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Risk Assessment and Research Synthesis methodologies in food safety: two effective tools to provide scientific evidence into the Decision Making Process.

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RISK ASSESSMENT AND RESEARCH SYNTHESIS METHODOLOGIES IN FOOD SAFETY: TWO EFFECTIVE TOOLS TO PROVIDE SCIENTIFIC EVIDENCE INTO THE DECISION MAKING PROCESS

by

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RISK ASSESSMENT AND RESEARCH SYNTHESIS METHODOLOGIES IN FOOD SAFETY: TWO EFFECTIVE TOOLS TO PROVIDE SCIENTIFIC EVIDENCE INTO THE DECISION MAKING PROCESS

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The food supply chain is a complex and diverse system. Some food products need minimum processing to reach the consumers, while others involve several different processes, countries and suppliers, can take several months to be on the table of the end consumer. Regarding food safety, the public health of consumers is at stake and the consequences of outbreaks could prove disastrous. This has been recognized as a matter of global importance for the food industry and authorities around the world since several efforts to improve quality, safety and trade of food have arisen since the early 1960s. The birth of the Codex Alimentarius Commission, a joint organism lead by the World Health Organization and the Food and Agriculture Organization, marks a milestone, creating the first organization dedicated to proposing international food safety standards and to foster fair global food trade.

All these organizations agree that the use of solid scientific evidence in the decision making process is the cornerstone in creating a safe global food supply chain. Although there is widespread consensus about this, developing countries usually encounter heavy difficulties in accomplishing these objectives due to obstacles such as low funding to

sample their food products, a weak regulatory system, insufficient technology and scientific capabilities. Therefore, addressing the question “how can we provide tools for these countries to strengthen their capacities to create scientific evidence based regulations with the consideration of these limitations?” is in great need. In this project two case studies were used to show that risk assessment, in conjunction with the use of research synthesis methodologies, are two approaches that can be used by the food industry and governments to provide effective scientific insights into their respective decision making processes. The focus of this research project is food safety in Chile, thus the analysis, results and overall direction will be narrowed to the perspective of this developing country.

ACKNOWLEDGEMENTS

In first place, this experience would not have been possible if Dr. Bing Wang and John Rupnow have not believed in me. For that I am really grateful. It was an honor to have worked together. Thanks to my Committee Members, Dr. Jennifer Clarke, George Gray and Rolando Flores; they were wonderful mentors always eager to answer my questions and guide me throughout this project.

My family were always supporting me in every possible aspect of this adventure in the United States. Having such a loving, happy and united family has created a mark in my personality and soul, which I carry with great pride anywhere I go. Thank you, for you are the reason I am happy in life and able to reflect that on others.

Living abroad can be sometimes hard for many reasons. But I want to acknowledge that the people I met during this two years living in Lincoln have made this journey incredibly enjoyable and rewarding. The American friends I made here, John, Michaela, Michael, John Jimma, Josh, Brian, Becca, Kim and Emika, among many, gave me the best impression of this beautiful country and its people. Keep being so unique, smart and loving. I hope our paths cross again and I will call you my friends forever!

Thanks to all my coworkers, Anand, Ece, Yangyunna, Sapna and Bin. All the best in your academic and personal lives. I am sure every single one of you will turn into incredible scientists!

I can’t close this dedication without naming the most amazing group of individuals that gathered in Lincoln, Nebraska. The almighty Cabroncos! With an unquenchable thirst for music (country and heavy metal), adventures and paellas, this group were my first close

friends. All the trips together, the fun times, the culinary meetings and the scientific/philosophical conversations we had made my stay here so awesome. Carolina, Denis, Ivo, Felipe, Isabel, Michael, K, Hugo and of course, our beloved leader, Isidro Campos, thank you all for being such wonderful people and being my friends.

To everyone that somehow affected my life in a positive, which I am proud to say it has been every single person here in the U.S., thanks.

And always remember;

"Don't worry about a thing, 'cause every little thing gonna be alright"

Bob Marley

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CHAPTER 1: FOOD SAFETY, TRADE, AND THE NEED FOR SCIENCE IN POLICY

I. INTRODUCTION

A. A brief history of food safety

Food has played a pivotal role in the development of mankind, in both the nutritional and cultural dimensions. Food safety practices can be tracked to prehistoric times, starting with the Chinese that developed the first preservation methods for vegetables in 4000 BC (Uemura and Bari, 2016), which provided them means to attain higher levels of food safety. As eating patterns and foods changed and evolved over time, food safety laws started to appear (Uemura and Bari, 2016).

The first food laws can be seen in the book of Leviticus around 2000 BC and in the Quran by 570 AC (Hutt and Hutt, 1984). Although these were targeting food adulteration, as with food preservation, the population indirectly received the first benefits of food safety practices. In spite of food safety being a very old subject which almost every early civilization was addressing to some extent, it was not until the 19th century that comprehensive food legislations were adopted (Uemura and Bari, 2016). Figure 1.1 uses the United States (U.S.) as an example to show important milestones in the history related to food safety, starting from the late 1800's.

B. About this study

The purpose of this study is to demonstrate how the two systematic approaches, risk assessment and research synthesis methodologies, can be utilized by food industry and regulatory authorities to provide effective scientific insights to inform the process of

designing intervention strategies, regulations, policies or laws. In this chapter, the foundation of why we need science in the decision making process is going to be explained, from the perspective of domestic food safety protection and international trade. The whole work will revolve around how Chile – a developing country in terms of the International Monetary Fund (IMF, 2016) – can harness the potential of these two tools and include them in their decision making process for food safety matters. This will be achieved by comparing the epidemiological status, current trade situation and use of science by the two most powerful actors in food trade, the United States of America and the European Union (EU), compared to the Chilean reality. These nations were selected since they are the leaders in food safety sciences and technologies, employ more advanced regulatory frameworks and, as we will see later, are the most important trading partners to Chile. The epidemiological status was surveyed to have a broad understanding of the range of deaths and illnesses caused by food in each nation. The integration of science into the decision making process is something that in developing countries is hard to achieve. Thus, it is important to have a look in developed countries and understand how they are achieving this. Finally, a description is given of the tools that this thesis is proposing should be used in order to achieve the food safety protection objective.

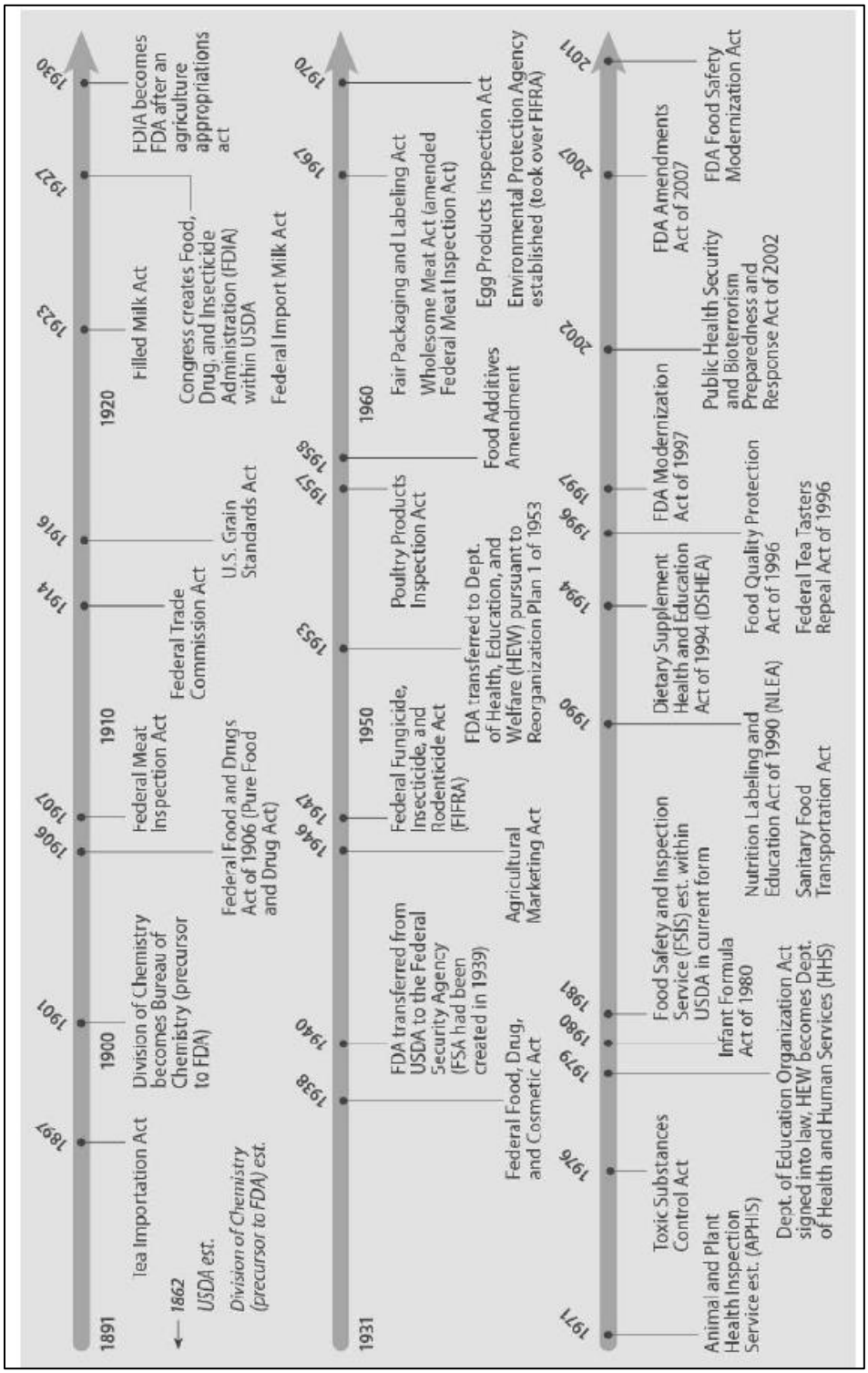

Figure 1.1 Important milestones in food safety in the United States (adopted from Reneé Johnson, 2014)

II. FOOD SAFETY – SELECTED NATIONS COMPARISON

A. United States

i. Economics and food trade

According to 2016 estimates by the IMF, the United States (US) Gross Domestic Product (GDP) is US$ 18,562 billion, making the U.S. the second largest economy in the world after China. Its GDP per capita is US$ 56,084 ranking 11 worldwide (IMF, 2016).

The United States Department of Agriculture (USDA) has predicted for 2017 an export forecast for agricultural trade of US$ 133.0 billion and imports of US$ 113.5 billion, worth 1.327% of the total GDP. As shown in Figure 1.2 and Figure 1.3, a considerable portion of trade corresponds to human food.

The total local retail and food services sales for 2015 were US$ 1,511 billion, which is worth 8.14% of the total GDP. (Economic Research Service, 2016)

ii. Food safety epidemiology situation

The Center for Disease Control and Prevention (CDC) has estimated that yearly, 48 million people get sick, 128,000 are hospitalized and 3,000 die due to foodborne illnesses (CDC, 2016). However, these numbers are underestimated due to the surveillance methods used, under-diagnosis because of variations in medical care seeking, specimen submission, laboratory testing and sensitivity (CDC, 2016).

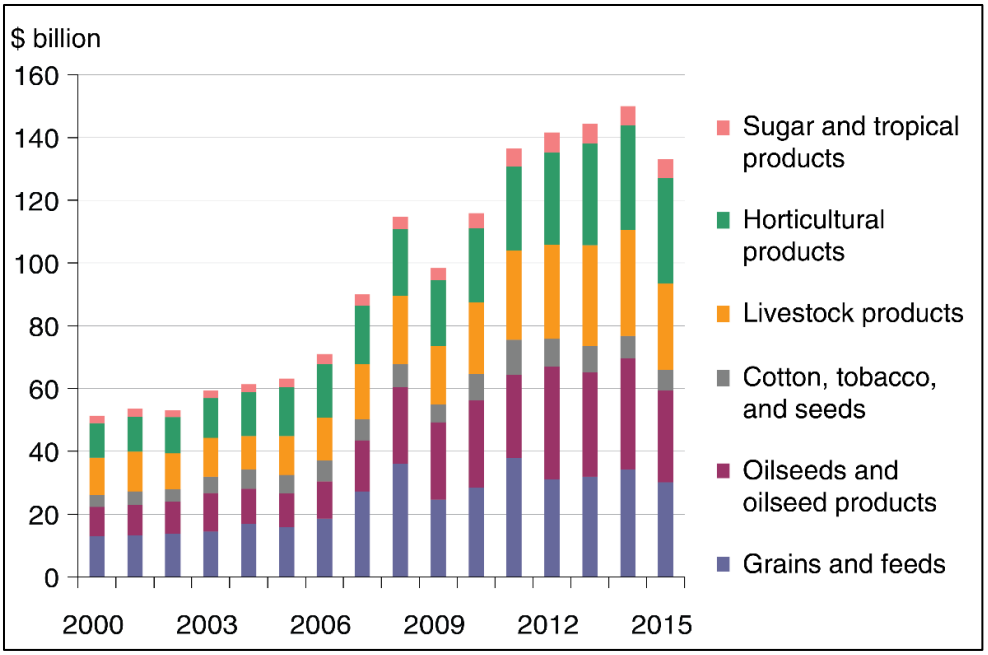

Figure 1.2 The U.S. agricultural exports evolution from 2000-2015. Data retrieved from the Economic Research Service.

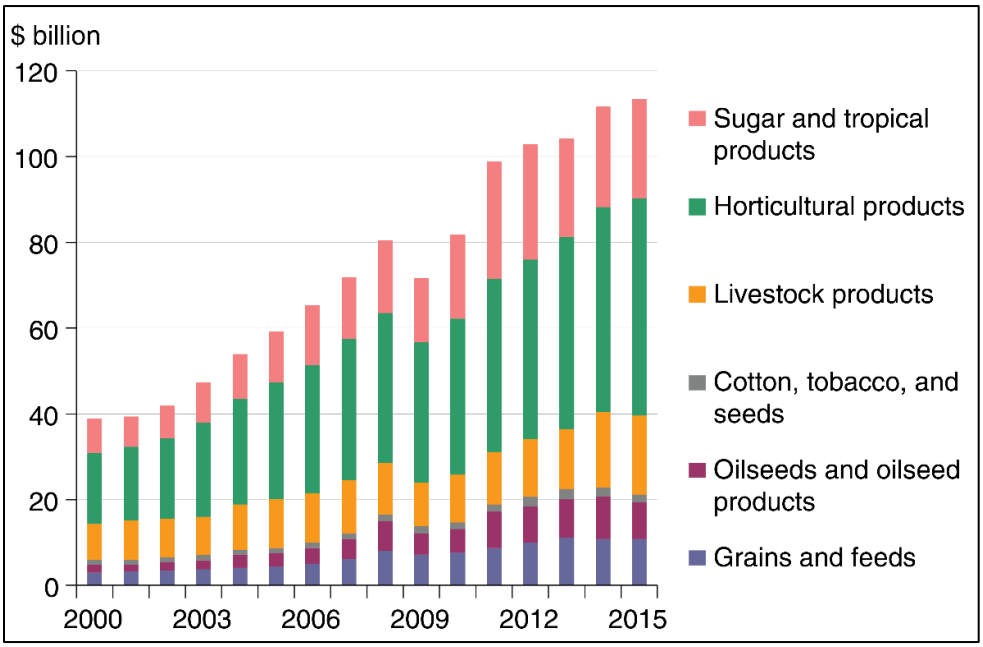

Figure 1.3. The U.S. agricultural imports evolution from 2000-2015. Data retrieved from the Economic Research Service.

iii. Regulatory framework

Food safety responsibilities are divided among several different agencies in the U.S. The USDA and Food and Drug Administration (FDA) have direct enforcing and regulation power over different sets of foods, while the CDC is the supporting agency that collects data on foodborne illnesses and supports foodborne disease surveillance and response (Foodsafety.gov, 2016)

Meat, poultry and egg products are under the jurisdiction of USDA, through its Food Safety and Inspection Service (FSIS). Any other types of food are regulated by FDA.

B. European Union

i. Economics and trade

The European Union is a political and economic union of 28 member states. If treated as a single country, according to the IMF for 2016, its GDP is US$ 16.673 billion, ranking the third largest economy of the world.

