Methodological Advances to Improve the Cost-effectiveness of Monitoring for Mycotoxins in Cereal Grains

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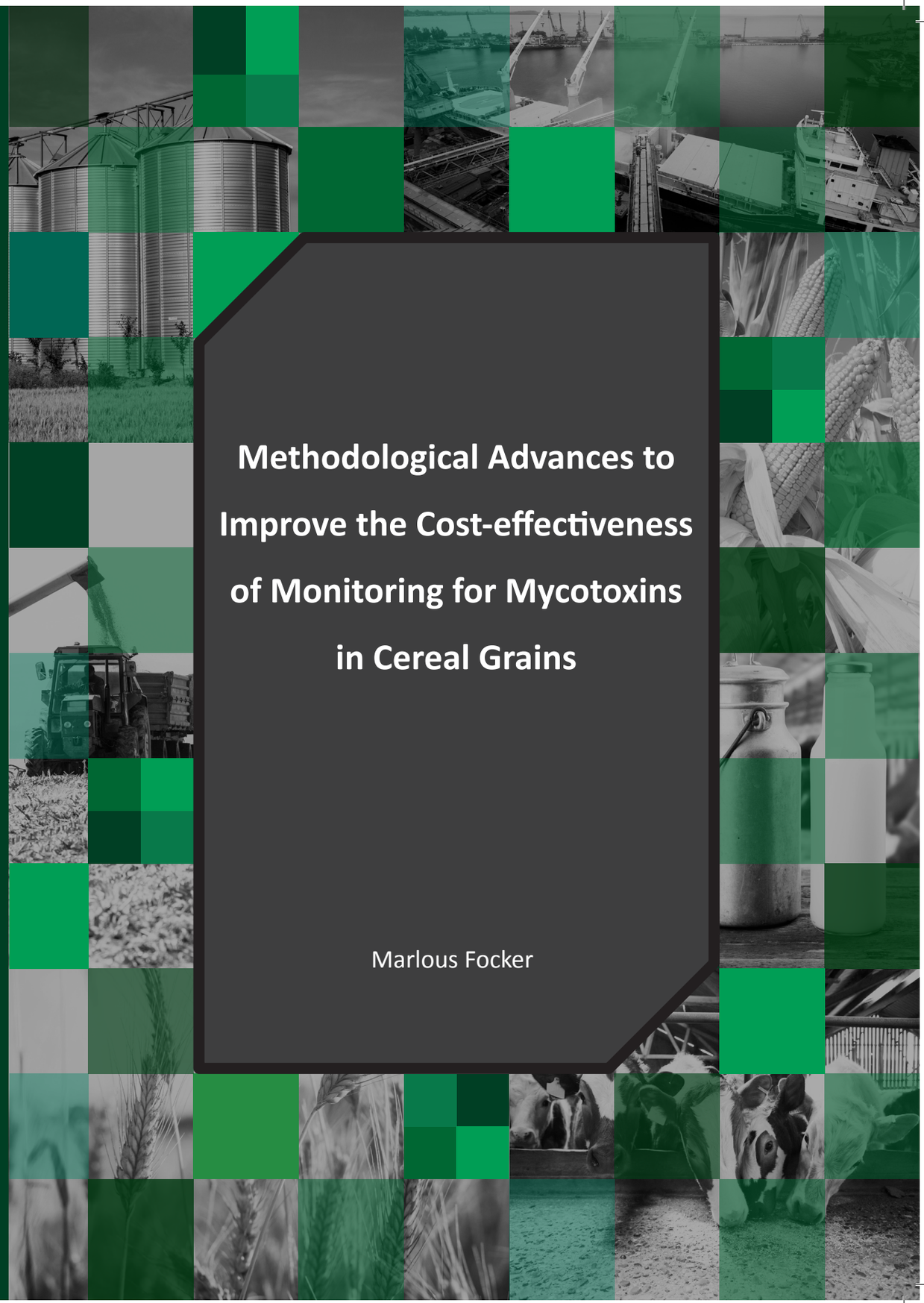

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This research was conducted under the auspices of the Wageningen School of Social Sciences (WASS).

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Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 11 December 2019
at 4 p.m. in the Aula.

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Methodological Advances to Improve the Cost-effectiveness of Monitoring for Mycotoxins in Cereal Grains
150 pages

PhD thesis, Wageningen University, Wageningen, the Netherlands (2019)
With references, with summary in English

ISBN: 978-94-6395-173-9
DOI: https://doi.org/10.18174/503936

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Chapter 1

Introduction

1.1. Background

1.1.1. Mycotoxins

Mycotoxins are toxins produced by certain species of filamentous fungi under favourable environmental conditions. Most fungi are able to produce multiple mycotoxins, and one mycotoxin can be produced by different fungi. Mycotoxins are toxic to animals (especially vertebrates) and human (Zain, 2011). Exposure of humans or animals to mycotoxins through ingestion of contaminated foods and feeds can lead to two types of negative health effects: acute effects with a rapid onset and an obvious toxic response, or chronic effects, seen after a low dose exposure over a longer period of time. Examples of chronic effects are immune suppression or cancer in human and a lower productivity in animals. Chronic exposure to mycotoxins accounts for the major burden on animal and human health, even though acute effects are the best known (Bennett and Klich, 2003).

Mycotoxins are a very diverse group of chemicals: they are produced by different fungal species, have diverse chemical structures and have different biological effects (Bennett and Klich, 2003). This thesis focusses on two main mycotoxins in Europe, based on their occurrence and/or on their toxicity: aflatoxins and deoxynivalenol (DON). DON is the most prevalent mycotoxin in crops in temperate climates observed in Europe, North America or Oceania. For example, DON was found in 63% of the samples in Europe in 2018 (BIOMIN, 2018). DON is mainly produced by *Fusarium* species and is prevalent in small grain cereals like wheat and barley. After exposure to DON, symptoms such as vomiting, abdominal pain, and diarrhoea can be observed in human, and reduced feed intake, weight loss and reproductive disorders can be observed in pigs (Maresca, 2013).

Aflatoxins are not the most prevalent mycotoxins in crops grown in Europe but they can lead to the most severe toxic effects in animals and human. Aflatoxins are a group of mycotoxins; the major ones being aflatoxins B_1 (AFB_1), B_2, G_1, G_2 and M_1 (AFM_1). Aflatoxins are classified by the IARC (International Agency for Research on Cancer) in group 1 as human carcinogen (IARC, 2012). Aflatoxins are produced by several *Aspergillus* species, of which *Aspergillus flavus* is most common in cereals and *Aspergillus parasiticus* in nuts (Prandini *et al.*, 2009). Aflatoxins are prevalent in food crops in tropical and sub-tropical areas, such as South Asia, South America and Africa (BIOMIN, 2018). In addition, during the last decade, aflatoxins have been observed in high frequency in Southern Europe. They were present in 55% of the cereal samples in 2012, in 40% of the samples in 2014, in 38% of the samples in 2016, and in 21% of the samples in 2018 (BIOMIN, 2012; 2015; 2016; 2018). Furthermore, climate change is expected to increase the probability of aflatoxins being present in food crops (Battilani *et al.*, 2016; van der Fels-Klerx and Camenzuli, 2016). Although aflatoxins

are mainly observed in cereal grains and nuts, aflatoxins can carry over to dairy products. When cows consume feed contaminated with AFB_1, this toxin is metabolised in the cow's body into a hydroxylated form: AFM_1; this form is then excreted in the dairy cow's milk (van der Fels-Klerx and Camenzuli, 2016).

In the European Union (EU), legal limits are set for the maximal presence of aflatoxins and DON in food crops and food products (EU, 2006a). The margin of exposure (MOE) – the ratio between the dose at which a small but measurable adverse effect is first observed and the level of exposure to the substance – for aflatoxins, for the Dutch population, was estimated to be in the range of 57 – 210, depending on the age group. This MOE is below 10,000, which is, the minimum value above which a potential health risk is negligible (RIVM, 2017). For DON in wheat, a Tolerable Daily Intake (TDI) of 1µg/kg BW/day was estimated; this TDI is frequently exceeded in Europe for the groups of infants, toddlers and other children (EFSA, 2017). The low MOE for aflatoxins and the frequent exceedance of the TDI for DON show that legal limits are necessary to protect the human population for a too high exposure to these mycotoxins.

In the EU, for feed products, legal limits are set for the maximal presence of AFB_1 (EU, 2002a) and guidance limits are set for the presence of DON in raw materials and feed products (EU, 2006b). For aflatoxins, adverse health effects are seen in animals when the concentration is above 1,500µg/kg feed. The European Food Safety Authority (EFSA) concluded that the legal limit of 20µg/kg for animal feed adequately protects animal health with a factor of 75 between the concentration where adverse health effects are seen and the limit of 20µg/kg (EFSA, 2004). EFSA estimated that chronic animal health risk for DON is low. However, for pigs, a possible risk related to chronic adverse health effects was identified at the 95^{th} percentile of dietary concentrations (EFSA, 2017).

Mycotoxins are heat stable and cannot completely be eliminated during processing of feed and food (Kabak, 2009). Therefore, high mycotoxin concentrations in raw cereals have to be avoided. In the arable fields, mycotoxins can be controlled, although in a limited way, by applying Good Agricultural Practices such as an appropriate crop rotation, the use of a resistant cultivar, the use of fungicides, and the drying of the crops directly after harvest (Waseem *et al.*, 2014). However, local weather is one of the main factors determining fungal infection of the crop and production of mycotoxins. With favourable weather conditions, i.e. warm and dry summers for aflatoxins, and cool and humid summers for DON , the presence of mycotoxins is often unavoidable. Hence, contaminated batches have to be identified and removed from the feed and food supply chain by regulations, monitoring and tracing (Bennett and Klich, 2003).

1.1.2. Monitoring

Effective monitoring systems need to be in place to check raw materials for food and feed and their derived products as part of Hazard Analysis and Critical Control Points (HACCP) programs in the feed and food industry and for compliance with the legal limits and standards. Monitoring of mycotoxins in cereal grains is done by regular collection of samples from batches along the supply chain and the chemical analysis of the individual or aggregated samples with a detection method for the presence of mycotoxins. A batch is here defined as a quantity of cereals that is contained in the same silo/truck/ship compartment. A sampling and analytical (S&A) plan consists of three steps: first, the sample collection step, in which multiple samples are collected from the batch; second, the sample preparation step, in which the collected samples are aggregated and mixed; and, third, the analysis of a sub-sample with a mycotoxin detection method. Since the contaminated particles (e.g. kernels) are not distributed uniformly throughout the batch, the final sample should be an aggregation of several or many small (incremental) samples collected from as many different places in the batch as possible (Whitaker, 2006). Due to heterogeneous distribution of the mycotoxins in the batch and the imprecision of the detection method, uncertainty is associated with a sampling and analytical plan. To express the degree of uncertainty of a S&A plan, the variance between the mycotoxin concentrations of the samples is used. The total variance of a sampling and analytical plan is the sum of the variances of each of the three steps: sample collection, sample preparation and the analysis. The smaller the variance, the higher the performance of the sampling and analytical plan. In the first step of sample collection, the more samples are collected, the lower the variance associated with this step. In the second sample preparation step, the better the final sample is mixed and ground, the lower the variance associated with this step. In the third step, i.e. the chemical analysis for the presence of mycotoxins, the better an detection method is able to identify the true mycotoxin concentration, the lower the analytical variance. The sum of the variances due to sample collection, sample preparation the analysis determines the total variance of the sampling and analytical plan (Whitaker, 2006). For foodstuffs, the criteria that a S&A plan should fulfil for official purposes are described in the EU Regulation No 401/2006 (EU, 2006c). Regulation No 152/2009 describes the criteria for S&A plans for official control of mycotoxins in feed (EU, 2009).

The size of the analytical variance mainly depends on the performance of the detection method used: instrumental methods like LC-MS/MS tend to result in a lower variance than faster, non-instrumental detection methods such as Enzyme-Linked Immunosorbent Assay (ELISA) or Lateral Flow Devices (LFDs). ELISA and LFDs are immunological tests that are based on the interaction between antibodies and mycotoxins: there can easily be cross-

reactivity, for example, between DON derivatives and the antibody, and thus overestimation of the concentration of DON in the sample. The matrix – referring to the components present in the cereal sample other than the mycotoxin – can also influence the specific binding of the antibody and the mycotoxin. The EU does not impose a specific detection method for mycotoxins in food and feed but specific guidelines for validation and verification for mycotoxin screening methods, regarding, amongst others, the concentration range, the recovery, the reproducibility and repeatability, the sensitivity and selectivity, are set in Commission regulation (EU) No 2014/519, amending Regulation No 401/2006. These guidelines were applied to validate some ELISA and LFD methods (Lattanzio *et al*., 2019).

1.1.3. Economic consequences of mycotoxins

Monitoring plans for mycotoxins in food and feed products and their raw materials should be as effective as possible to limit the probability of contaminated batches entering the market, and of related negative health effects after human an animal exposure. With effectiveness we here mean the probability of identifying the true mean mycotoxin concentration in a batch. However, monitoring for mycotoxins is costly and time consuming, so the direct costs of monitoring, e.g. the costs to collect samples and costs of the detection method, should be considered, but also the indirect costs, such as the costs due to batches being downgraded from food to feed, or human and animal health costs.

The economic aspect of monitoring for mycotoxins has not been widely discussed in scientific literature. Mitchell *et al.* (2016) touched upon this topic by estimating the potential losses due to aflatoxins in the United States. These authors included the probabilities of false positive and false negative test results when monitoring batches (Mitchell *et al.,* 2016). However, costs of different monitoring schemes were not compared and indirect costs of imperfect monitoring, such as potential recall costs in case contaminated batches are identified in the downstream stages of the supply chain, were not considered. To the best of our knowledge, no other studies about the economic aspects of monitoring for mycotoxins are publically available.

Other economic consequences of mycotoxin contaminated food and feed batches, not directly related to monitoring, have been described. First, several research groups estimated the direct economic impact of batches contaminated with mycotoxins, such as related to border rejections and to cereal batches downgraded from food to feed. Examples are the studies of Vardon *et al*. (2003) and Mitchell *et al*. (2016) estimating the total annual losses in the United States due to aflatoxins, fumonisins and DON (Vardon *et al.,* 2003; Mitchell *et al*., 2016). Second, the indirect economic impact of contaminated batches with mycotoxins, such as the impact on human health, was estimated. Most work in this area has been done

on aflatoxins, since this toxin leads to acute health effects and possible human health end-points, such as acute aflatoxicosis, hepatocellular carcinoma (HCC), immunosuppression and stunted growth in children, can thus be identified (IARC, 2012). Liu and Wu (2010) and Liu *et al.* (2012) estimated the global burden of aflatoxin-related HCC (Liu *et al.*, 2012; Liu and Wu, 2010). Third, a few studies estimated the economic impact due to feed contaminated with mycotoxins on animal health (Wu and Munkvold, 2008; Wu, 2007). Fourth, a few papers described the cost-effectiveness of control measures taking into account the direct economic consequences for developed countries, and taking into account both the direct economic consequences and the indirect economic consequences, such as the impact on human health, in low income countries (Wu, 2004; Wu and Khlangwiset, 2010; Zorn *et al.*, 2017).

1.2. Problem statement

Mycotoxins are highly heterogeneously distributed in cereal grain batches. In the major part of the batch, the concentration is often low, whereas only a few spots are highly contaminated. Because of this heterogeneous distribution, samples collected at different locations in the batch will have different mycotoxin concentrations. One of the challenges is to collect the right number of samples to reflect the overall mean concentration of the batch. Another challenge is to accurately determine the concentration from the final sample. However, the most accurate analytical methods are usually also the most resource demanding methods.

The resources available for mycotoxin monitoring in cereal supply chains are limited and, therefore choices have to be made to effectively allocate the resources available between the number of sample to collect, the control points in the supply chain where to collect samples, and the method of analysis of the samples. Given pre-defined available resources, the more resources are spent on the analysis, the less is available for the collection of the samples, and vice versa. For mycotoxins that are not only produced in the field but can also be produced under sub-optimal conditions during storage and transport, such as aflatoxins, an extra challenge in the monitoring of these mycotoxins is to check at the right control points along the supply chain. So, resources for monitoring have to be divided over the steps of sample collection, preparation and analysis, and choices have to be made so to optimise the effectiveness of the sampling and analytical plan given a pre-set budget. Also, total resources that are spent on monitoring should be in line with the potential economic impact of mycotoxins under imperfect monitoring.

1.3. Objectives of the thesis

The overall objective of this thesis was to develop methods for cost-effective monitoring of mycotoxins in batches of cereal grains along the supply chain. This main objective was broken down into the following four sub-objectives:

1) To estimate the direct financial impact due to maize batches contaminated with aflatoxins and a monitoring scheme that does not detect the contaminated batches effectively.

2) To identify methods for evaluating the cost-effectiveness of monitoring hazards in the life sciences, assess the strengths and weakness of these methods and discuss these methods in the context of food safety;

3) To develop a method for designing cost-effective sampling and analytical plans for mycotoxins in a cereal batch;

4) To develop a method for providing insights into the most critical control points, and the number of samples required, to obtain a pre-defined concentration of aflatoxins at the end of the maize supply chain;

1.4. Outline of the thesis

This thesis consists of six chapters, of which Chapters 2, 3, 4, and 5 focus on the four sub-objectives described in section 1.3, and Chapter 6 presents a synthesis of the results.
Chapter 2 addresses the first objective, with a focus on the 2013 aflatoxin incident in Europe. This incident was caused by elevated concentrations of aflatoxins in maize grown in South Europe. This aflatoxin-contaminated maize was transported to other countries in Europe where it was processed into, amongst others, compound feed for dairy cows, which eventually led to milk exceeding the EU legal limit for AFM_1. This incident was described and the financial losses were estimated for the most important stakeholders in the Netherlands.
Chapter 3 addresses the second objective and presents a literature review describing the methods published in scientific literature to design cost-effective monitoring schemes for hazards in animals (diseases), plants (pests), soil, water, food, and animal feed. Next, these methods were assessed in terms of their applicability to food safety hazards, in this case mycotoxins, so that this chapter served as a starting point for the next two chapters.
Chapter 4 addresses the third objective of the thesis (see section 1.3) and develops a method for determining the optimal number of samples to collect from a batch and the detection method to use given a pre-set budget. This chapter focusses on two mycotoxin-crop combinations, being DON in a wheat batch and aflatoxins in a (kernel) maize batch.

Chapter 5 addresses the fourth objective of this thesis, focussing on the case of aflatoxins in the maize supply chain in the Netherlands. Most of the maize used for feed and food production in the Netherlands is imported, so the supply chain includes several transport steps. This chapter determines the optimal control points along the maize supply chain, the number of batches to sample at these control points, and the number of samples to collect from each batch to identify contaminated batches and to obtain an aflatoxin concentration in the final batches that is below a certain pre-set limit.

Finally, Chapter 6 synthesises the results of the previous chapters and provides recommendations for business, policy makers and further research. Figure 1.1 summarises the outline of the thesis as well as the links between the different chapters.

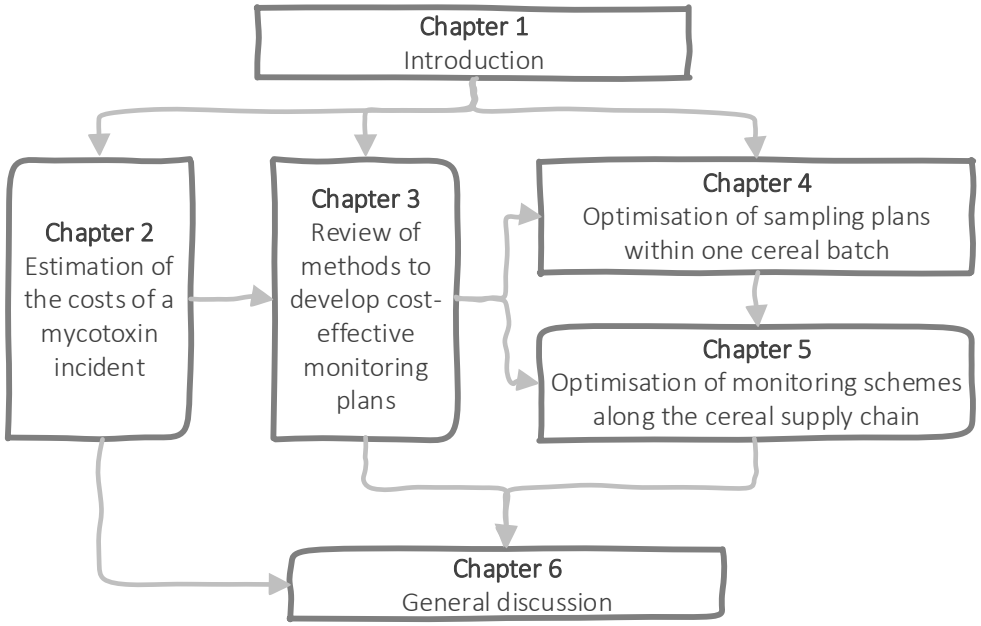

Figure 1.1. Outline and links between the chapters of this thesis

Chapter 2

Financial Losses for Dutch stakeholders during the 2013 Aflatoxin Incident in Europe

M. Focker, H.J. van der Fels-Klerx, A.G.J.M. Oude Lansink

(Submitted to a peer reviewed journal)

Abstract

Early 2013, high concentrations of aflatoxin M_1 were found in the bulk milk of a few dairy farms in the Netherlands and Germany. These high concentrations were due to aflatoxin B_1 contaminated maize from the Black Sea area that was processed into compound feed, which was fed to dairy cows. Since the contamination was discovered in the downstream stages of the supply chain, multiple countries and stakeholders were involved and recalls of the feed were necessary, which resulted into financial losses. The aim of this study was to estimate the financial losses related to the 2013 aflatoxin incident for three stakeholders, being the maize traders, the feed industry and the dairy sector, in the Netherlands, and its neighbouring countries Germany and Belgium. First, the sequence of events of the incident was retrieved. Data on the incident and financial losses were collected from literature, news items on the internet and in-depth interviews. A Monte Carlo simulation model was built to combine the scarce and uncertain data to estimate the financial losses for each stakeholder. The estimated total direct financial losses of this incident for all three countries and all three stakeholders was estimated to be in the range of tens of millions of euros. The largest share, about 70%, of the total losses were endured by the maize traders. These losses were due to imported maize exceeding the legal limit for aflatoxins which was not yet sold to the feed industry. About 30% of the total losses were for the feed industry. These losses were due to contaminated feed that was sold and delivered to dairy farms. Recalls and replacements of the contaminated feed were organised. Since the contamination was discovered before any dairy milk was sold to consumers, less than 1% of the total losses were for the dairy sector.

2.1. Introduction

Aflatoxins are a worldwide issue for human and animal health as well as the economy. Although aflatoxins are more likely to be a problem in tropical and sub-tropical areas, the annual losses due to aflatoxins in US corn have been estimated to be about USD 163 million (Wu, 2006). Even though Europe might not be seen as a high risk area, in Southern Europe, aflatoxins were detected in 55% of the cereal samples collected in 2012, in 40% of the samples in 2014, in 38% of the samples in 2016, and in 21% of the samples in 2018 (BIOMIN, 2012; 2015; 2016; 2018). Furthermore, climate change may lead to an increased probability of the presence of aflatoxins (van der Fels-Klerx *et al.*, 2019). One of the most important aflatoxins is aflatoxin B_1 (AFB_1), which is frequently found in cereals such as maize and in nuts. When exposed to AFB_1 for a longer period of time, it can lead to complications such as immunotoxicity, hepatotoxicity, and teratogenicity in human (Kumar *et al.*, 2017). Furthermore, when cows are fed with feed contaminated with AFB_1, the toxin is metabolised in the cow's body and excreted in the milk as the metabolite aflatoxin M_1 (AFM_1) (van der Fels-Klerx and Camenzuli, 2016). In most countries, maximum (legal) limits are set for aflatoxins as a group and/or for AFB_1 alone in food and feed products and/or for AFM_1 in milk. In the European Union (EU), different limits are set for aflatoxins for different types of products. For example, in food, the legal limit for aflatoxins for all cereals and all products derived from cereals, including processed cereal products for AFB_1 is 2µg/kg. The legal limit for AFM_1 in milk is 0.05µg/kg (EU, 2006a). The legal limit for AFB_1 in most feed products is 20µg/kg, however, the limit for compound feed for dairy cattle is 5µg/kg (EU, 2002a). Many feed companies in the Netherland use a lower limit for maize used in compound feed for dairy cows: 2.5µg/kg (SecureFeed, 2018).

In February and March 2013, as part of regular monitoring, AFM_1 was found to be present in the milk tanks of dairy farms in the Netherlands and in Germany. The source of this contamination was the maize-based compound feed for dairy cows; feed producers had (unintentionally) used maize that was contaminated with aflatoxins as ingredient in their compound feed production. During the maize growing season of 2012, high aflatoxin concentrations were observed in South-East Europe. Between July 2012 and July 2013, 17 RASFF alerts were published notifying AFB_1 concentrations above the EU legal limit in maize intended to be used as feed ingredient. The contaminated maize originated from Serbia, Bulgaria, Romania, Hungary, Ukraine, Spain, Italy, Greece and Poland. Aflatoxins can already be present in the cultivation stage of the crops and, in case of improper conditions, can continue to be produced during transport and storage. During compound feed formulation, the aflatoxin concentration usually will decrease since maize is just one of the

ingredients used in the compound feed formulation. However, in case of high concentrations in the maize ingredient, the compound feed can be (highly) contaminated.
To the best of our knowledge, the full storyline including the estimated financial losses of any aflatoxin related incident, including the 2013 aflatoxin incident in Europe, has not been described before. The financial losses of this incident for the Serbian dairy sector incident has been described by Popovic *et al.* (Popovic *et al.*, 2017). However, no information was available on the financial losses endured by other stakeholders and other countries involved. Estimation of the financial losses of this incident could give insight into the financial losses for different groups of stakeholders and how much could be spent on prevention and control measures. The objective of this study was to estimate the direct financial losses of the 2013 aflatoxin incident for the Netherlands. As part of this objective, the events of the incident for the Netherlands and the two involved neighbouring countries, Germany and Belgium, were described. Germany and Belgium were included because of the intense cross-border trade.

2.2. Methods

2.2.1. Study demarcation

An illustration of the maize to milk supply chain is presented in Figure 2.1. The origin of the contaminated maize was the Black sea area, mainly Ukraine, Serbia, Romania, Hungary, and Bulgaria. The maize was then transported by ship to the Netherlands, Germany or Belgium where the maize was stored or directly transported to compound feed companies for further processing. The feed produced was transported to dairy farms where it was fed to dairy cows.

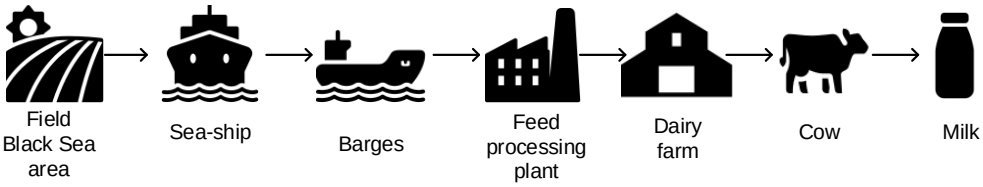

Figure 2.1. The maize to milk supply chain (free icons by Icons8: https://icons8.com/)

The three main stakeholders involved from the Netherlands, Belgium and Germany were included in this study; they were the maize traders, the feed producers and the dairy farms using the feed. Other stakeholders were not included in this study; amongst others, they include farmers growing the maize, the pig, poultry and cattle farmers, excluding dairy farmers, using the contaminated feed, and the authorities. Farmers growing the maize were not included in this study since they were not located in the Netherlands, Belgium or

Germany and were therefore not within the focus of the study, even though, during the growing season of 2012, high aflatoxin concentrations were found in the Black Sea area and, consequently, the farmers as well as the dairy sector in this area, did suffer from the contamination. This has been described previously (Popovic *et al.*, 2017). Furthermore, we assumed that the pig, poultry and cattle farms, excluding dairy cattle farms, did not suffer from any significant losses since the feed for these animals did not exceed the EU legal limit and carry-over of AFB_1 from the feed to meat and eggs is very low (BfR, 2013c).

2.2.2. Information and data collection

Data were obtained from the literature and through experts interviews. First, we searched for scientific articles or reviews about the 2013 aflatoxin incident in the Netherlands, Germany and Belgium, using several databases: Scopus, Web of Science, Pubmed, Agris, and Science Direct. The keywords used were: "aflatoxin" AND "milk" AND "2013" AND ("Netherlands" OR "Germany" OR "Belgium"). Second, we searched for news items on the internet for farmers and the general public, and statements and reports written by authorities. The search was performed using Google NL. The keywords "aflatoxin" AND "milk" AND "2013" were used. Since these references were written in the language of the country, the search was done in three languages: Dutch ("aflatoxine melk 2013"), German ("aflatoxine milch 2013") and French ("aflatoxine lait 2013"). Third, we searched for RASFF notifications of batches of maize intended to be used as feed materials that exceeded the legal limit for feed, as notified in the RASFF portal in the period 01/08/2012 to 01/07/2013. The product category was set to feed materials, the hazard category to mycotoxins, and the risk decision to serious. In order to complement the literature search and/or confirm the data found, five in-depth expert interviews were held. The experts, from trading companies and the feed industry, were interviewed about quantities of contaminated maize imported, quantities of contaminated maize processed, quantities of dairy cow feed produced, and the quantity of feed recalled. They were also asked about the prices of one tonne of maize, one tonne of feed, the costs for extra testing, recalling feed and destroying maize and/or feed. A pre-defined questionnaire was used as the basis for the interviews. All interviews were done by the first author of this paper.

