by MCSA.

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Appendix 1: Data and descriptive statistics of the volume

The data of the volume is obtained from Eurostat database. Eurostat is an official statistical database of European Union, which aims to provide high-quality statistics for Europe. According to the production volumes, the top five kinds of cereals were wheat, maize, barley, oats and rye. In the current study, the total volumes of these five kinds of cereals between 2004 and 2016 were used for analyzing and summarized in **Table A1-1**. These total volumes of cereals include both the imported volume and the volume of domestic production.

Table A1-1. Descriptive statistics of the volume of cereals

Year	**Cereal**				
	Wheat	Maize	Barley	Rye	Oats
2004	4955.36	2547.86	1438.67	132.39	52.31
2005	4639.45	2548.97	1260.38	217.20	33.89
2006	5535.72	2720.13	1813.67	162.70	68.67
2007	6013.82	3759.05	1727.63	111.35	48.02
2008	6261.25	4050.51	1781.57	163.61	40.01
2009	6342.88	3391.20	1867.91	391.61	28.42
2010	6582.11	3182.58	2218.21	115.65	50.40
2011	4892.88	3761.56	1863.12	115.17	54.55
2012	5379.32	4284.81	1825.90	240.91	59.41
2013	5318.90	4531.67	2163.49	300.33	81.00
2014	5797.90	5614.97	2090.45	209.53	105.07
2015	7237.26	5018.25	1736.09	128.32	87.69
2016	6098.62	4321.86	2235.40	91.18	103.40

Mean	5773.50	3825.65	1847.88	183.07	62.53
Standard Deviation	716.40	908.32	276.24	83.92	24.05

Sources: Agriculture, fishery and forestry statistics, main results – 2010 – 11 2012 edition; 2013 edition; 2014 edition; 2015 edition (EUROSTAT, 2013, 2014, 2015, 2016); Crop statistics of agricultural production (EUROSTAT, 2018a); International trade in goods-detailed data (EUROSTAT, 2018b).

Appendix 2: Descriptive statistics of the prevalence rate

Since the previous national monitoring data of prevalence rate was owned by national reference laboratory and was not published, the descriptive statistics were collected only from public reports and relevant literature. The definition of prevalence rate is the percentage of positive samples (above the LOD values) over the total number of samples. The overviews of prevalence rates of each mycotoxin in cereals were presented in **Table A2-1, A2-2, A2-3, A2-4, A2-5**. In these tables, some prevalence rates that collected from neighbouring western European countries were used as the data of the Netherlands since the Dutch data was not available.

Table A2-1. Descriptive statistics of the prevalence rate of aflatoxins

Cereal	Number of positive samples	Total number of samples	Prevalence rate	Reference
Wheat	117	405	29%	(Andrade & Caldas, 2015)
	0	11	0%	(Binder et al., 2007)
Maize	435	1858	23%	(Andrade & Caldas, 2015)
	3	14	21%	(Binder et al., 2007)
	7	94	7%	(Boon et al., 2009)
Barley	0	3	0%	(Binder et al., 2007)
	122	123	100%	(Ibáñez-Vea et al., 2012)
	44	44	100%	(Ibáñez-Vea et al., 2012)
	68	68	100%	(Ibáñez-Vea et al., 2012)
Oats	0	0	0%	(Binder et al., 2007)

Table A2-2. Descriptive statistics of the prevalence rate of DON

Cereal	Number of positive samples	Total number of samples	Prevalence rate	Reference
Wheat	227	487	47%	(Boon et al., 2009)
	157	254	62%	(Binder et al., 2007)
	:	:	71.4%	(Van Der Fels-Klerx, Klemsdal, et al., 2012)
Maize	197	244	81%	(Binder et al., 2007)
	:	:	87.5%	(Van Der Fels-Klerx, Klemsdal, et al., 2012)
	57	86	66%	(Van der Fels-Klerx, De Rijk, et al., 2012)
	15	21	71%	(Van der Fels-Klerx, De Rijk, et al., 2012)
Barley	:	:	35.7%	(Van Der Fels-Klerx, Klemsdal, et al., 2012)
	81	161	50%	(Binder et al., 2007)
	9	35	26%	(Boon et al., 2009)
Rye	2	39	5%	(Boon et al., 2009)
	3	139	2%	(Van Der Fels-Klerx, Klemsdal, et al., 2012)
Oats	41	50	82%	(Binder et al., 2007)