According to the Agricultural and Rural Development Department of the European Commission, for 2015, the agri-food exports ascended to US$ 129 billion, while imports were worth US$ 113 billion. This is equal to 1.45% of the total GDP. As shown in Figure 1.4, an important part of the exports and imports correspond to human food.

ii. Food safety epidemiology situation

The World Health Organization has estimated that the number of foodborne illnesses in the EU is approximately 2,431 cases per 100,000 persons and the number of deaths is 0.4 in every 100,000 (WHO, 2010). Taking into consideration the EU population of about

508 million (European Union, 2016), the cases calculated are lower in foodborne adverse outcomes compared to the U.S.: 12 million illnesses and 2,000 deaths.

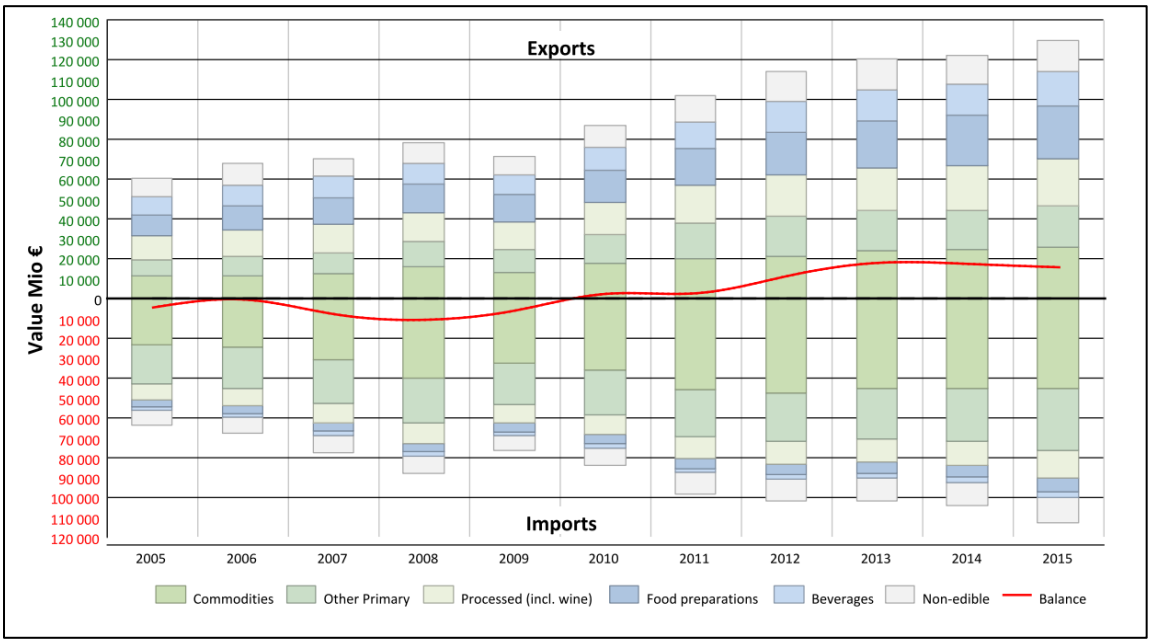

Figure 1.4. Evolution of agri-food related imports and exports in the EU. It is important to note that the "commodities" class includes live livestock and some other non-edible items. (Agricultural and Rural Development, 2016)

iii. Regulatory framework

Each member state is allowed to have its own food safety agencies, research and outreach efforts. There is, nonetheless, a general guideline called "The General Food Law". Under Regulation (EC) No 178/2002, the General Food Law is defined as "the foundation of food and feed law. It sets outs an overarching and coherent framework for the development of food and feed legislation both at Union and national levels. To this end, it lays down general principles, requirements and procedures that underpin decision making in matters of food and feed safety, covering all stages of food and feed production and distribution." (European Commission, 2016)

This regulation also creates the European Food Safety Authority (EFSA), which is an independent agency that provides scientific advice and support to member states regarding food safety and public health matters. It is important to highlight that EFSA does not enforce food safety, which is still a responsibility of each member state.

C. Chile

i. Economics and trade

According to the IMF, for 2016 the GDP for Chile is of US$ 422,422 billion. With a population of about 18 million, the GDP per capita is about US$ 23,507.

Table 1.1 shows the main economic activities of Chile and its corresponding share of the GDP. With a total of US$ 5.749 million, Agriculture and forestry exports make 6.43% of the exports. In particular, US$ 4.738 million correspond to fruit exports and the rest to other agri-food related items (Chilean Central Bank data for 2011). Figures 1.5, 1.6 and 1.7 shows worldwide trade, to the U.S., and to the EU, respectively.

ii. Food safety epidemiology situation

The latest epidemiology report from the Health Ministry in Chile indicated that in 2015, there were 5,901 diagnosed foodborne illnesses and 119 hospitalizations (Chilean Ministry of Health, 2015). The number of deaths attributable to foodborne illnesses is not available. The estimated number of illnesses is also not available.

Table 1.1. Largest economic activities and its contribution to the GDP in Chile for 2011. Agriculture is showed as it is the class that includes agri-food related items.

Economic Activity	Percentage of the GDP
Mining	15.2%
Business Services	13%
Manufacturing industry	10.9%
Personal services (health, education, others)	10.6%
Retail	7.9%
Agriculture and forestry	2.8%
Remaining activities	39.6%

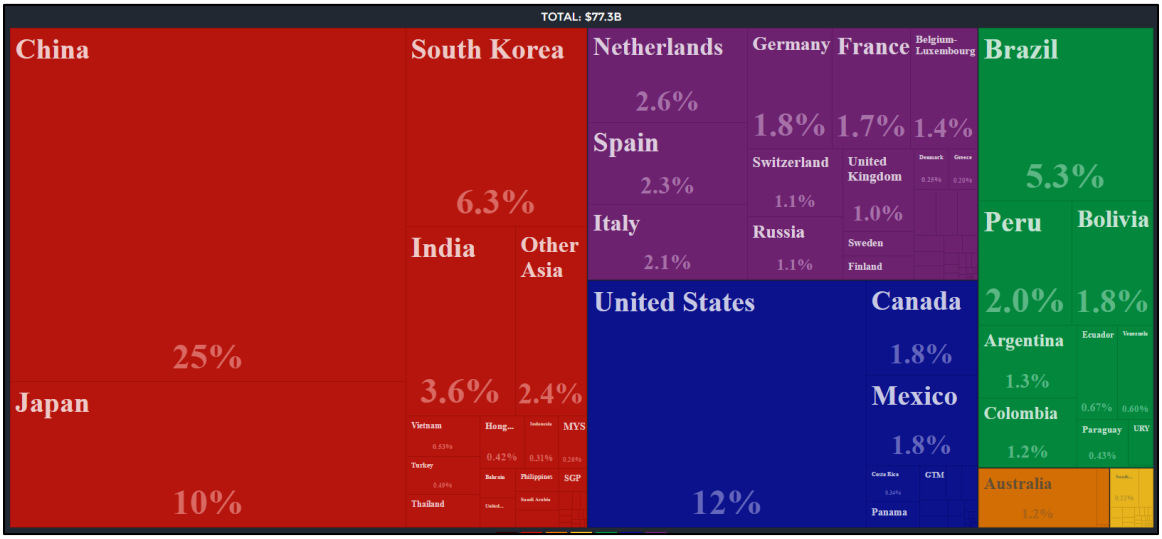

Figure 1.5. Interactive graph showing Chile's largest trading partners in 2014, in terms of exports. Data taken from the Observatory of Economic Complexity from the Massachusetts Institute of Technology.

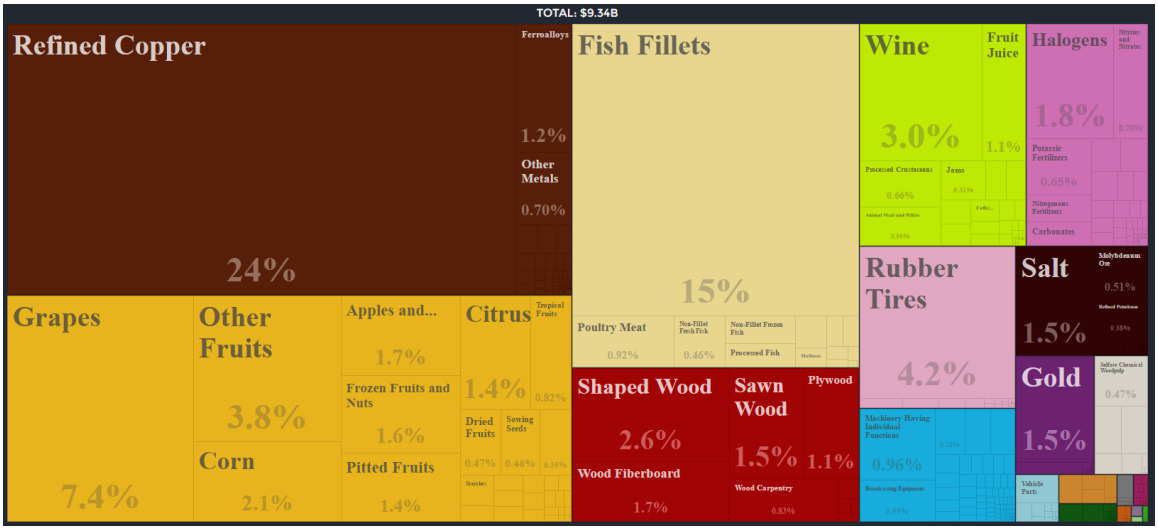

Figure 1.6. Interactive graph showing Chile's exports to the US in 2014. Data taken from the Observatory of Economic Complexity from the Massachusetts Institute of Technology.

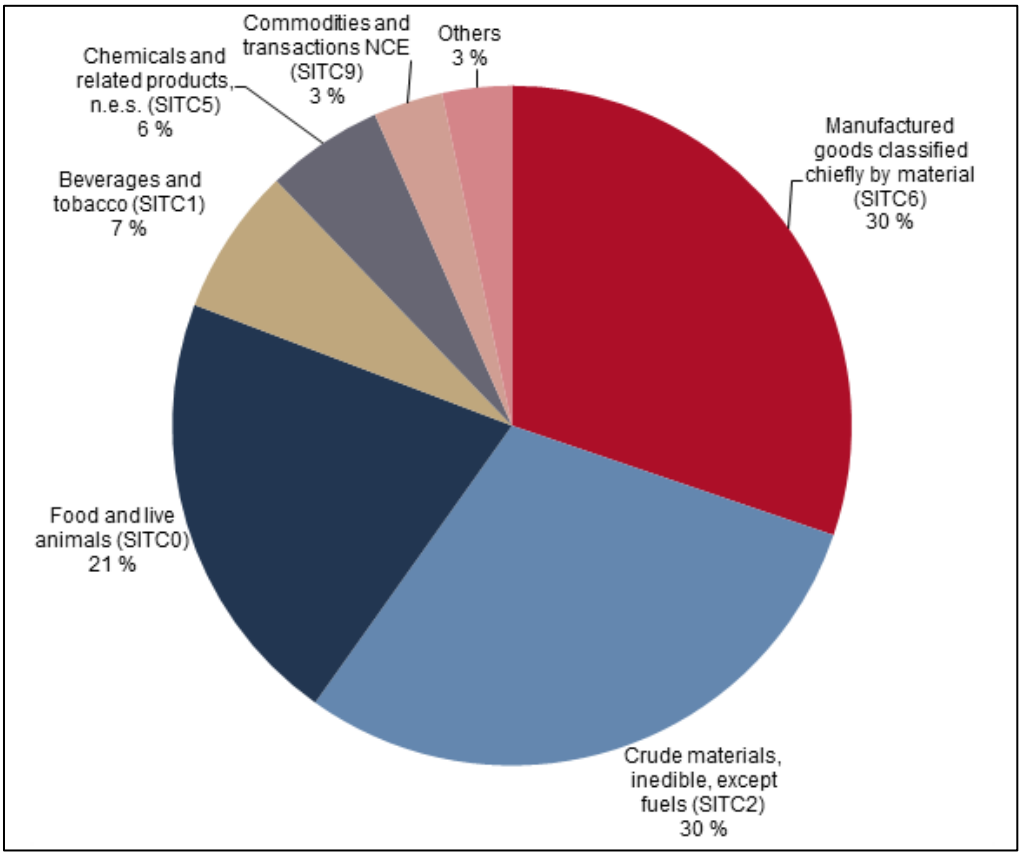

Figure 1.7. Imports of the EU from Chile in 2014. Data taken from Eurostat webpage.

Proposal of an estimated number of illnesses in Chile, based on diagnosed cases.

As a foodborne illness estimate is missing in Chile's statistics, data from CDC was extracted and adjusted to Chile's numbers with the purpose of doing a comparison. Table 1.2 shows the comparisons between the nations of interest based on the population.

Table 1.2. Foodborne illnesses comparison chart from selected countries.

Country	Total population	Yearly estimated illnesses (% of total population)	Yearly estimated deaths (% of total population)
United States	324,099,593[a]	48,000,000 (14%)	3,000 (0.0009%)
European Union	510,056,011[b]	12,000,000 (2.35%)	2,000 (0.0004%)
Chile	18,006,407[c]	1,513,800 (11.89%)[d]	No data

a. United States Census Bureau. Retrieved on October 13, 2016

b. Eurostat – Population on 1 January 2016". European Commission. Retrieved on October 13, 2016.

c. Chilean National Statistics Institute. Retrieved on October 13, 2016.

d. Derived in this study based on CDC's adjustment factor.

The latest CDC report on foodborne illnesses indicated that the number of diagnosed foodborne illnesses for 2006 was 142,481. Scallan et al 2011 proposed an estimate of 37,220,098 foodborne illness cases, based on the number of diagnosed cases. Therefore, with the latest technology and science available, there is a 261.2 factor difference between the foodborne illnesses estimate and the number of actual diagnosed cases. This factor was used to estimate Chilean foodborne illnesses based on the number of diagnosed cases. Caution should be used when using this number as there are several differences in laboratory technology, scientific capacities, pathogen prevalence and

dietary differences between the U.S. and Chile that makes this estimate only valid when looking at the data from a general perspective.

iii. Regulatory framework

The only agency that enforces food safety in Chile is the Ministry of Health, through the SEREMIS (Regional Health Secretariat), which are regional independent secretariats with full legal power. Nonetheless, other agencies have compliance authority - but they cannot recall a food product. Figure 1.8 indicates the organization of this multi-sectorial management of food safety in Chile.

The Chilean Food Safety and Quality Agency (ACHIPIA) is a scientific advice and support agency, created with the model of EFSA in mind. The main difference is that ACHIPIA gives integral scientific support to the three agencies involved in food safety: the Service for Livestock and Agriculture (SAG) and the National Service of Fisheries (SERNAPESCA) and SEREMIS, instead of to member states.

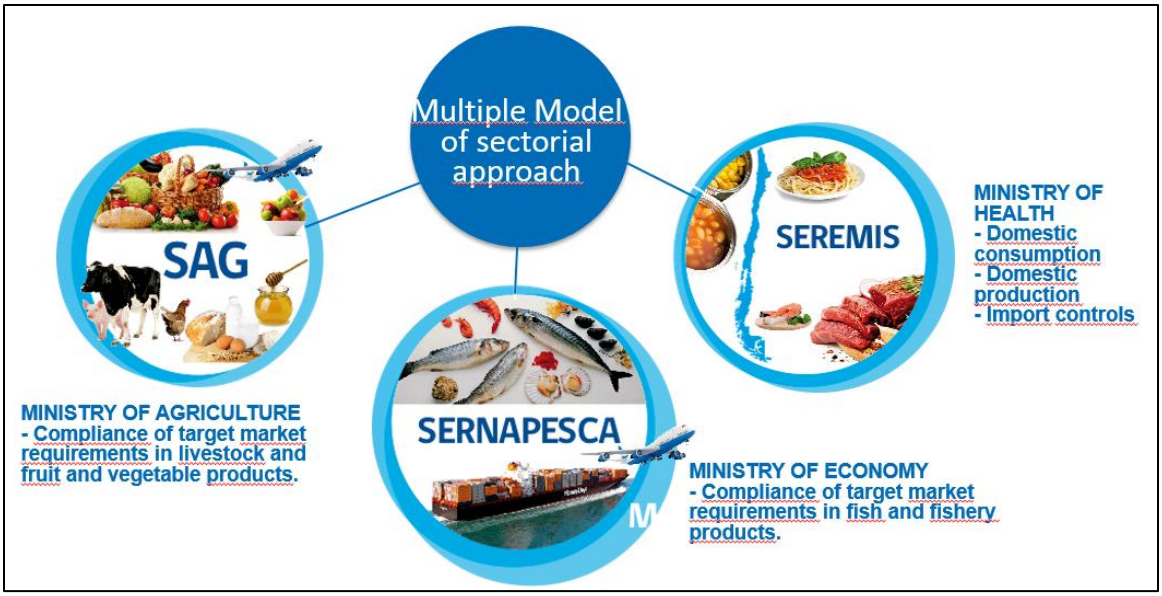

Figure 1.8. Diagram of the food safety management system in Chile. Courtesy of the Chilean Agency for Food Safety and Quality.