2.2.3. Estimation of the financial losses

The financial losses for the traders were based on the quantity of contaminated maize imported, not processed into feed but sold as biogas instead:

$$\text{Financial losses traders} = import * (100 - p\_feed) * (c\_import - c\_biogas) \quad (1)$$

where *import* is the quantity of contaminated maize imported in tonnes, *p_feed* is the percentage maize that was processed into feed, *c_import* the value of one tonne of imported maize destined for feed, and *c_biogas* the value of one tonne of imported maize destined for biogas.

In order to estimate the financial losses for the feed industry, information was needed on the number of tonnes dairy cattle feed produced with using the imported contaminated maize. This was estimated with Equation (2).

$$dairy\_feed = import * (p\_feed/100) * (p\_dairy\_feed/100) * (1/p\_maize/100) \quad (2)$$

In Equation (2), *dairy_feed* is the quantity, in tonnes, of dairy cattle feed produced with the contaminated maize, *import* is the quantity of contaminated maize imported in tonnes, *p_feed* is the percentage maize that was processed into feed, *p_dairy_feed* is the percentage of feed produced destined for dairy cattle, *p_maize* is the percentage of maize incorporated into the compound feed.

Then, information was needed on the volume of feed (tonnes) that was recalled. This was estimated with Equation (3):

$$t\_recall = (p\_recall/100) * dairy\_feed \quad (3)$$

where *t_recall* are the number of tonnes of feed recalled, *p_recall* the percentage of feed delivered to the dairy farmers that was recalled, and *dairy_feed* the quantity, in tonnes, of feed produced, calculated with Equation (2).

The total recall costs, including the destruction and the replacement of the feed, were estimated by the following equation:

$$tc\_recall = t\_recall * (c\_recall + c\_destr + c\_feed) \quad (4)$$

where *tc_recall* the total costs to recall, destroy and replace the feed, *t_recall* the quantity of feed recalled, in tonnes, *c_recall* the costs, per tonne, to recall the feed, *c_destr* the costs, per tonne, to destroy the feed, and *c_feed*, the costs, per tonne, to replace the feed.

Next, the costs for extra testing of the raw maize and feed were estimated using:

$$tc\_test\_feed = c\_test\_feed * dairy\_feed \quad (5)$$

where *tc_test_feed* are the costs for testing the raw maize and feed, *c_test_feed* are the average costs per tonne of feed and *dairy_feed* the quantity, in tonnes, of dairy cattle feed produced.

The costs to compensate the dairy farmers who received contaminated feed were estimated by the following equation:

$$tc\_comp = c\_comp * dairy\_feed \quad (6)$$

where *tc_comp* are the total costs for the feed industry to compensate the dairy farmers who received the feed, *c_comp* the compensation costs per tonne of feed, and *dairy_feed* the quantity of feed produced.

The total direct financial losses for the feed industry were estimated with Equation (7).

$$\text{Financial losses feed industry} = tc\_recall + tc\_test\_feed + tc\_comp \quad (7)$$

In Equation (7), *tc_recall* are the total costs to recall the feed, *tc_test_feed* are the costs for extra testing, and *tc_comp* are the total costs to compensate the dairy farmers.

To estimate the financial losses for the dairy industry, the assumption was made that if the milk produced at a particular farm was above the legal limit, the farm could not sell any milk for some time after the discovery of the contamination. Farms receiving contaminated feed but not having the milk exceeding the legal limit did not suffer any major financial losses. The costs for the milk testing was most of the times declared to the feed industry, and added to the financial losses for the feed industry. Furthermore, no milk was lost. The test results were available the same day. The financial losses for the dairy sector were computed using Equation (8).

$$\text{Financial losses dairy sector} = nb\_farms\_cont * nb\_days * nb\_cow * l\_milk * c\_milk \quad (8)$$

Where *nb_farms_cont* are the number of farms at which AFM_1 was found above the legal limit, *nb_days* was the number of days after the discovery that the dairy farms were unable to sell their milk, *nb_cows* the number of cows per farm, *l_milk* the litres milk produced per cow per day, and *c_milk*, the selling price of one litre of milk for a farmer.

A Monte Carlo simulation was used to estimate the financial losses per stakeholder and the total financial losses of the incident for the Netherlands. We chose a Monte Carlo simulation, with 10,000 iterations, to include the uncertainty of data collected on some of the input parameters: the number of tonnes of contaminated maize imported, the value of imported

maize destined for feed, the percentage of maize used for feed, the percentage of maize used for dairy cattle feed, the cost-price to produce compound feed, the percentage of feed recalled, the costs to recall feed, the costs to destroy contaminated feed, the costs to test feed for AFB_1, the costs to compensate dairy farmers, the number of cows per dairy farm, the selling price for milk . For each iteration, a number was picked from the distributions describing the variables of the model. The following input parameters were considered to be deterministic values: the value of maize intended for use as biogas, the number of dairy farms exceeding the legal AFM_1 level and the number of days a farm was blocked in case contaminated milk was found. A summary of all model variables is found in Table 2.1.

2.3. Results

Three scientific articles related to the 2013 aflatoxin incident were found. De Rijk *et al.* (2015) used one batch of maize involved in the 2013 aflatoxin incident to investigate the efficiency of the EU sampling procedures (de Rijk *et al.*, 2015). Van der Fels-Klerx and Camenzuli (2016) used the concentrations observed during the 2013 aflatoxin incident to model the possible AFM_1 concentrations in milk (van der Fels-Klerx and Camenzuli, 2016). Popovic *et al.* (2017) described the financial losses of the 2013 aflatoxin incident for the Serbian dairy sector. None of these scientific papers described the events of the 2013 aflatoxin incident in detail and neither of them estimated the financial losses for either the Netherlands, Belgium or Germany.

Relatively many news items, reports and announcements were available on the web. Based on this grey literature, we were able to describe the events, as presented in Section 5.4.1. Section 5.4.2 lists the RASSF notifications in which the Netherlands was involved. Section 5.4.3 presents the financial losses of this incident. The data used in the model calculations came from the grey literature collected as well as from the expert interviews conducted.

2.3.1. Description of the events based on grey literature

In the Netherlands, in February 2013, AFM_1 was found to be present in the milk tanks of two dairy farms (Boerenbusiness, 2013b; NRC, 2013). Six weeks later, AFM_1 was again found to be present in the milk of two other Dutch farms (Veeteelt, 2013). In Germany, in March 2013, 0.057µg AFM_1 per kilogram milk was discovered in a milk tank in Lower Saxony. In North Rhine Westphalia, 0.10µg/kg, i.e. twice the EU legal limit for AFM_1 in milk, was detected in a milk tank by the State Office for Nature, Environment and Consumer protection (LANUV) (BfR, 2013a; b). AFM_1 was found in the milk of four other dairy farms in Emsland, Germany. The milk did, however, not exceed the EU legal limit (Boerenbusiness, 2013e).

The high levels of AFM_1 found in the Netherlands and Germany were the results of contaminated compound feed that was fed to the dairy cows. This compound feed included

maize contaminated with AFB_1 coming from the Black Sea area (Boerenbusiness, 2013b). Three contaminated maize batches were identified: one ship containing maize from Serbia which entered Germany in the port of Brake, one ship with maize from Serbia which entered the Netherlands in the port of Rotterdam, and one ship with maize from Romania which entered Belgium in the port of Ghent (EVMI, 2013).

Batch 1

Before February 2013, in Germany, 45,000 tonnes of AFB_1-contaminated maize from Serbia were imported in the port of Brake in North Germany, via the port of Constanza in Romania. After detection of high concentrations of AFB_1 in the maize batch, about 10,000 tons were blocked in Brake, and 25,000 tonnes were stored in a warehouse in Bremen and were blocked there. The remaining 10,000 tonnes of maize were already delivered and processed into feed in Lower Saxony and in the Netherlands (BfR, 2013a; Boerderij, 2013; EVMI, 2013; Niedersächsisches Ministerium für Ernährung Landwirtschaft und Verbraucherschutz, 2013; Zeit, 2013). The compound feed produced by Dutch feed producers, using the contaminated maize, was mostly delivered and fed to pigs in the Netherlands (EVMI, 2013).

The feed producers were ordered to send their sales lists to the Lower Saxony State office for Consumer Protection and Food Safety (LAVES), on February 20th. From these lists, it was estimated that about 6,500 pig, poultry and cattle farms, including about 1,000 dairy farms in Germany, received feed produced using the contaminated maize. Adding to the fact that the feed producers needed to send their sales lists to LAVES, they had to inform their clients, organise recalls (at the dairy farms), and report back to LAVES (Kyiv Post, 2013; Niedersächsisches Ministerium für Ernährung Landwirtschaft und Verbraucherschutz, 2013).

On March 4th, BfR published a literature review stating eggs and meat were not at risk to be contaminated with aflatoxins. The risk for livers and kidneys could not be excluded and these animal derived products had to be put on hold. A risk for AFM_1-contaminated milk was identified and the dairy farmers were not allowed to sell their milk, unless analytical test results showed the milk was not contaminated with AFM_1 (BfR, 2013c). On March 4th, still about 40 German farms were excluded from the milk collection (Niedersächsisches Ministerium für Ernährung Landwirtschaft und Verbraucherschutz, 2013). On March 5th, BfR published a risk assessment for aflatoxins in livers and kidneys; these products were identified not to pose a risk to human health and the ban on the sale of these two products was lifted. Furthermore, all dairy farms were cleared and could sell their milk again (BfR, 2013a).

Batch 2

On February 20th, a 45,000t contaminated maize batch from Serbia entered the Netherlands via the harbour of Rotterdam. About 1,000 tonnes were blocked in Rotterdam. About 1,000 tonnes were delivered to feed producers in Germany on February 21. On March 6th, about 1,200 tonnes were delivered to Germany but could be traced and blocked before being processed (Boerenbusiness, 2013f). Another source states that 35,000 tonnes were stored in Bremen, Germany (NOS, 2013a), and about 10,000 tonnes were processed by feed producers and delivered to about 3,600 farms in Germany and in the Netherlands (Blik op Nieuws, 2013; NRC, 2013). However, an estimate of the Dutch news agency NOS pointed at 6,500 farms (NOS, 2013b). The feed produced was delivered to 87 dairy farms, which needed to be tested for the presence of AFM_1 before they could sell their milk again. After three days the testing results showed no AFM_1 concentrations above the EU legal limit in the milk tested, and the affected farms could sell their milk again (Niedersächsisches Ministerium für Ernährung Landwirtschaft und Verbraucherschutz, 2013).

Batch 3

One 53,000t contaminated maize batch from Romania was delivered to Belgium in the port of Ghent. The batch was then transported to the Netherlands. The largest part of the batch was blocked before further processing. Part of the batch was processed into feed for pigs, poultry and, though to a lower extent, for cattle. The AFB_1 concentration in the compound feed did not exceed the legal limit. Furthermore, the milk samples collected from dairy farms using the feed did not show high levels of AFM_1 (EVMI, 2013; VRT, 2013). It was stated that the feed was not used in Belgium. All samples of raw materials, feed products and milk analysed in Belgium were compliant to the EU limits for AFM_1 (FASFC, 2013).

Measures taken

Dutch feed producers arranged recalls of their feed. The recalls took approximately two weeks. First, compound feed with high inclusion rates of maize from Serbia, Romania and Hungary was recalled and, next, feed with lower percentages of maize from the Balkan area was recalled as well (Boerenbusiness, 2013a; d). TRUST FEED, the umbrella organisation of compound feed producers in the Netherlands, states that the produced feed did not exceed the EU legal limit for AFB_1, the feed contained on average 1µg/kg AFM_1, and the recalls were a preventive measure (TRUST FEED, 2013). The contaminated maize was not used for feed, nor for biogas, and was either destroyed or sold to the United States (Boerenbusiness, 2013c).

On March 8th, LAVES published a general order that maize from Serbia may only be sold under strict controls and should be mixed with other animal feed in Lower Saxony and

Bremen. LAVES needed to check these products before they were placed on the market. On March 23rd, a general feed-related law for the protection against risks related to AFB_1 in feed from maize harvested in Bulgaria, Romania and Poland in 2012 was issued, complementing the already existing general ruling on feed maize from Serbia (Niedersächsisches Ministerium für Ernährung Landwirtschaft und Verbraucherschutz, 2013). The Dutch organisation TRUST FEED gave advice not to include maize from Serbia, Romania and Hungary into dairy cattle feed. Furthermore, TRUST FEED stated that the feed producers intensified the monitoring program for aflatoxins of incoming maize batches. In addition to sampling when unloading the ship, extra samples were collected when the batches arrived at the processing plants (TRUST FEED, 2013).

2.3.2. RASFF notifications

Three contaminated sea ships were reported in the news items mentioned before, however, 17 RASFF notifications were made between 01/08/2012 and 01/07/2013. If only the notifications involving the Netherlands were considered, eight notifications were left. One batch was returned to the consignor, two batches were used for other purposes than feed or food, two batches were officially blocked, for two batches the recipients had to be informed, and for one batch the decision was unknown. The list and details of the notifications is presented in Table 2.1.

Table 2.1. List of RASFF notifications notifying feed batches contaminated with AFB_1 distributed to the Netherlands

Date	Origin	Distribution	Type of check	AFB_1 (µg/kg)	decision
01/03/2013	RO	NL, DE, via BE	Company own check	57.6 – 71.3	Informing recipients
01/03/2013	RS	DE, NL, US, via RO	Official control	204, 112, 38, 21	Official detention
04/03/2013	RS, RO, BG, PL	DE, UK, via NL	Company's own check	37.1	Official detention
08/03/2013	RO, RS, BG	BE, FR, DE, NL	Company's own check	1.9 – 158.5	/
13/03/2013	RO, BG	DE via NL	Company own check	22.4 – 26.7	Use for other purpose than food/feed
19/03/2013	HU	DE, NL, AT	Company own check	117.5 – 102.5	Return to consignor
29/03/2013	UA	BE, FR, DE, NL	Company's own check	32.1	Informing authorities
16/04/2013	UA	BE, DE, via CH and NL	Company's own check	35.4	Use for other purpose than food/feed

Note: AT: Austria, BE: Belgium, BG: Bulgaria, CH: Switzerland, DE: Germany, FR: France, HU: Hungary, NL: the Netherlands, PL: Poland, RO: Romania, RS: Republic of Serbia, UA: Ukraine, UK: United Kingdom, US: United States

2.3.3. Estimation of the financial losses

Table 2.2. Data collected to estimate the financial losses of the 2013 aflatoxin incident for stakeholders in the Netherlands

Variable	Estimation	Source
Tonnes of contaminated maize imported from Black Sea in 2012 (t)	143,000 225,000 300,000	45,000t + 45,000t + 53,000t 3 ships with capacity between 70,000t – 80,000t (1) 4 ships (2 with decision 'official detention' and 2 with decision 'informing recipients' (RASFF, 2013)
Value maize from the Black Sea in 2012 (€/t)	220 – 260 255 – 244 – 246 279 – 255	FOB Black sea region (Potori and Józsa, 2014), €20/t for maritime transport added (2) Prices for Nov 2012 – Jan 2013 (3) Prices for Aug and Dec 2012 (Agrimatie, 2012)
Value maize intended for use as biogas (€/t)		(Boerenbond, 2014)
Percentage imported maize processed (%)		10,000t (batch 1) + 10,000t (batch 2), which is 22% (NVWA, 2014) (5)
Percentage of total feed production used as dairy cattle feed (%)		the Netherlands, 2013 Germany, 2013 Belgium, 2013 (FEFAC, 2018)
Percentage of maize in dairy cattle feed (%)		(Devun *et al.*, 2014; van der Fels-Klerx and Camenzuli, 2016) (4)
Cost price compound feed (€/t)		(Remmelink, 2018) (4)
Percentage feed recalled		(4) (5) (6)
Recall costs (€/t)		(4) (5) (6)
Costs destruction feed (€/t)		(4) (5) (6)
Costs testing feed for AFB_1 (€/t)		(4) (5) (6)
Costs compensation dairy farms (€/t feed)		(4) (5) (6)
Number of dairy farms exceeding the legal AFM_1 level		(BfR, 2013c; Veeteelt, 2013)
Number of days a farm was blocked in case of contaminated milk		(2)
Number of dairy cows per farm		(CBS, 2017; NZO, 2016)
Litres of milk produced (l/day)		(The Daily Milk, 2017) (5)
Price per litre milk (€/l)		(De Volkskrant, 2013) (Eurostat, 2018)

(1) Oral communication Dutch Association Feed Companies
(2) Own estimation
(3) Oral communication trading company
(4) Oral communication Dutch Feed company
(5) Calculation based on information available
(6) Assumption: RSD of 25%

Table 2.2 presents the collected data based on news items on the web, RASFF notifications, scientific literature, some publicly available statistics, and expert interviews. The experts interviewed are or were working in the feed industry, trading companies or the Netherlands food safety authority. In case only one data point was available, and this data point was uncertain, a relative standard deviation (RSD) of 25% around that data point was assumed. Based on these collected data, input values of the model parameters, as shown in Table 2.3, were determined.

Table 2.3. Input parameters of the model with their estimations or distributions used to estimate the financial losses of the 2013 aflatoxin incident for the Netherlands

Variable	**abbreviation**	**Unit**	**Estimation or distribution**
Tonnes of contaminated maize imported from Black Sea in 2012	import	t	triangular(143,000; 300,000; 225,00)
Value maize from the Black Sea in 2012	c_import	€/t	normal(251; 17)
Value maize intended for use as biogas	c_biogas	€/t	30
Percentage imported maize used for feed	p_feed	%	normal(0.22; 0.055)
Percentage of total feed production used as dairy cattle feed	p_dairy_feed	%	triangular(0.10; 22; 0.28)
Percentage of maize in dairy cattle feed	p_maize	%	uniform(0.10; 0.20)
Cost price compound feed	c_feed	€/t	triangular(220; 279; 251)
Percentage feed recalled	p_recall	%	normal(0.60; 0.15)
Costs to recall feed	c_recall	€/t	normal(58; 14.5)
Costs destruction feed	c_destr	€/t	normal(5; 1.25)
Costs testing feed for AFB_1	c_test_feed	€/t	normal(5; 1.25)
Costs compensation dairy farms	c_comp	€/t feed	normal(84; 21)
Number of dairy farms exceeding the legal AFM1 level	nb_farms_cont	nb	6
Number of days a farm was blocked in case of contaminated milk	nb_days	nb	14
Number of dairy cows per farm	nb_cows	nb	triangular(1; 400; 083)
Litres of milk produced	l_milk	l/cow/day	21
Price per litre milk	c_milk	€/l	uniform(0.34; 0.41)

The estimation of the direct financial losses related to the 2013 aflatoxin incident in the Netherlands, resulting from the Monte Carlo simulations, are presented in Figure 2.2 and Table 2.4.

Table 2.4. The estimated financial losses for the maize traders, the feed producers, the dairy sector and the total financial losses related to the 2013 aflatoxin incident

	mean	**median**	**5% percentile**	**95% percentile**
Direct financial losses maize traders	36,754,000	37,000,000	16,501,000	63,192,000
Direct financial losses feed producers	13,705,000	15,468,000	1,225,000	69,620,000
Direct financial losses dairy sector	98,000	107,000	2,000	286,000
Direct total financial losses	51,477,000	52,574,000	36,751,000	71,740,000

Figure 2.2 and Table 2.4 show the distributions of the estimated direct financial losses for the maize traders, the feed producers, the dairy farms and the total estimated financial losses. The largest percentage of the direct financial losses, about 71%, is for the maize traders, with an average estimated cost of about €37 million, with the 5th percentile being €17 million and the 95th percentile €63 million. The direct financial losses for the feed producers are in the order of €14 million, between €1 and €70 million. The direct financial losses for the dairy sector are almost negligible with a mean of €98,000. The total financial losses are in the order of €51 million, with the 5th percentile being €37 million and the 95th percentile being €72 million.

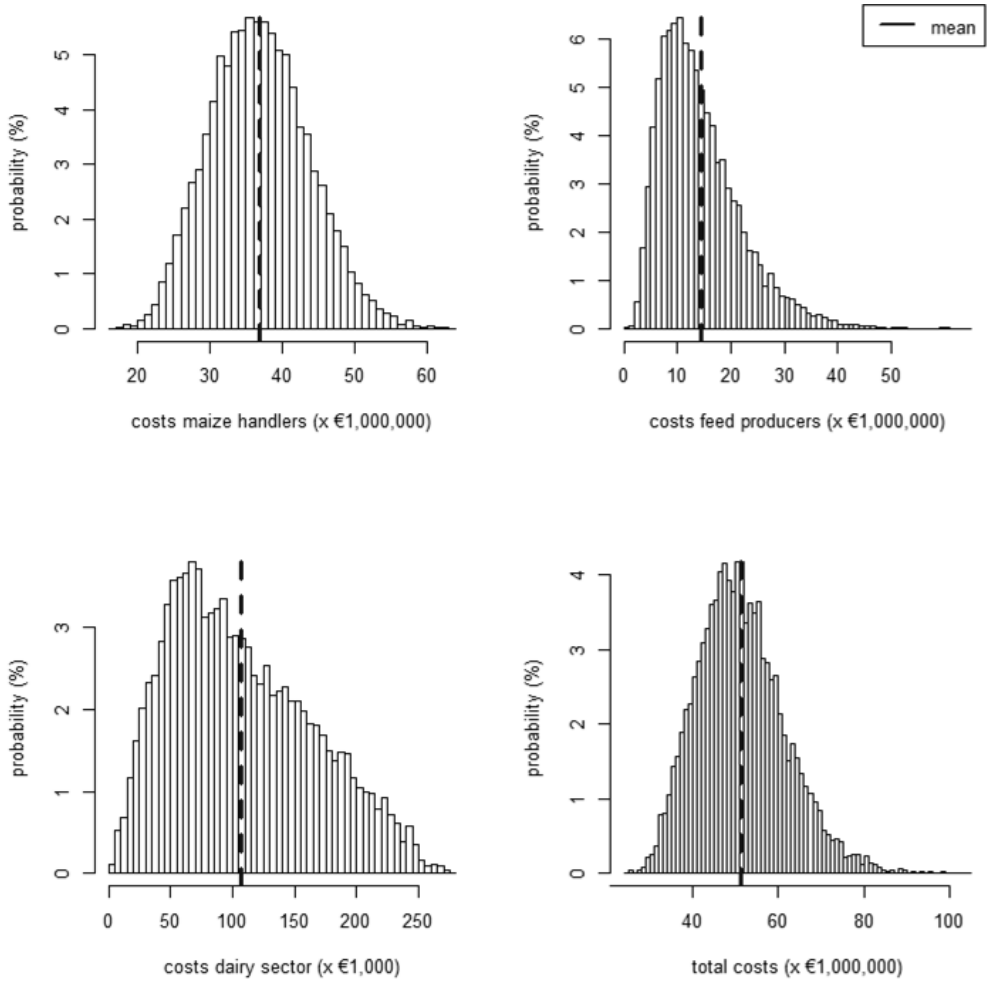

Figure 2.2. Estimated direct financial losses for the maize traders, the feed producers and the dairy sector in the Netherlands, Belgium and Germany due to the 2013 aflatoxin incident

2.4. Discussion

Information was collected from various sources to estimate the financial losses of the 2013 aflatoxin incident for the most heavily involved stakeholders (the maize traders, the feed producers and the dairy farms) in the Netherlands, Germany and Belgium, with a focus on the Netherlands. Germany and Belgium were included where needed because of the intense cross-border trade. Several contaminated ships were imported from the Black sea area into these three countries and were intended to be used in feed production. Since only a small percentage of maize is incorporated in the feed and the EU limit for AFB_1 in feed is 20µg/kg

(EU, 2002a), the probability of the concentration of AFB_1 being above this limit is in general small. For example, if the inclusion rate of maize in compound feed is 20%, the maize needs to have an AFB_1 concentration of at least 100µg/kg for the compound feed to have a concentration above 20µg/kg. In this example we did not take into account the limited decrease of the concentration during processing, and possible AFB_1 contamination of other ingredients used in the compound feed formulation. Given that the EU limit for compound feed for dairy cows feed is 5µg/kg (EU, 2002a), the probability of the AFB_1 concentration exceeding this limit is much higher. Again, inclusion of 20% of maize in the compound feed, i.e. maize with a concentration of at least 25µg/kg, will lead to feed with a concentration of 5µg/kg or higher. Furthermore, AFB_1 is metabolised in the cow's body and is excreted as AFM_1 in the milk. Since AFM_1 is an unwanted toxic compound, contaminated maize can lead to both dairy cow feed exceeding the EU limit for AFB_1 and dairy cows' milk exceeding the EU limit for AFM_1 (van der Fels-Klerx and Camenzuli, 2016).

Farmers in the Black sea area in Europe suffered a lot from the high aflatoxin concentration in maize during the growing season of 2012. In Serbia, the crisis lasted for two years and affected all dairy companies in the country. Popovic *et al.* (2017) estimated that the total direct and indirect economical losses of the Serbian farm-level dairy sector during the crisis mounted to €74.4 – €96.2 million depending on the scenario. Serbia has about 158,000 dairy farms and is a net exporter of dairy products. During the crisis, in Serbia, the consumption of dairy products decreased by 11.4%, up to even 26.6% in the city Belgrade. Export of dairy products decreased as well, e.g., the export of liquid dairy products was halved (Popovic *et al.*, 2017).

In the Netherlands, Belgium and Germany, the largest share (about 71%) of the total financial losses, were for the maize traders. The incident was initially discovered in the milk of a handful of dairy farms in February 2013. However, it was quickly traced back to contaminated maize used in feed production. Due to extra testing of the incoming maize batches, the incident remained relatively small, and mostly at the level of the maize traders. Since the AFB_1 concentrations discovered in maize in several ship compartments were above the EU legal limit for maize intended for feed production, the maize had to be returned to the supplier, used for other purposes, or destroyed. This study showed this had led to high direct financial losses. The financial losses for the feed industry, about 28% of the total financial losses, were mostly due to the maize which had already been processed before discovering the high AFM_1 concentrations in the milk. About 22% of the maize coming from the three imported shipments was processed into feed for pigs, poultry and cattle. Only a small percentage of this maize was used in compound feed for dairy cattle. The direct financial losses for the dairy sector, estimated at less than 1% of the total financial losses, were negligible compared to the total financial losses. However, for the individual farms

having their milk exceeding the EU limit, the financial losses could be high: two weeks of closure of the farm being equivalent to roughly 3.8% of the yearly income (2 weeks divided by 52 weeks).

In this study, we considered only the direct financial losses of the 2013 aflatoxin incident. We assumed that the indirect financial losses, such as a lower export of dairy products and a lower domestic consumption of dairy products, were negligible for the Netherlands, Germany and Belgium since no contaminated milk had reached the markets. From 2012 to 2013, the export of Dutch dairy products increased by 12% in general. In 2013, 60% was exported to five countries: Germany, Belgium, France the UK and Russia. In 2012, about 187 million of dairy products were exported to Russia. In 2013, this export increased to 300 million (Business Insider Nerderland, 2014). From these numbers, we conclude that the export volume did not suffer from the 2013 aflatoxin incident. Since the milk sold on the market did not exceed the EU legal limit for AFM_1, there were no expected risks for the consumers. In Serbia, the situation was different: it was estimated that the loss for the dairy sector mounted to €96.2 million (Popovic *et al.*, 2017).

Furthermore, Wu (2008) estimated the average direct financial losses for an EU border rejection of a cereal batch because of mycotoxin contamination, to be between €8,900 and €13,400 for extra costs, including sampling, testing, transportation, storage, labour costs, and reprocessing (Wu, 2008). Additional costs can occur depending on the destination of the batch for, since it might still be suitable for feed instead of food. These administrative costs are not included in this study, and could be added to the financial losses for the maize traders.

Other indirect financial losses, not considered in this study, did have an impact on the Netherlands. These are financial losses due to the changes in the Dutch monitoring program for aflatoxin in maize. Indeed, the feed industry in the Netherlands has intensified their monitoring program for aflatoxins in maize after the 2013 aflatoxin incident. Extra sample collection was required when unloading batches in the harbour, at the level of the ships transporting the maize to the feed producers. Furthermore, after the 2013 aflatoxin incident, countries exporting maize to the Netherlands are classified each year into low, medium and high risk countries for aflatoxin contamination. Each category of countries has its own monitoring plan, which leads to extra costs. Adding to that, since the 2013 aflatoxin incident, up until early 2019, compound feed producers test one batch of feed that contains maize per week for the presence of aflatoxins, the frequency is now lowered to once a month (SecureFeed, 2018). Given that there are 40 locations producing feed for dairy cattle in The Netherlands and aflatoxin testing costs of a batch are between €300 and €1,100 when 20 or 100 samples are collected respectively (Focker *et al.*, 2019), this would lead to an extra

€624,000 to €2,288,000 per year for the feed industry, when one batch per week per location is tested.

The estimation of the financial losses of the 2013 aflatoxin incident was based on scarce data that had to be obtained from different sources, and combined. In order to account for the uncertainty of the input data, a Monte Carlo simulation was performed. Since for some variables, we could collect only one data point, a relative standard deviation of 25% was added to this data point. However, uncertainty still remains and the results presented in this study are only an approximation of the financial losses for the three stakeholders in the Netherlands and the neighbouring countries Germany and Belgium.