: Not available

Table A2-3. Descriptive statistics of the prevalence rate of ZEA

Cereal	Number of positive samples	Total number of samples	Prevalence rate	Reference
Wheat	44	86	51%	(Van der Fels-Klerx, De Rijk, et al., 2012)
	12	21	57%	(Van der Fels-Klerx, De Rijk, et al., 2012)
	:	:	8.7%	(Van Der Fels-Klerx, Klemsdal, et al., 2012)
	44	48	92%	(Binder et al., 2007)
Maize	59	93	63%	(Binder et al., 2007)
	:	:	37.4%	(Van Der Fels-Klerx, Klemsdal, et al., 2012)
Barley	:	:	6.7%	(Van Der Fels-Klerx, Klemsdal, et al., 2012)
	9	81	11%	(Binder et al., 2007)
	48	123	39%	(Ibáñez-Vea et al., 2012)
	16	44	36%	(Ibáñez-Vea et al., 2012)
	28	68	41%	(Ibáñez-Vea et al., 2012)
Oats	0	11	0%	(Binder et al., 2007)

: Not available

Table A2-4. Descriptive statistics of the prevalence rate of Fumonisin B_1

Cereal	Number of positive samples	Total number of samples	Prevalence rate	Reference
Wheat	1	1	100%	(Binder et al., 2007)
	2	340	1%	(Boon et al., 2009)
Maize	9	16	56%	(Binder et al., 2007)
	71	97	73%	(Boon et al., 2009)
Barley	0	1	0%	(Binder et al., 2007)
	1	30	3%	(Boon et al., 2009)
Rye	1	138	1%	(Boon et al., 2009)
Oats	0	1	0%	(Binder et al., 2007)

Table A2-5. Descriptive statistics of the prevalence rate of OTA

Cereal	Number of positive samples	Total number of samples	Prevalence rate	Reference
Wheat	12	91	13%	(Boon et al., 2009)
	5	12	42%	(Binder et al., 2007)
Maize	0	11	0%	(Binder et al., 2007)
Barley	72	123	58%	(Ibáñez-Vea et al., 2012)
	28	44	64%	(Ibáñez-Vea et al., 2012)
	36	68	53%	(Ibáñez-Vea et al., 2012)
	1	31	3%	(Binder et al., 2007)
Rye	2	6	33%	(Boon et al., 2009)
Oats	0	0	0%	(Binder et al., 2007)

Appendix 3: Probability of the carcinogenic risk

The probability of the carcinogenic risk was classified based on the results of the ratio (EDI/TDI) according to **Table 3-2**. The EDI values of Dutch were assessed in the previous assessment reports and used in the current study for calculations. In order to have an overview of EDIs, the specific EDI values of each mycotoxin-cereal combination and the corresponding probabilities were summarized in **Table A3-1**.

Table A3-1. Probability determination of the carcinogenic risk

Mycotoxin	Cereal	Population	EDI value (ng/kg bw/day)	Reference	Probability
DON	Wheat	Adults	10.3	(Gareis, 2003)	1
		Children (1-4yrs)	77	(Gareis, 2003)	1
		Children (1-6yrs)	49	(Gareis, 2003)	1
	Maize	Adults	30	(Gareis, 2003)	1
		Children (1-4yrs)	104	(Gareis, 2003)	10
		Children (1-6yrs)	95	(Gareis, 2003)	1
	Barley	Adults	25	(Gareis, 2003)	1
		Children (1-4yrs)	0	(Gareis, 2003)	0.01
		Children (1-6yrs)	0	(Gareis, 2003)	0.01
	Oats	Adults	0	(Gareis, 2003)	0.01
		Children (1-4yrs)	1	(Gareis, 2003)	0.1
		Children (1-6yrs)	1	(Gareis, 2003)	0.1
	Rye	Adults	6	(Gareis, 2003)	0.1