D. International Organizations

There are some international organizations that are worth mentioning mainly because of their significant impact on the development of standardized food safety standards, epidemiologic data generation, education and scientific integration into regulatory issues.

i. WHO and FAO

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) are both entities from the United Nations. Although their missions are different, they share a common goal in terms of food safety. That is the reason why, even though the WHO and the FAO have their own food safety capacity building, outreach and support teams, they co-manage the Codex Alimentarius Commission (CAC), which is a food standards creation program. In the CAC sessions, all member nations participate and

scientific evidence is taken with high regard, to promote fair international trade and safe food.

ii. ILSI

The International Life Sciences Institute (ILSI) is a nonprofit scientific organization whose mission is: “to provide science that improves human health and well-being and safeguards the environment” (ILSI, 2016). ILSI advocates for better and transparent scientific advice in topics such as food and environment. It has stable funding sources, which are mainly agri-food related industries.

E. Conclusion

This section introduced the three actors in this Chapter from a trade, food safety and regulatory perspective. The US and the EU are the most important trade partners along with China for Chilean agri-food exports. It is essential to understand how they manage their food safety issues and what their current epidemiological situation is.

“As previously noted, not everything is run by the government. Instead, key international actors such as the FAO, WHO, and ILSI contribute to the harmonization of food safety standards, placing great efforts on ensuring a safe food supply while simultaneously promoting fair global food trade.

This section is fundamental to understand the key players in food safety around the world and to understand the structure of this thesis. The next section explains how these recently introduced countries and organization take into consideration the scientific support in their decision making process and regulation design.

III. SCIENCE INTO THE DECISION MAKING PROCESS: A GLOBAL REVIEW

A. United States

i. National Academy of Sciences

The National Academy of Sciences (NAS) is a private, non-profit society, founded in 1863 with its mission of "providing independent, objective advice to the nation on matters related to science and technology" (NAS, 2016). Any governmental departments can call upon the NAS for scientific advice. More than 6,000 experts have served in different policy studies and reports, on matters of critical importance to the society.

The NAS is constantly collaborating with the Government in order to provide the best independent scientific advice that would ultimately be used in the design of public policies. An example of such is the request of the US Congress on November 22, 2015 to the NAS to create a Forensic Sciences Committee, with the objectives of, among others: (National Criminal Justice Reference Service, 2015)

(1) Assess the present and future resource needs of the forensic science community, to include State and local crime labs, medical examiners, and coroners;

(2) Make recommendations for maximizing the use of forensic technologies and techniques to solve crimes, investigate deaths, and protect the public;

(3) Make recommendations for programs that will increase the number of qualified forensic scientists and medical examiners available to work in public crime laboratories;

This kind of collaborations explains how important the link is with the scientists in the U.S. and how evidence is taken strongly into account when dealing with public policies.

ii. FDA

The FDA's mission is to: "protect and advance public health by helping to speed innovations that provide our nation with safe and effective medical products and that keep our food safe. The Agency achieves this by applying the latest technology and science-based standards to the regulatory challenges presented by drugs, biologics (vaccines, blood products, cell and gene therapy products, and tissues), medical devices, food additives, and, since 2009, tobacco." (FDA, 2016)

Science is fundamental in the creation of regulations for the FDA, as there is recognition that science-based standards are essential to providing effective public health. There are several examples on how the FDA does that, but in the food safety area, the most important is the Food Safety Modernization Act (FSMA). The main objective of FSMA is to shift the food production system from being reactive to being preventative, with a risk-focus.

FSMA was born from several scientific risk assessments of the potential contamination routes and recent foodborne outbreaks. For example, Section 105 of FSMA, which contains the rule "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption" was initially created after the findings of the ''Draft Qualitative Assessment of Risk to Public Health from On-Farm Contamination of Produce". (FDA, 2016)

iii. Joint Organisms and Homeland Security Centers of Excellence

The need to create better science and to extend the scientific knowledge to the public is taken in high regard by the US agencies. For food safety issues, it is of paramount

importance to leverage the resources given by Academia and to create synergies using Government-Academia alliances.

One of the successful experiences is the FDA's Joint Institute for Food Safety and Applied Nutrition (JIFSAN), which is a collaborative project with the University of Maryland. Its mission is to "be a premier source of scientific information and education programs on food safety and applied nutrition that enables the development of sound public health policy and reduces the incidence of food-related illness." (JIFSAN, 2016). Established in 1996, one of its numerous achievement is to have delivered in-country international training programs over 70 times in 24 different countries. These training programs range from Good Agricultural Practices to seafood HACCP trainings. (JIFSAN, 2016)

The second successful collaborative program worth mentioning is the Homeland Security Centers of Excellence. The "DHS S&T Centers of Excellence (COEs) develop multidisciplinary, customer-driven, homeland security science and technology solutions and help train the next generation of homeland security experts." (DHS, 2016). There are eight centers for excellence that focus on protecting the US from external and internal attacks on any critical supply chain or infrastructure. Regarding food safety, the "Food Protection and Defense Institute (FPDI), led by the University of Minnesota, defends the safety and security of the food system by conducting research to protect vulnerabilities in the food supply chain". (DHS, 2016)

B. European Union

i. EFSA

The work of EFSA is mainly focused on answering member states, European Commission and Parliament. The scientific advice comes from the Scientific Panels and Scientific Committee, organisms that adhere to several working principles such as transparency, cooperation and independence. There is a structured process on how EFSA conducts science and a quality assurance system that "continually monitors and strengthens the quality of EFSA's scientific work" (EFSA, 2016).

Among the myriad number of activities that EFSA conducts, there is a multi-annual project called: "Promoting Methods for Evidence Use in Science" that defines principles, processes and methods for the use of evidence in scientific assessment. (EFSA, 2015).

Moreover, the project: "Application of systematic review methodology to food and feed safety assessments to support decision making" was performed in 2010. (EFSA, 2010). These kinds of activities indicate the high regard which the EU holds for scientific evidence in the decision making process.

C. Chile

1. ACHIPIA: Scientist Network

ACHIPIA has set the Risk Analysis Process as the prime resource to integrate science into its advisory responsibilities. The Scientist Network has become one of the main sources for local data and expert elicitations.

The Food Safety Scientist Network was created in 2014 to establish an effective link between ACHIPIA and the scientific community. Its activities range from local data

collection, expert elicitation panels and an Advisory Scientific Committee that manages all the collaboration between the agency and the scientific community and sets the priorities for the Network. (ACHIPIA, 2016)

During 2016, five expert elicitations have been conducted and more than ten Scientific Opinions had been submitted to international fora such as the Codex Alimentarius Commission and EFSA.

D. International Organizations

i. WHO, FAO and the Codex Alimentarius Commission

The World Health Organization and the Food and Agriculture Organization were pioneers in integrating science into their decision making process through the Joint FAO/WHO Expert Committees. These committees provide independent scientific advice upon request to WHO and FAO. The oldest is the JECFA, which stands for Joint FAO/WHO Expert Committee on Food Additives and was founded in 1956 (WHO, 2016). There are two other committees, the JEMRA - Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment – and the JMPR - Joint FAO/WHO Meetings on Pesticide Residues – that are currently working and collaborating with the FAO and WHO. Later, in 1963, when the Codex Alimentarius Commission (CAC) was established, these committees found an improved meaning and mission, turning into the prime resource of scientific advice and priority setting for the CAC.

With the creation of the World Trade Organization in 1995, the major multilateral food agreement was signed: the Sanitary and Phytosanitary (SPS) agreement, which: “sets out the basic rules for food safety and animal and plant health standards.” (WTO, 2016)

For food safety, the key success of these negotiations was the acknowledgment of the Codex Alimentarius Commission as the definitive resource of scientific information for food international standard setting and the harmonization of food laws. Specifically: "Harmonization with international food safety standards means basing national requirements on the standards developed by the FAO/WHO Joint Codex Alimentarius Commission. Codex standards are not "lowest common denominator" standards. They are based on the input of leading scientists in the field and national experts on food safety." (WTO, 2016)

IV. TECHNOLOGIES AND TOOLS IN FOOD SAFETY

A. Food Safety Risk Assessment

Risk Assessment is the "scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, iii) exposure assessment, and (iv) risk characterization". (Codex Alimentarius Procedural Manual, 24th edition, 2016). The World Health Organization defines it more specifically as "the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards" (WHO, 2016). It is embedded in a broad food safety framework called risk analysis, which is the "process consisting of three components: risk assessment, risk management and risk communication". (Codex Alimentarius Procedural Manual, 24th edition, 2016). Risk Analysis is the modern focus that Governments are undertaking to manage Food Safety issues.

The first mentions of risk assessments on public health in the scientific literature start around the late 1960's. It is not until 1983 that the National Research Council (NRC), by

request of the United States Congress, wrote the book: “Risk Assessment in the Federal Government: Managing the Process”. This book contains the first guidelines and scientific opinions on how to use risk assessment and its related tools to “strengthen the reliability and objectivity of scientific assessment that forms the basis for federal regulatory policies applicable to carcinogens and other public health hazards”. (Risk Assessment in the Federal Government: Managing the Process, NRC, 1983). This book is the cornerstone of all the subsequent work on how scientific advice can be useful to the Regulatory Agencies, with the objective of creating science-based regulations and guidelines.

The WTO recognizes the value of Risk Assessment and considers it nowadays as an essential source of evidence for managing food safety issues, not only at a national level but international as well. The SPS agreement, for example, ensures that all international standards are science based, which is reflected in the first paragraph of Article 5 of the SPS agreement text: “Members shall ensure that their sanitary or phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risks to human, animal or plant life or health, taking into account risk assessment techniques developed by the relevant international organizations.”

B. Research Synthesis Methodologies

i. Literature Review

Harvard University describes a literature review as an: “assessment of a body of research that addresses a research question” Its purpose is to “1) Identify questions a body of research does not answer, and 2) Make a case for why further study of research questions is important to a field”. (Harvard Graduate School of Education, 2016)

The Cochrane Collaboration explains that literature reviews are usually characterized by the use of informal, unsystematic and subjective methods to collect and interpret information. Thus, they are subject to the author’s bias, statistically, incomplete or incorrect analysis and potentially inconsistent conclusions that may suit the author’s experiences or overall direction of the review.

ii. Systematic Review

The Systematic Review, on the other hand, is a literature review that collects and critically appraises several different research studies or papers, following a pre-specified procedure and criteria. The Cochrane Collaboration defines it as: “a high-level overview of primary research on a particular research question that tries to identify, select, synthesize and appraise all high quality research evidence relevant to that question in order to answer it”.

Figure 1.9 shows the main differences between literature review and systematic review.

There are a number of successful experiences of Systematic Reviews informing the decision making process, most of them in the Health Care management area (Lavis et al, 2015, Mays et al, 2005 and Keown et al, 2008).

	Systematic Review	Literature Review
Definition	High-level overview of primary research on a focused question that identifies, selects, synthesizes, and appraises all high quality research evidence relevant to that question.	Qualitatively summarizes evidence on a topic using informal or subjective methods to collect and interpret studies.
Goals	Answer a focused clinical question Eliminate bias	Provide summary or overview of topic
Question	Clearly defined and answerable clinical question Recommend using PICO as a guide	Can be a general topic or a specific question
Components	Pre-specified eligibility criteria Systematic search strategy Assessment of the validity of findings Interpretation and presentation of results Reference list	Introduction Methods Discussion Conclusion Reference list
Number of Authors	Three or more	One or more
Timeline	Months to years Average eighteen months	Weeks to months
Requirements	Thorough knowledge of topic Perform searches of all relevant databases Statistical analysis resources (for meta-analysis)	Understanding of topic Perform searches of one or more databases
Value	Connects practicing clinicians to high quality evidence Supports evidence-based practice	Provides summary of literature on a topic

Figure 1.9. Comparison chart between Systematic Review and Literature Reviews. Adopted from Lynn Kish, MLIS. University of Southern California.

iii. Meta-Analysis

The purpose of a meta-analysis is to provide an estimate of an effect or observation across two or more studies. George Washington University defines it as: “A subset of systematic reviews; a method for systematically combining pertinent qualitative and quantitative study data from several selected studies to develop a single conclusion that has greater statistical power” (Himmelfarb Health Sciences Library, 2016)

Usually meta-analyses are conducted within the Systematic Review framework. It is a widely used tool in epidemiology but it has been lately used very frequently in the agri-food public health sector. (Sargeant et al, 2006).

iv. Others

Young and colleagues (2013), defined other two research synthesis methodologies: 1) The scoping reviews and 2) Structured rapid reviews. Scoping reviews are usually performed to summarize the state of knowledge in a certain area, to identify data gaps and to prioritize questions in a systematic review (Young et al, 2013). They are usually policy-driven so they are aimed to answer specific questions. On the other hand, structured rapid reviews are short, accelerated systematic reviews aiming to quickly inform decision-making officers for policy and practice (Gannan et al, 2010)

V. CONCLUSIONS

During this chapter, the current economical and food safety and science situation was described for the United States, European Union and Chile. These concepts set the foundation to understand why it is important to develop tools and resources for developing countries such as Chile, when evidence-based policies are needed.

Systematic Review and Risk Assessment are two tools widely used in the agri-food public-health sector. The outputs are several and they can be used for many purposes. Throughout this thesis, it will be shown that these two processes can be effectively conducted by developing countries and that the outputs are easily interpretable and ready to be integrated as a source of valuable information for decision makers or politicians.

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CHAPTER 2: RISK ASSESSMENT COLLABORATION PROJECT

I. ABSTRACT

Risk Assessment is a widely used tool for many fields. It is especially important for food safety as it has been recognized by numerous governments and international organizations as the main scientific evidence provider to the risk managers or decision making bodies. Risk Assessment has reached an unprecedented relevance for food trade, as the World Trade Organization recognizes it as the main dispute resolution system when two nations differ in the setting of a certain food safety standard. Thus, it is very important for all nations to be able to conduct Risk Assessments and create regulations and policies that are based on these results. It is, however, complicated for developing nations to achieve this. A number of factors such as a fragmented regulatory system and insufficient scientific capabilities and technology, among others, make this process hard to perform. In this project, we demonstrate that collaborations between the Academia and Government are essential to narrow these gaps. Specifically, the Chilean Food Quality and Safety Agency (ACHIPIA) engaged in a collaborative project with the University of Nebraska-Lincoln to assess the risk on the production of raspberries destined to export to the United States. The results indicate that the most important factors contributing to the bacterial and viral concentration are the water used for pesticide applications and that a considerable effort must be done to improve the data quantity and quality. This Risk Assessment project provides simple and straightforward recommendations to the Chilean policy makers to effectively focus their financial and human resources to solve issues that are significantly affecting the contamination of raspberries. This collaboration was a pilot experience and a number of lessons were learned during the process, such as the need to

improve the Food Safety Scientist Network from ACHIPIA and to further bolster Government-Academia alliances, since they are very effective in narrowing the gap between science and policy.

II. INTRODUCTION

One of the main roles of the Chilean Food Quality and Safety Agency, ACHIPIA, is to support the incorporation of a risk analysis framework in the context of a National Food Quality and Safety System (SINCA). ACHIPIA is currently undergoing a design phase of the structure and operation of a risk analysis process in its internal procedures. To achieve this, it has been coordinating the development of several pilot programs in collaboration with food safety scientists throughout the world (ACHIPIA, 2016). The long-term goal is to build the capacity to implement a risk analysis framework to provide evidence-based decisions in the agri-food sector in Chile. The results of these studies will provide essential and new scientific information to the public services to advance SINCA and enforce food safety for both domestic consumption and international trade.

To achieve its goal, ACHIPIA signed a cooperation agreement with the University of Nebraska-Lincoln, Department of Food Science and Technology (UNL-FDST), with the specific objective to support and strengthen ACHIPIA’s capacities to conduct research projects in a variety of issues related to food quality and safety, especially within the food safety risk analysis scope.

Risk Assessment Collaborative Project

Risk Assessment is one of the three components of the Risk Analysis process. The other two are Risk Management and Risk Communication. It is the main tool that provides scientific evidence to the Risk Managers.