2.5. Conclusion

To conclude, imported maize with an aflatoxin concentration above the EU legal limit for feed lead to high financial losses, first of all for the maize traders, and also for the feed producers in case the maize has already been processed. Increasing the frequency of monitoring, in the upstream stages of the maize supply chain, could help avoid financial losses for stakeholders in the downstream stages of the maize supply chain, such as the feed producers and the dairy sector.

Chapter 3

Systematic Review of Methods to Determine the Cost-effectiveness of Monitoring Plans for Chemical and Biological Hazards in the Life Sciences

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Published in: Comprehensive Reviews in Food Science and Food Safety 17(3): 633-645. https://doi.org/10.1111/1541-4337.12340

Abstract

This study reviews the methods used to determine the cost-effectiveness of monitoring plans for hazards in animals (diseases), plants (pests), soil, water, food, and animal feed, and assesses their applicability to food safety hazards. The review describes the strengths and weaknesses of each method, provides examples of different applications, and concludes with comments about their applicability to food safety. A systematic literature search identified publications assessing the cost-effectiveness of monitoring plans in the life sciences. Publications were classified into four groups depending on their subject: food safety, environmental hazards, animal diseases, or pests. Publications were reviewed according to the type of model and input data used, and the types of costs included. Three types of models were used: statistical models, simulation models, and optimisation models. Input data were either experimental, historical, or simulated data. Publications differed according to the costs included. More than half the publications only included monitoring costs, whereas other publications included monitoring and management costs, or all costs and benefits. Only a few publications were found in the food safety category and all were relatively recent studies. This suggests that cost-effectiveness analysis of monitoring strategies in food safety is just starting and more research is needed to improve the cost-effectiveness of monitoring hazards in foods.

3.1. Introduction

Contaminated food sold on the market has the potential to cause serious health damage to humans upon consumption. In addition, companies supplying contaminated food to the market run the risk of high recall and disposal costs, reputational damage, and loss of consumers' confidence in the company, potentially resulting in temporary or even structurally, lower sales (Hussain and Dawson 2013; Thomas *et al.,* 2015). Furthermore, the General Food Law in the European Union stipulates that "Food shall not be placed on the market if it is unsafe" (EU, 2002b). It is therefore in the interest of consumers, food companies, and the government to strictly monitor food hazards, to ensure that these hazards do not become a risk for public health. A monitoring plan should consider not only accuracy, but also the budget available for monitoring, which is generally limited. Given limited resources, a batch of food or a daily production cannot be tested endlessly. This highlights the importance of monitoring in a cost-effective way to achieve the highest accuracy for a given budget.

Prior to undertaking this research, we observed that only very few studies had addressed the cost-effective monitoring of hazards in food. Monitoring schemes in the field of food safety tend to focus only on accuracy and not on costs. Including the costs in the design of the monitoring plan may improve accuracy at the same cost, or it may lead to cost savings while achieving the same accuracy (Lascano-Alcoser *et al.,* 2013). To assess the cost-effectiveness of monitoring hazards in food, adequate methods to determine cost-effectiveness are needed. A large body of research addresses cost-effective monitoring of hazards in other fields, such as animal and plant diseases and soil and water contaminants. Methods used in these fields might also be applicable to food safety issues, where many hazards are monitored but cost-effective monitoring schemes are frequently lacking. A review of the methods used to determine the cost-effectiveness of monitoring in different fields can provide insight into the applicability of methods for the field of food safety. Including different fields is particularly relevant because researchers tend to focus on publications from their own discipline and different disciplines develop their own methodological procedures.

The objective of this study was to identify and review methods for evaluating the cost-effectiveness of monitoring hazards in the life sciences by performing a systematic literature search and review. The review aimed to assess the strengths and weaknesses of all available methods, to provide examples, and to discuss the methods in the context of food safety.

3.2. Materials and Methods

3.2.1. Literature search

This review focused on studies assessing the cost-effectiveness of monitoring biological and chemical hazards in the life sciences, excluding human diseases. The review was therefore restricted to studies related to plant and animal pests and diseases, food and feed safety, and soil and water contaminants. A hazard was defined in this study as: a chemical, biological, or physical component or species that has the potential to cause damage to human, plant, or animal health.

A systematic literature search was conducted to identify methods currently used to assess the cost-effectiveness of a monitoring plan. The search method was developed beforehand. Electronic databases were used to gather sources from the scientific literature. The selected databases were Scopus, PubMed, and CAB Abstracts. These three databases were expected to provide a sufficiently large initial sample of peer-reviewed publications. Scopus is a very large database that includes peer-reviewed publications on a variety of topics: science, including medicine, technology, social sciences, arts, and the humanities (Elsevier 2017). CAB Abstracts focuses on the life sciences and includes topics such as agriculture, applied economics and sociology, animal sciences, plant sciences, environmental sciences, biotechnology, chemistry, climate change, food sciences, human nutrition, leisure and tourism, pharmacology, microbiology, natural resources management, and veterinary medicine (CAB Abstracts 2017). PubMed also focuses on the life sciences.

The research question was formulated prior to the literature search as: What methods are used to determine the cost-effectiveness of monitoring plans for hazardous components in the life sciences? Relevant keywords were also identified prior to the search: “monitoring”, ‘hazardous components”, and “cost-effectiveness”. The keyword “hazardous components” was not included in the search string because it had too many synonyms and the search would miss important information if some synonyms were omitted from the search string. To find the best search strings, several publications from different fields were first read to identify different terms used for a specific subject. For example, synonyms frequently used for monitoring were “surveillance”, “sampling”, “testing”, and “screening”. Synonyms used for the concept of costs were “cost“, “economic“, and “resources“. Finally, synonyms used for the concept of effectiveness were “effective“, “efficiency“, “efficient“, “optimal“, “optimisation“, and “allocation“. The initial search string used to search the three databases was: “(monitoring OR sampling OR testing OR surveillance OR screening) AND ((cost? OR economic* OR resource*) AND (effective* OR efficien* OR optim* OR allocation))”, using the wildcards “*” for 0 to n characters, and “?” for 0 or 1 character. Table 3.1 shows the exact

search terms used and provides an explanation of the terms added to the initial search string in the different databases.

Table 3.1. Search strings used in the different databases utilised for this study

Database	**Search string**
Scopus	(TITLE(monitoring OR sampling OR testing OR surveillance OR screening) AND TITLE-ABS-KEY ((cost* OR economic* OR resource*) AND (effective* OR efficien* OR optim* OR allocation))) AND PUBYEAR > 1999 AND PUBYEAR < 2017 AND (LIMIT-TO(DOCTYPE,"ar") OR LIMIT-TO(DOCTYPE,"ch")) AND (LIMIT-TO (LANGUAGE,"English")) AND (LIMIT-TO(SUBJAREA,"ENVI") OR LIMIT-TO (SUBJAREA,"AGRI") OR LIMIT-TO(SUBJAREA,"SOCI"))
PubMed	(((monitoring[Title] OR sampling[Title] OR testing[Title] OR surveillance[Title] OR screening[Title])) AND ((cost? OR economic* OR resource*) AND (effective* OR efficien* OR optimiz* OR allocation))) AND ("2000/01/01"[PDat] : "2016/12/31"[PDat]) AND Animals[Mesh:noexp]
CAB Abstracts	TI (monitoring OR sampling OR testing OR surveillance OR screening) AND ((cost? OR economic* OR resource*) AND (effective* OR efficien* OR optim* OR allocation)) NOT SU (man OR human) NOT SU (gene* OR DNA OR RNA) Limiters: Publication year: 2000-2016, Publication type: journal article, book, Broad category: Animal Sciences, Plant Sciences, Ecology & Environmental Sciences, Agricultural Economics & Rural studies Language: English.

As the databases covered a broad area of subjects, and as the keywords did not specify the field, extra limiters were added. In PubMed, one can choose between "Human" or "other animals". The limiter "other animals" was chosen to eliminate the large amount of human health studies. In Scopus, the subject was limited by choosing the topics "agricultural and biological sciences", "social sciences" and "environmental science" and the document type was set to articles or book chapters. In CAB Abstracts, the broad category was set to animal sciences, plant sciences, ecology & environmental sciences, agricultural economics & rural studies, the publication type to journal article and books, and the language to English. As CAB Abstracts included a detailed list of subjects for each article, it was used to remove a large amount of articles about human diseases by adding the string "NOT SU (man OR human)" and to remove many articles about DNA testing or resistance screening by adding the string: "NOT SU (gene* OR DNA OR RNA).

The records obtained from the initial database searches were then included or excluded according to the following six criteria.

(1) Type of publication: Published peer-reviewed primary research papers and book chapters.

(2) Language: Studies written in English.

(3) Date: Studies published between January 2000 and December 2016.

(4) Subject: The studies were about monitoring hazards, where hazards followed the definition used in this research. Studies that were not about monitoring hazards, such as resistance screening and biodiversity monitoring, were excluded. Validation studies of a particular detection method were excluded, as these do not include cost calculations. Natural disasters (floods, fires, or rain) were also excluded, as these hazards were not considered to have the same direct impact on plant, animal, or human health as a disease would. Studies assessing the number of wildlife were also excluded, as this was considered biodiversity monitoring. However, the review included studies on monitoring pests with the potential of harming plants (forest pests) or humans (mosquitoes) directly, or indirectly as a vector of a disease (mosquitoes). Lastly, studies on human diseases were excluded. Although much research is available on the cost-effective monitoring of diseases in human populations, this field has different ethical considerations.

(5) Assessment of costs and effectiveness: Both the costs and the effectiveness of monitoring plans were quantitatively assessed, or assessed with at least a few qualitative classes. Only stating that the method was cost-effective in the introduction or conclusion was not considered sufficient to include the publication. Effectiveness was defined as the extent to which a target was achieved. Costs could be expressed in monetary terms, in time, or in other resources.

(6) Key words: The search was restricted to references with the key word “monitoring” in the title, or the synonyms “surveillance”, “sampling”, “testing” and “screening”. The words “cost” and “effectiveness” were also required, but these terms could be present in the title, abstract, or the keywords. The following synonyms were included: “cost”, “economic”, “resources”, “effective”, “efficiency”, “optimal”, “optimization” and “allocation”.

The search in the three databases using these search strings was expected to provide a set of relevant publications covering a large fraction of all available publications. This set

became a starting point to find additional relevant publications, by using the snowballing technique to find publications in the reference lists of the selected publications.
The citation manager Endnote was used to combine the references found in the search and to remove the duplicates. The remaining references were then sorted into two groups based on the title: a group containing references with relevant titles and references for which the relevance was unclear after reading the title, and a group containing irrelevant titles for this study. Irrelevant titles were titles that did not meet the following inclusion criteria: English title, addressing monitoring hazards except human diseases. After discarding the references with irrelevant titles, the remaining references were filtered based on their abstracts. Again, two groups of references were made: a group of studies with a relevant abstract and another group of studies with irrelevant abstracts that did not meet the following inclusion criteria: addressing monitoring hazards expect for human diseases, the idea of effectiveness and costs were mentioned. Finally, the full text of the remaining relevant publications was read, focusing on the methods section. References were excluded at this stage if the full text could not be accessed, if the text was not in English, or if there were no quantitative calculations or qualitative assessments with classes of the effectiveness or costs of a monitoring plan (criterion 5).
After gathering the list of relevant references using the systematic database search, the snowballing technique was applied. The reference lists in the selected publications were screened to find additional relevant publications. The procedure to determine relevance was identical to the procedure followed for the database search. The relevant publications were then added to the set obtained from the database search.

3.2.2. Classification of the relevant publications

The final set of selected studies was inserted in an Excel file. The publications were divided into four categories according to subject: animal diseases, food and feed safety (hereafter termed food safety), environmental hazards (soil and water), or plant diseases/pests/invasive species (hereafter termed pests). The hazard studied and the methods used were recorded in the Excel file for each publication. The methods used in the studies were classified according to four criteria:

(1) Input data: experimental/historical or simulated.

(2) Type of model: simulation model, optimisation model, or statistical model.

(3) Treatment of uncertainty: deterministic or stochastic model.

(4) Cost items taken into account: only monitoring costs, monitoring and management costs, all direct costs and benefits, or all costs and benefits including indirect effects (for example market effects).

(1) Input data can be experimental, historical, or simulated. Experimental and historical data differ according to how they are generated: experimental data are generated from an experiment that controls for the influence of external factors, whereas historical data are collected from real life situations where these influences are not controlled. We were only interested in whether the results were based on actual or simulated data, and therefore experimental and historical data were considered as a single category. Advantages of using historical and experimental data are that the results are based on real-life data and no assumptions are made. However, a large-scale experiment or a large dataset with historical data is needed to obtain statistically significant results. Alternatively, input data can be simulated or calculated. For example, the sensitivity of a surveillance program can be assessed with a model. Although the parameters are usually based on historical data, the data used to draw conclusions are calculated or simulated. Advantages are the potential to incorporate uncertainty, for example by using a distribution to represent a parameter, and the ability to model situations where no data are available. The main disadvantage is that results are based on an assumption, an approximation, or a calculation with a formula or model, which might not reflect reality.

(2) Studies can use a statistical model, a simulation model, or an optimisation model. A statistical model is defined as an empirical model based on data (observations). A statistical model describes and summarises data, and it uses the data to approximate reality and draw conclusions and to explore the relationships between variables (Ott and Longnecker 2010). A simulation model is a mathematical model that predicts the impact of some inputs and decisions on an output parameter (Winsberg, 2003). Performance of different monitoring programs can be compared by changing input parameters. In contrast, an optimisation model aims to find optimal levels for some (decision) variables. For example, an optimisation model can be used to minimise the total costs of a surveillance program by choosing the optimal set of monitoring activity levels. No advantages and disadvantages of the three model types can be summarised, since the methods have different aims.

(3) The models were classified as either deterministic or stochastic models according to how they treated uncertainty. Models with both deterministic and stochastic parts were considered as stochastic models in this research. A deterministic model has a unique set of outputs, which are determined by the input parameter values and the initial conditions. An average or expected result can be determined (Bolker, 2007). A worst- or best-case scenario

can be estimated by considering more extreme values for the input parameters. A deterministic model can provide results and predictions quickly and the results are also easy to interpret. However, these results do not include variability or uncertainty in the model or its inputs, and therefore, the likelihood of each outcome cannot be assessed.
A stochastic model includes random variables with associated probabilities to account for natural variability and uncertainty. A random variable can, for example, be represented by a discrete or continuous probability distribution. An expected value can be found in this way (Dijkhuizen and Morris 1997). Another option is to use a set of simulated random input variables and to record the output. This procedure is repeated many times with different sets of input variables. The final output is a distribution of outputs from the different simulations, enabling the probability of an outcome to be assessed. A Monte Carlo simulation is frequently used to generate random samples from distributions, and it shows all the possible outcomes and their likelihoods (Fortin and Langevin, 2012; Martins *et al.*, 2017). The advantage of a stochastic model is that it includes model and input variability and uncertainty and generates a distribution of possible outcomes. However, for complicated systems, only a limited number of events can be integrated in the stochastic simulation, making it difficult to draw a clear conclusion.

(4) Finally, the methods were compared according to the cost items that were taken into account: only monitoring costs, monitoring and management costs, all direct costs and benefits or all (direct and indirect) costs and benefits. Monitoring costs can be measured as the time needed to sample and analyse, or as the material and labour costs of the analytical test. Including only monitoring costs is straightforward and requires the least data. However, this approach does not include the consequences of better or worse monitoring, even though these costs can be more important than the monitoring costs alone. Another approach is to consider both monitoring and management costs: management costs are the costs of actions that need to be taken after a contamination has been detected, for example culling, recall, eradication.
A third approach is to consider all the costs and benefits of monitoring by using the partial budgeting method. Partial budgeting can be used to compare the costs and benefits of alternative monitoring plans. It focuses on the added returns, the reduced costs, the added costs, and the reduced returns of each monitoring plan (Dijkhuizen and Morris, 1997). An example of added returns is the improved productivity due to fewer animal diseases (Tambi *et al.*, 2004); an example of reduced costs is the avoided recall cost; an example of added costs is the costs of the monitoring program and the management costs in case a contamination is found; and finally an example of reduced returns might be related to slower production in case of lengthy monitoring plans. The net effect can be summarised as the

total benefits minus the total costs. Partial budgeting enables both costs and benefits to be compared. However, the method requires benefits to be expressed in monetary terms, which might not always be easy to obtain.

Finally, the market effects of a contamination can be estimated using a partial equilibrium model, a model of supply and demand in a market. This type of model only considers markets that are directly affected, that is, it excludes interactions between different markets in an economy. A partial equilibrium model accounts for the effects of an outbreak, contamination, or pest invasion on supply and demand (Surkov *et al.*, 2009). This method is complex and requires a detailed background in economics. Table 3.2 provides a summary of the advantages and disadvantages of the different approaches.

After classifying the publications according to the type of data and models used and the cost categories included in the analyses, these aspects were compared across the different categories of hazards. The results section presents this comparison and provides examples of the different types of methods from different fields.

Table 3.2. Summary of the advantages and disadvantages of the different methods discussed in this study

	Method	Advantages	Disadvantages
Input data	Actual	+ Results are straight-forward + No assumptions	- An experiment has to be set up, or a dataset with historical data is needed. - The results are only based on the results of the experiment or on the historical data.
	Simulated	+ Uncertainty can be incorporated: a parameter can be represented by a distribution for example.	- Based on assumptions, calculations, formulas, approximations: might not be exactly the same as in real-life.
Uncertainty	Deterministic	+ Easier to interpret than a stochastic model	- Results are determined, fixed, no uncertainty is incorporated. - The likelihood of each outcome is not assessed.
	Stochastic	+ Uncertainty is included + All the possible outcomes are shown and how likely they are.	- In complicated systems, only a limited amount of events can be integrated in the simulation - The conclusions are not always straight-forward.
Assessment of costs	Only monitoring costs	+ The easiest way to compare different monitoring strategies + Not much data is needed	- Damage costs or benefits are not considered, which might be more important than the sampling costs
	Monitoring and management costs	+ Management costs are considered as well: might be less with better monitoring	- Benefits are not considered
	All direct costs and benefits	+ All different aspects of the costs are considered + Costs and benefits can be easily compared	- Data is needed on benefits in terms of costs
	All (direct and indirect) costs and benefits	+ All aspects are taken into account	- Data needed - More complex than the other approaches: an economical background is needed

3.3. Results and Discussion

This section starts with presenting the results of the literature search and the results of the classification of the relevant publications. After that, the methods used in the publications harvested are discussed and examples are given for each method. The section on discussing the methods is divided into three parts: a) input data, b) model types and c) assessment of costs. Then, the fourth section, on food safety, discusses the applicability of the different methods to food safety hazards. The final paragraph of this chapter discusses the limitations of the search method used.

3.3.1. Literature search

The database search was conducted in January 2017. A total of 8,914 publications were found in the three different databases (4,212 in Scopus, 3,747 in CAB Abstracts, and 955 in PubMed). After removing the duplicates, 7,207 results remained. These results were filtered based on their titles, resulting in the exclusion of 6,425 titles. The set of remaining relevant titles was then filtered by reading the abstracts: as a result, 606 abstracts were excluded. Finally, the full texts of the remaining 176 publications were read and a final set of 78 publications was selected. The snowballing technique was then applied. After screening the reference lists of the 78 selected publications, 79 additional titles were considered relevant. After reading the abstracts, 28 of these 79 publications remained. Finally, after reading the full texts, 21 additional publications were considered relevant and were added to the 78 publications previously found. The analysis in the remainder of the study is based on these 99 references. The selection process and the exclusion criteria are depicted in Figure 3.1.

3.3.2. Classification of the relevant publications

The different methods used in the different categories are summarised in Table 3.3. Only nine publications were classified in the category food safety, whereas the categories of animal diseases and pests had 35 publications each. Finally, 17 publications belonged to the category environmental hazards. Three publications did not fit within a category and were therefore placed in a separate group of general publications. The 99 references are listed according to category in the Appendix.

The results of the literature search confirmed our initial observation that very little research has been undertaken on cost-effective monitoring of hazards in food safety. The publications on monitoring food safety were from 2007 onwards, and therefore more recent than publications from other fields. This research originated from only three countries: Denmark

(four references), the United States (three references), and The Netherlands (two references). These results show that research on cost-effective monitoring in food safety is scarse and relatively recent. The methods used in this category are similar to the methods used in other categories, except that no studies using experimental data with a statistical model were found.

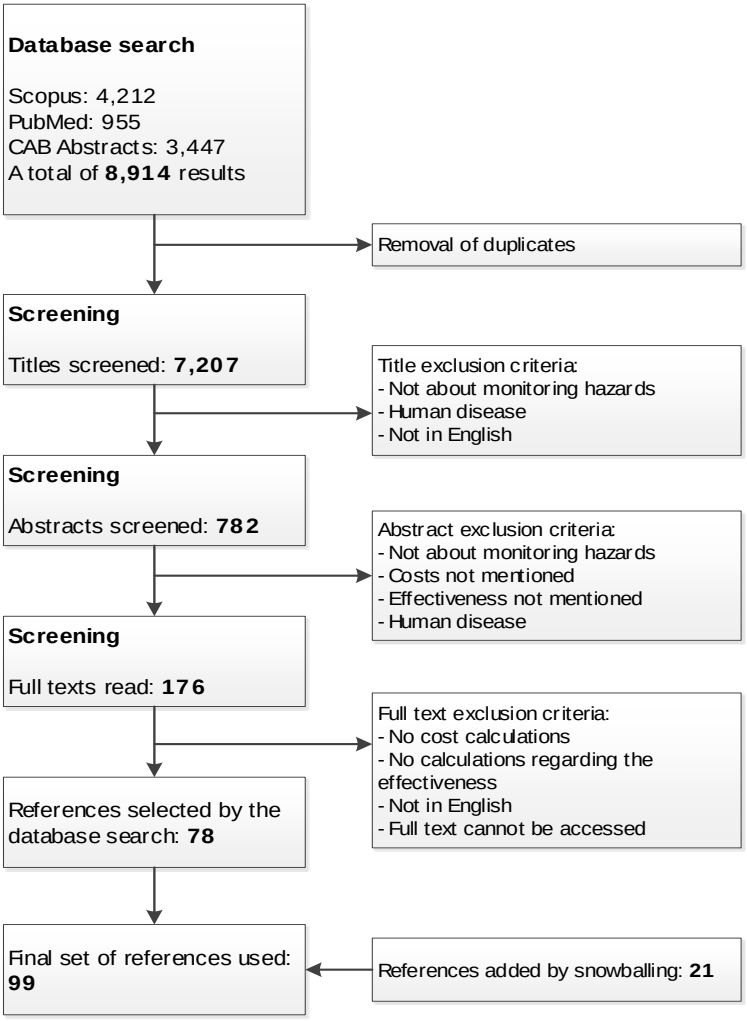

Figure 3.1. The selection process of the relevant literature harvested for this study (the search was finalised early January 2017)

Table 3.3. The different methods used to assess the cost-effectiveness of a monitoring plan

		Animal diseases	**Food and Feed Safety**	**Environmental hazards** (soil and water)	**Pests**	**Total** (including general papers)
Number of publications		35	9	17	35	99
Input data	Experimental/ historical data	3 (9%)	0 (0%)	2 (12%)	15 (43%)	20 (20%)
	Simulated	32 (91%)	9 (100%)	15 (88%)	20 (57%)	79 (80%)
Uncertainty	Deterministic	7 (20%)	2 (22%)	5 (29%)	22 (63%)	38 (38%)
	Stochastic	28 (80%)	7 (78%)	12 (71%)	13 (37%)	61 (62%)
Model type	Statistical	3 (9%)	0 (0%)	2 (12%)	14 (40%)	19 (19%)
	Simulation	27 (77%)	4 (44%)	7 (41%)	7 (20%)	47 (47%)
	Optimisation	5 (14%)	5 (56%)	8 (47%)	14 (40%)	33 (33%)
Assessment of costs	Only monitoring costs	20 (57%)	5 (56%)	11 (65%)	17 (49%)	56 (57%)
	Monitoring and management costs	6 (17%)	1 (11%)	3 (18%)	8 (23%)	18 (18%)
	All direct costs and benefits	9 (26%)	3 (33%)	3 (18%)	9 (26%)	24 (24%)
	All (direct and indirect) costs and benefits	0 (0%)	0 (0%)	0 (0%)	1 (3%)	1 (1%)

3.3.3. Analysis of the methods used

The pests category differed from the other categories, because studies in this category used more experimental data, deterministic models, and statistical models than studies in the other categories. In the pests category, experiments are relatively easily designed and conducted. Examples of experiments include experiments to test different insect traps, to compare different water sampling methods, or to compare the performance of different sampling techniques for insects. In contrast, setting up an experiment with artificial contamination from a hazard is less feasible in the categories of environmental sciences, animal diseases, and food safety. Stochastic simulation models were the most frequently used models in the category animal diseases, because it is difficult to experiment with diseases. Stochastic variables were frequently used in this category.

The cost items considered had a similar distribution in all categories: approximately half of the references in each category only considered the monitoring costs, a quarter included management costs, and the remaining quarter estimated both the costs and benefits. In the following subsections, the input data, models, and the assessment of costs are discussed in more detail with the use of examples.

The following sections describe the input data used, the model types and the assessment of costs using examples from the publications retrieved by the search. These examples are chosen in order to represent at best all fields and all different methods deemed relevant from the literature study.

Input data

Experimental and historical data were used more often in the pests category than in the other categories. This type of data was used in 15 of the 35 publications. Puckett (2013) designed an experiment to test the effectiveness of six different traps for phorid flies. The design was as follows: a grid was set on an aerial map, 20 sampling blocks (replicates) were selected, six sampling points within each block were chosen, and the traps were set. The traps were then brought to the lab for further assessment. Tunks *et al.* (2005) designed an experiment to compare diffusion samplers with conventional samplers for groundwater contamination. In the research by Hodgson *et al.* (2004), ten commercial soybean fields were sampled and analysed for pests for a period of three years.

Simulated data were the preferred type of data in all categories. For example, a scenario tree model was frequently used in the category animal diseases to estimate the sensitivity of a monitoring plan. The review shows that very few historical data were available; assumptions and formulas were used to fill the gaps. Future research could be improved if

large datasets become available, which can be shared and combined to improve the reliability of the research conducted.

Model types

Three categories of models were differentiated: statistical models, simulation models, and optimisation models. Statistical models were used in 14 of the 35 publications in the pests category. Rosado *et al.* (2014) compared different sampling plans for pest mites on nuts. They analysed the results of their experiment using different statistical tools and models: T test, ANOVA, Tukey's test, and linear regression. An example of a statistical model in the category animal diseases is the study of van Schaik *et al.* (2007), who compared the sensitivity of two paratuberculosis tests: a faecal culture on pooled samples versus a less sensitive ELISA test. Blood and faecal samples were collected from commercial farms in Chile. The study used Fisher's exact test to determine the difference in sensitivities.

In general, simulation models were used most frequently (47%) and statistical models were used least frequently (19%). However, the proportions differed across the categories of hazards. Simulation models were used most frequently in the category animal diseases (77%), a large percentage of these were scenario tree models. Simulation models were used to simulate the transmission of a disease in animals, the spread of a pest population, the spread of a chemical in soil or water, and the probability that a monitoring plan detects a hazard.

Optimisation models were used in one third of all publications, and they were used relatively more frequently in the categories food safety (five of the nine publications) and environmental hazards (eight of the 17 publications). Methods used to solve these optimisation problems were genetic algorithms, simulated annealing, linear programming, and stochastic dynamic programming (SDP). Simulated annealing was used by Nunes *et al.* (2004) to optimise groundwater monitoring. One station at a time was replaced and the result was evaluated with regard to the objective function and whether the Metropolis criterion was fulfilled. A decision was then made to either keep or reject the change. Simulated annealing was, however, only able to find optimal local solutions (Nunes *et al.*, 2004). A genetic algorithm is a method used to solve an optimisation problem and is based on the Darwinian principles of natural evolution (McCall, 2005). Genetic algorithms were frequently used in the category environmental hazards, for example by Reed *et al.* (2000) and Reed *et al.* (2001) to optimise groundwater monitoring. In these studies, fitness values, which are measures of quality, were assigned to each sampling plan and were used to determine which sampling plans were allowed to reproduce and make a new population. Several iterations were made and a new population was created each time until the genetic algorithm converged (Reed *et al.*, 2000). NGSA-II is a second-generation non-dominated

sorting genetic algorithm developed by Deb *et al.* (2000) to rapidly solve multi-objective optimisation problems (Deb *et al.*, 2002). This technique was used by Reed and Minsker (2004) to optimise groundwater monitoring. The objectives of this optimisation problem were to minimise the sampling costs, maximise the relative accuracy of local concentration estimates, maximise the relative accuracy of global mass estimates, and minimise the estimation uncertainty.

SDP is a method used to optimise an objective function for a fixed period. Moore *et al.* (2010) used this method to find an optimal budget allocation for surveillance of invasive species on an island. The model calculated the optimal allocation for a fixed time period by applying an iterative backward process. By starting at the final time spot, the process accounted for future expected costs of invasive species. The SDP stepped back to the previous time period and calculated the expected costs for each decision and repeated this for each time period. The optimal decision for each state was then found (Moore *et al.*, 2010).