		Children (1-4yrs)	17	(Gareis, 2003)	1
		Children (1-6yrs)	14	(Gareis, 2003)	1
ZEA	Wheat	Adults	20.3	(Gareis, 2003)	10
		Children (1-4yrs)	45.1	(Gareis, 2003)	10
		Children (1-6yrs)	48.6	(Gareis, 2003)	10
	Maize	Adults	0.4	(Gareis, 2003)	0.1
		Children (1-4yrs)	1.3	(Gareis, 2003)	0.1
		Children (1-6yrs)	1.2	(Gareis, 2003)	0.1
FB_1	Maize	Adults	3.1	(Gareis, 2003)	0.1
		Children (1-4yrs)	0	(Gareis, 2003)	0.01
		Children (1-6yrs)	0	(Gareis, 2003)	0.01
OTA	Wheat	Whole population	1.28	(Miraglia & Brera, 2002)	1
		Whole population	14.68	(Miraglia & Brera, 2002)	10

Since daily intakes of aflatoxins were reported very low, aflatoxins were not considered as a health risk for Dutch people (G. Bakker et al., 2009). However, since the RASFF published lots of notifications on aflatoxins, the probability of carcinogenic risk of aflatoxins was assumed as 0.1 (medium).

Appendix 4: Carcinogenic consequence factors

According to the carcinogenicity descriptions of mycotoxins in **Table 2-4** and the classifications of carcinogenic consequences in **Table 3-2**, the carcinogenic consequence factors of mycotoxins were presented in **Table A4-1**.

Table A4-1. Classifications of the carcinogenic consequence factor of mycotoxins

Mycotoxin	Carcinogenic consequence factor
Aflatoxins	10 (Severe)
Deoxynivalenol	0.1 (Medium)
Zearalenone	0.1 (Medium)
Fumonisins	1 (High)
Ochratoxin A	1 (High)

Appendix 5: Possible results of MCSA

Since the results of MCSA are simulations, the specific scenario would randomly present in the @RISK 7.0 software. Specific values of RBSSs can only be screen-printed. In order to have an intuitive overview, three possible scenarios were presented in Figure A5-1, A5-2, and A5-3.

without budget limitation						
risk-based sample size		AFLATOXIN	DON	ZEA	FB1	OTA
	Wheat	447045	1950423	1582191	689270	1274429
	Maize	387968	1974584	470730	650191	4747
	Barley	199833	111012	52059	3199	442922
	Oats	458	24385	9	125	349
	Rye	4265	1421	1446	533	13174
with budget limitation						
risk-based sample size		AFLATOXIN	DON	ZEA	FB1	OTA
	Wheat	22	95	77	34	62
	Maize	19	96	23	32	0
	Barley	10	5	3	0	22
	Oats	0	1	0	0	0
	Rye	0	0	0	0	1

Figure A5-1. Risk-based sample sizes of MCSA (possible scenario 1)

without budget limitation

		AFLATOXIN	DON	ZEA	FB1	OTA
risk-based sample size	Wheat	636463	1767319	1793507	2265115	1746238
	Maize	191009	1006900	440884	765937	5124
	Barley	658090	124613	43456	20089	128427
	Oats	708	15000	253	230	160
	Rye	6380	3659	248	532	40629

with budget limitation

		AFLATOXIN	DON	ZEA	FB1	OTA
risk-based sample size	Wheat	27	76	77	97	75
	Maize	8	43	19	33	0
	Barley	28	5	2	1	6
	Oats	0	1	0	0	0
	Rye	0	0	0	0	2

Figure A5-2. Risk-based sample sizes of MCSA (possible scenario 2)

without budget limitation

		AFLATOXIN	DON	ZEA	FB1	OTA
risk-based sample size	Wheat	686486	1669706	2969314	462257	1283135
	Maize	221889	1399034	469940	591683	5268
	Barley	718338	211803	37513	9093	204501
	Oats	142	7024	104	104	54
	Rye	4272	855	152	356	26394

with budget limitation

		AFLATOXIN	DON	ZEA	FB1	OTA
risk-based sample size	Wheat	31	76	135	21	58
	Maize	10	64	21	27	0
	Barley	33	10	2	0	9
	Oats	0	0	0	0	0
	Rye	0	0	0	0	1

Figure A5-3. Risk-based sample sizes of MCSA (possible scenario 3)