The first activity under this cooperation agreement was to conduct a risk assessment project of the Raspberries Official Control Program (ROCP), which is enforced by the Livestock and Agriculture Service of Chile (SAG). SAG, through ACHIPIA, reached out to UNL-FDST to advance the current ROCP through a risk-based project for the raspberry safety protection. Three parties, including SAG, ACHIPIA and UNL-FDST, were involved in this collaborative risk assessment project, with the agreement that the research group at UNL-FDST will conduct the specific risk assessment project under the risk management objectives discussed among the three parties, based on the information shared by SAG. The results of this assessment will be taken by ACHIPIA and SAG to evaluate and improve the effectiveness of the ROCP.

Raspberries Official Control Program (ROCP)

The ROCP was designed to verify the fitness for human consumption and complete traceability of the raspberries produced in Chile destined for export to the United States of America, by establishing the auditable requirements to guarantee the safety of the raspberries.

Two outbreaks related to raspberries set the first alarm to Chile’s producers, though they had been systematically increasing their exports. The first one was the detection of

Cyclospora on raspberries from Guatemala in 1995 (Ho et al, 2002) and later a Calicivirus outbreak in Canada in 1997 (Berger, 2016). Though the two outbreaks were not linked to Chilean raspberry exports, in consultation with different stakeholders Resolution N°3410 was enacted in 2002 by the Chilean Ministry of Agriculture, which created the ROCP. The ROCP was designed under a public consultation meeting, where many different stakeholders had the chance to comment and work together with government agencies. The ROCP has two main objectives: 1) verify the traceability of the raspberries and 2) guarantee the safety for human consumption. These two objectives are accomplished using on-site audits of the participants of the ROCP. The ROCP covers participants in the administrative regions VI-X (Figure 2.1), which are located in the mid-south part of Chile and covers the majority of raspberry producers in the country.

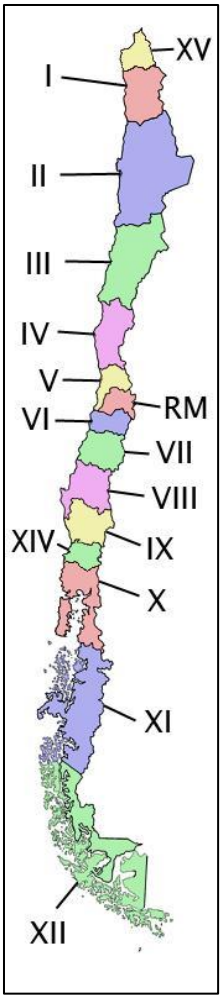

Figure 2.1. Political map of Chile showing the region numbers. Taken from Icarito encyclopedia.

Most of the participants of ROCP are small family oriented farmers. Every owner of a raspberry farm who wishes to export their raspberries has to be accredited by SAG, otherwise their exports will be halted by Chile's custom before leaving the country. This accreditation consists in the completion of a small, farmer-tailored Good Agricultural Practices Program (GAP). This limited GAP focuses on the most common issues for small farmers, such as water quality, hygiene measures for harvesters and animal controls on the farm. (SAG auditor Manual, 2008). With the compliance of the GAP program, the

farmers will be accredited and automatically included in the registry held by SAG, which enables the export of their raspberries. The accreditation is active for one year and is required to be renewed annually to stay in the registry.

Need for risk assessment in ROCP

Though ROCP has been running almost for 15 years, there is limited knowledge about the real hazards and risk factors, since these were not formally evaluated, based on the information collected through the auditing program conducted by SAG (SAG's personal indication). Consequently, there is no chance to propose improvements to the program or to the raspberry production process.

Risk Assessment is a tool that allows this kind of evaluation and furthermore, the progression to a risk-based program where they can propose improvements in controlling hazards that are significantly affecting the contamination. This will allow the SAG to better allocate their human and financial resources as well as to improve the exports amounts and raspberry safety.

Specific Objectives of the Risk Assessment project (Project Profile between ACHIPIA and UNL, 2015)

1) Assess the risks of *E. coli* and Hepatitis A in the frozen and fresh raspberry production chain;
2) Identify risk-based interventions to control microbial contamination in raspberry end products;
3) Develop a collaborative model between academia and a regulatory agency for food safety protection.

III. MATERIALS AND METHODS

The project started from the development of a project profile, which consisted of the problem formulation, project scope and outline, and the role and responsibilities of involved parties (Project Profile, 2015) in detail. Briefly, a list with all the activities, expected outcomes and responsibilities is shown in Table 2.1. The list was agreed by all parties serving as the roadmap of this project.

Table 2.1. Activity list agreed upon the project profile.

Expected result		***Activity***		***Responsible***
1. Description of the production, storage and packing stages of the frozen raspberries process.	i.	Visit to a farm and packaging facility.	i.	SAG-ACHIPIA
	ii.	Create a process flow using the information of the visit.	ii.	ACHIPIA
	iii.	Complement the process flow with the activities done by SAG under the ROCP program.	iii.	ACHIPIA
2. Microbiological risk assessment of the process.	i.	Detailed description of the current actions taken by SAG in the ROCP.	i.	SAG
	ii.	ROCP results evaluation with current available information.	ii.	SAG
	iii.	Collect the data generated by ROCP during the last and current season.	iii.	SAG-ACHIPIA
	iv.	Identification and prioritization of hazards.	iv.	SAG-ACHIPIA
	v.	Data analysis regarding ROCP management and water quality tests.	v.	UNL
	vi.	Define the risk assessment model to be used and the information needed.	vi.	UNL
	vii.	Expert identification for expert panel/elicitation.	vii.	ACHIPIA SAG
	viii.	Mitigation measures identification.	viii.	UNL
	ix.	Development of the risk assessment.	ix.	UNL
	x.	Preliminary report of the risk assessment.	x.	ACHIPIA-SAG
	xi.	Comments session on the preliminary report.	xi.	UNL
	xii.	Final report of the risk assessment.	xii.	UNL
	xiii.	Translation of the final report.	xiii.	ACHIPIA
	xiv.	Proposal of scientific publications.	xiv.	SAG-ACHIPIA-UNL
	xv.	Validation of the publications.		
	xvi.	Workshop		

The following section summarizes the process of conducting the raspberry risk assessment project, including the main steps as follows:

1. Create and administer on-farm, collection center and packaging practices survey;
2. Build raspberry supply chain flow chart;
3. Collect data from literature and survey;
4. Fill data gaps with expert elicitation;
5. Build quantitative risk assessment model;
6. Run Monte Carlo simulation;
7. Scenario analysis; and
8. Result inference.

III.1) On-farm, collection center and packaging practices survey

A non-scheduled data collection activity additional to the activities planned in the project profile was conducted in early 2016 (February-March), which is usually the time for raspberry harvest and SAG audits conducted more intensively. During December 2015, before the harvest season of 2016, the UNL-FDST group provided a list of data needed for the development of the quantitative risk assessment model, and drafted three surveys in English to collect data regarding the practices on the farm, at collection center and packing plants. The draft surveys were discussed and finalized between UNL-FDST and ACHIPIA experts, translated into Spanish by ACHIPIA and distributed by SAG to the raspberry farmers registered in ROPC. The objectives of the surveys were to 1) obtain a real picture of the current practices of raspberry supply in Chile, 2) collect data that can be incorporated in the quantitative risk assessment to simulate how the practices can

influence the introduction and transmission of the microbial loads towards the end products. Therefore, these surveys provided the fundamental to narrow the data gap and significant insights on the process from a local perspective. The surveys are provided in English in Annex I, II, and III focusing on practices on farm, at collection center and packing plant, respectively.

III.2) Build raspberry supply chain flow chart

A three step module process was established based on the preliminary data: Farm, Collection Center and Packing Plant (shown in Figure 2.2). A general overview of the process is as follows: at the farm, raspberries are planted, irrigated, applied pesticides and fertilizers and finally harvested during summer (January-March). The Collection Center is a place where raspberries from different farmers are gathered and sold as one package to a Packaging Plant. The Packaging Plant is the place where raspberries are visually inspected and selected for export (best quality), sent to juice and other processed fruits (lower quality) or discarded.

The end products of interest include both fresh and frozen raspberries. In discussion with SAG and ACHIPIA, the contamination of *Escherichia coli* and Hepatitis A virus was studied as they had previous border detections (SAG's personal indication). To understand their behavior and to identify potential contamination and reduction stages, we used the information contained in the surveys to model each event. The data collected through the survey were vast and sometimes too complicated to be integrated in the risk assessment model, especially because there are no mathematical models available in the literature to relate the data. Therefore, data that were determined as not significantly impacting the microbial contamination in raspberries were excluded.

III.2.1) Justification to exclude data points

a. Irrigation practices

The Expert Elicitation indicated that the possibility of contamination with the irrigation water is insignificant. Raspberries are extremely sensitive to the contamination with the fungal species *Botryotinia fuckeliana*, which causes a gray mold disease almost always when the fruits are exposed to high humidity situations. In the situations where the fruits are touched by irrigation water, they would be spoiled immediately due to this fungi and would not be harvested.

b. Frequency of pesticide application and type of application system

Water used to dilute the pesticide is considered as a potential risk factor to introduce microbial contamination during the growth of the fruits through the pesticide application. No data were found on the cumulative impact of multiple pesticide applications on the microbial loads in fresh produce at the pre-harvest stage. The only similar information found was in Petterson et al (2001), which showed that the last irrigation is the most significant in terms of contamination. So, the last pesticide application was used in the model. The transfer mechanisms or transfer rates were not found.

d. Hygiene of harvest trays

Cannon et al (2014) evaluated the persistence and transfer of enteric viruses in food-contact surfaces and in foods. However, contamination data for viruses in the harvesting trays as well as transfer rates for bacteria could not be found. Quadros Rodrigues et al (2014) investigated the bacterial contamination on the harvesting tray, however, the transfer rate from harvesting tray to the fruits were not found.

e. Food contact surface hygiene at packing plant

Butot et al (2008) found the bacterial contamination reduction due to the use of chlorine in food contact surfaces. No data was found on the distribution of food contact surfaces contamination so it was impossible to model this step.

III.3) Collect data from literature and survey

As mentioned earlier, the Chilean farmers were surveyed and information production practices was collected. The surveys were received in Spanish, translated and answers collected in an Excel spreadsheet. For the farm module, 226 surveys were received, 23 for the collection center and 36 for the processing plant.

Literature searches were conducted using UNL's library resources, mainly the Web of Science database. Data was fitted by @risk (Palisade Corporation, 2016) and integrated in the risk assessment model with the proposed distribution. Tables 2.2-2.9 summarize the information collected and the sources.

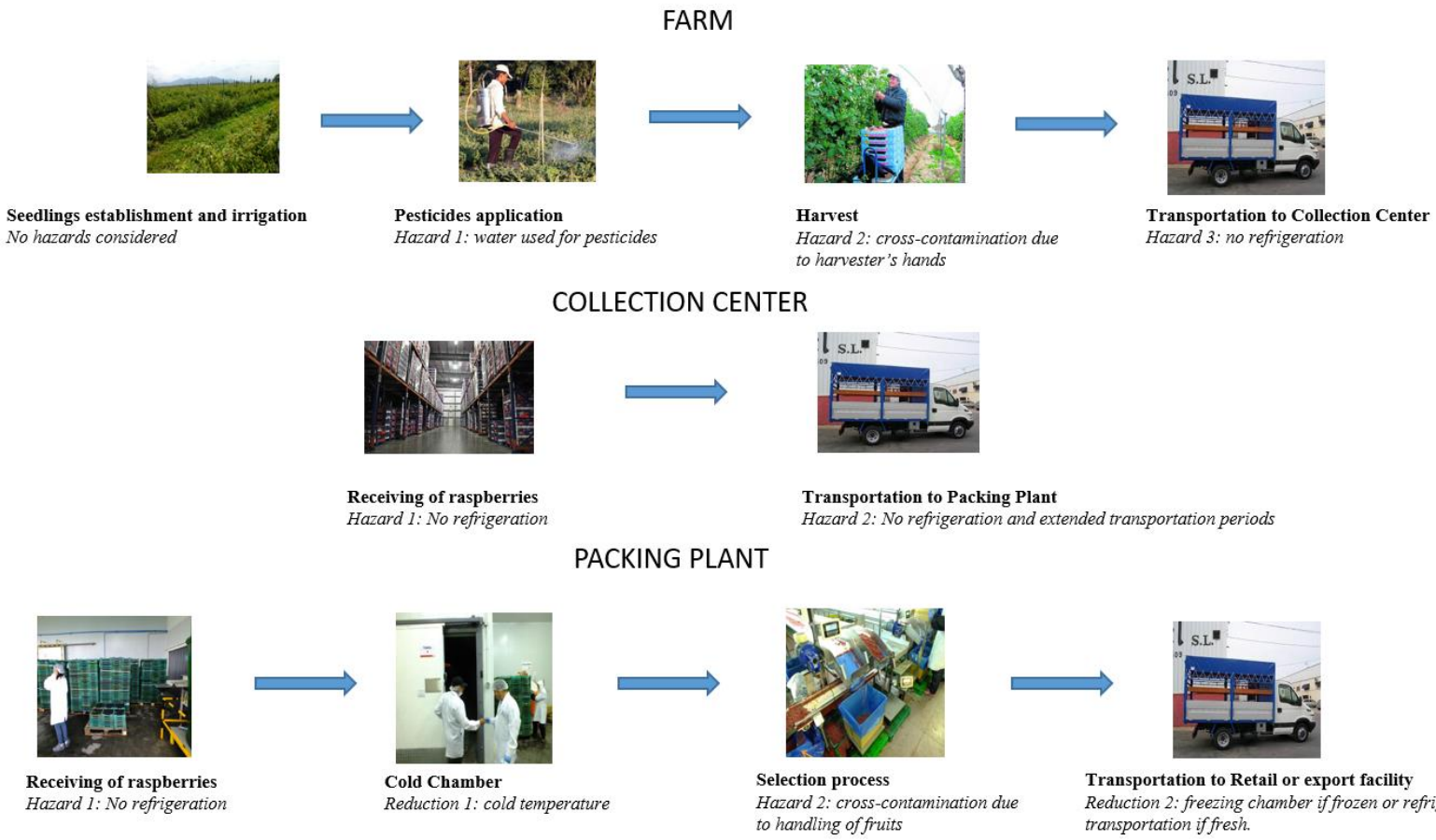

Figure 2.2. Flow chart of the processing steps for raspberries including potential hazards and reduction steps at the packing plant.

III.4) Fill data gaps with expert elicitation

A spreadsheet was designed to collect missing data and was sent to the Food Scientists Network, managed by ACHIPIA. The spreadsheet is shown in Annex IV.

III.5) Build quantitative risk assessment model

Tables 2.2-2.9 list the inputs used in the risk assessment model. Based on the information collected, different equations were constructed to model each one of the steps in the risk assessment model.

Table 2.2. List of parameters, values and distributions used in the farm module for both fresh and frozen raspberries.

Parameter (information source)	***Description***	***Distribution/Unit***	***Reference***
w_t_pest (Survey)	Type of water used for pesticide applications 1 – Groundwater 2 – Surface 3 – Potable	Discrete 1 – 71% 2 – 15% 3 – 14%	@ risk fit from survey
Cw_1 (Lit. search)	Bacterial groundwater contamination	Uniform (0,1000) CFU/L	GDWQ, 3rd Edition
Cw_2 (Lit. search)	Bacterial Surface water contamination	Pareto (1.31,2900) CFU/L	@risk fit from de Roda Husman et al., 2006
Cw_3 (Lit. search)	Bacterial Potable water contamination	Uniform (0.01,0.1) CFU/L	Chilean potable water regulation "Nch 409"
Cw_4 (Lit. search)	Viral groundwater contamination	Uniform (0,2) PDU/L	GDWQ, 3rd Edition
Cw_5 (Lit. search)	Viral Surface water contamination	Uniform (0.01,10) PDU/L	GDWQ, 3rd Edition
Cw_6 (Lit. search)	Viral Potable water contamination	Uniform (0.006-4) PDU/L	Borchard et al, 2012
T_{ap} *(Survey)*	How much times goes by between the last application and the harvest?	Laplace (30,21.88) Days	@ risk fit from surveys
D *(Lit. search)*	Bacterial and viral decay rate	Triangular (0.008,0.019,0.039) Log CFU/day Log PDU/day	Danyluk et al, 2011
Bac_transf (Lit. search)	Percentage of bacterial transfer per 0.5gr	Uniform (0.000081, 0.00011)	Gerba et al, 2005 and 2011
Vir_transf (Lit. search)	Percentage of virus transfer per 0.5gr	Uniform (0.021, 0.031)	Gerba et al, 2005 and 2011
Prev_hands (Lit. search)	Bacterial prevalence in harvesters hands	Beta (7,41)	Aceituno et al, 2016

Table 2.2 (Continuation) List of parameters, values and distributions used in the farm module for both fresh and frozen raspberries.