Linear programming was used, for example, by Lascano-Alcoser *et al.* (2013) to determine the optimal level of monitoring activities for dioxins in milk. Linear programming requires the specification of a linear objective function to be minimised or maximised by changing decision variables, given some constraints (for example, limited resources). The feasible region contains the solutions that satisfy all the constraints. Within the feasible region, the optimal solution has the most favourable value for the objective function. The simplex method considers the corner points to find the optimal solution (Dijkhuizen and Morris, 1997). Yemshanov *et al.* (2015) circumvented the requirement for a linear objective function by using a piecewise approximation of a nonlinear objective function.

Multi-objective optimisation models were encountered a few times. Bode *et al.* (2016) estimated Pareto-optimal solutions for their multi-objective optimisation model to optimise groundwater monitoring. The first objective was to detect with maximum probability all possible future contaminations. The second objective function was a maximum early-warning time for installing counteractions. The third objective function was to minimise costs. The first two objective functions relied on model-based predictions and Monte Carlo simulations were used to include uncertainty.

Vos *et al.* (2015) combined a stochastic simulation model with an optimisation model. The simulation model simulated the introduction of paratuberculosis into an importing country through importation of live animals. The model was run for 50,000 iterations. Model outputs were the number of imported infected animals and the number of detected infected animals (taking into account the sensitivity and specificity of the test). Two effectiveness parameters were considered: the number of infected animals detected and the net economic benefit. The model either minimised losses or maximised the number of infected animals detected. The optimised parameter was the percentage of tested animals from each imported group.

The constraint was the total number of animals sampled (maximum costs for testing). Costs of testing, management costs, and possible costs due to an outbreak were taken into account.

Deterministic simulation models were the most frequently used method in the pests category, used in 22 of the 35 publications. Bogich *et al.* (2008) developed a deterministic simulation model to determine the optimal trap density for an invasive species, the gyspy moth in the USA. Colonies were represented by circular regions, and if the region overlapped, with a survey point, then the colony was detected and eradicated; if it was not detected, it continued to grow. All colonies had to be eradicated at the end of the program. The model could determine the optimal trap density, the minimum total cost (trap costs and eradication costs) for the whole program, and the minimum total cost per year for programs from one to 20 years. Examples of deterministic models were also found in the category animal diseases. Boden *et al.* (2012) compared three control strategies for scrapies using a deterministic transmission model to simulate the within-flock transmission of scrapies for low, medium, and high prevalance flocks. Massaro *et al.* (2013) used a deterministic simulation model to estimate the transmission of paratuberculosis between cows placed in different states included in the model: heifers, calves, adults, susceptible, exposed, low shedding, and high shedding. Revenues were the slaughter value of the culled animals, the value of the sold animal, and the value of milk produced. Expenditures were the costs for ELISA or EVELISA, the costs of replacing culled animals, and the overhead and operating costs. The model ran for a period of 10 years and compared three strategies: no testing, ELISA, or EVELISA. The most cost-efficient surveillance system was the system with the highest average revenue per cow.

Overall, 62% of all models used were stochastic. In the category animal diseases, 80% of the models had at least one stochastic variable and stochastic simulation models were common. A Monte Carlo simulation was frequently used to generate random samples from distributions. Values were sampled at random from the input probability distributions. Each combination of input variables was an iteration, hundreds or thousands of iterations were performed to create a probability distribution of the outcome. Scenario-tree models were common stochastic simulation models in the category animal diseases. A flowchart of the disease progression helped to show each step of the process and enabled easy simulation of the effect of different monitoring systems on the outcome. Nodes represented events that had to occur to achieve the outcome and the branches each had a different probability. For example, the JohneSSim model was a stochastic and dynamic simulation model of paratuberculosis within a herd, which simulated the herd dynamics, the disease dynamics within the herd, the control of paratuberculosis, and the economic consequences at herd-level for a period of 20 years (Weber *et al.*, 2004). In the category environmental hazards, a

stochastic simulation model was used to compare an alternative groundwater monitoring approach with the conventional system. The model simulated the release of a contaminant plume and estimated the detection of the contamination and the size of the plume at detection time. The Monte Carlo approach was used to add uncertainty (Yenigul *et al.*, 2006). In the food safety category, Alban *et al.* (2016) used a scenario tree to evaluate the performance of a monitoring system for the detection of antimicrobial residues. Monte Carlo simulations were used. Finally, in the pests category, Cacho *et al.* (2010) used a stochastic simulation model to simulate the spread of an invasive population and study the interactions between active and passive surveillance.

Assessment of costs

More than half of the selected publications only assessed the monitoring costs. Monitoring and management costs were assessed in approximately one fifth of the publications The remaining quarter of the publications assessed both costs and benefits using partial budgeting. Examples of management costs were the costs of eradicating the pest, the costs of culling animals, or the costs of tracing back the contamination. A single publication used a partial equilibrium model to analyse the effects of a hazard on the market (Surkov *et al.,*2009).

Different indicators were used to compare the effectiveness and costs of a monitoring strategy. The benefit-cost ratio (BCR) relates the costs of monitoring to the benefits, both of which are expressed in monetary units. In case part or all of the costs and benefits occur in the future, then they should be discounted so that the ratio represents the present value of costs and benefits (Dijkhuizen and Morris 1997). The net present value (NPV) can be used to measure the profitability of a monitoring strategy, and it represents the difference between the present value of the cash inflows (benefits in monetary units) and the present value of the cash outflows (costs). The future benefits and costs are discounted so that the NPV represents the value of the monitoring program at present (Dijkhuizen and Morris 1997). If the discount rate is high, a monitoring program with a high initial cost and benefit in the future is penalised, because the money earned today is worth more than money earned in the future. The NPV method was used to assess the monitoring of rinderpest in cattle in Africa. The direct costs of economic losses were compared with the baseline scenario of no intervention. The annual costs and benefits were projected over time and discounted at 12% over a 12-year period to compute the BCR as a measure of economic efficiency and NPV as a measure of economic feasibility (Tambi *et al.*, 2004). Tunks *et al.* (2005) calculated the return on investment for the issue of groundwater contamination: the potential cost savings were divided by the implementation costs of passive diffusion samplers. To calculate a BCR,

an NPV, or a return on investment, a partial budgeting approach is required to identify and quantify all the costs and benefits in monetary terms.

The cost-effectiveness ratio relates the total costs of a program, or the costs relative to an alternative solution, to the effectiveness of the program. This ratio is used when the benefits are not easily quantifiable in monetary units (Dijkhuizen and Morris 1997). The effectiveness or benefits are not expressed in monetary units but can be measured in different ways. Examples of effectiveness measures include the probability of disease transmission (Häsler *et al.*, 2012), the probability to detect disease (Knight-Jones *et al.*, 2010; Tavornpanich *et al.*, 2006; Tavornpanich *et al.*, 2008), or the proportion of correct decisions (Paula-Moraes *et al.*, 2011). The monitoring costs alone are sufficient to calculate a cost-effectiveness ratio.

To conclude, more than half of the publications only assessed the monitoring costs. This is because this approach is straightforward and requires only limited data. Calculating the damages and management costs requires more information than may be readily available. A further explanation may be found in the focus of the authors, as many of the publications were written by groups with a focus on life sciences rather than economics. A partial equilibrium model was only encountered once and was developed by a group specialised in business economics.

3.3.4. Food Safety

Few references were found in the food safety category, and all were relatively recent studies. This suggests that cost-effectiveness of monitoring strategies in food safety is only starting to be investigated and more research is needed to improve the cost-effectiveness of current monitoring strategies. The methods identified in this literature review can be applied to improve research on cost-effective monitoring of hazards in food. We discussed the applicability of the different methods to food safety, based on what was observed in this research as well as the strengths and weaknesses of each method summarised in Table 3.2. Since performing experiments with hazards in food safety is neither easy nor ethical to perform, similar to animal diseases, little experimental data will be available or produced. No publications using experimental data were found in this research. Calculated and simulated data will be more applicable to food safety hazards.

Statistical methods are less applicable to food safety. As stated in the previous paragraph, little experimental data will be available. Simulation models, as used in four out of the nine publications, are an appropriate type of model to predict the outcome of different monitoring methods. Simulation models are a way to compare different scenarios and predict outcomes. Optimisation models, used in five out of the nine publications, can be used to allocate a budget or to optimise a current monitoring plan. All different ways of solving

optimisation models, encountered in this research and described in the previous section can be used: genetic algorithms, simulated annealing, linear programming, and stochastic dynamic programming (SDP). The effectiveness can be optimised for a fixed budget or the costs can be reduced by achieving the same effectiveness at a lower cost. If simulated or calculated data is used, a stochastic model, as used in seven out of the nine publications, is preferred to a deterministic model, since, in that way, uncertainty is included and different ranges of input data can be explored.

With regards to assessing the costs of a monitoring plan, assessing all direct costs and benefits, using partial budgeting seems to be the most comprehensive way of assessing the costs of a monitoring plan. This was however done in only three out of the nine publications.

Since the costs and benefits might not be the same for the different stakeholders (farmers, producers, consumers), multi-criteria decision analysis can be used to help choose between alternative solutions, taking into account preferences of different stakeholders. The stakeholders' interests are represented by weights attached to the criteria used to rate the alternatives. This technique was used by Guo *et al.* (2014) to optimise the monitoring of swine fever for different stakeholder preferences.

These methods can be applied to mycotoxin monitoring for example: mycotoxin contamination in cereals, fruits, and nuts leads to huge economic losses and potential serious health problems for both humans and animals. Therefore, finding a cost-effective monitoring system is crucial.

3.3.5. Limitations of the search method

This review covered published peer-reviewed primary research papers and published book chapters only; the search did not provide an exhaustive list of methods used in all scientific research. However, by restricting the review to published material, only scientifically validated methods were gathered, which provided a broad overview of the methods in different fields. Only studies written in English were considered. This was considered acceptable because English is widely accepted as the *lingua franca* in science. The search was restricted to references published between January 2000 and December 2016 to provide an overview of the most recent methods, techniques, and applications, thereby capturing the state-of-the-art in these fields. References were searched with keywords. The choice of keywords was crucial to gather relevant studies. Omitting potential synonyms in the search string might have led to missed relevant references. To address this, we added synonyms encountered in the publications of our initial searches.

3.4. Conclusion

Our hypothesis that monitoring schemes in the field of food safety rarely include an economic assessment was confirmed by the search results. As the budget allocation available for monitoring is usually limited, it is important to optimise monitoring plans taking into account both costs and effectiveness. In this way, the effectiveness can be optimised for a particular budget or a monitoring scheme can be developed that achieves the same effectiveness at lower cost. The methods encountered in this review can be adapted and used in the field of food safety. Stochastic simulation models, using both historical and simulated data, and optimisation models are proven to be suitable. Although used in only one third of the publications encountered in food safety, considering all directs costs and benefits seems to be more comprehensive since the benefits of monitoring, such as an avoided recall of contaminated food placed on the market, can be more important than the costs. A relatively new development that might enrich research on cost-effective monitoring of hazards is the combination of expertise, knowledge, and models from different fields. An example of such interaction is the modelling of zoonoses where plant and animal diseases and food safety all interact and should therefore be modelled together.

Appendix: Results of the systematic literature search

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Chapter 4

Cost-effective Sampling and Analysis for Mycotoxins in a Cereal Batch

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Published in: Risk Analysis 39(4): 926-939. https://doi.org/10.1111/risa.1320

Abstract

The presence of hazards (e.g., contaminants, pathogens) in food/feed, water, plants or animals can lead to major economic losses related to human and animal health or the rejection of batches of food or feed. Monitoring these hazards is important but can lead to high costs. This study aimed to find the most cost-effective sampling and analysis (S&A) plan in the cases of the mycotoxins deoxynivalenol (DON) in a wheat batch and aflatoxins (AFB_1) in a maize batch. An optimisation model was constructed, maximising the number of correct decisions for accepting/rejecting a batch of cereals, with a budget as major constraint. The decision variables were the choice of the detection method: instrumental method (e.g. Liquid Chromatography combined with Mass-Spectrometry (LC-MS/MS)), Enzyme-Linked-Immuno-Assay (ELISA) or Lateral Flow Devices (LFD), the number of incremental samples collected from the batch, and the number of aliquots analysed. S&A plans using ELISA, showed to be slightly more cost-effective than S&A plans using the other two detection methods. However, for DON in wheat, the difference between the optimal S&A plans using the three different detection methods was minimal. For AFB_1 in maize, the cost-effectiveness of the S&A plan using instrumental methods or ELISA were comparable whereas the S&A plan considering on-site detection with LFDs was least cost-effective. In case of non-official controls, which do not have to follow official regulations for sampling and analysis, on-site detection with ELISA for both AFB_1 in maize and DON in wheat, or with LFDs for DON in wheat could provide cost-effective alternatives.

4.1. Introduction

A huge amount of hazardous agents, such as chemical contaminants (e.g. heavy metals, pesticides) and pathogens are observed in the environment, our food and drinking water, in animals and in plants. The agricultural and food industry should avoid high concentrations of these hazardous agents to protect human, animal and plant health. In the agricultural and food industry, regular monitoring is performed as part of quality management systems, such as Hazard Analysis Critical Control Point (HACCP) programs to check whether the concentration of the hazardous agent complies with pre-defined thresholds.

Governmental monitoring aims to verify that the presence of the hazardous agent in plants, animals or foods is well below legal limits, and to obtain an overview of the current presence of the agent. As part of such monitoring systems, samples are collected and analysed for the potential presence of the agent. Since most hazardous agents are not homogeneously distributed throughout the particular population (e.g. herd of animals) or unit (e.g. batch of grains), which means that there is variability in time and/or space, and several random samples have to be collected from the population. These collected samples can, for certain products, then be pooled into one aggregate sample, of which one or a few aliquots can be analysed, and lastly, the volume of the aliquots can be varied. However, the number of samples that is collected, the number of aliquots that is analysed, and the choice of the detection method is restricted by the available budget (B). An optimal sampling and analytical (S&A) plan should be applied taking both the effectiveness and the costs into account.

This study used mycotoxins in cereals as a case study to develop an optimal, cost-effective S&A plan, for a truckload or ship compartment of cereals at arrival. Mycotoxins are secondary metabolites, produced by fungi, in crops and derived food and feed products. The presence of mycotoxins in cereal batches can lead to severe negative health effects and to major economic losses (Marin *et al.*, 2013). An extreme case scenario would be a contaminated batch entering the food supply chain and leading to a major food safety incident. An example is the case of 2013, when milk produced in the Netherlands and Germany was found to be contaminated with aflatoxin M_1. The cause appeared to be imported maize severely contaminated with aflatoxins that was used as dairy cows' feed. Recalls of the feed were carried out (De Rijk *et al.*, 2015).

The S&A plans used for official control for mycotoxins by competent authorities in Europe are described in Regulation (EU) No 401/2006 (EU, 2006c). These S&A plans consist of collecting incremental samples at different locations within the batch, combining these collected samples into one aggregate sample and extracting one aliquot from the aggregate sample for chemical analysis. In Regulation (EU) No 401/2006 an aliquot is referred to as

a laboratory sample (EU, 2006c). The aliquot should then be analysed with an detection method that fulfils predefined performance criteria (EU, 2006c). Several instrumental methods, for example Liquid Chromatography coupled to Mass-Spectrometry (LC-MS/MS), fulfil these criteria (Spanjer *et al.*, 2008). These instrumental methods are performed in an appropriately equipped laboratory and are nowadays often used since it allows the simultaneous analysis of multiple mycotoxins (Berthiller *et al.*, 2017).

The S&A plan used for official controls has to comply with the regulatory framework. However, food and feed companies could apply an alternative S&A plan, using a cheaper and faster detection method. The review of Berthiller *et al*. (2017) on recent updates on mycotoxin analysis, frequently mentions the rapid methods Enzyme-Linked-Immuno-Assay (ELISA) and Lateral Flow Devices (LFDs) (Berthiller *et al.*, 2017) as alternative methods to instrumental methods for the food industry. ELISA has to be performed in a laboratory. However, because this method requires less time and uses cheaper materials, more incremental samples and/or more aliquots can be analysed for the same budget than when using instrumental methods. Some ELISA tests have been validated for specific toxins in specific matrices (Alshannaq and Yu, 2017). LFDs can be used on site, making it attractive for food and feed companies. Each incremental sample can be directly analysed by means of this method, and results are directly available. Some LFDs have been validated for specific matrices and concentrations as screening tests (Lattanzio *et al.*, 2014). However, the reliability of LFDs depends on the matrix, over and under-estimation, as well as cross-reactivity is frequently observed (Krska and Molinelli, 2009). These rapids methods tend to have a larger relative standard deviation (RSD) between test results of the same samples. This high RSD makes these methods less precise than instrumental methods (Meneely *et al.*, 2011). However, since these methods are cheaper, more aliquots can be analysed with the same budget, which lowers the total analytical variance.

The aim of this study was to develop a cost-effective S&A plan for two mycotoxin-crop combinations: DON in a batch of wheat, and AFB_1 in a batch of maize. These combinations were selected since these are two of the most relevant mycotoxin-grain combinations within Europe given the EU regulations, the occurrence of the toxins and volumes of these grains used for feed and food production. The S&A plan was defined by the combination of the number of incremental samples per batch, the number of aliquots analysed and the detection method used. The most cost-effective S&A plan was considered to be the plan which would lead to the highest number of correct decisions on rejecting/accepting batches within the given budget. Batches are rejected in case the test results are above the limits set in EU regulations.

4.2. Methods

An optimisation model was constructed to determine the most cost-effective S&A plan for mycotoxins in a cereal batch. This model optimised the effectiveness of the S&A plan by choosing one of the three pre-defined S&A plans, each using a different detection method, and by optimising the number of incremental samples collected from the batch and the number of aliquots analysed. The financial budget available for sampling and analysis was used as constraint. The following section, section 3.3.1, details the assumptions the model was based on. Thereafter, section 3.3.2 presents the optimisation model.

4.2.1. Assumptions

Sampling and analytical (S&A) plans

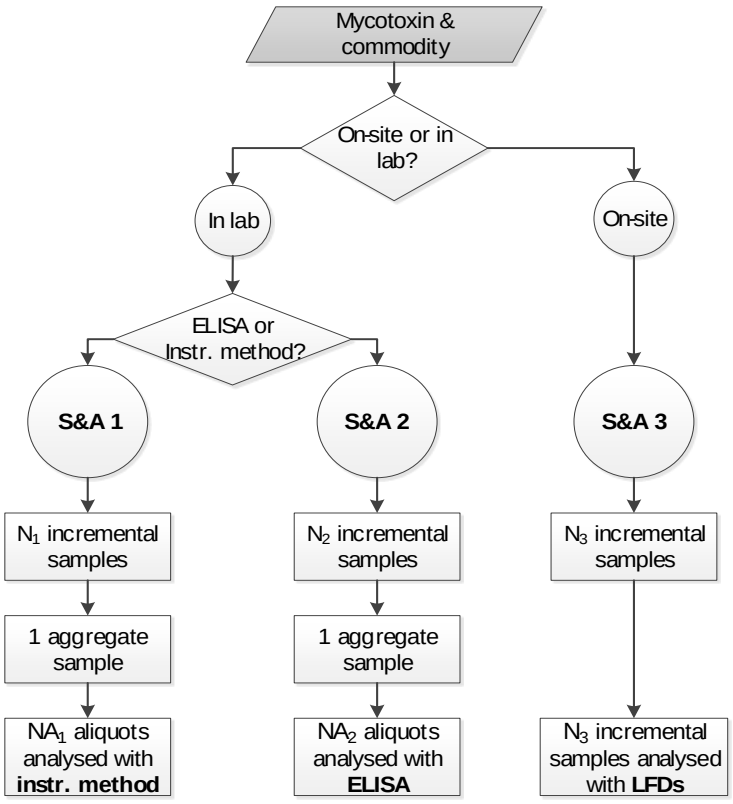

Figure 4.1. Flowchart of the different sampling and analytical plans

Figure 4.1 presents a flowchart of the three different pre-defined S&A plans considered in this study. The three detection methods considered were instrumental methods, ELISA and

LFDs. Instrumental methods and ELISA were performed in a laboratory. All incremental samples collected were pooled into one aggregate sample, and one or more aliquots per aggregate sample were analysed by means of an instrumental method for the first S&A plan or ELISA for the second S&A plan. The third S&A plan was based on LFDs: each incremental sample collected from the batch was directly analysed on-site, and the analytical result was immediately available. In the third S&A plan, we assumed that the incremental samples collected were not pooled together into one aggregate sample because of insufficient equipment on-site to grind and homogenise a large aggregate sample. Instead, all incremental samples collected were analysed individually with a LFD.

The variance of a sampling and analytical plan

Comparison of the effectiveness of the S&A plans was based on the variance associated with the measured concentration. The larger the variance, the less effective a S&A plan is in discriminating compliant and non-compliant batches. The total variance of a S&A plan was calculated as the sum of the variance due to the sample collection, the sample preparation and the analysis of the sample (Whitaker, 2003). Variance due to sample collection is related to the fact that mycotoxins are not homogeneously distributed throughout the batch (De Rijk *et al.*, 2015). The variance from the preparation of the laboratory sample is related to the fact that only a small sample is taken from the large aggregate sample made from all the incremental samples collected from the batch. The detection method used encompasses variance as well: the analytical variance (Whitaker, 2003). The formulas to calculate the variance due to the sample collection and sample preparation of each S&A plan were based on formulas used in literature (FAO, 2014). The variance due to the analysis was based on the relative standard deviation for repeatability (RSD_r) of each detection method considered, measured within a lab and reported in papers or reports (Astoreca *et al.*, 2017; CODA-CERVA, 2009; 2011; Lupo *et al.*, 2010a; Lupo *et al.*, 2010b; Tangni *et al.*, 2010; Van Asselt *et al.*, 2012; van der Fels-Klerx and de Rijk, 2014) and listed in Table A.2 in Appendix A. The RSD_r gives an estimation of the precision of an detection method with comparable conditions, so within a lab and with the same operator. The accuracy, which is a combination of precision and trueness (or bias), can be measured by the toxin recovery, the relative standard deviation for reproducibility (RSD_R) measured between-labs and the RSD_r. The choice was made to focus on the precision of an detection method only (RSD_r). By not considering other characteristics than the RSD_r, we assumed that all detection methods were equally unbiased over the full range of concentrations and that the matrix effect (toxin recovery) was the same for all detection methods. In order to calculate the total variance of a S&A plan, the necessary inputs were: the mycotoxin and the commodity of interest, the expected average batch concentration, the number of incremental samples collected, the

number of aliquots analysed, and the detection method used. The formulas used to calculate the total variance of the S&A plan are presented in Table 4.1 and explained in more detail in Table A.1 in Appendix A.

Table 4.1. Parameters of the model

	Explanation	Formula/assumption
Index		
i	The choice of the detection method	i=1, 2 or 3 for S&A plans 1, 2 and 3, respectively
Decision variables		
α_i	Dummy variable	$\alpha_i \in \{0;1\}$
N_i	The number of samples collected from the batch with method i	
NA_i	The number of aliquots analysed with method i	$N_3 = NA_3$
Model parameters		
C	The batch concentration	in mg/kg for DON, in µg/kg for AFB_1
Cr	The natural logarithm of the limit	ln(1.25) for DON in wheat (EU, 2006a) ln(5) for AFB_1 in maize (EU, 2006a)
T	The total number of batches that are sampled	T=1,000
$f(C)_{DON}$	The fraction of T wheat batches with concentration C	$\frac{GAMMA.DIST(C\,;1.03\,;2.12)}{\sum_{c=0.01}^{C=5} GAMMA.DIST(C\,;1.03\,;2.12)}$
$f(C)_{AFLA}$	The fraction of T maize batches with concentration C	$\frac{GAMMA.DIST(C\,;0.61\,;0.27)}{\sum_{c=0.1}^{C=50} GAMMA.DIST(C\,;0.61\,;0.27)}$
$Var(C)_{1\text{-}DON}$ [a]	Variance of the sampling plan for DON in wheat with an instrumental method	$\frac{1.18}{N_1}C^{0.833} + 0.0033C^{0.833} + \frac{(0.05C)^2}{NA_1}$
$Var(C)_{2\text{-}DON}$ [a]	Variance of the sampling plan for DON in wheat with ELISA	$\frac{1.18}{N_2}C^{0.833} + 0.0033C^{0.833} + \frac{(0.9C)^2}{NA_1}$
$Var(C)_{3\text{-}DON}$ [a]	Variance of the sampling plan for DON in wheat with LFDs	$\frac{1.18}{N_3}C^{0.833} + \frac{(0.13C)^2}{NA_3}$
$Var(C)_{1\text{-}AFLA}$ [a]	Variance of the sampling plan for aflatoxins in maize with an instrumental method	$\frac{128.4}{N_1}C^{0.98} + 0.1254C^{0.98} + \frac{(0.09C)^2}{NA_1}$
$Var(C)_{2\text{-}AFLA}$ [a]	Variance of the sampling plan for aflatoxins in maize with ELISA	$\frac{128.4}{N_2}C^{0.98} + 0.1254C^{0.98} + \frac{(0.16C)^2}{NA_2}$
$Var(C)_{3\text{-}AFLA}$ [a]	Variance of the sampling plan for aflatoxins in maize with LFDs	$\frac{128.4}{N_3}C^{0.98} + \frac{(0.22C)^2}{NA_3}$
µ	Mean of the Lognormal distribution	$\ln(C) - \frac{\sigma^2}{2}$ (Lyman *et al.*, 2011)

σ^2	Variance of the Lognormal distribution	$\ln(\frac{var(C)}{C^2}+1)$ (Lyman *et al.*, 2011)
$PA(C)_i$	Probability to accept the batch	NORM.DIST(Cr, μ, σ^2, TRUE)
CBA_i	The number of compliant batches that are accepted	$\sum_{C=0}^{Cr} T * f(C) * PA(C)_i$
$NCBR_i$	The number of non-compliant batches that are rejected	$\sum_{C>Cr}^{Cmax} T * f(C) * (1 - PA(C)_i)$

[a] Explained in more detail in Table A.1 in Appendix A

When is a batch compliant?

In order to decide whether the batch was compliant to the Regulation No181/2006, the result given by the detection method, or the mean of the results in case of analysing multiple aliquots, was considered. Regulation No 401/2006 suggests to take into account the measurement uncertainty when deciding if a batch is complaint or not. If the result is above the limit but the limit is within the uncertainty range, or if the mean result is below the limit and the limit is within the uncertainty range, the batch can be accepted (EU, 2006c). The measurement uncertainty associated with the analytical result reported includes all effects that cause the dispersion of the results. This is not the same as the precision of the detection method, in this study based on the RSD_r. However, the precision of a detection method has an influence on the uncertainty of an analytical result (Stroka and van Egmond, 2006). For sake of simplicity, the measurement uncertainty was not considered in this study.

The limit for AFB_1 used in this study was 5μg/kg. This is the legal limit for "maize and rice to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs" as stated in Section 2 of the annex of Regulation No 181/2006 (EU, 2006a). For DON, the limit of 1,250μg/kg was considered, which is the limit for "unprocessed cereals other than durum wheat, oats and maize", described again in the annex of Regulation No 181/2006 (EU, 2006a).

The batches considered

The AFB_1 concentrations of 8,496 maize batches, analysed between 2001 and 2016, were retrieved from the Dutch database of the Program for the Quality of Agricultural Products (KAP). After fitting several distributions to the data (Delignette-Muller *et al.*, 2010), the gamma distribution was chosen, with a shape of 0.61 and a rate of 0.27. The concentrations between 0.1 and 50μg/kg were considered in this study; this range was based on the detection limit and AFB_1 occurrence in Europe. This gamma distribution was used to calculate the frequencies of the concentrations within this range.

The DON concentrations of 4,113 wheat batches, analysed between 2001 and 2016 as part of the Dutch monitoring program were gathered from the KAP database. Several

distributions were fitted to the data (Delignette-Muller *et al.*, 2010). The gamma distribution was chosen, with a shape of 1.02 and a rate of 2.12. Based on detection limits and occurrence in Europe, concentrations between 100 and 5,000µg/kg were considered in this study. This gamma distribution was used to calculate the frequency of the concentrations within this concentration range.

The costs of S&A plans

The costs of sampling and analysing were assumed to have a fixed cost component and some variable cost components per incremental sample collected and per aliquot analysed. The costs of the detection methods were based on data collected from literature and personal communications, and were assumed to be the same for DON and AFB_1. Equation (1) gives the total costs (TC) of the S&A plan:

$$TC = \sum_{i=1}^{i=3} \alpha_i (a_i + b_i N_i + c_i NA_i) \quad (1)$$

Where TC are the total costs, i was equal to 1 for S&A plan 1, 2 for S&A plan 2, and 3 for S&A plan 3. α_i was a dummy variable representing the choice of the S&A plan, a_i were the fixed costs for S&A plan i, b_i were the variable costs per incremental sample collected for S&A plan i, c_i were the variable costs per aliquot analysed for S&A plan i, N_i the number of samples collected with S&A plan i, and NA_i the number of aliquots analysed with S&A plan i. The variable and fixed costs included both material costs and labour costs. The prices were assumed to be same for DON and AFB_1, even though slight variations can be expected. The costs used as input parameters for the model are listed in Table 4.2, and were based on data collected and calculations shown in Table B.1 and Table B.2 in Appendix B.