Parameter (information source)	***Description***	***Distribution/Unit***	***Reference***
F_prod (Lit. search)	Transferred proportion per touch from produce to hand	Beta(15.64,41.94)	Verhaelen et al, 2013
W_harv (Lit. search)	Surface area of hands that touch the produce	2.1 cm^2	Verhaelen et al, 2013
W_hand (Lit. search)	Total surface area of one side of one hand	245 cm^2	USEPA, 2011
W_prod (Lit. search)	Surface area of produce	Normal (1064,167) mm^2	Bouwknegt et al, 2015
F_hand (Lit. search)	Transferred proportion per touch from hand to produce	Lognormal(-8.34,0.58)	Verhaelen et al, 2013
C_harv_vir (Lit. search)	Virus number on harvester's hand	Gamma(0.14,54.6) PDU/hand	Bouwknegt et al, 2015
C_harv_bac (Lit. search)	Bacterial number in harvester's hands	Uniform(1,1.9) CFU/cm^2	Quadros Rodrigues et al, 2014
transp_time (Survey)	How long does it take from the Farm to the Collection Center	Loglogistic(0.0014937 ,0.044281,1.7081) Days	@risk fit from survey
transp_temp (Survey)	At which temperature are the raspberries usually transported?	Triangular(11.256,28, 28) °C	@risk from survey

Table 2.3. List of parameters, values and distributions used in the collection center module for fresh raspberries.

Parameter (information source)	***Description***	***Distribution/Unit***	***Reference***
Time_cc (Survey)	Average time raspberries stay in the Collection Center	Triangular (0.041667,0.041667,0.33716) Days	@ risk fit from survey
Temp_cc (Survey)	What is the average temperature of the Collection Center?	Extreme Value(24.3522,5.1304) °C	@ risk fit from survey
transp_temp (Survey)	What it the temperature in the transport?	Triangular (-7.6691,27,27) °C	@ risk fit from survey
transp_time (Survey)	Time taken from the Collection Center to the Packing Facility	Exponential (0.060343) Days	@ risk fit from survey

Table 2.4. List of parameters, values and distributions used in the collection center module for frozen raspberries.

Parameter (information source)	***Description***	***Distribution/Unit***	***Reference***
Time_cc_frz (Survey)	Average time raspberries stay in the Collection Center	Uniform (30,40) Days	Survey
Temp_cc_frz (Survey)	What is the average temperature of the Collection Center?	Uniform (-22.5,-18) °C	Survey
transp_temp_frz (Survey)	What it the temperature in the transport?	Uniform (-22.5,-20) °C	Survey
transp_time_frz (Survey)	Time taken from the Collection Center to the Packing Facility	Uniform (0.0007,0.0834) Days	Survey

Table 2.5. List of parameters, values and distributions used in the packing plant module for fresh raspberries.

Parameter (information source)	***Description***	***Distribution/Unit***	***Reference***
wait_time_rec (Survey)	Waiting time when receiving the raspberries	Exponential (0.010305) Days	@ risk fit from survey
wait_temp_rec (Survey)	Average temperature in the receiving	Triangular (0.050215,27,27) °C	@ risk fit from survey
cold_time (Survey)	Time that the fruits stays at the Cold Chamber	Triangular (-0.0093158,0.083333,0.56261) Days	@ risk fit from survey
cold_temp (Survey)	Target temperature in the Cold Chamber	Exponential (0.79688) °C	@ risk fit from survey
C_food_vir (Lit. search)	Virus number on handler's hand	Gamma(0.67,1.62) PDU/hand	Bouwknegt et al, 2015
π_{food} *(Lit.Search)*	Proportion of the food handler's hand touching the produce	Uniform (0,1)	Bouwknegt et al, 2015
C_food_bac (Lit. search)	Bacterial number in handler's hands	Uniform(1,1.9) CFU/cm^2	Quadros Rodrigues et al, 2014
pack_time (Survey)	Time taken from selection to transport	Loglogistic (0.0043615,0.0080573,1.7482) Days	@ risk fit from survey
pack_temp (Survey)	What is the temperature inside the Packing area	Logistic (7.6448,1.4959) °C	@ risk fit from survey
time_transp (Survey)	Time taken to destination.	Pareto(0.77518,0.083333) days	@ risk fit from survey
temp_transp (Survey)	Temperature of the cooling truck during transport	Loglogistic(-23.0679,4.6603,4.4384) °C	@ risk fit from survey

Table 2.6. List of parameters, values and distributions used in the packing plant module for frozen raspberries.

Parameter	Description	Distribution/Unit	Reference
wait_time_rec (Survey)	The wait time in the receiving	Laplace (0.021,0.0164) Days	@ risk fit from survey
wait_temp_rec (Survey)	Average temperature in the receiving	Triangular (0.050215,27,27) °C	@ risk fit from survey
cold_time (Survey)	Time that the fruits stays at the Cold Chamber	Triangular (-0.0093158,0.083333,0.56261) Days	@ risk fit from survey
cold_temp (Survey)	Target temperature in the Cold Chamber	Exponential (0.79688) °C	@ risk fit from survey
C_food_vir (Lit. search)	Virus number on handler's hand	Gamma(0.67,1.62) PDU/hand	Bouwknegt et al, 2015
C_food_bac (Lit. search)	Bacterial number in handler's hands	Uniform(1,1.9) CFU/cm^2	Quadros Rodrigues et al, 2014
pack_time (Survey)	Time taken from selection to freeze chamber	Loglogistic (0.0043615,0.0080573,1.7482) Days	@ risk fit from survey
π_{food} (Lit.Search)	Proportion of the food handler's hand touching the produce	Uniform (0,1)	Bouwknegt et al, 2015
pack_temp (Survey)	What is the temperature inside the Packing area	Logistic (7.6448,1.4959) °C	@ risk fit from survey
Frz_temp (Survey)	The target temperature is	Uniform (-35,-25) °C	@ risk fit from survey
Frz_time (Survey)	Time at freezing chamber	Inverse Gaussian (16.348,1.3124) days	@ risk fit from survey
time_transp (Survey)	Time taken to destination.	Pareto(0.77518,0.083333) days	@ risk fit from survey
temp_transp (Survey)	Temperature of the cooling truck during transport	Loglogistic(-23.0679,4.6603,4.4384) °C	@ risk fit from survey

Pre-harvest contamination (Farm module)

The objective of this module is to understand how the contamination from the water is being transferred to the crops during the pesticide application. The concentration on the raspberry during the pre-harvest stage (C_{ph}) were calculated as a function of the concentration in the raspberry after the last pesticide application (C_{ap}), the time between the last application and harvest (T_{ap}) and the decay rate (D) using the following calculations proposed by Danyluk et al (2011):

$$C_{ph} = C_{ap} - T_{ap} * D \quad (1)$$

Gerba and collegues calculated the transfer rate of bacteria and viruses during a pesticide application (Gerba et al, 2005). This information was used to calculate C_{ap}, which is the product of concentration of the water used (C_w) and the bacterial or viral transfer rate (*Bac_transf* and *Vir_transf*).

Cross-contamination at harvest (Farm module)

To assess the potential contamination contribution due to harvesting practices of raspberries, the Bouwknegt et al (2015) model was used. The number of bacteria or viruses per gram *(N_{harv})* of raspberry during harvest was calculated as

$$N_{harv} = C_{ph} - f_{prod}\frac{W_{harv}}{W_{prod}}C_{ph} + f_{hand}\frac{W_{harv}}{W_{hand}}C_{harv} \quad (2)$$

with F_{hand} being the proportion of viruses transferred from hand to raspberries. The size of a hand *(W_{hand})* corresponds to the total surface area of a harvesters' hand. (USEPA,

2011) W_{harv} is the area of the hand that actually touches the raspberries. Finally, C_{harv} is the concentration of bacteria or viruses in the hand.

Growth model (Farm, Collection Center and Packing Plant modules)

One of the main effects on the bacterial populations is the growth due to temperature abuse and the reduction due to freezing and cooling practices. Danyluk and colleagues (2011) studied the growth parameters of *E. coli* O157:H7 in leafy greens and proposed a growth model. Survival of *E. coli* O157:H7 was studied as well in strawberries during cooling and freezing temperatures (Harris et al (2011)). Based on data extracted from these two publications that were found the most similar to this research, three models were created based on the temperature of the process under modelling: over 8°C, between 0°C and 8°C, and under 0°C . Tables 2.7, 2.8 and 2.9 indicate the summarized parameters and values.

For the cooling and freezing temperatures, a maximum reduction (r_{max}) was proposed based on the data from Harris et al (2001). Additionally, the first days of freezing have a stronger reduction in bacterial populations, so two different reduction rates (r_1 and r_2) were proposed based upon the freezing times. For less than 8 days, r_1 was used and for more than 8 days, r_2 was used.

Table 2.7. Bacterial growth model parameters for temperatures over 8°C.

Parameter ID	Parameter Description	Equation	Value/Distribution/ Calculation	Unit
μ	Growth rate	$(b*(T-T_0))^2$	-	Log CFU
T	Temperature of modelled step	-	See Table 5,6,7,8 and 9	°C
T_0[1]	Temperature constant 1	-	2.628	sqrt(log cfu/day/°C)
b[1]	Temperature constant 2	-	0.0616	°C
t	Time of the modelled step	-	See Table 5,6,7,8 and 9	Days
Ci	initial concentration	-	From previous step	Log CFU/gr
-	Final concentration	Ci+ μ*t	-	Log CFU/gr

[1] Equations and constants are adopted from Danyluk et al., 2011.

Table 2.8. Growth model parameters for temperatures between 0°C and 8°C.

Parameter ID	Parameter Description	Equation	Value/Distribution/ Calculation	Unit
r[1]	Reduction per day	-	0.18l	Logs/day
r_{max}[1]	Maximum log reduction	-	1.225	Logs
t	Time of the modelled step	-	See Table 5,6,7,8 and 9	Days
Ci	Initial concentration	-	From previous step	Log CFU/gr
-	Final concentration	Ci-r*t or Ci- r_{max}	-	Log CFU/gr

[1]Parameters derived from Danyluk et al, 2011 data.

Table 2.9. Growth model parameters for temperatures below 0°C.

Parameter ID	Parameter Description	Equation	Value/Distribution/ Calculation	Unit
r_1[a]	Reduction per day, less than 8 days	-	0.18l	Logs/day
r_2[a]	Reduction per day, more than 8 days	-	1.225	Logs
r_{max}[a]	Maximum reduction	-	1.6	Logs
t	Time of the modelled step	-	See Table 5,6,7,8 and 9	Days
Ci	Initial concentration	-	From previous step	Log CFU/gr
-	Final concentration	Ci-r1*t, if t<8 or Ci-r2*t, if t>8 or Ci- rmax	-	Log CFU/gr

[a]Parameters derived from Danyluk et al, 2011 data.

Cross-contamination due to handling (Packing Plant module)

Similar to the harvesting module, the Bouwknegt et al (2015) model was used for the handling of raspberries during selection in the packaging plant. The selection process consists of workers manually handling raspberries to assess their visual quality. The number of bacteria or viruses per gram *(n_{touch})* of raspberry during the selection process was calculated as

$$n_{touch} = C_{cc} - f_{prod}\pi_{food}C_{cc} + f_{hand}\frac{W_{food}}{W_{hand}}C_{food} \quad (3)$$

with C_{cc} being the concentration in the raspberry after the Collection Center, which is the previous step to the Packaging Plant where raspberries are stored and selected. C_{food} is the concentration of viruses or bacteria in the handler's hands, π_{food} is the proportion of the food handler's hand touching the produce and W_{food} is the touching surface of a handler's hand which is the same as W_{harv} at the Farm.

III.6) Run Monte Carlo simulation

Once the model was developed, the Monte Carlo simulation using Latin Hypercube sampling for 10,000 iterations was performed to obtain stochastic estimates of the output variables, namely, bacterial and viral contamination loads in both fresh and frozen raspberry products, using Microsoft Excel add-on package @Risk (version 7.0, Palisade Corporation, New York, USA). Sensitivity analysis was conducted to evaluate the importance of input variables on the changes in contamination risks, represented in tornado charts.

III.7) Scenario analysis

The efficacy of microbial control interventions that can be potentially adopted at different points along the raspberry supply chain were evaluated through a scenario analysis. A total of 13 scenarios were run in the model, including a baseline scenario for comparative purposes using the data mentioned above for the estimate of "no intervention" scenario and 10 other alternative scenarios to predict the food safety protection in end raspberry products if a specific intervention technology or regulation would be adopted. For each scenario, the model was run for 10,000 iterations to generate the mean risk estimates. All the scenario analysis were conducted on fresh raspberries. The list of scenarios evaluated is shown in Table 2.10 for water interventions and in Table 2.11 for the reduction of time when raspberries are stored at the collection centers.

Previous studies show that water is one of the prime sources of contamination for berries and leafy greens (Bern et al, 1999 and Ashbolt et al, 2001). As shown in the on-farm practice survey, raspberry farms in Chile mainly rely on three types of water sources with

microbial safety level in the order of portable water as the cleanest source, followed by ground water and surface water. Therefore, one of the water intervention actions evaluated in this study is changing the use of potable water and/or ground water instead of surface water with the improvement of public water treatment and supply infrastructure in Chile. The changes in water sources were modeled by increasing the proportions of raspberry farms using potable and/or ground water in the model. To control the microbial loads in the water sources, the introduction of ultraviolet light is the other intervention actions evaluated in this thesis, because it has been shown that ultraviolet lamps are easy to install and operate in less expensive costs and do not create harmful byproducts (Pariseau et al, 2010). Ultraviolet light has been demonstrated to reduce bacterial and viral contamination in water by 2-4 logs (Chang et al, 1985 and Pariseau et al, 2010). Combinations of the two water intervention actions were also evaluated. Relative changes in mean risk estimates of each alternative scenario were calculated, compared to the baseline scenario.

Table 2.10. Water uses scenario analysis for bacterial contamination.

Scenario	Water contamination	Water type			Water type change
		Occurrence of groundwater use (GW)	Occurrence of surface water use (SW)	Occurrence of potable water use (PW)	
Baseline	Contamination as current	71%	15%	14%	Current occurrence
A	No intervention	86%	0%	14%	100% SW→GW
B	No intervention	42%	8%	50%	50% SW → GW & 50% GW → PW
C	No intervention	5%	5%	90%	GW&SW→PW
D	UV light intervention	71%	15%	14%	Current occurrence
A+D	UV light intervention	86%	0%	14%	100% SW→GW
B+D	UV light intervention	42%	8%	50%	50% SW → GW & 50% GW → PW

Table 2.11. Transportation reduction time scenarios for bacterial contamination.

Scenario	Transport time from farm to collection center	Transport time from collection center to packing plant
Baseline	0-9 hours	0.5-8 hours
E	1 hour	Baseline
F	Baseline	1 hour
E+F	1 hour	1 hour

Table 2.12 Temperature reduction at Collection Center scenarios

Scenario	Temperature at Collection Center
Baseline	0.5-30 °C
G	50 % reduction
H	4-8 °C (fully implemented refrigeration system)

Table 2.13 Pesticide applications time scenarios

Scenario	Harvest time after last application
Baseline	0-120 days
I	25% increase
J	50% increase

IV. RESULTS AND DISCUSSION

Risk estimates of current practices

Figures 2.3 and 2.4 show the contamination distribution at the end of the process for fresh raspberries for *E.coli* and Hepatitis A, respectively. The bacterial contamination for frozen raspberries is shown in Figure 2.5. Data for viral contamination in the frozen chain is not shown because the only parameters changed are the freezing practices, which does not result in difference from fresh fruits. Note that the baseline scenario was not an accurate representation of the current risk estimate of contamination in raspberry products, since some initial input parameters were populated with data extracted from studies conducted in countries other than Chile.

For the fresh raspberries, bacterial contamination mean was -1.89 log CFU/gr. The majority of the results (95% probability interval) for 10,000 iterations ranged between -5.48 and 0.13 log CFU/gr with the maximum value over 8 logs. The contamination mean for the frozen raspberries was -4.44 log CFU/gr.

The viral contamination mean for fresh raspberries was -2.07 log PDU/gr. The majority of the results (95% probability interval) for 10,000 iterations ranged between -3.67 and -0.93 log PDU/gr with a maximum value of 0.03 log PDU/gr.