Table 4.2. Costs used in the model

parameter	Costs (€)	details
a_1	367	Fixed costs for S&A plan 1, consisting of: Sample collection: €160 Transport: €15/30kg Storage: €96/day
a_2	285	Fixed costs for S&A plan 2, consisting of: Sample collection: €160 Transport: €15/30kg Storage: €96/day Labour costs for ELISA €14/plate
a_3	160	Fixed costs for S&A plan 3, consisting of: Sample collection: €160
b_1	10	Variable costs (labour costs) per sample collected for S&A plan 1
b_2	10	Variable costs (labour costs) per sample collected for S&A plan 2
b_3	30	Variable costs (labour and material costs) per sample collected and analysed for S&A plan 3
c_1	100	Variable costs per aliquot analysed with an instrumental method
c_2	8	Variable costs (material cost) per aliquot analysed with ELISA

For the sample collection, a fixed cost of €160 was considered, which was used to hire a trained person to collect the samples (NVWA, 2016). In addition, variable costs of €10 per incremental sample collected of labour costs were added. Material costs for maize and wheat were assumed to be negligible. However for higher value commodities, material costs should be included. Both the fixed costs and the variable costs of the sample collection phase were considered to be the same for all three S&A plans.

When S&A plans 1 or 2 were chosen, the samples were sent to a laboratory for analysis. Therefore, transport and storage costs were taken into account. Transport costs per sample were considered to be €15 (Post NL, 2017). Storage costs were considered to be €96 per day for the entire cereal batch, which was stored until the results were available. Price estimation is detailed in Table 4.2. When the first S&A plan, based on instrumental methods, was selected, two days of storage were assumed (€192), and when the second S&A plan, based on ELISA was selected, one day of storage was assumed (€96). When the third S&A plan, based on on-site detection with LFDs, was selected, no storage costs were added.

In addition to sampling, storage and transport costs, the analyses brought additional costs. The costs for one chemical analysis with an instrumental method was considered to be €100.

The costs for one analysis with ELISA was considered to be 8€ per sample of material costs, which was an average of the costs of different ELISA tests, listed in Table B.2 in Appendix B, and a fixed cost of €14 for labour, considered to be the average cost. Multiple samples could be analysed with one ELISA plate, therefore analysing one or 10 aliquots took the same time led to the same labour costs. The cost for one analysis with a LFD was considered to be €20, including both material costs and labour costs. The costs were assumed to be the same for DON and AFB_1.

4.2.2. The optimisation model

An optimisation model was developed that maximised the number of correct decisions by varying the choice of the S&A plan (α_i), the number of incremental samples collected (N_i) and the number of aliquots analysed (NA_i).

$$Max \sum_{i=1}^{i=3} \alpha_{i\,(CBA_i+NCBR_i)} \tag{2a}$$

Subject to:

$$TC \leq B \tag{2b}$$

$$\alpha_i \in \{0\ ;\ 1\}\ and\ \sum_{i=1}^{i=3} \alpha_i = 1 \tag{2c}$$

$$if\ i \in \{1\ ;\ 2\}: NA_i\ \leq 10 \tag{2d}$$

$$N_i, NA_i\ > 0\ and\ integers \tag{2e}$$

The objective function of this optimisation model was to maximise the number of correct decisions, equal to the sum of the number of the 'compliant batches accepted' *(CBA_i)* and 'non-compliant batches rejected' *($NCBR_i$)* (FAO, 1993) (Equation 2a). This sum depended on the probability to accept a batch, which was the probability that the measured concentration was lower than the threshold, knowing the true batch concentration (FAO, 1993). The probability to accept a batch with concentration *C* depended on the total variance of the S&A plan. The first and major constraint of the model was that the total costs (TC) had to be below the pre-defined budget (B) (Equation 2b). The dummy variable α_i took the value of 1 or 0 depending on the choice of the S&A plan. Since only one method could be used, these dummy variables summed up to 1 (Equation 2c). If S&A plans 1 or 2 were chosen, the maximal number of aliquots was set to 10 (Equation 2d). Finally the number of incremental samples collected and aliquots analysed were restricted to positive and integer numbers (Equation 2e). Table 4.1 presents all parameters and formulas used. The optimisation model was solved using the add-in Solver from the What-if Tools of Excel 2010.

A sensitivity analysis was performed to assess the sensitivity of the results to changes in input parameters. The costs for sampling, storage and analysis, the RSD_r of the detection methods, and the initial mycotoxin concentration were increased and/or decreased, and the number of correct decisions was optimised again after each change.

4.3. Results

To put into perspective the budgets discussed in this study, we estimated the price to sample and analyse a 100t (tonne) cereal batch according to the EU Regulation, using the prices assumed in the model. According to Regulation (EU) No 401/2006 for the sampling and analysis of mycotoxins in a 100t unprocessed cereal batch, 100 incremental samples are needed, one aggregate sample and one aliquot analysed with an instrumental method. This S&A plan costed €1,467. This S&A led to an estimated 98.1% of correctly classified wheat batches entering the Netherlands and 91.1% of correctly classified maize batches entering the Netherlands. To achieve the same effectiveness, companies could choose a S&A plan based on ELISA, collecting 100 incremental samples and analysing three aliquots with ELISA, for a total cost of €1,309.

4.3.1. DON in wheat

Table 4.3 shows the outcomes of the optimisation model for DON in wheat for available budgets ranging from €200 to €2,000. For example, for a €500 budget, the dummy variable α_2 is equal to 1, meaning that the second S&A plan, based on ELISA was chosen. N_2 was equal to 20, thus 20 incremental samples were collected. NA_2 was equal 2, thus two aliquots were analysed. Finally, the number of correct decisions was expected to be 96.2%. The S&A plans based on ELISA were the optimal S&A plans for budgets between €500 and €2,000. For budgets below 500€, only the S&A plan based on LFDs could be selected due to high storage costs of the other S&A plans. However, below 500€ the effectiveness rapidly decreased.

Table 4.3. The Optimal S&A plan (α), the optimal number of samples collected (N), the optimal number of aliquots analysed (NA) and the associated percent of correct decisions, for different budgets (DON in wheat, limit of 1,250µg/kg)

Budget (€)	α_1	α_2	α_3	N_1	N_2	N_3	NA_1	NA_2	Correct decisions (%)
200	0	0	1	0	0	1	0	0	89.7
500	0	1	0	0	19	0	0	3	96.2
1,000	0	1	0	0	65	0	0	8	97.9
1,500	0	1	0	0	113	0	0	10	98.3
2,000	0	1	0	0	163	0	0	10	98.5

Figure 4.2 showing the estimated percentage of correct decisions for the three S&A plans for a range of budgets and Table 4.4 showing the outcomes of the optimisation model for all three S&A plans separately, allow us to compare the effectiveness of different S&A plans. Figure 4.2 shows that for a budget above €500, ELISA was the optimal detection method, even though for a budget of €800 or more, the three S&A plans did not differ much in effectiveness. In addition to the minimal difference between the three S&A plans, for budgets above €800, the effectiveness did not improve much: with a S&A plan based on ELISA, 97.5% of correct decisions were estimated for a €800 budget and 98.5% correct decisions were estimated for a budget of €2,000 (Table 4.4).

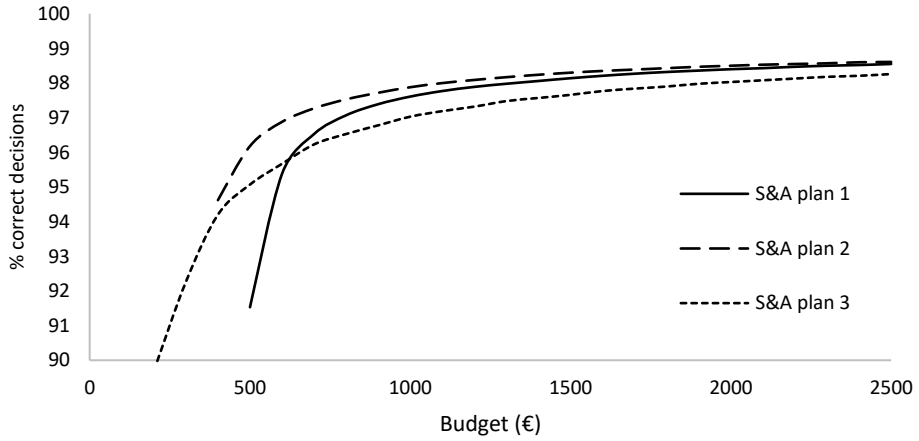

Figure 4.2. Percentage of correct decisions using the optimal sampling plan for three different detection methods (DON in wheat, limit of 1,250µg/kg)

Table 4.4. Correct decisions (%) with optimal sampling plans (DON in wheat, limit of 1,250µg/kg)

	S&A plan 1			S&A plan 2			S&A plan 3	
Budget (€)	**Correct (%)**	**N_1**	**NA_1**	**Correct (%)**	**N_2**	**NA_2**	**Correct (%)**	**N_3**
200							89.7	1
600	95.4	13	1	96.9	29	3	95.7	14
800	97.1	33	1	97.5	48	4	96.5	21
1,000	97.6	53	1	97.9	65	8	97.0	28
1,200	97.9	73	1	98.1	85	8	97.3	34
1,400	98.1	93	1	98.2	104	9	97.6	41
1,600	98.2	103	2	98.4	123	10	97.8	48
2,000	98.4	143	2	98.5	163	10	98.0	61

4.3.2. AFB_1 in maize

To estimate the aflatoxin concentration in a maize batch, for budgets between €500 and €2,000, the optimal S&A plan was the one based on ELISA (Table 4.5). Table 4.5 shows the optimal number of incremental samples to collect and aliquots to analyse. For a €1,000 budget and higher budgets, the difference of correct decisions is minimal for the second S&A plan based on ELISA and the first S&A plan based on an instrumental method (Figure 4.3 and Table 4.5). Using an instrumental method or ELISA, approximately the same number of incremental samples were collected. However, using ELSA, multiple aliquots had to be analysed in order to achieve the same low analytical variance as with an instrumental method (Table 4.6).

Table 4.5. The Optimal S&A plan (α), the optimal number of samples collected (N), the optimal number of aliquots analysed (NA) and the associated percent of correct decisions, for different budgets (AFB_1 in maize, limit of 5µg/kg)

Budget (€)	**α_1**	**α_2**	**α_3**	**N_1**	**N_2**	**N_3**	**NA_1**	**NA_2**	**Correct decisions (%)**
200	0	0	1	0	0	1	0	0	85.5
500	0	1	0	0	22	0	0	1	87.9
1,000	0	1	0	0	71	0	0	3	90.3
1,500	0	1	0	0	120	0	0	4	91.5
2,000	0	1	0	0	169	0	0	5	92.4

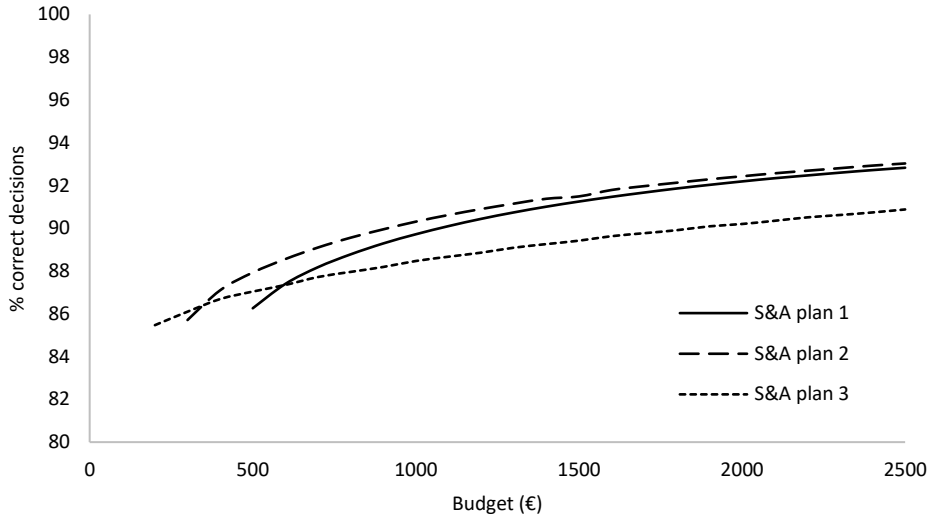

Figure 4.3. Percentage of correct decisions using the optimal sampling plan for three different detection methods (AFB_1 in maize, limit of 5µg/kg)

Table 4.6. Correct decisions (%) with optimal sampling plans (AFB_1 in maize, limit of 5µg/kg)

	S&A plan 1			S&A plan 2			S&A plan 3	
Budget (€)	**Correct (%)**	**N_1**	**NA_1**	**Correct (%)**	**N_2**	**NA_2**	**Correct (%)**	**N_3**
200							85.5	1
600	87.4	15	1	88.6	32	1	87.4	14
800	88.8	35	1	89.6	51	3	88.0	21
1,000	89.7	55	1	90.3	71	3	88.5	28
1,200	90.1	65	1	90.9	91	3	88.9	34
1,400	91.0	95	1	91.4	111	3	89.3	41
1,600	91.5	105	1	91.8	130	4	89.6	48
2,000	92.2	155	1	92.4	169	5	90.2	61

4.3.3. Sensitivity analysis

Results of the sensitivity analyses are shown in Tables 4.7 and 4.8 for the cases of DON in wheat and a AFB_1 in maize, respectively. For both cases, lower costs for sampling, i.e. €80 fixed cost and €5 per incremental sample collected instead of €160 fixed costs and €10 per sample collected, did not influence the choice of the optimal S&A plan. Lower sample collection costs could be realised if for instance the owner's private personnel and material were used.

Table 4.7. Sensitivity analysis DON in wheat, limit of 1,250µg/kg

Budget	**€200**	**€500**	**€1,000**	**€1,500**
Normal situation	S&A 3 89.7%	S&A 2 96.2%	S&A 2 97.9%	S&A 2 98.3%
Sampling is cheaper (fixed costs of €80, €5/sample)	S&A 3 92.3%	S&A 2 97.5%	S&A 2 98.3%	S&A 2 98.6%
Storage is more expensive (€300 for ELISA, €600 for instrumental method)	S&A 3 89.7%	**S&A 3** 95.1%	S&A 2 97.5%	S&A 2 98.2%
No storage costs	S&A 3 87.7%	S&A 2 96.8%	S&A 2 98.0%	S&A 2 98.4%
Instrumental method is cheaper (€50)	S&A 3 89.7%	S&A 2 96.2%	S&A 2 97.9%	S&A 2 98.3%
ELISA is more expensive (fixed costs of €80, €10/sample)	S&A 3 94.0%	S&A 2 95.3%	S&A 2 97.8%	S&A 2 93.3%
LFDs are performing better (same RSD_r as instrumental method: 5%)	S&A 3 89.7%	S&A 2 96.2%	S&A 2 97.9%	S&A 2 98.3%
ELISA is performing worse (RSD_r of 25%)	S&A 3 89.7%	S&A 2 95.7%	**S&A 1** 97.6%	**S&A 1** 98.2%
ELISA and LFD (Bias +15% or -15%)	S&A 3 90.3%	**S&A 3** 94.9%	**S&A 1** 97.6%	**S&A 1** 98.1%
Year with high DON levels (mode of 1,000 instead of 500µg/kg)	S&A 3 70.6%	S&A 2 88.0%	S&A 2 92.7%	S&A 2 94.1%

If storing batches were more expensive, i.e. €300 instead of €96 storage costs per day for example to incur an extra penalty if the batch had to be put on hold, the results changed for a €500 budget. For this budget, for both DON and AFB_1, the third S&A plan, based on LFDs became the optimal method instead of the second S&A plan based on ELISA. This increase in storage costs only moved the intersection point where ELISA performed better than LFDs to a larger budget. When the storage costs for the first and second S&A plans were not considered at all, ELISA remained the optimal method for budgets ≥ €500. More incremental samples could be collected, resulting in a slight increase of the percentages of correct decisions. The results were the same for DON in wheat and AFB_1 in maize.

If the price for one analysis with an instrumental method were halved, the results for both DON and AFB_1 did not change. If the price for one analysis with ELISA was higher, i.e., €80 fixed costs and €10 per aliquots instead of €14 fixed costs and €8 per aliquot, the second S&A plan based on ELISA remained optimal for budgets ≥ €500. The percentage of correct decision was a little lower since less budget could be spent on collecting incremental samples.

If LFDs had the same RSD_r as instrumental methods, the results remained exactly the same. The results did not change with a small increase or decrease in the RSD_r for LFDs, since the

most important component of the total variance was the variance due to the sample collection. If we considered that ELISA had a large RSD_r: 25% instead of 9% for DON and 30% instead of 16% for AFB_1, the choice of the optimal S&A plan did not change in the case of AFB_1 in maize. The percentage of correct decisions was hardly impacted. For the case of DON in wheat, for budgets ≥ €1,000, the optimal S&A plan became the first, based on instrumental methods.

Table 4.8. Sensitivity analysis AFB_1 in maize, limit of 5µg/kg

Budget	**€200**	**€500**	**€1,000**	**€1,500**
Normal situation	S&A 3 85.5%	S&A 2 87.9%	S&A 2 90.3%	S&A 2 91.5%
Sampling is cheaper (fixed costs of €80, €5/incremental sample)	S&A 3 86.1%	S&A 2 89.9%	S&A 2 92.2%	S&A 2 93.3%
Storage is more expensive (€600 for S&A plan 1, €300 for S&A plan 2)	S&A 3 85.5%	**S&A 3** 87.0%	S&A 2 89.5%	S&A 2 91.1%
No storage costs	S&A 3 85.5%	S&A 2 88.5%	S&A 2 90.6%	S&A 2 91.8%
Instrumental method is cheaper (€50/aliquot)	S&A 3 85.5%	S&A 2 87.9%	S&A 2 90.3%	S&A 2 91.5%
ELISA is more expensive (fixed costs of €80, €10/aliquot)	S&A 3 85.5%	S&A 2 87.4%	S&A 2 90.0%	S&A 2 91.4%
LFDs are performing better (same RSD_r as instrumental method: 9%)	S&A 3 85.5%	S&A 2 87.9%	S&A 2 89.5%	S&A 2 91.1%
ELISA is performing worse (RSD_r of 30%)	S&A 3 85.5%	S&A 2 87.9%	S&A 2 90.2%	S&A 2 91.5%
ELISA and LFD (Bias +15% or -15%)	S&A 3 85.2%	S&A 2 87.1%	**S&A 1** 91.5%	**S&A 1** 92.2%

We considered the scenario that the rapid detection methods, ELISA and LFDs, could give a biased result: the true result could be underestimated by 15% or overestimated by 15%. A possible bias impacted the results for both AFB_1 and DON. In the case of AFB_1in maize the optimal S&A plan remained the same for budgets up to €500, the percentages of correct decisions estimated were lower due to the biased results. In the case of DON in wheat, the third S&A plan based on LFDs was optimal for budgets up to €500. Again, the percentages of correct decisions estimated were lower due to the biased results. For budgets ≥ €1,000, for both AFB_1 and DON, the first S&A plan, based on instrumental method became optimal. The last scenario considered was a year with very high DON concentrations in wheat batches, with a mode of 1,000µg/kg, close to the EU legal limit of 1,250µg/kg for unprocessed wheat

(EU, 2006a), instead of 500µg/kg. The percentage of correct decisions decreased, since more batches sampled had a concentration closer to the limit and were therefore harder to classify (accept/reject). However, the optimal S&A plan did not change.

4.4. Discussion

A vast amount of money is spent on monitoring a wide range of contaminants and pathogens in a wide variety of plants, animals and food/feed. Therefore designing cost-effective S&A plans is of utmost importance. For each hazard, the most cost-effective S&A plan will be different. DON and AFB_1 differ in their heterogeneous distribution within a batch (Cheli *et al.*, 2009); this leads to three observed differences. First, more incremental samples have to be collected from the batch for AFB_1 than for DON to achieve the same effectiveness. Second, on-site detection methods are less suitable for the detection of AFB_1 than for DON. Third, a lower budget for the detection of DON than for the detection of AFB_1 is required to achieve the same effectiveness. DON is produced pre-harvest whereas AFB_1 are produced both pre- and postharvest, and hence more heterogeneously distributed throughout the batch; one of the reasons is that localised hotspots can be formed during improper storage of maize batches. The variance due to the sample collection was larger for AFB_1 than for DON. Collecting many incremental samples, combining these samples into an aggregate sample and analysing only a few aliquots was the most cost-effective S&A plan in the case of AFB_1. For example, in the case of DON in wheat, the model chose a higher number of aliquots analysed for a €1,500 budget: 113 incremental samples and ten aliquots from the aggregate sample analysed with ELISA (Table 4.3). For the case of AFB_1 in maize, for the same €1,500, the optimal solution was to collect more incremental samples: 120, and analyse only four aliquots with ELISA (Table 4.5). For more heterogeneously distributed mycotoxins, such as AFB_1, collecting as many incremental samples as the budget allows contributes more to lowering the variance of the S&A plan than analysing many aliquots.

A S&A plan based on on-site analysis was not the optimal S&A plan to achieve the highest effectiveness. We assumed that all incremental samples collected were all analysed with an LFD. We decided that incremental samples collected could not be combined in an aggregate sample because the analysis was done on-site and no tools were available to grind and prepare a large aggregate sample before the analysis. Analysing all incremental samples separately with a LFD led to high detection costs. Therefore, less incremental samples could be collected. The variance due to the sample collection is the main component of the total variance of the sampling plan (Whitaker, 2004) and is even more important for AFB_1 than for DON. Therefore, the difference between the S&A plan based on LFDs and the S&A plan based on ELISA, was small in the case of DON in wheat, i.e. 0.9% for a €1,000 budget. The

difference between the optimal S&A plan based on ELISA and the S&A plan based on LFDs was larger for AFB_1 in maize, i.e. 2.1% for a €1,000 budget. LFDs seem to be a useful tool to screen for DON by industry since the results are available the same day, avoiding storage of the batches while waiting for the outcome of the detection method.

Since the variance due to the sample collection is larger for AFB_1 than for DON, for ≥ €1,000 budgets, the percentage of correct decisions hardly increased for DON in wheat. Therefore, it might not be cost-effective to spend more budget than €1,000 for sampling and analysing a batch. Spending more to collect extra samples improved the percentage correct decisions more for AFB_1 in maize than for DON in wheat.

For both, AFB_1 in maize and DON in wheat, the results of this study showed that the S&A plan using ELISA was slightly more cost-effective than the S&A plan using instrumental methods. However, multiple aliquots had to be analysed with ELISA in order to lower the variance due to the analysis. One advantage of instrumental methods is that multiple mycotoxins can be detected simultaneously, which does not hold for most LFDs or ELISA. This aspect of multiple analyses was, however, not considered in this study. Another advantage of instrumental methods is that they are less prone to variance: LFDs and ELISA can have a very large RSD_r depending on the persons that perform these rapid methods and on how well-trained they are. Instrumental methods are usually performed by well-trained persons and therefore the RSD_r is lower. S&A plans based on ELISA or LFDs might not be able to estimate very effectively the concentration of the batch, due to the large RSD_r of the results. However, analysing multiple aliquots will lower the variance due to the detection method. In addition, the aim was not to effectively estimate the batch concentration but to classify the batches correctly below or above the pre-set limit. LFDs could be used by food or feed companies as a first screening for DON in wheat. However, it should be stressed again that the use of most S&A plans using LFDs considered in this study, are not suitable for official controls according to the EU regulations, because of the low amount of samples collected.

In this study, the mean of the test results was used to decide whether or not the batch was accepted, but one could decide to have all test results be below the limit to accept the batch. For example, in the case AFB_1 in maize, 20 incremental samples would be collected from the batch, and each sample would be analysed with an LFD. If the mean of these 20 results would be used to take the decision, 92.9% of the batches with a mycotoxin concentration below the limit would be accepted, and 57.6% of the batches with a high mycotoxin contamination would be rejected. If the results of all 20 LFDs need to be below the pre-set limit for AFB_1, the false negatives would be eliminated: 100% of the batches with a high concentration would be rejected. However, a large number of false positives would be expected: only about half (47%) of the compliant batches would be accepted (Figure 4.4).

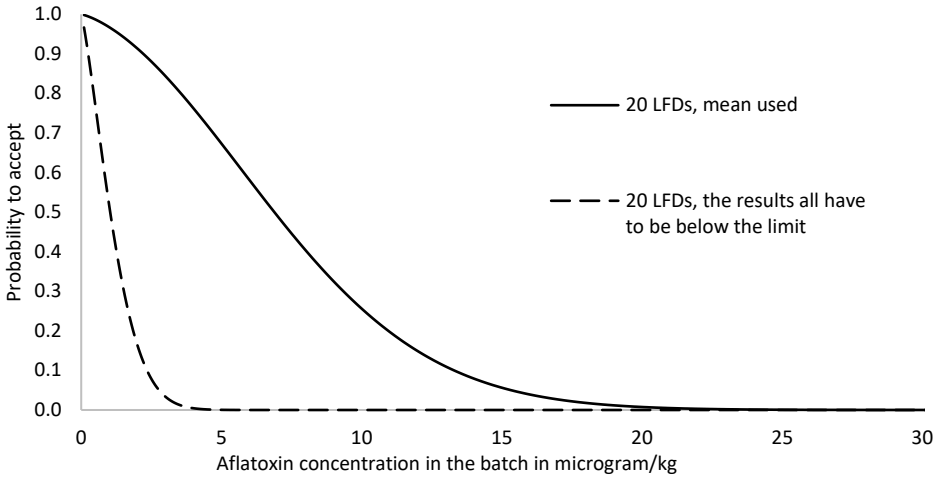

Figure 4.4. Operating Characteristic (OC) curves for different amounts of samples that have to be below the limit, AFB_1 in maize

This research combined available studies in literature to develop a model that estimated the number of correct decisions taken with a number of incoming batches having different concentrations. Despite some assumptions and uncertainties in the model (such as RSD_r of the tests, prices for sampling, storage and testing, not taking into account the measurement uncertainty when deciding if a batch is compliant), we believed that the model was quite robust, which was shown in the sensitivity analysis. Slight variations in the input parameter values barely impacted the results. Only much higher or lower storage costs, or a possible bias of a rapid detection method, may lead to a change in the optimal S&A plan for certain budgets. The results were based on concentrations of batches imported or produced in the Netherlands in the last years. In countries where the concentrations of the batches are all close to the limit, the percentage of correct decisions made are expected to be much lower. However, the sensitivity analysis showed that the choice of the S&A was not impacted. According to Regulation No 401/2006, the measurement uncertainty of the detection methods should be taken into account when deciding whether a batch is compliant (EU, 2006c). In this study, this uncertainty was not considered is estimating the number of correct decisions made by each S&A plan. If this uncertainty would have been taken into account, an detection method with greater uncertainty measurement would be less likely to reject a non-compliant batch than an detection method with a smaller measurement uncertainty. For this reason, fast detection methods like ELISA and LFD methods could perform less well than is shown in this study. This is a first study that optimises S&A plans for monitoring using an optimisation modelling technique. In future studies, the full

measurement uncertainty, which contains much more than just the RSD_r currently considered should be incorporated.

4.5. Conclusion

To our best knowledge this is the first study that aims to optimise S&A plans for mycotoxins in cereals, based on cost-effectiveness calculations, having a fixed budget as constraint. An optimal S&A plan, which does not exceed the available budget for sampling and analysis, has a low chance for misclassifying batches, so a low chance to accept a batch with a true concentration above the pre-set limit and a low chance to reject a batch that has a true concentration below the pre-set limit, and does not exceed the budget available for sampling and analysing. For AFB_1, it was crucial to collect as many incremental samples as possible. The S&A plans based on ELISA and instrumental methods were most cost-effective. For DON in wheat batches, the difference between the different S&A plan considered was minimal.
We consider the results of this research to be very helpful for food and feed companies as to make cost-effective decisions on their preferred S&A plans. This research focused on mycotoxins in grains, but the methodology can be used in a wide range of applications to develop cost-effective S&A plans for other contaminants and pathogens in food, water, the environment, plants or animals.

Appendix A: The variance of a sampling and analytical plan

Table A.1. Variance components of a S&A plan

Crop/ toxin	Detec-tion method	Variable	Sample collection variance [a]	Sample preparation variance [a]	Analytical variance [b]	Distribu-tion test results
Wheat DON	LC-MS/MS	$var(C)_{1\_DON}$ =	$1.18/N_1*C^{0.833}$+	$0.0033*C^{0.833}$+	$((0.05*C)^2)/NA_1$	Log-normal
	ELISA	$var(C)_{2\_DON}$ =	$1.18/N_2*C^{0.833}$ +	$0.0033*C^{0.833}$+	$((0.09*C)^2)/NA_2$	
	LFD	$var(C)_{3\_DON}$ =	$1.18/N_3*C^{0.833}$	+	$((0.13*C)^2)/N_3$	
Maize Aflatoxin	LC-MS/MS	$var(C)_{1\_AFLA}$ =	$128.4/N_1*C^{0.98}$+	$0.1254*C^{1.27}$+	$((0.09*C)^2)/NA_1$	
	ELISA	$var(C)_{2\_AFLA}$ =	$128.4/N_2*C^{0.98}$+	$0.1254*C^{1.27}$+	$((0.16*C)^2)/NA_2$	
	LFD	$var(C)_{3\_AFLA}$ =	$128.4/N_3*C^{0.98}$	+	$((0.22*C)^2/N_3$	

[a] The formulas for the sample collection and sample preparation variances were adapted from the mycotoxin sampling tool of the FAO (2014). An incremental sample was considered to be 100g. A subsample taken from the aggregate sample and prepared for analysis was considered to be 500g when an instrumental method or ELISA are used.
[b] The variability due to the analysis was derived from the Relative Standard Deviation (RSD_r) calculated during validation studies of different LFD and ELISA methods.
Assumption: var = $(RSD_r*C)^2$

The variance due to the sampling step was calculated with a formula adapted from the formula offered by the FAO. The FAO calculated the sample collection variance for DON in wheat as shown in Equation (A1) (FAO, 2014):

$$var(C)_{DON} = (13{,}620/N_i) * 0.026 * C^{0.833} \quad \text{(A1)}$$

where N_i is the number of incremental samples collected from the batch and C the true batch concentration. The aggregate sample size was expressed in number of raw wheat kernels (30,000 kernels/kg). This was first converted to a sample size in kg, and resulted into Equation (A2):

$$var(C)_{DON} = (0.454/N_i) * 0.026 * C^{0.833} = (0.118/N_i) * C^{0.833} \quad \text{(A2)}$$

Finally the aggregate sample size in kg was transformed to the number of incremental samples collected, considered to be 100g each, which resulted into Equation (A3):

$$var(C)_{DON} = (1.18/N_i) * C^{0.833} \quad \text{(A3)}$$

The same procedure was used for the sample collection variance in maize. The sample collection variance was a function of the concentration of the batch (C) and the number of incremental samples collected, N_i.

The variance due to the sample preparation step was calculated with formulas presented by the FAO as well (FAO, 2014). The test portion size was considered to be 500g. When LFDs were considered, no sample preparation variance was included as no aggregate samples were made.

The variance due to the analysis step was estimated based on the relative standard deviation for repeatability (RSD_r) measured in validation studies of the detection methods or simply found in literature. The values taken where within-lab standard deviations. The assumption was made that the relative standard deviation is the same at low and high concentrations. The exact data collected is presented in Table A.2. The equation used for the analytical variance is the following:

$$var(C) = (RSD_r * C)^2 / NA_i \quad \text{(A4)}$$

where RSD_r is the mean Relative Standard Deviation for repeatability of the detection method considered, C the batch concentration and NA_i the number of aliquots analysed with this method.

Table A.2 Relative Standard Deviation (RSD) of the detection methods, data collected from literature

Detection method	RSD_r [a] (%)	Reference	Mean RSD_r
LC-MS/MS DON	4.9	(Van Asselt *et al.*, 2012)	
HPLC DON	1.57	(Astoreca *et al.*, 2017)	
LC-MS/MS aflatoxins	8.2	(Van Asselt *et al.*, 2012)	
HPLC aflatoxins	6.4 – 12.3	(Lupo *et al.*, 2010a)	
ELISA DON			
Veratox DON 5/5 (Neogen)	9.0	(Tangni *et al.*, 2010)	
Veratox DON 2/3 (Neogen)	<10	(Lupo *et al.*, 2010b)	
DON EIA (Europroxima)	3	(Tangni *et al.*, 2010)	8.8%
Agraquant (Romer Labs)	1.9	(Tangni *et al.*, 2010)	
Agraquant, DON EIA, Veratox	<20	(CODA-CERVA, 2009)	
ELISA aflatoxins			
Veratox total aflatoxins (Neogen)	8.2 – 17.2	(Lupo *et al.*, 2010b)	
Ridascreen total aflatoxins (R-Biopharm)	6.8 – 14	(Lupo *et al.*, 2010b)	15.2%
Ridascreen, AgraQuant, Veratox	13 – 32	(CODA-CERVA, 2011)	
LFD DON			
Reveal Q+ (Neogen)	3.3	(Astoreca *et al.*, 2017)	
Rosa Fast5 DON (Charm)	10.7	(van der Fels-Klerx and de Rijk, 2014)	
RIDA quick DON (R-Biopharm)	12.2	(van der Fels-Klerx and de Rijk, 2014)	
Donsensor (Unisensor)	19.1	(van der Fels-Klerx and de Rijk, 2014)	12.3%
Reveal Q+ (Neogen)	8.4	(van der Fels-Klerx and de Rijk, 2014)	
Rosa, Agrastrip	<20	(CODA-CERVA, 2009)	
LFD Aflatoxins			
Rosa Aflatoxins (Charm)	16 – 28	CODA-CERVA (2011)	22%

[a] RSD_r = Relative Standard Deviation for repeatability. Multiple measurements were performed of a sample with known concentration in the same lab, the standard deviation, divided by the concentration was reported as RSD_r.

Appendix B: Data collected: costs

Table B.1. Costs for transport, sampling and storage

Description	Value [a]	Unit	Reference
General costs			
Labour costs	40	€/h	(Van der Fels-Klerx and Van Wagenberg, 2014)
Estimated transport costs to the lab if no on-site analysis	15	€/package	(Post NL, 2017)
Sampling costs			
Starting price (fixed cost)	160	€	(NVWA, 2016)
Labour costs	30	€/15 min	
Labour	5	min/sample	Own estimation
Variable cost	10	€/sample	Calculation
Storage costs			
Labour	2	h	Own estimation
Depreciation (5%)	300 – 500 – 1,500	€/year	(Agrifirm, 2017)
Interest rate (5%)	300 – 500 – 1,500	€/year	
Electricity in EU	0.114	€/kWh	(Eurostat, 2016)
Ventilation	2.2	kWh	(Agrifirm, 2017)
Electricity costs	6	€/day	Calculation
Total costs	90 – 103	€/day	Calculation

[a] All values are rounded to whole numbers

Table B.2. Costs for each detection methods

Description	Value [a]	Unit	Reference
	55 – 70	€/sample	Personal communication commercial lab
Instrumental method	250	€/sample	(NWVA, 2016)
	100	€/sample	(Van der Fels-Klerx and Van Wagenberg, 2014)
LFD Aflatoxins (Based on ROSA AFQ by Charm Sciences)			
Material costs	184	€/ 20 samples	Personal communication Charm Sciences
Time for analysis	15	min/sample	Charm Sciences
Time to read and store results	5	min/sample	Own estimation
Total cost per sample	23	€/sample	Calculation
ELISA Aflatoxins (Based on Veratox Aflatoxin by Neogen Corp.)			
Material costs	8	€/sample	Personal communication Neogen Corp
Time needed for analysis	15	min/plate	Average
Time to read and store results	5	min/sample	Own estimation
Labour costs	14	€/sample	Calculation
Total costs for one sample	22	€/sample	Calculation
Total costs for 10 samples	94	€/10 samples	Calculation
LFD DON (Multiple tests considered)			
Material costs	8 – 10	€/ sample	(CODA-CERVA, 2009)
Time for analysis	11 – 12	min/sample	(CODA-CERVA, 2009)
Time to read and store results	5	min/sample	Own estimation
Total cost per sample	17 – 20	€/sample	Calculation
ELISA DON (Multiple tests considered)			
Material costs	6 – 8	€/ sample	(CODA-CERVA, 2009)
Time for analysis	10 – 20	min/plate	(CODA-CERVA, 2009)
Time to read and store results	5	min/plate	Own estimation
Labour costs	9 – 15	€/plate	Calculation
Total cost for one sample	17 – 21	€/sample	Calculation
Total costs for 10 samples	89 – 75	€/ 10 samples	Calculation

[a] All values are rounded to whole numbers

Chapter 5

Optimisation of the Aflatoxin Monitoring Costs along the Maize Supply Chain

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Published in: Risk Analysis. https://doi.org/10.1111/risa.13364

Abstract

An optimisation model was used to gain insight into cost-effective monitoring plans for aflatoxins along the maize supply chain. The model was based on a typical Dutch maize chain, with maize grown in the Black Sea region, and transported by ship to the Netherlands for use as an ingredient in compound feed for dairy cattle. Six different scenarios, with different aflatoxin concentrations at harvest and possible aflatoxin production during transport, were used. By minimising the costs and using parameters such as the concentration, the variance of the sampling plan, and the monitoring and replacement costs, the model optimised the control points (e.g. after harvest, before or after transport by sea ship), the number of batches sampled at the control point, and the number of samples per batch. This optimisation approach led to an end-of-chain aflatoxin concentration below the pre-determined limit. The model showed that, when post-harvest aflatoxin production was not possible, it was most cost-effective to collect samples from all batches and replace contaminated batches directly after the harvest, since the replacement costs were the lowest in the upstream stages of the maize supply chain. When there was aflatoxin production during storage, it was most cost-effective to collect samples and replace contaminated batches after storage and transport, in the downstream stages of the maize supply chain, to avoid the duplicate before and after monitoring and replacement costs. In the downstream stages of the maize supply chain, more stakeholders are involved leading to higher replacement and recall costs.

5.1. Introduction

Mycotoxins, toxins produced by fungi on various food and feed products such as cereals and nuts, present a worldwide food and feed safety concern, which can lead to several health problems in humans and animals, as well as major economic losses for farmers, the industry, and society (Marin *et al.*, 2013). The Food and Agriculture Organization estimated that approximately one quarter of all cereal products worldwide are contaminated by mycotoxins (Boutrif and Canet, 1998). Furthermore, according to the Rapid Alert System for Food and Feed (RASFF), mycotoxins, in particular aflatoxins, are the main reason for European border rejections (Marin *et al.*, 2013). The high percentage of contaminated batches and the high number of border rejections show that mycotoxins are common, especially in cereals and nuts. Mycotoxin presence in cereal commodities could lead to direct market losses (associated with lost trade or lost revenue due to batches that are downgraded or even destroyed), losses related to human health costs, and less productive animals (IARC, 2012).

Proper monitoring of batches throughout the cereal food supply chain reduces the probability that a contaminated product will be sold for feed or food production, and the consequences thereof, such as expensive recalls or, even worse, reduced animal or human health. Cereal batches are monitored by collecting and analysing multiple samples from the batch. Since mycotoxins are heterogeneously distributed (Cheli *et al.*, 2009) samples collected at different locations in a cereal batch are expected to have different mycotoxin concentrations. Therefore, collecting multiple samples to estimate the mycotoxin concentration in the batch is essential for a true estimate of the batch concentration. When more samples are collected, the sampling plan becomes more accurate (Johansson *et al.*, 2000a).

For official monitoring, EU regulations describe the minimum number of samples to collect, e.g. Regulation No 152/2009 defines the sampling and detection methods for feed products (EU, 2009). In the case of a private company, the number of samples required for routine checks, customer demands, or certification systems depends on the private system in place, and/or the available budget. Companies often want to limit their budgets for mycotoxin monitoring in cereals, and do not have to follow the sampling requirements stated in the regulations accurately. For companies, the choice between monitoring frequency and costs is often difficult: the optimal monitoring plan for a private company is a cost-effective monitoring plan. This, in turn, is a plan that results in a low probability of contamination of the end product, without extremely high sampling and analysis costs.

A few earlier studies have used optimisation modelling to optimise monitoring plans for various types of hazards in food and feed products (Ferrier and Buzby, 2013; Lascano-

Alcoser *et al.*, 2014; Lascano-Alcoser *et al.*, 2013; St-Pierre and Cobanov, 2007). In this study, a single-objective constrained optimisation problem, solved by linear approximation, was applied to the case of mycotoxin monitoring along the cereal chain. The objective is cost minimisation, and the main constraint is an end-of-chain contamination level below the pre-determined limit. To monitor mycotoxins along the cereal chain, from the fields to the end product, samples can be collected at different control points (CPs) (e.g., field, storage silo, trucks, processing plant, end product). This study provides insight into the most critical CPs, and the number of samples required to fulfil the concentration criterion at the end of the chain. We assumed that there was one budget available for monitoring the entire maize chain, for example, that one big company was responsible for the entire chain. This paper focusses in particular on AFB_1 in the maize chain. AFB_1 is of great interest, since it is the most toxic mycotoxin, is heterogeneously distributed throughout the batch, and can be formed on the field as well as during transport and storage in case of improper conditions.

5.2. Methods

5.2.1. The Maize Chain

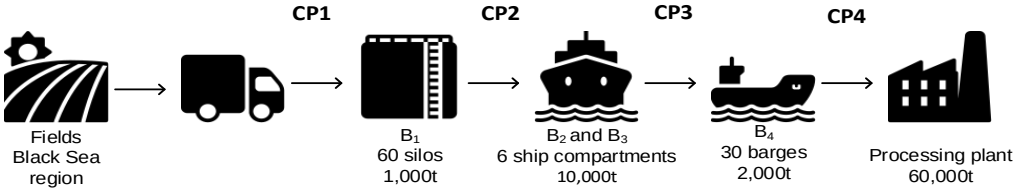

*Figure 5.1. A typical Dutch maize supply chain * Note: CP_1: control point 1, CP_2: control point 2, CP_3: control point 3, CP_4: control point 4 (Free icons by Icons8: https://icons8.com/)*

The optimisation model was based on a representative but simplified Dutch maize chain, in which maize grown in South East Europe was transported by ship to the Netherlands and processed into compound feed for dairy cows (Figure 5.1). In this chain, 60,000t (tonnes) of maize was followed from the fields in South East Europe to the storage silos, through sea shipment, and finally to the barges en route to the processing plant in the Netherlands. The model assumed that, on average, a field has a 100t production capacity. Maize from 600 fields were assumed to be transported to 60 large storage silos, each with a 1,000t storage capacity (B_1). The content of these storage silos was transferred to a 60,000t sea-going ship with 6 compartments of 10,000t each (B_2 and B_3). At the harbour, the maize was transferred to 30 smaller 2,000t barges (B_4), destined for the processing plants, which are mostly situated along the rivers. All fields, silos, ship compartments, and barges had the same capacity, and other possible (smaller) transport steps were not considered.

We assumed that the processing plants need a pre-defined amount of maize; therefore, if the AFB_1 concentration of a batch exceeded the threshold and the batch was rejected, it had to be replaced. For the purpose of this study, a batch was defined as maize of the same origin and conditions: for example, a batch was the content of one silo or the content of one compartment in the ship. During mixing, the number of batches and their size varied along the supply chain.

5.2.2. The Control Points (CP_i)

Four control points (CP) to monitor for AFB_1 were chosen, following current practices. The first control point (CP_1) was set at the point where the storage silos were loaded after harvest; in case of contamination, the content of the silo was replaced. CP_2 was set at the point where the sea-going ship was loaded; in case of contamination, the content of the cargo space was replaced. CP_3 was set at the point where the ship was unloaded in the country of destination; since the tests results last a couple of days, in case of contamination, the barges with the content of the contaminated cargo spaces were replaced. The last control point, CP_4, was set at the point where the barges were unloaded at the final destination; in case of contamination, the content of the barges was replaced. For simplicity, it was assumed that the same number of samples were collected from each silo, compartment, or barge.

5.2.3. Scenarios

We considered six scenarios, with the characteristics as shown in Table 5.1. Two scenarios (S1, S2) started with a low in-field AFB_1 concentration of 1µg/kg, two scenarios (S3, S4) started with a higher (4µg/kg) AFB_1 concentration, and the two last scenarios (S5, S6) started with a very high (10µg/kg) in-field AFB_1 concentration. In some scenarios, the storage conditions during transport by ship were assumed to be sub-optimal, that is, the water activity and temperature were not controlled well. During this period, the *aspergillus* fungi could grow and produce AFB_1. Based on the results of Abdel-Hadi *et al.* (2012), we assumed the following sub-optimal conditions: a temperature between 25 and 30°C and a water activity of 0.95, and consequently a AFB_1 production of 100µg/kg in the sections of the ship compartments contaminated with the fungi (*prod*). AFB_1 was only produced in hot-spots in ship compartments that were contaminated by the fungi and had high water activity and temperature. We assumed that these hot-spots comprised 5% of the total compartment volume.

Table 5.1. Characteristics of the six scenarios considered

	S1	S2	S3	S4	S5	S6
Initial AFB_1 concentration in fields (µg/kg) c_1	1	1	4	4	10	10
AFB_1 production during transport by ship in 5% of the batch (µg/kg) prod	0	100	0	100	0	100

5.2.4. Sampling and Analysis

Since we assumed that the processing plants required a fixed amount of maize, batches that exceeded the pre-defined maximum limit after sampling and analysis were replaced. The concentrations of the batches after sampling, analysis, and replacement *(cm_i)* were based on the probability of accepting and rejecting a batch; cm_i reflects the average concentration after monitoring of all batches at CP_i. This is the average concentration of batches, in cases where the same batches were monitored an infinite number of times. This average was calculated as follows:

$$cm_i = c_i * PA_i + m * (1 - PA_i) \quad (1)$$

with c_i being the initial concentration of the batches at CP_i before sampling and analysis; PA_i the probability to accept this batch; m the mean AFB_1 concentration of the replacing batch; and $1\text{-}PA_i$ the probability to reject a batch. If no samples were collected, PA_i was equal to 1 and, therefore, the concentration after sampling and analysis remained the same as the initial concentration.

The probability to accept a batch is the probability that the measured batch concentration is below the pre-determined limit, based on a lognormal distribution (Equation 2).

$$PA_i = \mathrm{P}(\mu_i \leq \ln(\mathrm{lim}) | \mathbf{c_i}) \quad (2)$$

with μ_i being the mean of distribution of test results at CP_i; *lim* the pre-determined limit in µg/kg; and c_i the mean AFB_1 concentration at CP_i before sampling and analysis.

The test result followed a lognormal distribution with mean μ_i and variance σ_i^2, calculated with Equations (3) and (4) respectively:

$$\mu_i = \ln(c_i) - \frac{\sigma_i^2}{2} \text{ (Lyman } \textit{et al.}\text{, 2011)} \quad (3)$$

$$\sigma_i^2 = ln\left(\frac{var(c_i)}{c_i^2} + 1\right)$$ (Lyman *et al.*, 2011) (4)

with c_i being the mean AFB_1 concentration at CP_i before sampling and analysis, and $var(c_i)$ the total variance of the sampling and analysis plan, which is a function of c_i and the number of samples collected per batch (ns_i) (Equation 5).

$$var(c_i) = \frac{128.4}{ns_i} * c_i^{0.98} + (0.50 * c_i)^2 \quad (5)$$

This variance includes the variance due to sample collection (Johansson *et al.*, 2000b), as well as the variance due to analysis. Variance due to sample preparation was in this case integrated in the variance due to analysis. We assumed that ns_i 100g samples were collected from each batch at CP_i and combined into one aggregate sample, which was analysed in a laboratory with an instrumental method such as LC-MS/MS. A total uncertainty of 50% was associated with sample preparation and analysis. This was based on the measurement uncertainty for an LC-MS/MS-based multi-mycotoxin assay estimated by Stadler *et al.* (2018). The total variance of the sampling plan was based on the variance due to collecting multiple samples from the batch during unloading or loading of the silos, ships, or barges, as well as the analytical variance.

In this study, we assumed a pre-determined limit of 2.5µg/kg for AFB_1 in maize intended for dairy-cow feed, and did not consider the measurement uncertainty when deciding to reject or accept the batch. The AFB_1 limit for dairy cattle feed listed in Directive 2002/32/EC is 5µg/kg (EU, 2002a).

5.3.5. Monitoring and Replacement Costs

The sample collection and LC-MS/MS analysis costs of a sample are €10 and €100, respectively, in the case where samples are aggregated for analysis. These analysis costs are quoted per analysed batch, and are only accounted for when samples were collected from that batch. The costs at each CP_i were calculated with Equation (6):

$$costs_i = 10 * ns_i * n_i + 100 * n_i + r_i * (1 - PA_i) * n_i \quad (6)$$

where n_i represents the number of batches (silos, ship compartments, and barges) sampled and analysed at CP_i; ns_i is the number of samples collected from each batch that is sampled at CP_i; r_i represents the replacement costs for one batch at CP_i; and PA_i represents the probability that the batch will be accepted.

We assumed that the costs for sample collection and analysis were the same at each CP. Replacement costs at CP_1 were considered to be an average FOB (Free on Board) export price expected for 2019 from the Black Sea region of €160/t. At the subsequent CPs along

the chain, recall and replacement costs increased, since the transport costs of the replaced batch had to be added. At CP_2, we assumed that the batch was on its way to the country of destination, and an additional 20€/t was added as transport cost. At CP_3 and CP_4, an additional €10 was added at each CP as transport and transhipment costs. More details and cost references are shown in Table 5.2.

Table 5.2. Replacement costs

Item	Value	Reference/calculation
Replacement costs maize at harbour in the Black sea region (€/t)	160	Predicted average for 2019: Low: $178, high: $189.50 (CME group, 2019) $1 = €0.86 (rate 03/07/2018)
Transport costs from Black sea ports to NL (€/t)	20	Average: $24/t in 2017 (Medstone SA, 2017) $1 = €0.86 (rate 03/07/2018)
Replacement costs maize at harbour in country of destination (€/t)	180	160+20
Transport costs by barge (€/t)	10	€5/t transport costs (Bureau Voorlichting Binnenvaart, 2006) €5/t transshipment costs (assumption)
Replacement costs maize arriving at processing plant (€/t)	190	180+10
CP_1: Replacement costs one batch of 1,000t (silo) (€)	160,000	1,000*160
CP_2: Replacement costs one batch of 10,000t (ship compartment) (€)	1,800,000	10,000*180
CP_3: Replacement costs one batch of 10,000t (five 2,000t barges) (€)	1,900,000	10,000*190
CP_4: Replacement costs one batch of 2,000t (barge) (€)	380,000	2,000*190

5.2.6. The Optimisation Model

An optimisation model was constructed to minimise the total costs for monitoring and replacing the batches that exceeded the pre-determined limit, with the constraint that the AFB_1 concentration in the batch was below the pre-determined limit at the end of the chain.

Min:

$$\sum_{i=1}^{i=4} 10 * ns_i * n_i + 100 * n_i + r_i * (1 - PA_i) * n_i \quad (7)$$

Subject to:

$$0 \leq n_i \leq B_i \; and \; integer \quad (8)$$

$$0 \leq ns_i \leq 200 \; and \; integer \quad (9)$$

$$cm_4 \leq lim \quad (10)$$

With:

$$cm_4 = \frac{n_4}{B_4} * \; cm_3 * PA_4 + \frac{1-n_4}{B_4} * m * (1 - PA_4) \quad (11)$$

$$cm_3 = \frac{n_3}{B_3} * (cm_2 + prod) * PA_4 + \frac{1-n_3}{B_3} * m * (1 - PA_3) \quad (12)$$

$$cm_2 = \frac{n_2}{B_2} * cm_1 * PA_2 + \frac{1-n_2}{B_2} * m * (1 - PA_2) \quad (13)$$

$$cm_1 = \frac{n_1}{B_1} * c_1 * PA_1 + \frac{1-n_1}{B_1} * m * (1 - PA_1) \quad (14)$$

where n_i is the number of batches (silos, ship compartments, or barges) sampled and analysed at CP_i; ns_i is the number of samples collected from each batch, between 0 and 200, at CP_i; r_i reflects the replacement costs for one batch at CP_i; PA_i is the probability to accept the batch and keep it in the chain depending on n_i and ns_i; B_i is the number of batches at CP_i; *lim* is the pre-determined limit for AFB_1; c_i is the average AFB_1 concentration at CP_i before sampling and analysis; cm_i is the average AFB_1 concentration at CP_i after monitoring and replacement; and m is the AFB_1 concentration of the replacing batch. The parameters of the model can be found in Table 5.3. The COBYLA algorithm from the R package "nloptr", version 1.0.4 - Constraint Optimisation by Linear Approximation - was used to solve the problem.

Table 5.3. The optimisation model's parameters

Variable	Explanation	Estimate, formula or distribution
lim	Limit set by a compound feed company for AFB_1 in maize intended to be used in dairy cow feed (µg/kg)	2.5
m	Mean AFB_1 concentration of the replacement batch (µg/kg)	1
CP_i	Control point i	$i \in \{1\,;2\,;3\,;4\}$
n_j	Number of batches (silos, ship compartments or barges) checked at CP_i	
ns_j	Number of samples collected and analysed from the batches at CP_i	
c_i	AFB_1 concentration at CP_i before monitoring (µg/kg)	c_1 = 1 , 4 or 10
prod	AFB_1 production between CP_2 and CP_3 (µg/kg)	prod = 0 or 100
cm_i	AFB_1 concentration at CP_i after monitoring (µg/kg)	$(c_i + prod) * PA_i + m * (1 - PA_i)$
PA_i	Probability to accept the batches at CP_i	$P(\mu_i \leq \ln(\text{lim}) \mid c_i)$
μ_i	Mean lognormal distribution of the test results	$\ln(c_i) - \frac{\sigma_i^2}{2}$ (Lyman *et al.*, 2011)
σ_i^2	Standard deviation lognormal distribution of the test results	$\ln\left(\frac{var(c_i)}{c_i^2} + 1\right)$ (Lyman *et al.*, 2011)
$var(c_i)$	Variance of a sampling plan at CP_i, collecting ns samples and analysing one aliquot per batch with LC-MS/MS	$var(c_i) = \frac{128{,}4}{ns_i} * c_i^{0.98} + (0{,}50 * c_i)^2$
r_i	Recall and replacement costs for one batch (in case of rejection) at CP_i	r_1 = €160,000 r_2 = €1,800,000 r_3 = €1,900,000 r_4 = €380,000

5.3. Results

Table 5.4 shows the optimisation results for each scenario (S1, S2, S3, S4, S5, and S6): the optimal CP, the optimal number of batches checked at this CP, the number of samples collected from each batch, the costs for monitoring and replacing, the concentration at the time of sampling, the probability of accepting the batches, the concentration at the end of the chain, and the volume-based percentage of maize replaced.

Table 5.4. Optimal number of batches checked and optimal number of samples per batch at each CP for each scenario and the associated costs

	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Input: initial AFB_1 concentration and AFB_1 production during transport						
c_1	1	1	4	4	10	10
prod	0	100	0	100	0	100
Output: optimal number of batches checked and number of samples per batch						
$CP_1 – n_1$	0	0	60	0	60	0
$CP_1 – ns_1$	0	0	23	0	10	0
$CP_2 – n_2$	0	0	0	0	0	0
$CP_2 – ns_2$	0	0	0	0	0	0
$CP_3 – n_3$	0	6	0	6	0	6
$CP_3 – ns_3$	0	18	0	11	0	6
$CP_4 – n_4$	0	0	0	0	0	0
$CP_4 – ns_4$	0	0	0	0	0	0
Output: costs						
monitoring (€)	0	1,700	19,800	1,300	12,000	1,000
replacing (x €1,000)	0	7,978	4,842	9,305	8,132	10,410
Total (x €1,000)	0	7,994	4,862	9,317	8,144	10,419
Concentration at the time of sampling (µg/kg)	1.0	6.0	4.0	9.0	10	15
Concentration at the end of the chain (µg/kg)	1.0	2.5	2.5	2.5	2.4	2.2
PA (%)	100	30	50	18	15	9
% maize replaced	0	70	50	82	71	91

S1 had a low AFB_1 concentration and no AFB_1 production during transport, indicating that it was optimal not to collect any samples. S3 and S5 had high (4µg/kg) or very high (10µg/kg) AFB_1 concentrations in the fields, and no production during transport. For these scenarios, it was optimal to collect samples early in the chain, after harvest and before transport, at CP_1, since the replacement costs were the lowest, and the AFB_1 concentration did not change after this CP. In S2, S4, and S6, there was AFB_1 production during transport. For these scenarios, it was optimal to delay sample collection after transport at CP_3, when unloading the sea-going ship.

The number of samples collected per batch depended on the concentration: a high concentration at the time of sampling, for example 15µg/kg for S6, required only a few samples for contamination detection: six samples per ship compartment, with a 9%

probability of error. A concentration closer to the limit, for example, 4µg/kg for S3, required more samples to lower the concentration below the pre-determined limit: 23 samples per silo, with a 50% probability of error. The monitoring costs were the highest at CP_1 (€19,800 for S3 and €12,000 for S5), since the number of batches, in this case silos, was higher than the number of ship compartments at CP_3. The replacement costs were higher at CP_3 than at CP_1. Replacement of the batch at the end of the chain, a higher AFB_1 concentration and, consequently, a higher percentage of replaced maize, led to higher replacement costs.

Table 5.4 shows that the optimal CP was different, depending on whether AFB_1 production occurred during transport. Hence, no conclusion can be drawn from Table 5.4 with regards to the most critical CP. Collecting samples only at CP_1 would not lead to a satisfactory concentration at the end of the chain, in case of AFB_1 production later in the chain. The safest option would be to collect samples at CP_3 when unloading the sea-going ship. S3 needed the highest number of samples per batch (23 samples) to lower the average concentration to the pre-determined limit. If we collected 23 samples per ship compartment at CP_3 for all scenarios, the average concentration at the end of the chain for all scenarios would be below the pre-determined limit (Table 5.5). However, the costs would be higher than when tailoring the number of samples and the CP to each scenario.

Table 5.4 shows that for S2, collection of 18 samples from each ship compartment with an average concentration of 6µg/kg led to a probability to accept and retain the batch in the chain, that is, a 30% probability of error and, consequently, a probability of 70% to reject and replace the batch. Since we only considered the average concentration at the end of the chain, the average concentration after replacing 70% of the maize would be 2.5µg/kg, which is exactly the pre-determined limit. However, in individual cases, each tested compartment has a 30% chance to remain in the chain, and the end concentration could be 6µg/kg, which is above the pre-determined limit.

If a company prefers to lower the probability of error, more samples should be collected. As an example, we took a 5% probability of error at one CP. The results are shown in Table 5.6. Again, the closer the concentration was to the pre-determined limit, the higher the number of samples needed to correctly classify the batch as compliant or non-compliant. For a concentration of 6µg/kg (S2), 422 samples per batch were needed for a 5% probability of error. For a concentration of 4µg/kg, it was impossible to have a probability of error of less than 20%, since the concentration was too close to the pre-determined limit, and sample collection and analysis introduce significant uncertainty.