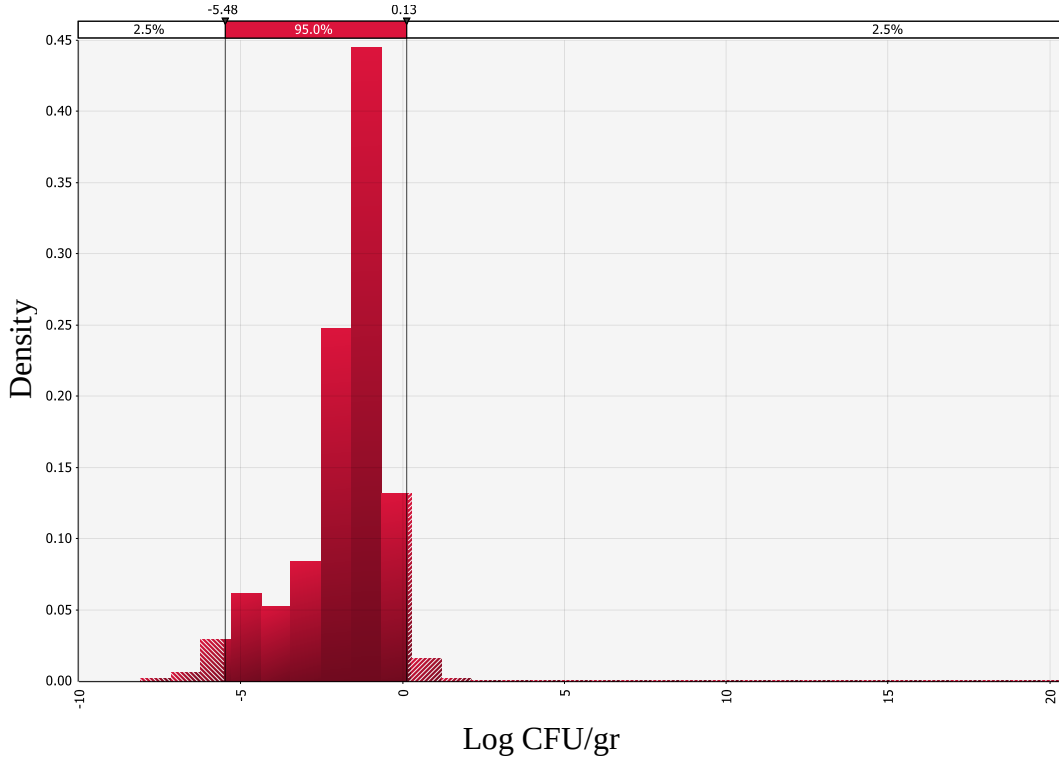

Figure 2.3. Bacterial Log CFU/gr contamination distribution of 10,000 iterations simulation for the fresh raspberry model. The 95% probability interval of the results are highlighted in the top portion of the plot.

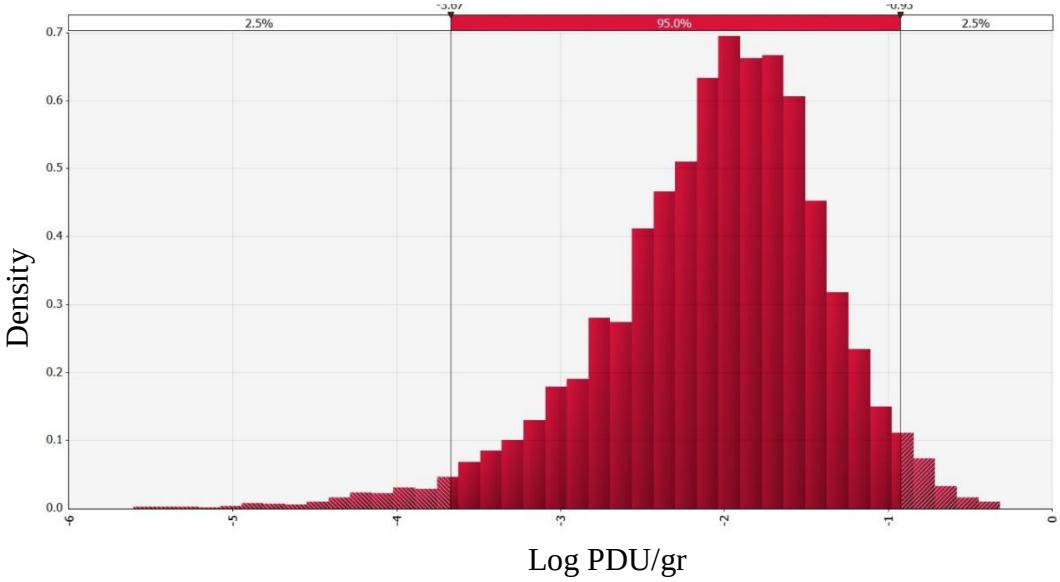

Figure 2.4. Viral log PDU/gr contamination distribution of 10.000 iterations simulation for the fresh raspberry model. The 95% proportion of the results are highlighted in the top portion of the plot.

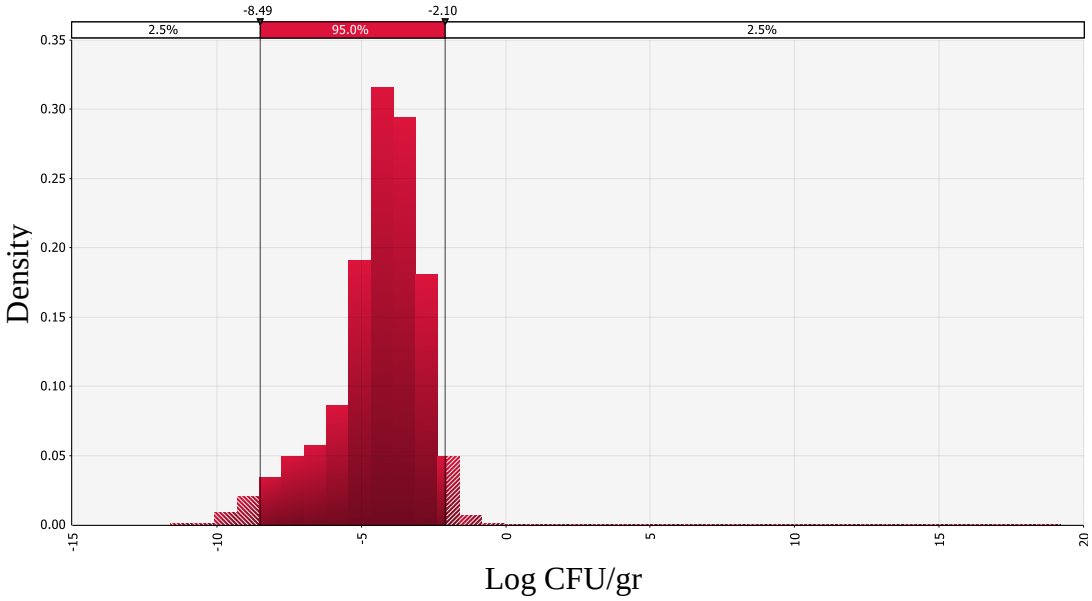

Figure 2.5. Bacterial log CFU/gr contamination distribution of 10.000 iterations simulation for the frozen raspberry model. The 95% proportion of the results are highlighted in the portion of the plot.

Expert elicitation

This project demonstrated that the Food Scientist Network is at its early development stage and that risk assessment procedures are still widely unknown, even to scientists. A number of questions were received indicating that the scientist were not understanding what was being asked, although examples were given. No data was received directly from the spreadsheet, but useful information was delivered, for example, that irrigation water should not be considered because the soft rot caused by *Botryotinia fuckeliana*.

Sensitivity analysis

The tornado plots shown in Figures 2.6, 2.7 and 2.8, indicate the inputs that have the largest impact in the simulations. For the bacterial contamination in the fresh chain, the three largest inputs that changes the results are the type of water used, times of transport

time from the Packing Plant and time after the last pesticide application. For the viral contamination, the three largest inputs that changes the results are the time after the last application, the groundwater contamination and the decay rate. Finally, for the frozen raspberry supply chain, the most important parameters are the type of water used, the freezing times and time of transport from the Packing Plant.

In all Monte Carlo simulations for every data set, one of the recurring significant parameters is the water used for pesticides applications. This is intuitive as several reports had indicated that water is one of the main vehicles for contamination of fresh produce (Herwaldt et al. 1997), especially in the case of water used for pesticide applications (Gerba et al. 2011, Caceres et al.1998; Herwaldt and Beach 1999). Initially irrigation water was also considered but later discarded due to the impossibility of harvesting a raspberry due to fungal spoilage associated with this event (Expert Elicitation, ACHIPIA 2016).

Freezing practices in the freezing chamber and the transport from the Packing Plant are also significant in the outputs since very low temperatures and extended periods of time reduces the bacterial load significantly (Harris et al, 2001).

As seen in Figure 2.8, time after the last application, groundwater contamination and the decay rate – all data related to pesticides applications – have the largest impact in viral concentrations. This is largely due to the fact that this stage is the only source of entry for viral contamination in this model.

Manipulation by harvester and handler hands does not show in the simulation as a significant factor. Due to the lack of the data, the prevalence data was not considered while is the most important parameter to study when assessing the impact of cross-contamination. The latter is especially important because with the model and data collected from different authors (Aceituno 2016, Quadros Rodriguez 2015 and Bouwknegt 2015) the net effect of touching a raspberry is an actual transfer of contamination from the raspberry to the hand, rather than the opposite direction.

All the results are within a low range, the fresh raspberry chain is the one with the highest counts of *E. coli*. The reason is that during the fresh raspberry chain, there are more waiting periods with higher temperatures. Nevertheless, the latter is not seen in the tornado plot in Figure 2.6, where one would expect these times and temperatures to have larger effects in the estimates. This is very likely due to the uncertainties linked to the water contamination data and transfers ratio to the fruit due to the pesticides applications.

There are significant uncertainties in the model that are classified in two categories: 1) non-local data and 2) non-optimized data. The first relates to the need to use data that has not been created from Chilean sources, such as the water contamination and the handler's hand contamination. The second class refers to data that was collected from other models and uses. Among others, the transfer rates proposed by Gerba et al (2005) were intended for lettuce not for raspberries, thus, this is an important limitation of the model.

Collection of data in terms of reducing uncertainty and in terms of having appropriate parameters closer to our research food matrix are invaluable to improving the quality of the risk estimates.

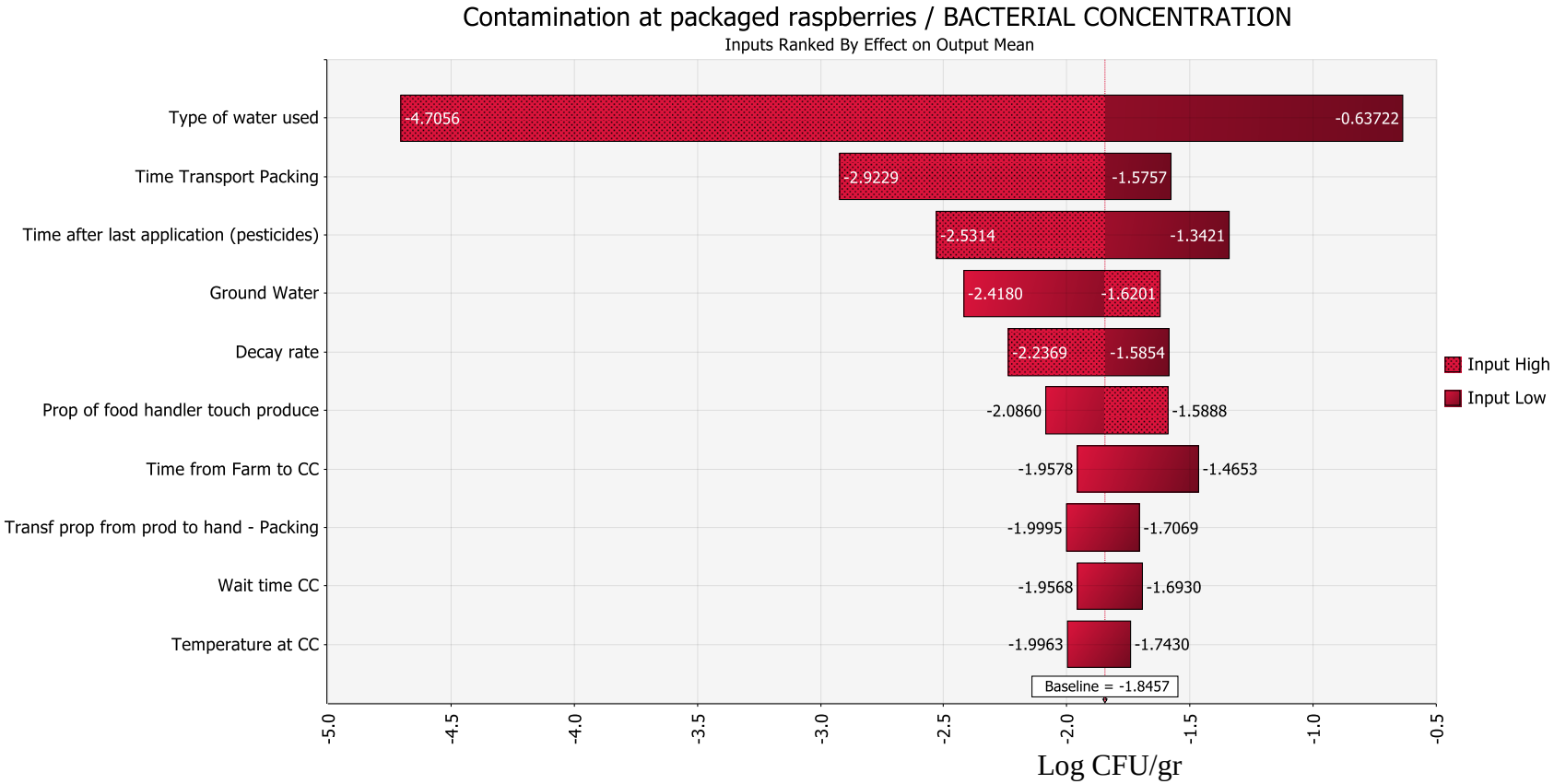

Figure 2.6. Tornado plot for the final bacterial concentration for the fresh raspberry model.

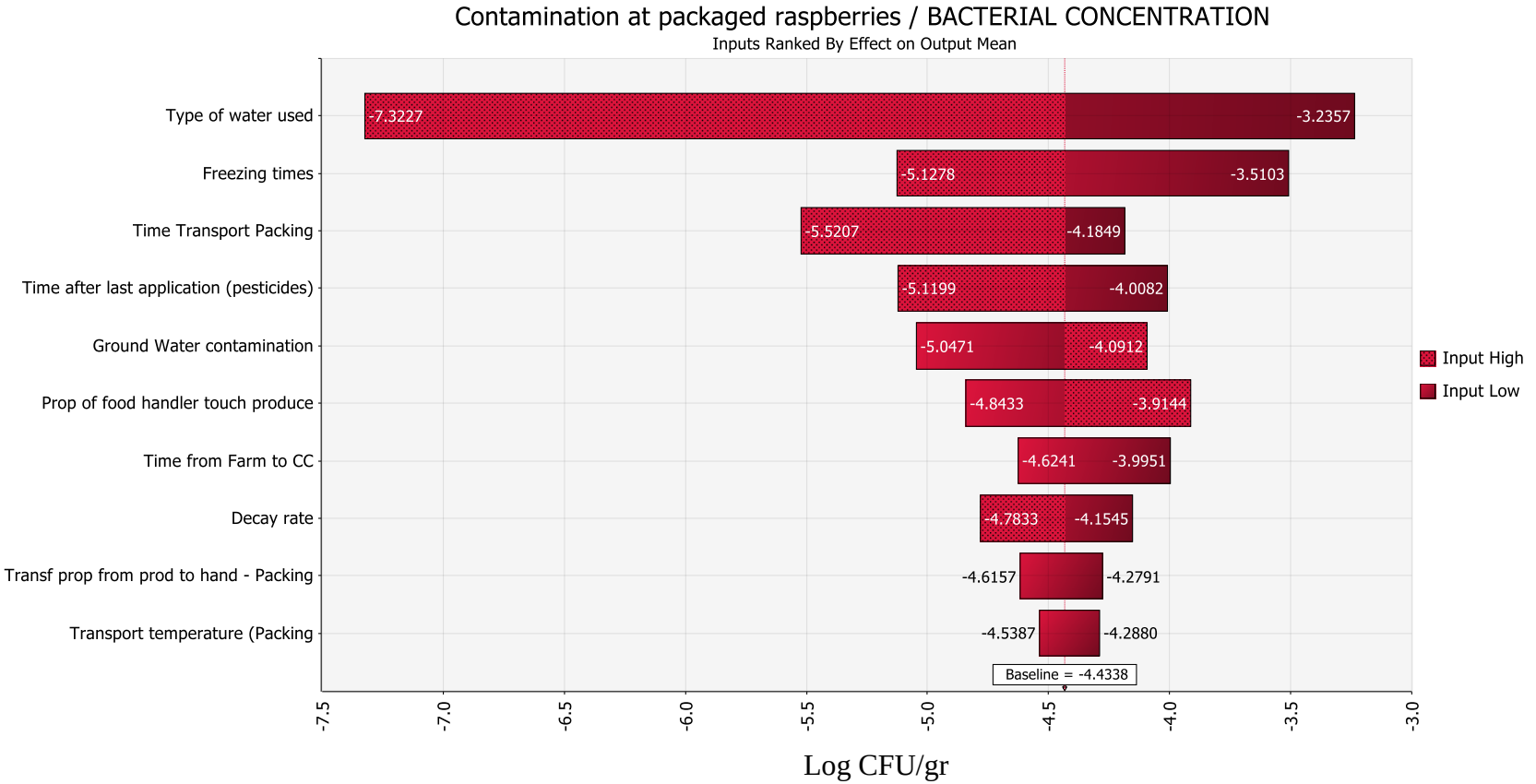

Figure 2.7. Tornado plot for the final bacterial concentration for the frozen raspberry model.