Table 5.4 indicates that it was optimal to test all batches at one CP. This was because all batches had the same average concentration. If this average concentration was above the pre-determined limit, all batches needed to be tested and replaced. For the case where only 50% of the batches could be tested at one CP, it was not always feasible to have an average

concentration at the end of the chain below the pre-determined limit (Table 5.7). More samples per batch, as well as more CPs, were needed (Table 5.7). The associated monitoring and replacement costs were both much higher (Table 5.7).

Table 5.5. Concentration at the end of the chain, PA and associated costs for each scenario, with 23 samples collected from each ship compartment during unloading

	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Input: initial AFB_1 concentration and AFB_1 production during transport						
c_1	1	1	4	4	10	10
prod	0	100	0	100	0	100
Input: all ship compartments checked at CP_3 with 23 samples per compartment						
$CP_3 – n_3$	6	6	6	6	6	6
$CP_3 – ns_3$	23	23	23	23	23	23
Output:						
Total costs (x €1,000)	266	8,526	5,752	10,479	10,781	11,320
Conc. at the end of the chain (µg/kg)	1.0	2.3	2.5	1.6	1.5	1.1

Table 5.6. Number of samples needed per batch at each CP to have a probability of error below 5%

	S1	S2	S3	S4	S5	S6
Input: initial AFB_1 concentration, AFB_1 production during transport and probability of error						
c_1	1	1	4	4	10	10
prod	0	100	0	100	0	100
Prob. error (%)	5	5	5	5	5	5
Output: optimal number of samples per batch						
$CP_1 – ns_1$	0	0	>500	0	25	0
$CP_2 – ns_2$	0	0	0	0	0	0
$CP_3 – ns_3$	0	422	0	35	0	9
$CP_4 – ns_4$	0	0	0	0	0	0
Output: costs						
monitoring (€)	0	25,900	>306,000	2,700	21,000	1,200
replacing (x €1,000)	0	10,830	7,650	10,832	9,131	10,905
Total (x €1,000)	0	10,856	>7,956	10,835	9,152	10,906
Output: concentrations, probability of error						
At the time of sampling (µg/kg)	1.0	6.0	4.0	9.0	10	15
At the end of the chain (µg/kg)	1.0	1.2	1.6	1.4	1.4	1.6
Prob. error (%)	0	5	20	5	5	4

Table 5.7. 50% or less of the batches are checked at each CP

	S1	S2	S3	S4	S5	S6
Input: initial AFB_1 concentration, AFB_1 production during transport and maximum number of batches checked						
c_f	1	1	4	4	10	10
$prod_s$	0	100	0	100	0	100
$CP_i – n_i$	$0 \le n_i \le B_i/2$					
Output: optimal number of samples per batch						
$CP_1 – n_1$	0	30	30	30	30	30
$CP_1 – ns_1$	0	9	167	200	107	200
$CP_2 – n_2$	0	2	3	3	3	3
$CP_2 – ns_2$	0	1	61	200	200	200
$CP_3 – n_3$	0	3	0	3	2	3
$CP_3 – n_3$	0	200	0	200	200	200
$CP_4 – n_4$	0	15	0	15	9	15
$CP_4 – ns_4$	0	200	0	200	31	200
Output: costs and end concentration						
Costs for monitoring (€)	0	95,500	47,800	118,100	38,400	118,100
Costs for replacing (x €1,000)	0	9,308	5,919	16,632	14,514	20,169
Concentration at the end of the chain (µg/kg)	1.0	2.7	2.5	3.0	2.5	3.2

5.4. Discussion

Optimisation models have been frequently used to optimise monitoring plans in the life sciences, such as for the detection of animal diseases, the detection of pests or invasive species, the detection of water contamination and, in some instances, for the detection of hazards in food and feed (Focker *et al.*, 2018). This research provides insight into the most critical CPs, and gives an indication of the number of samples needed to be collected to fulfil the concentration criterion at the end of the chain.

This study's first conclusion is that it is cost-effective to collect samples from all batches after harvest when no aflatoxin production was possible after harvest, since the replacement costs are the lowest at the beginning of the chain. The same result would be applicable to mycotoxins such as DON, which are produced in the fields and not during storage. If there is aflatoxin production during storage, it is cost-effective to sample, analyse, and replace contaminated batches after the aflatoxin production period, to avoid the double monitoring and replacement costs before and after storage and transport. This result would be applicable to other storage mycotoxins, such as Ochratoxin A.

Based on the optimisation model used in this study, samples were collected from all batches at one control point along the chain, rather than from half of the batches. This is because the average concentration of all batches was used at one point, which is the same as all batches having the same concentration. If a different concentration was seen in each batch, the results could have indicated that only half of the batches should be tested: testing and replacement of the half with a high concentration would be sufficient. Forecasting models for aflatoxins in maize at harvest could assist with deciding from which fields to collect samples. In this study, it is assumed that all fields have the same average concentration; however, in practice, concentrations will differ across fields, depending on the weather, location, and agricultural practices. The fields with high concentrations - and not necessarily those with low concentrations - should be tested.

The results of this study show the average outcome when the same scenario with the same batches are monitored for an infinite amount of times. As discussed before, the probability of error remains high in some cases. Increasing the number of samples reduces the chance that a batch with a high concentration is processed into an end product that will be consumed, but also results in higher costs. As can be seen from the results, more samples are required to correctly classify a batch with an AFB_1 concentration that is close to the pre-determined limit as contaminated or compliant, compared with a batch with an AFB_1 concentration that is much higher than the pre-set limit. A probability of error below 5% might not be achieved at one CP, even with a high number of samples collected, when the concentration is close to the pre-determined limit. When following Regulation No 152/2009

for the sampling and analysis methods for the official control of feed (EU, 2009), 100 samples should be collected from a ship compartment of 10,000t. Since the batch is very large, samples may be collected from only a part of the batch. For a silo of 1,000t, 100 samples should be collected. A minimum of 100 samples should be collected from a barge of 2,000t. These sample sizes are in most cases more than sufficient for a low probability of error. However, when the concentration is close to the pre-determined limits, 6µg/kg in S2 and 4µg/kg in S3, 100 samples per ship compartment were not sufficient to achieve a probability of error below 5%. In addition to increasing the number of samples from a batch, collecting and analysing samples at multiple CPs could decrease the overall probability to accept a contaminated batch, in cases where the AFB_1 concentration does not change along the chain.

The total costs estimated in this study, up to €10 million (Table 5.4), were much higher than only the costs for monitoring only, which went up to €19,800 (Table 5.4). First, this was because scenarios were considered where AFB_1 could be produced during transport, or cases where the concentration in the fields was high, resulting in replacement of a high percentage of maize. In addition, only the replacement costs per batch were considered, whereas the batch could be sold for other purposes in practice. Most rejected batches have an AFB_1 concentration above the considered limit of 2.5µg/kg, but below the EU limit for animal feed of 20µg/kg (EU, 2002a). These batches could thus be sold and used as pig and poultry feed, as such significantly lowering the replacement costs. Investment in good storage conditions would eliminate the high recall costs later in the chain, and would be the most cost-effective solution in the long term.

With an official control, described in Regulation No 152/2009 that prescribes the sampling and analysis methods for the official control of feed (EU, 2009), the analytical result is reported with a confidence interval that is based on the measurement uncertainty of the detection method. This measurement uncertainty includes different aspects: bias, recovery, and precision, both within and between laboratories. Only if the entire confidence interval, including the lower bound, is above the pre-determined limit, is a batch rejected. However, most private companies do not consider measurement uncertainty when buying maize. Furthermore, companies often have an internal pre-determined limit for AFB_1, which is lower than the EU limit; in this study, a pre-determined limit of 2.5µg/kg was considered.

5.5. Limitations of the Model

This study has some limitations, which are discussed below. First, the model was based on several assumptions: the maize supply chain, the costs for sampling and analysis, and the costs for replacing the batches. Further, the estimated probability to accept a batch

assumes that the test results followed a lognormal distribution. In addition, the variance due to sampling was estimated by Johansson *et al.* (2000a) using a limited number of batches, while the variance due to sample preparation and analysis was based on the work of Stadler *et al.* (2018). Furthermore, only six scenarios were considered, most of which are extreme scenarios which rarely occur. In practice, Scenarios 1 or 3 are the most likely to occur. Nevertheless, most assumptions are based on previous studies, and on what is observed in practice. Therefore, we believe that the model can still provide insights into cost-effective monitoring plans for mycotoxins along the cereal chain.

5.6. Conclusion

Since aflatoxins can accidently be produced during maritime transport under conditions of high temperatures and humidity, the harbour in the country of destination is a critical control point. The number of samples to be collected at the control point depends on the average batch concentration. With a high concentration, few samples will be sufficient for contamination detection; however, with a low concentration or a concentration close to the pre-determined limit, many samples are required. The replacement costs become higher and higher further along the chain: transport costs are added and, if contaminated maize is processed into feed, expensive recalls can be expected.

Chapter 6

General Discussion

6.1. Introduction

The presence of mycotoxins in cereals can lead to major economic losses for businesses and society (IARC, 2012). In developed countries, the majority of the losses are due to the mycotoxin concentration in cereal grains and derived feed and food products not complying with the legal limits of the importing country or the standards of the processing industry (Wu, 2004). However, as stated in Chapter 1, for several mycotoxins, legal limits for maximum concentration in food and feed are neccessary in order to protect human and animal health. Since mycotoxins are often heterogeneously distributed is raw materials and derived products, the sampling and analytical (S&A) plan can lead to false negatives and false positives, and batches with concentrations slightly above the legal limit can easily be accepted and enter the next stages of food and feed supply chains.

The economic consequences of monitoring for mycotoxins have not been widely addressed before, however, several papers have addressed the effectiveness of S&A plans for mycotoxins in cereals (Biselli *et al.*, 2008; Coker *et al.*, 2000; Mallmann *et al.*, 2014; Rivas Casado *et al.*, 2009; Whitaker, 2006). Furthermore, the FAO created an online sampling calculation tool showing the effectiveness of a chosen S&A plan for a list of mycotoxins and commodities, based on studies of Whitaker and co-workers (FAO, 2014). The costs associated with the S&A plans were, however, not considered in the before mentioned studies.

This thesis contributes to the interdisciplinary domain of food safety economics by developing methods that consider both the effectiveness and the costs of monitoring. The direct costs of monitoring such as the costs of sample collection and the costs of the chemical analysis, were considered, as well as the indirect costs, such as the potential losses due to recalls later in the chain in case of not detecting a too high contamination. Chapters 3, 4, and 5 developed methods for designing cost-effective monitoring plans for mycotoxins in the cereal supply chain, whereas Chapter 2 estimated the financial losses of the 2013 aflatoxin incident. This chapter starts in 6.2 with an additional comparison of several monitoring schemes and – to this end – integrates the results of Chapters 4 and 5, with the results of Chapter 2. Section 6.3 proceeds with a synthesis of all results and, next, provides a critical discussion of the methods used, the implications for policy makers and businesses, and the topics for further research. The chapter ends with the main conclusions of this thesis.

6.2. Financial impact of monitoring schemes for aflatoxins in maize at the harbour

Chapter 4 designed cost-effective S&A plans for a batch of cereals. Chapter 5 identified the most cost-effective control points along the chain, as well as the cost-effective number of samples to collect from batches at the particular control points. Chapter 5 concluded that, in terms of cost-effectiveness, minimal 18 samples per imported maize batch were necessary for one one the scenarios considered in that chapter. Chapter 5 did, however, only take into account the direct costs of monitoring and the costs of replacing a contaminated batch, and not any indirect costs such as the financial losses of an incident due to not-identifying contaminated batches, as, for example, the financial losses of the 2013 aflatoxin incident, as estimated in Chapter 2. This section presents an estimation of the financial effect of several monitoring schemes versus the financial effect of no-monitoring for aflatoxins in maize that is imported in the Netherlands for use infeed production, hereby integrating the results of Chapters 2, 4 and 5. We compared the financial effect of no-monitoring with the scenarios of collecting, respectively, 1, 18 and 100 samples per incoming maize batch at the harbour. The objective of this section was to investigate whether, when taking into account the financial losses of the 2013 aflatoxin incident, it would still be cost-effective to collect 18 samples per batch instead of 100 samples per batch as prescribed by the EU regulations.

6.2.1. Materials and methods

The Netherlands is a net importer of maize, each year about five million tonnes of maize are imported and only 200,000 tonnes are produced locally. Most of the imported maize is used for domestic consumption, with about 70% of the imported volume used for feed and 30% for food (World-Grain, 2016). In 2016, 67% of the maize import in the Netherlands came from Europe (equal to 3,238,600t), 16% from South America (equal to 776,100t), 4% from North America (equal to 191,600t) and 13% from other countries (Tridge, 2016). Our focus was on maize import that is destined for animal feed, which was equal to 70% of the imported maize. Most of the imported maize is transported via ships. It was assumed that a ship compartment had a 10,000t capacity. This would lead to an imported volume of 227 ship compartments from Europe, 55 ship compartments from South America and 13 compartments from North America.

The distribution of the aflatoxin concentrations in the incoming batches used in this section was based on data collected annually by Biomin (Biomin 2016; 2017; 2018). Biomin collected and analysed a high number of samples after harvest in the period 2016 – 2018,

and reported about the percentage of positive samples, the median concentration of the positive samples, and the maximum concentration found for aflatoxins. Data used in this section is shown in Table A.1, in the Appendix. By combining data about the imported quantities of maize from the different continents and Biomin's data about the aflatoxin concentrations in maize originating from these continents, the aflatoxin concentrations in the imported maize batches were simulated.

First, the scenario of no-monitoring for aflatoxins in maize was considered. Next, three scenarios with sampling at one control point before processing were considered: at ship compartment level at the harbour in the country of destination, with either 1, 18 or 100 samples collected per ship compartment. The limit of 20µg/kg was considered since this is the EU limit for AFB_1 in most animal feed products.

A partial budgeting model was used to assess the financial effect of different monitoring schemes taking into account both positive and negative financial effects of monitoring for mycotoxins. The positive effects included reduced costs and additionnal returns. Reduced costs were the result of reduced or no recalls, leading to recall costs avoided, and were related to the number of true positives. Additional resturns were the results of batches complying with the pre-set limit and correclty classified as compliant, and were related to the number of true negatives. The negative effects of monitoring consisted of the additional costs and the reduced returns. The additional costs consisted of, first, the costs for the sample collection and the chemical analysis, and second, the costs related to contaminated batches that were not detected and could potentially lead to expensive recalls later in the chain. The costs of potential recalls were related to the number of false negatives. Reduced returns, were the result of batches that could have been used for feed but, since they were incorreclty identified as non compliant with the pre-set limit, were downgraded to use in biogas production. The reduced returns were attributable to the number of false positives. Table 6.1 shows the summary of the partial budgeting model setup.

Table 6.1. Partial budgeting

Positive effects		**Negative effects**	
Reduced costs	· True positives	**Additional costs**	· Costs S&A · False negatives
Additional returns	· True negatives	**Reduced returns**	· False positives
Total positives effects: reduced costs + additional returns		**Total Negative effects:** additional costs + reduced returns	
Net effect: positive effects – negative effects			

One key assumption in the partial-budget calculations, that might not always hold in reality, was that batches with concentrations above 20µg/kg were always identified (detected) at

some in the downstream staged of the maize supply chain, before consumption. The number of true positives, true negatives, false positives and false negatives were based on the probability to accept a batch *(PA_i)*, which depended on the number of samples collected and the concentration of the batch. More information about the probability to accept a batch, including the formulas used can be found in Chapters 4 and 5. The additional returns were estimated to be €200 per tonne, the average value of one tonne of imported maize into the Netherlands (Chapter 2). The reduced costs were based on the recall costs of the 2013 aflatoxin incident, as calculated in Chapter 5. These recall costs were estimated to be on average of €230 per tonne (Chapter 2). The reduced costs were estimated to be on average €200 per tonne, equal to the recall cost per tonne avoided minus the value of one tonne of maize destined as biogas (€30) (Chapter 2). The reduced returned were estimated to be on average €170 per tonne, equal to the value of one tonne of maize that could be used for feed minus the value of one tonne of maize that was destined for biogas (Chapter 2). Stochastic inputs were used for most variables of the partial budget model. The variables used in the model can be found in Table A.2 in the Appendix.

6.2.2. Results and discussion

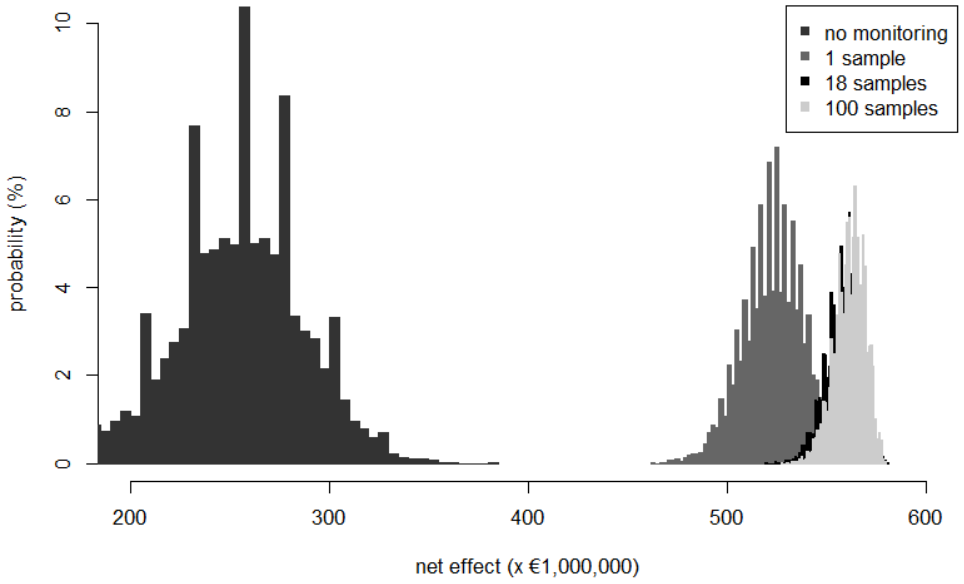

Figure 6.1. Results of the partial budgeting model for monitoring versus no monitoring

Figure 6.1 shows the results of the partial budgeting model. No-monitoring led to a lower positive financial effect than the monitoring schemes collecting 1, 18 or 100 samples per batch. The range of the financial effects of no-monitoring is wider than the distributions of

the net effects of monitoring. When monitoring is not performed, the financial effect entirely depends on the AFB_1 concentrations in batches that are imported; based on the data used, these concentrations are highly variable between batches that arrive in the harbour. Collecting one sample per batch led to a lower net effect than collecting more than one sample (18 or 100 samples). In addition, the distribution of the financial effect when collecting one sample per ship compartment is wider than the distribution of the financial effect when collecting more (18 or 100) samples, since collecting only one samples leads to highly variable results. The difference in positive financial effect between collecting 18 and 100 samples per ship compartment is less than 1% for the scenario considered in this section, and is therefore, small.

Table 6.2. Number of true negatives, true positives, false negatives and false positives with different monitoring schemes: averages of 10,000 iterations with minimum and maximum values shown in brackets

	No monitoring	**1 sample**	**18 samples**	**100 samples**
True negatives	214.0 (187-245)	213.9 (184-241)	212.8 (186-239)	212.8 (186-243)
True positives	0	67.5 (41-96)	77.7 (57-107)	78.5 (50-106)
False negatives	81.0 (50-108)	13.5 (2-28)	3.2 (0-12)	2.5 (0-10)
False positives	0	0.2 (0-4)	1.3 (0-7)	1.3 (0-7)
Total	295	295	295	295

Collecting 100 samples per incoming ship compartment resulted into more or less the same positive financial effect as collecting 18 samples per ship compartment (Figure 6.1). Furthermore, collecting 100 samples per batch instead of 18 led, on average, to the same number of false positives and, on average, only to 0.7 less false negative results (Table 6.2). Therefore, if we consider the flow of batches entering the country during one or more years, collecting 18 samples per batch would be a cost-effective monitoring strategy. However, when an individual batch is considered, collecting 18 samples can lead to a high probability of false negatives and/or false positives.

In this section, we used 20µg/kg as the limit for maize processed into feed, since usually only about 10 to 20% of the feed consists of maize and mycotoxins present in the maize will be diluted in the final product. If 20% of the feed consists of maize and the concentration of that maize is exactly 20µg/kg, the final feed product will have a concentration of 4µg/kg, which is just below the legal limit for dairy cow feed of 5µg/kg. In order to exceed the legal limit for other feed products of 20µg/kg, the maize incorporated in the feed has to be above 100µg/kg when a percentage of 20% maize in the feed in considered. In the Netherlands,

about 22% of the maize destined for feed is used for the production of compound feed for dairy cows. Since maize batches with AFB_1 concentrations above 100µg/kg are not frequently seen in Europe, in practice, 78% of the batches with AFB_1 concentrations above the legal limit remain undetected. However, industry should comply with the regulations set and, it is for this reason that we estimated the effect of monitoring by stating that true positives are a positive outcome of the monitoring system and not a negative outcome. When the partial budget model was run with the assumption that only 22% of the false negative test results led to recalls, monitoring still led to a higher positive financial effect than no-monitoring, and collecting 18 or 100 samples still led to a higher positive financial effect than collecting one sample per ship compartment. However, the differences between the positive financial effects observed between the monitoring schemes are less pronounced. The same result was obtained when the aflatoxin concentrations in the incoming batches are lower than the concentrations considered in this section: collecting between 18 and 100 samples per incoming ship compartment still led to a higher positive financial effect than collecting one sample per batch, however, the difference was less pronounced.

We also remark that this section used a supply chain approach, so this estimation shows the combined financial impact for both the maize importers and the feed producers. Different stakeholders invest in monitoring and/or get the benefits of it. Feed producers will benefit from reduced costs (related to the true positives) and will bear the additional costs related to the false negatives. The maize traders will suffer from the reduced returns related to both false and true positives. The results suggest that the total costs of monitoring along the supply chain should be shared between actors in the various supply chain stages, in order to obtain a cost-effective monitoring plan in the entire maize chain.

6.3. Synthesis of the results

A S&A plan for a batch of cereals consists of three steps. The first step is the sample collection step, in which incremental samples are collected from the batch. The second step is the sample preparation step in which the incremental samples are mixed and ground to form an aggregate sample, from which one or more aliquots are prepared for the analysis. The third step is the analysis of the aliquot with a mycotoxin detection method. This section starts with identifying the most critical step in obtaining precise results on the mycotoxin concentration of a cereal batch. Next, the cost-effectiveness of S&A plans aggregating incremental samples before chemical analysis are compared with S&A plans analysing each incremental sample individually. Then, the evidence in this thesis on the optimal number of samples to collect from a cereal batch, in terms of cost-effectiveness, is discussed. This

section ends which discussing the optimal control points along the cereal supply chain in terms of cost-effectiveness.

6.3.1. The sample collection step versus the analytical step

Chapter 4 of this thesis concluded that the sampling step is the most critical step from a S&A plan in obtaining a representative result on the mycotoxin concentration of a cereal batch. This conclusion coincides with results from other studies analysing the effectiveness of different sampling plans for DON in wheat and for aflatoxins in maize (Biselli *et al.*, 2008; Cheli *et al.*, 2009; Coker *et al.*, 2000; Coker *et al.*, 1995; Johansson *et al.*, 2000a; Mallmann *et al.*, 2014; Park *et al.*, 2007; Whitaker, 2006; Whitaker *et al.*, 2000). Since the sample collection step accounts by far for the largest percentage of the total variance, the detection method does not have a large influence on the effectiveness of the S&A plan. Therefore, a relatively low cost and easy to perform detection method, such as ELISA, is often a better detection method to use in comparison to the more resource-demanding instrumental methods, given that the resources saved with the chemical analysis are used for collecting and analysing more samples (Chapter 4).

6.3.2. Aggregating the incremental samples collected

Chapter 4 compared three different S&A plans. With a given budget, collecting incremental samples and analysing each sample individually on-site, was less effective than the S&A plans aggregating the incremental samples collected. Chapter 4 therefore concluded that analysing an aliquot from aggregated incremental samples, with any detection method is a mo-=re cost-effective method than analysing incremental samples separately for both DON in wheat and aflatoxin in maize. The same result was found by Biselli *et al.* (2008) who compared two different S&A plans for DON in a 26t wheat batch: the first S&A plan collected 100 incremental samples, aggregated all these samples, and analysed a subsample, whereas the second S&A plan analysed individually all 100 samples collected. Biselli *et al.* (2008) concluded that, for DON, the result of the analysis of the aggregate sample was in accordance with the average result of the analysis of each incremental sample separately, in case the aggregated sample is perfectly homogenised (Biselli *et al.*, 2008). Based on the results of this thesis as well as on the results of Biselli *et al.* (2008), we can conclude that aggregating incremental samples is a cost-effective method.

Analysing all samples separately could be used to decrease the number of false negatives when, instead of averaging the results of the incremental samples, all incremental samples collected have to test below the legal limit. This practice leads to the same outcome, less false negatives and, consequently more false positives, as lowering the pre-set limit

(Whitaker, 2006). Therefore, if one would like to decrease the number of false negatives, a cost-effective option would be to aggregate the samples collected and using a lower pre-set limit than the legal limit.

6.3.3. Optimal number of incremental samples

This thesis concludes that in terms of cost-effectiveness, less samples than prescribed by the EU regulations can be collected to obtain a reliable insight into the mycotoxin concentration of a cereal grain batch. Chapter 4 compared the optimal S&A plans for different budgets. For DON in wheat, the sampling and analytical plans using LC-MS/MS and collecting 13 and 103 samples showed a difference in the percentage of correct decisions of only 2.8% when we considered DON concentrations in wheat batches that are seen in practice in the Netherlands. For aflatoxins in maize, the difference in percentage of correct decisions when 15 or 105 samples were collected was 4.1%, which is larger than for DON in wheat. However, the difference in number of correct decisions when 55 or 105 samples were collected was only 1.8% (Chapter 4). Chapter 5 suggests that collecting between 18 and 23 samples per ship compartment of maize is cost-effective, depending on the scenario. These numbers are much lower than the minimum of 100 samples prescribed by the EU regulations for a large cereal batch.

Section 6.2 of this chapter compared the net financial effects of monitoring all ships entering the Netherlands with a different number of incremental samples collected from each ship compartment. This estimation included the financial impact of not detecting a contaminated batch, due to no-monitoring or imperfect monitoring, which would lead to the production of contaminated feed and, consequently, to expensive recalls as described in Chapter 2. The results showed that collecting at least one sample per incoming ship compartment greatly increased the net positive financial effect (versus no monitoring at all). These results also confirm the results of Chapters 4 and 5 by showing that the difference in net financial effect when collecting 18 or 100 samples per incoming maize containing ship compartment is very small.

The conclusion that collecting less samples than prescribed by the EU regulations is cost-effective is in line with a few previous studies. Coker *et al.* (1995) concluded that approximately 100 incremental samples are required to have an aggregate sample of 10kg that is representative for batches with large particles such as maize kernels. However, Rivas Casado *et al*. (2009) concluded that the accuracy of the S&A plans greatly increased up to 40 or 60 samples, depending on the simulation, but that the beneficial effect was negligible for 60 samples or more (Rivas Casado *et al.*, 2009). Biselli *et al*. (2008) compared S&A plans

and concluded that the increase in uncertainty when collecting 10 samples instead of 100 is not large.

To conclude, in terms of cost-effectiveness, less samples than prescribed by the EU regulations can be collected to obtain a reliable insights into the mycotoxin concentration of a cereal grain batch, but only when a flow of batches during a time period of one or multiple years is considered. For individual batches, collecting less samples than described in the EU regulations can lead to a large probability of misclassifying a batch when the batch concentration is close to the pre-set limit (Chapter 5).

6.3.4. Monitoring along the supply chain

Chapter 5 concluded that the cost-effective control point for aflatoxins along the maize supply chain is in the downstream stages of the maize supply chain, after the transport and storage steps during which (more) aflatoxins might be produced in case of sub-optimal storage conditions. When it was certain that aflatoxins would not be produced along the supply chain, for example with very good and controlled transport and storage conditions, it was cost-effective to monitor in the upstream stages of the maize supply chain, closely after harvest. At this point, the number of stakeholders involved is relatively small and the maize has not been transported yet. This result is in line with the work of Lascano-Alcoser *et al.* (2014) on cost-effective monitoring for dioxins in the pork chain. These authors concluded that for dioxins in the pork production chain, monitoring should focus on the upstream stages of the supply chain. The cost for tracing back the source of the contamination as well as the financial consequences of a contamination are smaller in the upstream stages of the supply chain than in the downstream stages of the supply chain (Lascano Alcoser *et al.*, 2014). Monitoring in the upstream stages of the food supply chain seems to be a cost-effective option for those contaminants that do not increase in concentration and are not introduced further along the supply chain such as dioxins and mycotoxins that are only produced in the field, like DON during wheat cultivation. For mycotoxins that can potentially be produced during storage, like aflatoxins, it is necessary to monitor in the downstream stages of the food supply chain.

Mycotoxins produced during storage tend to be more heterogeneously distributed in cereal batches than mycotoxins produced in the field. This is because mycotoxins produced during storage are produced in localised hotspots during storage and, in case of a standard silo with no mixer, no mixing has occurred during harvest as is the case for mycotoxins which are (locally) produced in the field. For these mycotoxins produced during storage, collecting a few samples only from a batch at one control point leads to a high probability of false negatives and false positives in case the mycotoxin concentration in the batch is close to the

pre-set limit. To increase the effectiveness of monitoring, samples should be collected and analysed at more than one control point (Chapter 5).