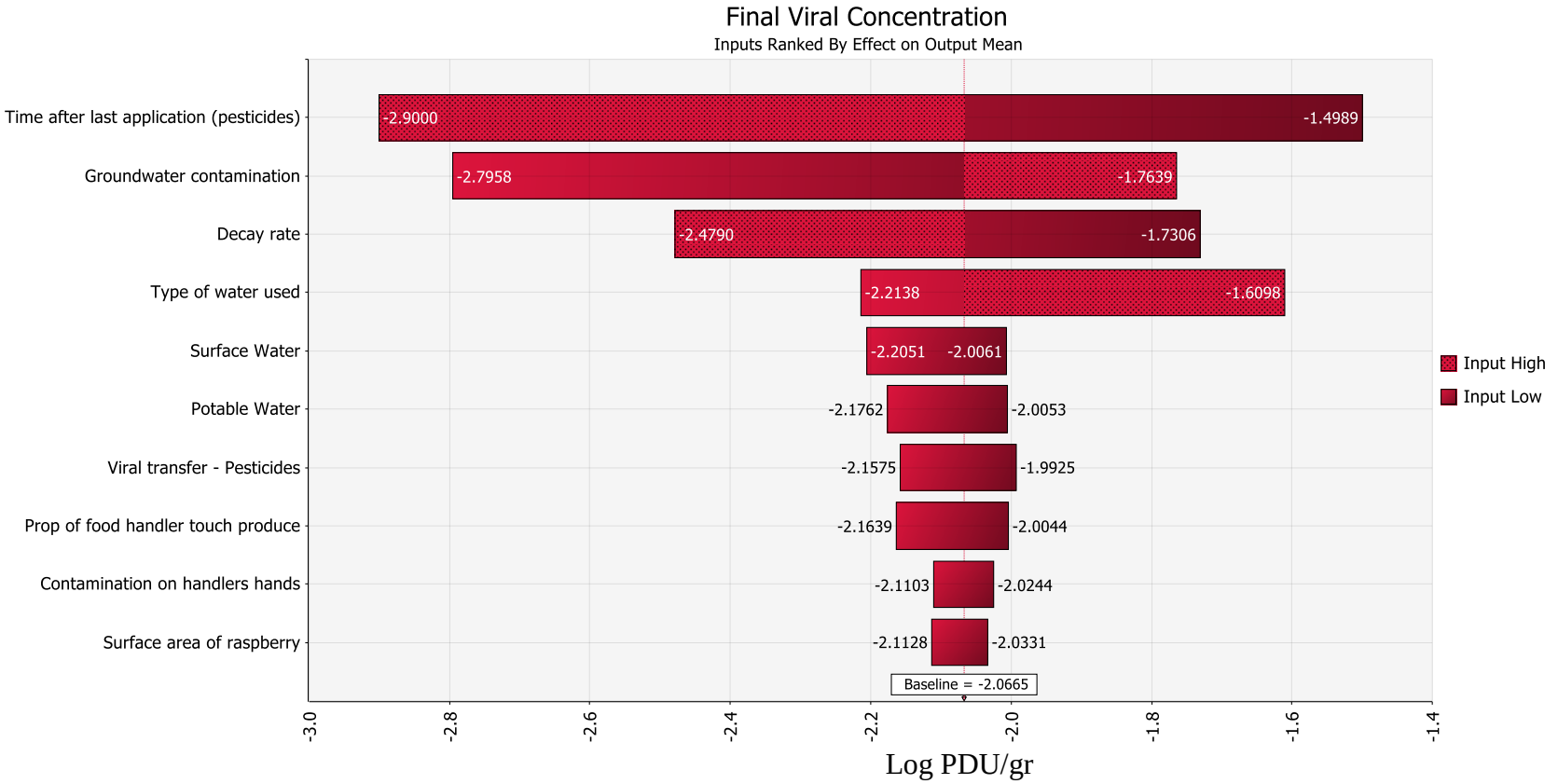

Figure 2.8. Tornado plot for the final viral concentration for the fresh raspberry model.

Scenario analysis and interventions evaluation

Table 2.14 summarizes the results from the different scenario analyses.

For the Scenarios A-C, changing the frequency of the type of water in use had a strong impact on the bacterial populations but not in the virus populations. Increasing the use of potable water reduced the bacterial populations by 66.35% and 136.96% for scenarios B and C, respectively. Viral populations were slightly affected by the changes in the frequency of use of the water sources.

Using UV-lamps had a much more marked effect, reducing bacterial populations to a similar level than when using mainly potable water (Scenario C). All scenarios with the UV lamp had at least 100% log reduction in bacterial populations and 50% viral.

Scenarios E and F had little effect on the simulations, resulting in reductions less than 6% in every case.

The scenario cases provides interesting insight on the production chain. As seen in Table 2.14, increasing the frequency of the use of potable water (Scenario C) is very effective for bacteria populations, but not for viruses. The minor increase in viruses may be due to the lack of data for potable water; the few data points collected from Borchard et al (2012), describes slightly higher concentration numbers compared to the global estimates of the WHO for groundwater. (GDWQ, 3rd Edition)

Table 2.14. Summary of the scenario analysis results.

Scenario	Mean contamination (log CFU/gr or log PDU/gr)	% change compared to baseline
Bacterial Viral *(Baseline)*	-1.84 -2.07	-
A	-2.01 -2.18	9.24% reduction 5.31% reduction
B	-3.06 -2.01	66.30% reduction 2.89% increase
C	-4.36 -1.91	136.96% reduction 7.73% increase
D	-4.29 -3.29	133.15% reduction 158.94% reduction
A+D	-5.41 -3.21	194.02% reduction 55.07% reduction
B+D	-4.30 -3.31	133.7% reduction 59.9% reduction
E *(Only bacterial)*	-1.90	3.26% reduction
F *(Only bacterial)*	-1.88	2.17% reduction
E+F *(Only bacterial)*	-1.94	5.43% reduction
G *(Only bacterial)*	-1.99	8.15% reduction
H *(Only bacterial)*	-2.03	10.33% reduction
I	-1.99 -2.26	8.15% reduction 9.18% reduction
J	-2.15 -2.44	16.85% reduction 17.87% reduction

On the other hand, the proposed ultraviolet lights intervention indicates significant reduction in both bacterial and viral populations. As shown in Table 2.14, the log

reduction achieved by this technology (Scenario D) for bacteria and virus are up to 133% and 159%, respectively. The effect on viruses is larger probably because there are no further grow stages as with bacteria.

The combination of both UV lamps and increasing potable water use (Scenarios A+D and B+D) does not seem to provide considerable further reduction, especially considering that the groundwater is increasing the virus counts (Scenarios B and C)

This technology is currently being applied in small farms in Chile (Expert Elicitation, ACHIPIA, 2016) so it arises as an interesting potential intervention.

As the receiving in the Collection Center is currently unrefrigerated, two scenarios were simulated (Scenarios G and H). The reduction achieved for a 50% decrease in temperature is 8.15%. Even implementing a refrigeration system in this step, which can be very costly, only reduces the contamination by 10.33%. The waiting time in this stage is very short (Table 2.3) so any temperature intervention would affect the final contamination considerably.

Although the time of application before the harvest appears to be an important input in the simulations (Figures 2.6 and 2.8), the reduction achieved for scenarios I and J is considerably smaller than previous scenarios. The practices associated with these last scenarios can be very resource consuming so it does not seem a practical intervention.

Data gaps identification

Several contamination routes were dropped due to the lack of models available to connect the Chilean data – mostly about frequency of use – or inexistent prevalence and concentration data for the selected microorganisms in raspberries. Animal contamination

on farm, harvester tray contamination, food contact surfaces and others were some datasets that had to be discarded due to these reasons. There is need to increase the data available, not only from an experimental perspective but from an observational point of view.

Nevertheless, there is much uncertainty as Chilean specific water contamination data was not obtained. Another uncertainty factor is the decay rate, Danyluk et al (2011) was the only author that proposed a usable estimate, although on spinach for an *Escherichia coli* surrogate.

The transfer rate used was estimated on lettuce, due to the lack of studies conducted in raspberries; data from experimental research was taken and applied. (Gerba et al, 2005)

Even considering these limitations, a comprehensive estimate was given for the behavior of the bacterial and viral populations in the fresh and frozen raspberry production chain.

There is a need for open access information and the creation of continuous surveillance systems that provide this kind of data to researchers. Academia-Government collaborations are useful to accomplish this objective, as shown in this study for some datasets.

Significance for regulators and evidence-based policies.

This collaborative project is the first in its kind in the realm of food safety in Chile.

ACHIPIA and SAG were effective collaborators and the outputs of this study are ready to be evaluated by risk managers or policy makers. The results are displayed in a simple way and very visual. There is no need to have specific expertise to critically analyze these

results and scientific evidence has been effectively provided to take well informed decisions.

V. CONCLUSIONS

Risk Assessment is a tool that has been used since the decade of the 1980's. It is a very well structured process that takes into consideration the data limitations and provides easy to understand information to risk managers. Although the process itself requires scientific expertise, this is when strategic alliances such as collaborations between Academia and Government are most useful.

In this particular Risk Assessment project the key findings from the perspective of Chile's government are:

1. Water quality needs to be improved as it is the main effector of contamination in raspberries.

2. Frozen raspberries are much safer in terms of bacterial contamination. Virus contamination is similar as in fresh raspberries.

3. Relatively cheap and easy to use technologies, such as ultraviolet light application, provide important contamination reduction. These interventions could be applied while a more definitive solution is developed, such as stronger regulation on water quality.

4. The use of Risk Assessment provides critical insight on the information gaps. There is a need for more research into water sources, raspberry-specific contamination transfer due to animal waste, and the prevalence of bacteria and viruses in the food operation premises, among others.

5. As stated previously, one of the objectives was to try the collaborative experience between Academia and ACHIPIA. Although no data was collected directly from the Expert Elicitation spreadsheet, very useful guidance and general comments were received. These kind of tools proved to be key in narrowing the gap between developing and developed countries when trying to integrate science into their decision making process.

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CHAPTER 3: SYSTEMATIC REVIEW OF SPORE FORMING BACTERIA IN MILK

I. ABSTRACT

Approximately one third of the produced fluid milk in the United States is lost annually. One important factor contributing to the loss is the contamination with spore-forming bacteria, which can not only survive the pasteurization process, but also grow under refrigeration conditions resulting in subsequent spoilage. The objective of this study is to describe the population dynamics of spore-forming bacteria and spores in milk from farm to packing plant through a systematic review approach. A database search was conducted to identify, appraise, and summarize primary research studies that describe the prevalence and/or concentration of spore-forming bacteria and spores at more than one production/processing point in the same study. Literature searches retrieved 9,778 citations, among which data were extracted from 31 relevant citations for meta-analysis. Due to variant milk sampling points recorded in citations, we standardized the sampling points by clustering similar ones as follows: Milking machine, Raw milk, Bulk tank, Transportation, Silo, Pasteurized milk and Packaged milk. Bacillus cereus was the most reported organism. Concentration data were more abundant with 582 data points for both vegetative cells and spores, compared to prevalence data with 68 points. In general, great heterogeneity was observed among studies in the contamination of milk samples. Spore concentrations remain stable until pasteurization, in a range of 0-2.5 log spores/ml. After pasteurization, spore concentrations decrease in accordance with an increase in vegetative cells. Although considerable research has been conducted on this topic, there are limited studies to holistically describe the population dynamics of spore-forming bacteria under

the current milk production system. Meta-regression analysis indicates that moderators Steps (in the milk chain), Season and Year of Publication explains 65.71% of heterogeneity for cells and 35.11% for spores. Findings of this study can provide insights regarding steps where spore-forming bacteria could be introduced for potential effective management, as well as further research needs to increase the quality and shelf life of milk products in the United States. This project demonstrated that the outputs of Systematic Review can feed the decision making process, through simple and clear recommendations to the risk managers using a high-level evidence synthesis analysis procedure.

II. INTRODUCTION

The previous chapter shows the useful application of risk assessment in food safety protection by a collaborative project of assessing microbial risks of raspberry products in Chile. In this chapter, the approach of systematic review is demonstrated via a case study of evaluating the changes in spore-forming bacteria along milk supply chain.

In the milk production process, contamination with microorganisms is the most important hurdle to overcome to provide safe milk products with long shelf life. Microorganisms that create spores, referred to as spore-forming bacteria throughout this paper, can persist along the downstream processing. This is due to its capacity of spores to resist pasteurization temperatures; leading to microbial growth and premature spoilage. Cotter and colleagues classified spore-forming bacteria in two groups. The first are the aerobic psychrotrophic thermophilic spore formers such as *B. cereus, Paenibacillus sp.* and *Geobacillus stearothermophilus.* The second ones are the anaerobic psychrotrophic

thermophilic spore-forming bacteria, such as *C. botulinum* and *C. perfringens.* (Cotter et al, 2015)

Spore-forming bacteria have been declared by the USDA and FDA to be the greatest threat to dairy products in terms of spoilage (Hull et al., 1992). The spores of these organisms, under the heat treatment of milk (e.g UHT), trigger the growth of its vegetative form. The subsequent growth of these microbes will generate the secretion of different thermostable lipolytic and hydrolytic enzymes that will breakdown the major constituents of milk (Samaržija et al, 2012). Under these circumstances, milk spoilage results and follows economic losses to farmers and processors. On the other hand, Gram-positive spore-forming bacteria such as *Bacillus cereus,* produce enterotoxins which can cause diarrhea and emetic disease due to food poisoning (Lindabk and Granum 2006). Therefore, the potential contamination of spore-forming bacteria is a very important issue that the dairy industry is aware of and constantly tries to address using exhaustive hygiene and preventive control programs, such as HACCP and Good Manufacturing Practices. Of particular interest to the milk industry are the spore-forming psychrotrophic bacteria, which are able to grow at 7˚C or less, regardless of their optimal temperature of growth (International Dairy Association, 1976) and synthetize thermoresistant spores.

Spore-forming bacteria can be introduced through multiple points along the liquid milk production chain. The initial contamination starts in the milking facilities. Teat skin is considered one of the major sources of spores in raw milk (McKinnon and Pettipher, 1983, Samaržija et al, 2012). It has also been documented that the number of spores present in milk is significantly correlated to the degree of soil contamination on teats (Christiansson et al, 1999), which indicates the significance of soil and dust attached to

the teat skin contributing to the spore-forming bacteria contamination in raw milk. The bulk milk storage tanks, pipelines and filling machines during processing procedures are also key contamination sources, via the formation of biofilms on the food-contact surfaces. Most of the spore-forming bacteria are able to create biofilms, which are very resistant to temperature and sanitation, therefore generating an additional hurdle to the industry.