6.4. Methodological issues

6.4.1. Modelling

The literature review in Chapter 3 showed that methods previously used in the life sciences to develop cost-effective monitoring strategies were simulation models, optimisation models and statistical models. The input data used in the models of these studies were either deterministic or stochastic in nature. In most studies, only the direct costs of monitoring were assessed, and only few studies also assessed the direct benefits (Chapter 3). Chapter 3 concluded that the methods suitable for assessing the cost-effectiveness of food safety monitoring were simulation and optimisation models with both deterministic and stochastic input data, since very few data are usually available. These methods were used in the remaining chapters of this thesis. Chapter 4 used a deterministic optimisation model considering the direct monitoring costs. Chapter 5 also used a deterministic optimisation model considering the direct costs associated with monitoring, and the costs of replacement of the contaminated batch. Section 6.2 used a simulation model with stochastic data and took into account the direct costs as well as the direct benefits of the different monitoring strategies considered.

The models developed in this thesis are a simplification of the reality and thus - by definition - not an exact representation of reality. Outcomes in practice will be different, and real life has mechanisms that are not captured in the models. The models can give insights into mechanisms (that are included in the models), make some approximations of the effectiveness and the costs of monitoring schemes, and make suggestions for cost-effective monitoring. The optimisation models developed in Chapters 4 and 5 are deterministic models, which means that uncertainty of the input data was not taken into account, and the model resulted into only one outcome. To obtain insights into the influence of the input parameters in the results, Chapter 4 performed a sensitivity analysis, changing some input parameters in the model. Chapter 5 did not include any uncertainty of the input parameters since varying the input data, or using stochastic variables, would lead to a different result for each iteration. One result for one iteration makes it impossible to describe the link between the input parameters of the model, representing a specific scenario, and the outcome of the model. In this case, more insight into the possible range of outcomes was obtained by describing specific scenarios and their associated results, their similarities and differences, rather than making a full description of all results possible. The model in Chapter 2, estimating the costs of the 2013 aflatoxin incident, did include stochastic input

variables, capturing the uncertainty of the input data and, in that way, described a wider range of possible results.

The first optimisation model that was developed within this thesis optimised sampling plans within a batch and focussed on two mycotoxin-crop combinations: DON in wheat and aflatoxins in maize, of which DON is produced in the field, and aflatoxins can be produced both in the field and during storage. The results of both models are mycotoxin-crop combination specific and, therefore, cannot be directly generalised to other mycotoxins and/or other crops. The second optimisation model developed in this thesis optimised monitoring schemes along the chain and focussed on aflatoxins in maize. The models and/or input variables can easily be adapted for other crops and mycotoxins: the supply chain might be different for another crop, the incoming mycotoxin concentrations are different, limits for each mycotoxin are different, and the degree of heterogeneity of the mycotoxin concentration in the batch, used to estimate the probability to accept a batch, is different for each mycotoxin-crop combination.

6.4.2. Input data

The major methodological issue throughout this thesis was the limited availability of data. Data about the costs of sample collection, the costs of chemical analysis, the transport costs, the recall costs, and the destruction costs were often not available in public sources and needed to be obtained by conducting interviews with experts. However, the information provided during personal communications is person-dependent. For example, laboratories charge different costs for the analysis, so depending on where the person interviewed works, the answer is different. To cover this issue, several experts were interviews for the costs of the analysis, several answers were collected from various experts, resulting in a range of costs, so that the input of the model did not depend on one interview. The sensitivity analysis performed in Chapter 4 showed that changing the costs of a detection method had a negligible impact on the results.

In Chapter 2, we based the recall costs on the costs to replace a contaminated cereal batch, the costs to remove the contaminated batch from the chain, and the costs to destroy the produced feed. This estimation of the recall costs was based on an in-depth interview with one feed producer. To account for this shortcoming, in Chapter 2, we used a normal distribution with the mean being the estimated value (expert answer) received and the standard deviation being 25% of this estimate value. To make the outcomes of the models more robust, more interviews should be conducted with cereal traders and the processing industry. More expert interviews will lead to more data and distributions or price ranges can be deduced from the collected data so that the outcomes will no longer rely on one data

point. Adding to that, more cost categories could have been added to the recall costs. For the maize traders, we could have added extra administration costs, described by Wu (Wu, 2008) as well as the difference in value of the maize is above the EU legal limit for dairy cattle feed but below the limit for other animal feed. However, data about these costs were not available for this research. For the processing industry, we could have added the costs of hiring extra employees, extra cleaning costs, and extra administration costs (financial administration, informing consumers, arranging recalls) due to discarding the contaminated batches. Again, data about these costs were not available for this research. These costs are however not expected to be major costs that influence the conclusions of this thesis.

The precision of a detection method is, like the costs of the analysis, different for each brand and each laboratory. Therefore, using the data from only one or two laboratories implies the outcomes rely on these one or two observations. In this thesis we used the precision and the costs of a few different brands of detection methods to account for this shortcoming. However, the sensitivity analysis performed in Chapter 4 showed that changing the precision of an detection method had a negligible impact on the results and did not influence the conclusions. The formula estimating the variance of a sampling plan, used throughout this thesis, was based on formulas developed by Johansson *et al.* in 2000 for aflatoxins in maize (Johansson *et al.*, 2000a) and by Whitaker *et al.* in 2000 for DON in wheat (Whitaker *et al.*, 2000). These formulas are integrated in the FAO sampling tool and are, therefore, widely used by industry. The variance due to the sample preparation, estimated in the research of Johansson *et al.* (2000) and Whitaker *et al.* (2000) is based on dry mixing as sample preparation method. However, if slurry mixing would be used to prepare the sample, the variance due to the sample preparation step would be lower than estimated by Johansson *et al*. and Whitaker *et al*. (Spanjer, 2006).

The formula to estimate the variance of a sampling plan for DON in wheat is different than the formula to estimate the variance of a sampling plan for aflatoxins in maize, since DON is less heterogeneously distributed than aflatoxins, and wheat has a smaller kernel size than maize. The results for the cost-effective sampling plans for DON in wheat and aflatoxins in maize are, therefore, also different (Chapter 4). From this difference, we can conclude that the robustness of the results of thesis relies on the formulas used to estimate the variance of a sampling plan. More experiments should be performed to be more certain about the accuracy of, in the first place, the formulas estimating the variance of a sampling plan, and in the second place, the results of this thesis.

6.5. Implications for policy makers and business

Collecting only one sample from a cereal batch leads to a very high probability of false negatives and false positives when classifying a batch as compliant or non-complaint with the legal limits, especially many false-negatives occur that can potentially lead to expensive recalls later in the supply chain (section 6.2). Collecting more than one sample, but less samples than is prescribed by the EU regulations, leads to a high probability of misclassifying a batch when the concentration of that batch is slightly above the pre-set limit, but not when this concentration is lower or much higher that the pre-set limit (Chapter 5). However, Chapters 4, 5, and 6 concluded that collecting less samples then prescribed by the EU regulations is cost-effective when a flow of batches during a longer time period is considered. Our results suggest that for routine monitoring performed by traders buying cereal batches, or the processing industry, collecting between 18 samples and 100 samples (number of samples that is prescribed by the EU regulations for a large batch), from all incoming batches, depending on the accuracy wished for and the available resources, is cost-effective. Since collecting fewer samples than is prescribed by the EU regulations can lead to a large probability of misclassifying a batch when the batch concentration is close to the legal limit, our results suggest to keep the current EU regulations regarding the sampling of feed and food products for official controls.

In general, to increase the cost-effectiveness of a S&A plan, our results suggest to allocate more budget to the sample collection step and less to the chemical analysis. When the budget is limited, a low-cost method, such as ELISA or LFD, to analyse a sub-sample from an aggregate sample, can be considered instead of the more expensive LC-MS/MS method, given that the money saved on the chemical analysis is spent on collecting extra incremental samples. Since the chemical analysis, regardless of the detection method used, is relatively costly, and most of the budget should be spent on collecting incremental samples, aggregating incremental samples and analysing one sub-sample instead of analysing each incremental sample collected is a cost-effective strategy (Chapter 4).

For mycotoxins that are only produced in the field, our results suggest that collecting samples from all batches in the upstream stages of the food supply chain is cost-effective. For mycotoxins with a high probability of an increase of the concentration during transport and storage, monitoring all batches in the downstream stages of the food supply chain is cost-effective. Since these mycotoxins produced during storage in localised hotspots are not mixed after harvest like mycotoxins only produced in the field, these mycotoxins are highly heterogeneously distributed. To increase the effectiveness of a monitoring plan, multiple control points, along the supply chain, after harvest, should be considered.

6.6. Further research

The estimation of the variance of a S&A plan throughout this thesis was based on one published experiment related to aflatoxins in maize, in which 45.4kg from in total 18 maize batches, were divided into 32 samples which were individually analysed for the presence of aflatoxins. The variance of a n for DON in wheat was based on one experiment published experiment, in which 20kg from in total 24 wheat batches were divided into 32 samples analysed individually for DON (Johansson *et al.*, 2000a; Whitaker *et al.*, 2000). Although, these two experiments sampled 18 and 24 batches, respectively, the volume of material sampled was relatively small. Since the results of this thesis highly depend on these estimations, more experiments should be performed, verifying results of these two experiments. Additional experiments should collect at least a hundred of incremental samples from individual batches with different mycotoxin concentrations, and analyse these incremental samples one by one with an instrumental method. The variance between the concentrations of the samples collected leads to the variance due to the sample collection step.

Furthermore, more research should be done to obtain insights into the variance between the mycotoxin concentration determined in individual samples collected from a single batch due to the sample preparation and the analysis steps. To this end, ring trials using different sample preparation and detection methods in different laboratories should continuously be organised and results should be published in scientific literature. Adding to that, Stadler *et al.* (2018) concluded that the lot-to-lot variance accounts for a large part of the variance of a sampling plan. These authors analysed the lot-to-lot variance for the LC-MS/MS detection method (Stadler *et al.*, 2018). More research on the lot-to-lot variation with other mycotoxin detection methods such as lateral flow devices and ELISA should be performed. Having multiple studies estimating the variance due to the sample collection, the sample preparation, the chemical analysis, and the lot-to-lot variation, will allow us to have a more complete and reliable way of estimating the total variance of a sampling and analysis plan, leading to more robust results accounting for a wide range of batch types, sample preparation methods and detection methods.

Chapter 4 and 5 focussed on specific mycotoxin-crop combinations. The most cost-effective monitoring scheme showed to be different for each mycotoxin. This is probably because of the degree of heterogeneity of the distribution which is different and the possibility of mycotoxin production during storage. The cost-effective monitoring schemes will be different for each crop as well: wheat or maize kernels are relatively small particles whereas nuts or figs are relatively larger particles, and particle size is known to influence the distribution of mycotoxins throughout the batch. Therefore, future research should focus on designing

optimal, cost-effective monitoring schemes for various mycotoxins in different crops instead of one mycotoxin-crop combination. Adding to that, research should also focus on simultaneously finding optimal monitoring schemes for different mycotoxins, or different contaminants in general, at the same time. Collecting samples for different contaminants simultaneously will lead to lower costs for the sample collection step at one control point in the supply chain. One example, in a different field than food safety, is the work of Guo *et al.* (2016) who presented a conceptual framework to allocate cost-effectively the resources for several animal diseases (Guo *et al.*, 2016).

The costs, both direct and indirect, of food and feed related incidents should more frequently be estimated for all stakeholders involved. Estimating the costs of these incidents can help policy makers and industrial risk managers to allocate the budget for prevention and control measures, and monitoring. If for a certain contaminant, the frequency of the incident and the incident costs are high, more resources can be spent on preventive and control measures than in case the frequency and the costs of the incident are negligible. Furthermore, the cost estimations could give insights into which stakeholders bear the costs of the incident, and which stakeholders benefit from increased prevention and control measures and better monitoring. These costs estimations could stress the need for sharing the costs of preventive and control measures as well as monitoring between the various actors and stages of the food supply chain.

In this thesis, human and/or animal health costs were not considered. Instead, this thesis assumed that all food and feed batches above the EU legal limit were detected in the downstream stages of the food supply chain, before consumption. In addition, this thesis assumed that batches with mycotoxin concentration equal or below the legal limits did not lead to human or animal health costs. Further research could integrate human and animal health costs in case the batches with concentrations above the legal limits are not detected before consumption.

6.7. Main conclusions

The main conclusions of this thesis are:

- Undetected contaminated maize batches (false negatives) in the upstream stages of the maize supply chain can lead to large economic consequences in the downstream stages of the maize supply chain (Chapter 2).
- It is cost-effective to allocate most of the budget of the S&A plan for mycotoxins in wheat and maize to the sample collection step, rather than to the chemical analysis (Chapter 4).

- Aggregating incremental samples and analysing a sub-sample from the aggregated sample is a cost-effective method for monitoring aflatoxins in maize and deoxynivalenol in wheat (Chapter 4).
- When an entire flow of batches during one or multiple years is considered, collecting less samples than prescribed by the EU regulations is cost-effective for monitoring deoxynivalenol in wheat and aflatoxins in maize (Chapters 5, 5 and 6).
- When the aflatoxin concentration in a maize batch is 1 to 1.6 times higher than the pre-set limit of 2.5µg/kg, 10 to 100 times more samples are needed to correctly classify batches than when the batch concentrations are lower or higher than 1.6 times the pre-set limit (Chapter 5).
- For deoxynivalenol, a mycotoxin produced in the field, monitoring in the upstream stages of the wheat supply chain is cost-effective whereas for aflatoxins, mycotoxins that can be produced both in the field and during storage, monitoring in the downstream stages of the maize supply chain is cost-effective (Chapter 5).

Appendix

Table A.1 Data from Biomin: aflatoxin concentrations in corn (BIOMIN, 2016; 2017; 2018)

	Year	% samples contaminated	Median positives (µg/kg)	Max (µg/kg)
Europe	2016	21	1	491
	2017	63	1	762
	2018	18	2	76
	Mean	34	1	443
North America	2016	3	3	15
	2017	5	9	139
	2018	9	15	280
	Mean	6	9	145
South America	2016	24	3	160
	2017	18	4	351
	2018	19	4	402
	Mean	20	4	304

Table A.2. Variables of the stochastic partial budget model used in section 6.1.

Symbol	Description	unit	Distribution or estimation	Source
ns	number of samples	Number	0, 1 18 or 100	
$c_{sampling}$	Costs sample collection	€ per sample	Normal(10; 2.5)	Chapters 4 and 5
$c_{analysis}$	Costs analysis	€ per sample	Normal(100; 25)	Chapters 4 and 5
c_{recall}	Costs recall	€/t	Normal(230; 46)	Chapter 2
m_{import}	Value imported maize for feed	€/t	€200	Chapter 2
m_{biogas}	Value imported maize for biogas	€/t	€30	Chapter 2
sc_{EU}	Number of ship compartments from Europe for feed	Number	324	Estimation, based on (Tridge, 2016)
c_{EU}	Aflatoxin concentration ship compartment from Europe	µg/kg	Bernoulli(1; 0.34) * Triangle(0.5; 443; 1)	Biomin(2016, 2017, 2018)
sc_{SA}	Number of ship compartments from South America for feed	Number	78	Estimation, based on (Tridge, 2016)
c_{SA}	Aflatoxin concentration ship compartment from South America	µg/kg	Bernoulli(1; 0.20) * Triangle(0.5; 304; 4)	Biomin(2016, 2017, 2018)
sc_{NA}	Number of ship compartments from North America for feed	Number	19	Estimation, based on (Tridge, 2016)
c_{NA}	Aflatoxin concentration ship compartment from North America	µg/kg	Bernoulli(1; 0.06) * Triangle(0.5; 145; 9)	Biomin(2016, 2017, 2018)
A_i	Batch *i* accepted	1 or 0	Bernoulli(1; PA_i)	
PA_i	Probability to accept batch i	between 0 and 1	See chapter 4	Chapter 4

For each ship compartment sc imported, if the concentration c was lower than 20µg/kg and A_i was equal to 1, meaning that the batch was accepted, this ship compartment resulted in a true negative. If the concentration c was lower than 20µg/kg and A_i was equal to 0, meaning that the batch was rejected, this ship compartment resulted in a false negative. If the concentration c was higher than 20µg/kg and A_i was equal to 1, meaning that the batch was accepted, this ship compartment resulted in a false positive. if the concentration c was higher than 20µg/kg and A_i was equal to 0, meaning that the batch was rejected, this ship compartment resulted in a true positive.

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Summary

Mycotoxins are toxic secondary metabolites produced by certain types of fungi under favourable conditions, and can be present in several food and feed commodities such as cereals and derived products. Mycotoxins can lead to adverse health effects in both animals and human. Therefore, legal limits and standards have been set for the maximum concentration of certain mycotoxins in various feed and food ingredients and products. An effective mycotoxin monitoring system needs to be in place to check for the presence of mycotoxin in raw materials for food and feed and derived products as part of Hazard Analysis and Critical Control Points (HACCP) programs in the feed and food industry, and for compliance with the legal limits. Monitoring is done by regular collection of samples from batches along the supply chain and the chemical analysis of these collected samples for the presence of mycotoxins. For official controls of both feed and food products, sampling and analytical (S&A) plans for mycotoxins are prescribed by the EU regulations. The monitoring procedures described in the EU regulations are, however, resource demanding because of the high number of samples that need to be collected and the detection methods that need to fulfil strict performance criteria.

Mycotoxins are often heterogeneously distributed in cereal grain batches; in the major part of the batch, the concentration is low, whereas only a few spots are highly contaminated. Because of this heterogeneous distribution, samples collected at different locations in the batch have different mycotoxin concentrations. One of the challenges in mycotoxin monitoring is to collect the right number of samples to reflect the mean concentration of the batch. Another challenge is to accurately determine the concentration of the mycotoxin in the final sample. However, the most accurate detection methods for mycotoxin analysis are usually also the most expensive and time consuming methods. The resources available for mycotoxin monitoring in a cereal supply chain are often limited and, therefore, choices have to be made to effectively distribute available resources between sample collection and the chemical analysis of the samples. The more resources are spent on the chemical analysis, the less is available for the collection of the samples, and vice versa. The overall objective of this thesis is to design methods to design cost-effective monitoring plans for mycotoxins in cereal grains.

Chapter 2 estimated the financial losses of the 2013 aflatoxin incident In Europe. In the summer of 2012, maize from the Black Sea area and highly contaminated with aflatoxins was transported and processed into dairy cow feed in the Netherlands. As a result, milk produced in the Netherlands exceeded EU limit for aflatoxin M_1. The financial losses of this incident were estimated for several stakeholders in the Netherlands and its neighbouring countries Belgium and Germany. The estimated financial losses were in the range of tens of

millions of euros for all stakeholders in these three countries. The largest percentage, about 70% of the costs, were for the maize traders importing and selling the maize. The remaining 30% of the costs were for the feed processing industry. These estimated costs gave an indication of the indirect costs of imperfect monitoring in the upstream stages of the maize supply chain.

Chapter 3 performed a literature review describing the methods previously used in the life sciences to develop cost-effective monitoring strategies, including monitoring for animal diseases, plant pests, hazards in the soil, water, food and animal feed. The pros and cons of these methods, as well as their applicability to food safety were assessed. Chapter 3 concluded that several methods were previously used to develop cost-effective monitoring strategies such as: simulation models, optimisation models and statistical models with a combination of using deterministic and stochastic input data.

Chapter 4 developed an optimisation model to design cost-effective S&A plans for mycotoxins in a cereal batch. Two case studies were used, being deoxynivalenol (DON) in a wheat batch and aflatoxins in a maize batch. This chapter concluded that the sampling step had the highest influence on the effectiveness of a S&A plan whereas the performance of the detection method had limited influence on the effectiveness of a S&A plan. Therefore, a detection method such as ELISA, which is lower in costs and easier to use than instrumental methods, was a suitable detection method given the money saved on the analysis was spent on collecting more samples to be analysed. Adding to that, given that one is interested in the mean mycotoxin concentration of a wheat or maize batch, Chapter 4 concluded that aggregating incremental samples and extracting a sub-sample from the aggregate sample for the chemical analysis is more cost-effective than analysing incremental samples separately for both DON and aflatoxins.

Chapter 5 developed an optimisation model to identify cost-effective monitoring strategies along the maize supply chain. This chapter focussed on the sample collection step of S&A plans only. This chapter concluded that, for aflatoxins, a mycotoxin produced both in the field and during storage in case of sub-optimal conditions, monitoring all batches in the downstream stages of the maize supply chain, thus the transport and storage steps, was cost-effective. The optimal number of samples to collect from the batches depended on the mycotoxin concentration at the time of sampling: when the mycotoxin concentration was slightly higher than the pre-set limit, more samples were needed than when the concentration was lower or much higher than the pre-set limit. The results of this chapter suggest that collecting less samples than prescribed by the EU regulations was cost-effective when considering a flow of multiple batches during a longer time period, e.g. one or more years. For individual batches, collecting less samples led to a high probability of

misclassifying the batch in case the concentration was slightly above to the pre-set limit and, hence, collecting as much samples as prescribed by the EU regulations is advisable.

Chapter 6 compared the financial effects of no-monitoring with monitoring schemes for aflatoxins collecting either 1, 18 or 100 samples per imported maize batch in the Netherlands. This chapter integrated the results of Chapters 4 and 5 with the results of Chapter 2. Chapter 6 showed that the net positive financial effect was higher when monitoring for aflatoxins was performed, compared to no monitoring at all. Therefore, independent of the monitoring plan applied, monitoring all incoming maize batches was cost-effective. Collecting 18 or 100 samples per batch, aggregating all these 18 or 100 samples, and analysing one sub-sample, led to a higher positive financial effect than collecting only one sample per batch. However, the difference between collecting 18 or 100 samples was small. We can, therefore, conclude from this chapter that collecting between 18 and 100 samples per batch, which is less then prescribed in the EU regulations, is cost-effective when a flow of imported batches are considered.

The main conclusions of this thesis are:

- Undetected contaminated maize batches (false negatives) in the upstream stages of the maize supply chain can lead to large economic consequences in the downstream stages of the maize supply chain (Chapter 2).
- It is cost-effective to allocate most of the budget of the S&A plan for mycotoxins in wheat and maize to the sample collection step, rather than to the chemical analysis (Chapter 4).
- Aggregating incremental samples and analysing a sub-sample from the aggregated sample is a cost-effective method for monitoring aflatoxins in maize and deoxynivalenol in wheat (Chapter 4).
- When an entire flow of batches during one or multiple years is considered, collecting less samples than prescribed by the EU regulations is cost-effective for monitoring deoxynivalenol in wheat and aflatoxins in maize (Chapters 4, 5 and 6).
- When the aflatoxin concentration in a maize batch is 1 to 1.6 times higher than the pre-set limit of 2.5µg/kg, 10 to 100 times more samples are needed to correctly classify batches than when the batch concentrations are lower or higher than 1.6 times the pre-set limit (Chapter 5).
- For deoxynivalenol, a mycotoxin produced in the field, monitoring in the upstream stages of the wheat supply chain is cost-effective whereas for aflatoxins, mycotoxins that can be produced both in the field and during storage, monitoring in the downstream stages of the maize supply chain is cost-effective (Chapter 5).

Acknowledgements

My official PhD journey began on May 1st 2016, but having written an MSc thesis on the same topic, it felt like I was just continuing my MSc thesis. Now, three and a half years later, my PhD thesis is finalised. I would like to thank many people that made it possible to finish this thesis.

First of all, I would like to express my gratitude to my supervisor and promotor Ine van der Fels-Klerx. First of all, you introduced me to the topic of both food safety economics as well as mycotoxins and supervised me during my master thesis. During the first months of my PhD journey you helped me a lot with starting up and giving me plenty of ideas for topics I could write papers on. I also appreciate the liberty and space you gave me during the process to figure things out on my own and make my own decisions. I would also like to express my gratitude to my promotor Alfons Oude Lansink for his constructive feedback. I appreciate the way you always bring in a positive manner your feedback. Despite your busy schedule, you were always very fast in answering emails and reading my draft papers. Even on Sundays or holidays you spent time reading my work.

Aside from the people directly involved in the creation of this thesis, I would like to thank all colleagues from the chain group at WFSR for creating a pleasant working environment, especially my colleagues in room 1058, Jen and Cheng. You were always there to help me, to give me advice, and most importantly, you were always there for nice chats. Thanks to Paulien, Jeanette, Viola, Martijn, Elise, Elise, Yvette, Rosan, and all others for the pleasant lunches at Impulse and the everyday same walk back to the office.

I would like to give a special thanks to my colleague Monique de Nijs. Thank you for reviewing one of my papers, thank you for always being so positive and encouraging, especially during the conference in Beijing we attended together.

Then I would like to thank all my colleagues and especially all the PhD students at BEC. I had very pleasant Mondays with PhD meetings, many cakes, lunch walks, nice barbecues and Christmas dinners with you.

A comfortable working environmental is crucial to deliver a PhD thesis but family and friends are equally as important. I would like to thank my parents who taught me to be independent and make my own choices. You made it possible for me to study in Wageningen in the first place. I would also like to thank you for helping me each time I needed to move from one home to another, for being supportive during my PhD, and being proud.

Then I would like to thank I would like to thank my sister Laura and my brother Yoann for being my paranymphs and being by my sides during my public defence.

Finally I would like to thank my partner Albert who is trying to teach me to believe in myself. Every day you are there to support me an tell me I am able to do everything. Even if you don't exactly know what I am doing sitting behind my computer all day long or what I am doing at a conference, you were always supportive and proud.

About the Author

Marlous Focker was born on May 11th 1992 in Meyrin, Switzerland. She obtained her Bachelor of Science with specialisation Biology at the University of Geneva in 2013. In 2016 she obtained her Master of Science at Wageningen University with specialisation Food Safety and with a minor in Nutrition. Her master thesis was on the topic of cost-effective sampling and analysis for mycotoxins in cereal grains at the Business Economics group of Wageningen University. After a six-month internship at the international Regulation department of the dairy company Elvir in France, she came back to Wageningen and started her PhD at Wageningen Food Safety Research in collaboration with the Business Economics group of Wageningen University, continuing on the same topic as her master thesis. She finished her PhD thesis in 2019. As of November 2019 she will be working as a postdoc at the Business Economics group of Wageningen University.

Marlous Focker

Wageningen School of Social Sciences (WASS)
Completed Training and Supervision Plan

Name of the learning activity	Department/Institute	Year	ECTS*
A) Project related competences			
Writing PhD proposal	Wageningen Food Safety Research (WFSR) Business Economics (BEC)	2016	6
'Cost-effective sampling and analysis of a cereal batch for mycotoxins'	Mycokey, Ghent, Belgium	2017	1
'Comparison of cost-effective sampling and analysis plans for DON in a wheat batch and aflatoxins in a maize batch'	World Mycotoxin Forum, Amsterdam, the Netherlands	2018	1
'Cost-effective sampling plans'	EU-China-Mycotoxin-Forum MyToolBox stakeholder meeting & training, Beijing, China	2019	1
'Optimization of aflatoxin monitoring along the maize supply chain'	European Association of Agricultural Economists (EAAE) PhD workshop, Uppsala, Sweden	2019	1
B) General research related competences			
Introduction course	WASS	2016	1
Advanced Agricultural Business Economics, BEC 30306	WUR	2016	6
Statistical programming with R	Utrecht University	2016	1.5
Systematic approaches to reviewing literature	WASS	2016	4
Dynamic models in R	PE&RC	2017	1.8
Scientific writing	Wageningen in'to Languages	2018	1.8
Attending PhD meetings in BEC group once per 2 weeks	BEC	2016-2019	4
Chain group meetings at WFSR once a month	WFSR	2016-2019	2
C) Career related competences/personal development			
Voice training (individual coaching)	Voicematters	2017	1
Presenting with impact	Wageningen in'to Languages	2019	1
Teaching: assisting course Food Safety Management (FHM-61312)	FHM	2017 and 2018	3
Teaching: assisting course Food Safety Economics (BEC-21306)	BEC	2018 and 2019	1
Total			**38.1**

*One credit according to ECTS is on average equivalent to 28 hours of study load

The work presented in this dissertation was conducted within the framework of the MyToolBox project, a European Union's Horizon 2020 Research and Innovation Program project, under grant agreement No 678012. In addition, this thesis received funding from the Ministry of Agriculture, Nature, and Food Quality in The Netherlands.

Layout and cover: Marlous Focker

Printing: ProefschriftMaken || www.proefschriftmaken.nl

Propositions

1. Collecting additional samples increases the effectiveness of monitoring for mycotoxins in maize and wheat more than increasing the precision of the detection method.
(this thesis)

2. Undetected contaminated batches in the upstream stages of a food supply chain can lead to large economic consequences in the downstream stages of the food supply chain.
(this thesis)

3. An individual's dietary choices have an effect on the sustainability of the planet.

4. Dark energy will never be able to drive the Milky Way apart.

5. Making a simulation model is like doing a lab experiment: it is simulating reality by simplifying it.

6. The human cardiovascular system has the same function as the plumbing system in houses.

Propositions belonging to the thesis, entitled

Methodological Advances to Improve the Cost-Effectiveness of Monitoring for Mycotoxins in Cereal Grains

Marlous Focker

Wageningen, 11 December 2019