Significant research has been conducted to develop the modern interventions to prevent microbiological contamination, which are contained at the farm and processing level. The application of Good Farm Managing Practices is critical to achieve low spore contamination of raw milk. While the dairy industry relies on pasteurization to achieve a reduction in the number of pathogenic and spoilage microorganism, pasteurization is ineffective against spores (Cotter et al., 2015, Gleeson et al., 2013). Usually the research focuses on specific points, but a limited number of studies have reported the cumulative impact of control efforts over the entire system. In addition, research papers quantifying the contamination of spore-forming bacteria in milk are available, but data with great heterogeneity may be reported depending on study design, size and quality. Holistic and systematic understanding of the dynamics of populations of spore-forming bacteria throughout the whole milk supply chain is a very valued information set that no research group has addressed, as most of the efforts are put in one or few steps.

In both situations, systematic review (SR) can facilitate the data collection conducted in a structured and comprehensive process to identify data gaps and to fully capture the naturally occurring variations among studies. Differing from narrative review, SR uses a structured research protocol to minimize selection bias and evaluate data quality. Data

extracted from independent studies selected in SR are commonly synthesized by meta-analysis (MA), which is a subset of SR to use statistical approaches to combine the results from multiple studies to develop a single conclusion with greater statistical power over individual studies. SR, together with MA, can independently address research questions by synthesizing relevant scientific evidence and also result in quantified estimates that are suitable for quantitative microbial risk assessment (QMRA) model parameterization to inform sound food safety risk management decision makings. The use of results from SR and MA will increase the confidence in the QMRA model input estimates and subsequent risk predictions, compared to using the "author-picked" data.

The present study was aimed at answering two research questions aided by SR: i) What are the magnitudes of the changes in prevalence and/or concentration of spore-forming bacteria and spores across steps along the pasteurized milk supply chain, and, ii) what are the factors that could explain the variability of prevalence and/concentration of spore-forming bacteria and spores in the intermediate and end milk products. Since the information to resolve these questions was collected in the farm-to-processing continuum, findings of this study will indicate the cumulative efficacy of the agricultural and manufacturing practices employed in the current milk supply system in controlling spore-forming bacteria. In this study, we report our first findings focused on spore-forming bacteria dynamics along the pasteurized liquid milk supply chain.

III. MATERIALS AND METHODS

2.1 Search strategy

In consultation with the University of Nebraska – Lincoln subject specialist for Food Science and Technology, Veterinary and Biomedical Sciences, a search strategy was

developed using different key words and syntax. The databases used were: Food Science and Technology Abstracts, Centre for Agriculture and Bioscience International database (CABI), MEDLINE®, BIOSIS Previews, Biological Abstracts and the Web of Science. The initial searches were narrow and specific, containing keywords that made reference to food products, spore-forming bacteria related terms and specific bacterial species. An initial screening of those results revealed that potential relevant manuscripts were being discarded. After testing several search strategies, a search strategy utilizing more general terms was determined appropriate to prevent losing relevant studies. A summary of the search strategy for each database is shown in Table 3.1. Proceedings of conferences were included when the full text was available. This study started on March 2015 and was finished in June 2016.

Table 3.1. Summary of the search strategies for the electronic databases.

Database name	Search strategy
CABI, Web of Science and Biological Abstracts	spore* OR "bacterial spores" OR sporeformer* OR "spore former" OR spore-former* OR sporeforming OR spore-forming OR "spore forming" OR endospore* AND "milk products" OR milk OR "ice cream" OR cheese* OR cream OR butter OR yogurt OR yoghurt OR dairy.
PubMed	milk OR milks OR "ice cream" OR "ice creams" OR cheese OR cheeses OR butter OR yogurt OR yoghurt OR cream OR dairy OR dairy products [MeSH] AND spore OR spores OR "spore forming" OR sporeform* OR spore-form* OR "spore former" OR "spore formers" OR endospore OR endospores OR spores, bacterial [MeSH]
Biosis Citation	milk OR milks OR "ice cream" OR "ice creams" OR cheese OR cheeses OR butter OR yogurt OR yoghurt OR cream OR dairy AND spore* OR “spore forming” OR sporeform* OR spore-form* OR "spore former" OR “spore formers” OR endospore*
Food Science and Technology Abstracts	spore* OR "bacterial spores" OR sporeformer* OR "spore former" OR spore-former* OR sporeforming OR spore-forming OR "spore forming" OR endospore* AND "dairy products" OR milk OR "ice cream" OR cheese* OR cream OR butter OR yogurt OR yoghurt OR dairy

2.2 Relevance screening

Two graduate-level students conducted independent relevance assessment of the initially retrieved publications in three steps: 1) title screening, 2) abstract screening, 3)full-text screening. The software EndNote X7® (Thomson Reuters, Toronto, Canada) was used to manage the references.

2.2.1 Title screening

Due to a large number of articles obtained and the broad search strategy selected, the title screening was first conducted to remove retrieval noise and evident non-relevant articles, such as “analysis of spore-forming bacteria in canned vegetables”.

2.2.2 Abstract screening

Primary research was included at this stage if the following information was covered, including 1) English language; 2) data from countries with similar milk production systems as the United States of America. (We consider all European countries, Australia, New Zealand and Canada as having close characteristics as the United States); 3) prevalence and/or concentration of; 4) cells and/or spores in milk samples on; 5) any step in the milk chain supply system. Reviews were collected to be used later as a quality check of our retrieved literature.

2.2.3 Full-text screening

The full-texts for the selected articles at the previous stage were collected for the final screening. Using the online resources, subscriptions and interlibrary load service available at the University of Nebraska-Lincoln, the full texts were downloaded and stored in the Endnote reference library. Any article whose corresponding manuscript was not retrievable was discarded at this stage.

Articles with available full-texts were further screened for data extraction and analysis, if the following information were reported, including 1) data of nationally-occurring contamination on, 2) at least one data point in the defined milk supply chain, 3) concentration and/or prevalence of spoilage sporeforming organisms in either raw or

pasteurized liquid milk with their respective sample sizes and 4) arithmetic mean concentration and/or prevalence with sample sizes reported. The variance and sample sizes are fundamental data needed to propose pooled estimates using MA tools. (Cochrane Collaboration webpage, 2016)

Articles were excluded if they pertained solely to detection or challenge studies to evaluate the efficacies of specific spore-forming bacteria/spore reduction. Our main focus was on observational research that studied the populations of spore-forming bacteria along the milk supply chain.

2.3 Data extraction

Relevant data were manually extracted, organized and stored in a spreadsheet. The following information from each selected articles was extracted: first author, year of publication, country where the study was conducted, study duration, study season, bacterial species, sample size (volume), sample number, production step involved, concentration/prevalence, detection method and its corresponding detection limit (when available) and statistical descriptors (when available) such as median, range, standard deviation, standard error and confidence intervals.

2.4 Standardization of milk supply steps

Due to the great heterogeneity of the studies, especially regarding sampling plans, the data extraction and grouping process yielded several different datasets within the milk supply chain. Different names among the manuscripts were combined into the same processing step, thus, developing a standardized process for the milk production chain was essential to group representative data and analyze it in a logical structure. Figure 3.1

shows the standardized steps and an explanation of the milk supply chain proposed in this study with their coverage.

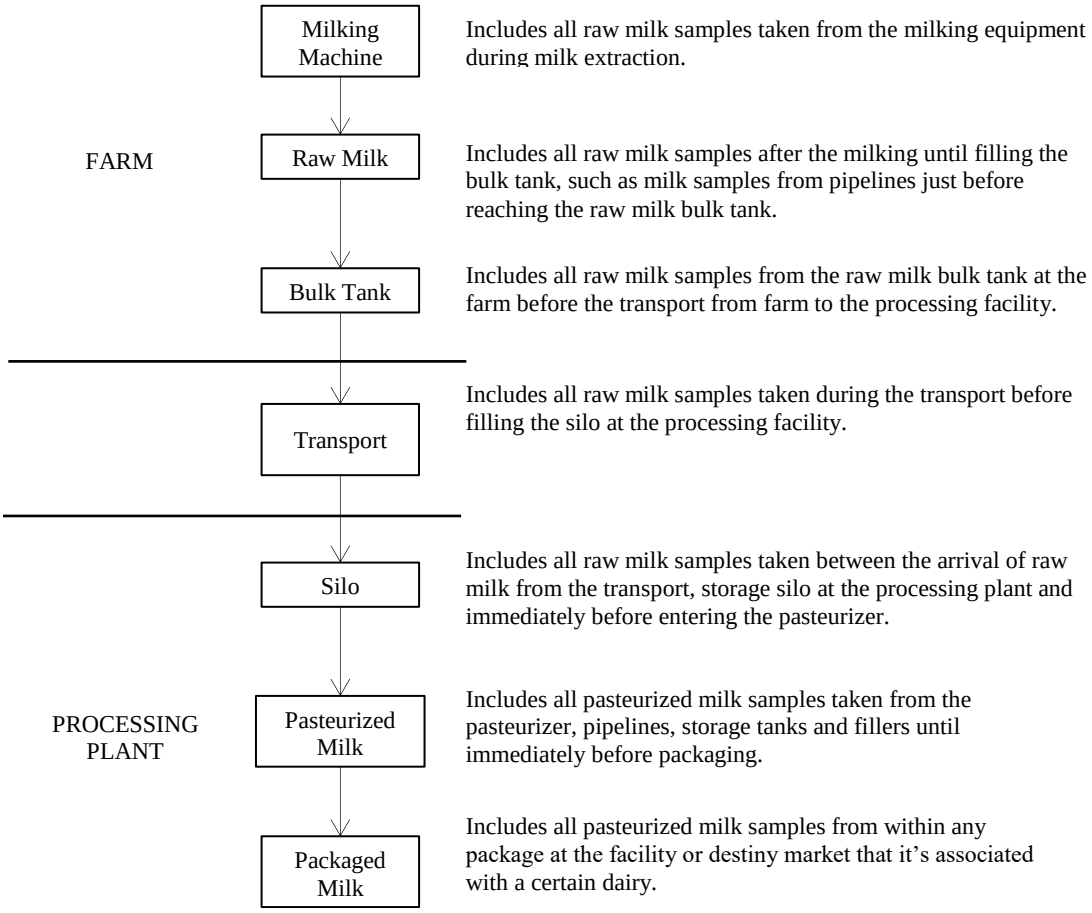

Figure 3.1. Flow chart of the standardized milk supply steps, with their coverage of samples described in the retrieved articles

2.5 Definitions

For the purpose of delivering straightforward and consistent discussion and conclusion, we propose the following definitions. A *citation* refers to a unique publication in which data from the primary research was collected, analyzed, and reported by the article

authors. Within a citation, data from multiple trials can be reported, which is referred to as a *study*. Multiple studies can be present in a single citation. Stating these differences is critical for the following descriptive and meta-analyses, which are based on the synthesis of studies within the same and also from different citations.

2.6 Data analysis

In spite of the large number of results and research in this topic, few studies were considered relevant to answer the research questions. The scarcity of statistical descriptors further limited qualification of selected articles for meta-analyses. Therefore, a descriptive approach was mainly used to analyze the data and informative plots were developed to describe the observed trends and data gaps. Dot plots, lattice plots and statistical descriptors such as minimum, maximum and quantiles were also obtained using the R statistical software package (Vienna, Austria).

A pooled estimate in each step is fundamental for data synthetizing studies. Due to the lack of statistical descriptors, specifically variance, we can't provide a pooled estimate of the concentrations. Nonetheless, we provided a weighted mean based on the sample size. Random effects Meta-analyses were conducted, when possible, for prevalence data to establish a proper combined estimate in each step. Random effects analysis, model selection and meta-regression analysis were performed in R 3.1.3 using the "Meta" and "Metafor" packages".

The Cochrane Collaboration defines the chi-squared test for heterogeneity (Q) as: "it assesses whether observed differences in results are compatible with chance alone". To quantify heterogeneity we used the I^2 statistic which is calculated as Higgins et al, 2003 proposes:

$$I^2 = 100\% * (Q - df)/Q \quad (1)$$

The purpose of meta-regression is to assess the impact of selected variables on the study effect size, in this case, prevalence and concentration. Figure 3.2 shows the model selection procedure. The model selection process and meta-regression analysis were conducted using a modified version of the method proposed by Islam, (Islam et al, 2014).

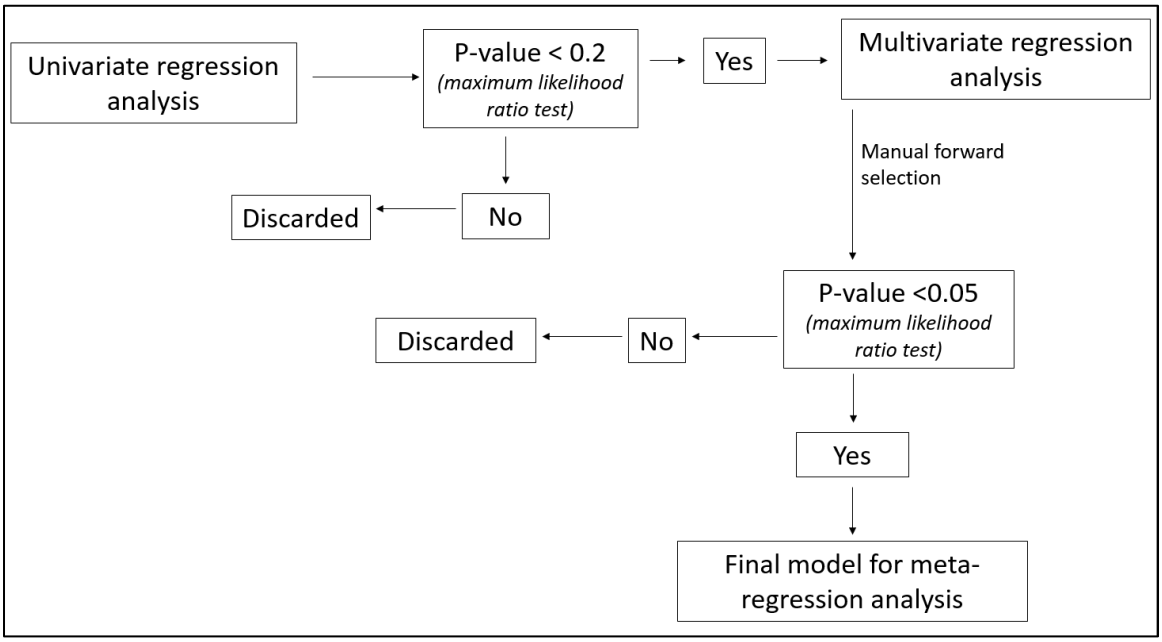

Figure 3.2. Model selection procedure

IV. RESULTS AND DISCUSSION

3.1 Systematic review process

Figure 3.3 summarizes the systematic review process conducted for this study. The search strategies retrieved 16,193 articles from six electronic databases. After deduplication, 8,553 unique articles remained for relevance screening. Of the 8,553 citations, 7,930 were excluded during the title and abstract screening because the articles

did not describe the primary research or were not deemed to be relevant based on the inclusion criteria that was pre-determined. Of the 623 articles that passed the title and abstract screening, another 503 articles were excluded either during or after full-text collection process. The articles were excluded because the full text was unavailable (89 articles) or did not pass the inclusion criteria (414 articles). Finally, 31 articles were deemed relevant and data was successfully extracted. Table 3.2 describes the data collected from each selected citation.

Table 3.2. Summary of the main characteristics of the citations that were included in the data extraction process.

Reference	Country	Production steps covered	Sample number	Cell stage	Spore-forming bacteria class/species	Analytical method	Concentration or prevalence
Buehner et al (2014)	United States	Raw milk – Bulk tank	738	Spores and cells	Thermophilic, Mesophilic and Total Spores. Thermophilic and thermoduric bacteria.	Spore count and Thermoduric bacteria count	Concentration
McAuley et al (2014)	Australia	Raw milk	15	Cells	*Bacillus cereus*	AS 5013.2-2007; Standards Australia 2007	Prevalence
Tabit et al (2011)	South Africa	Silo – Pasteurized milk – Packaged milk	Not available	Spores	*Bacillus sporothermodurans*	BHI agar plates	Concentration
Bartoszewicz et al (2008)	Poland	Silo – Pasteurized milk – Packaged milk	44	Spores	*Bacillus cereus*	Egg yolk precipitation on MYP medium	Concentration
Vissers et al (2007a)	Netherlands	Bulk tank	137	Spores	*Bacillus cereus*	Dutch standard 6875 (NEN-ISO, 1994)	Concentration
Vissers et al (2007b)	Netherlands	Raw milk	110	Spores	Mesophilic spores	Plate count milk agar	Concentration
Vissers et al (2007c)	Netherlands	Bulk tank	327	Spores	Butyric acid bacteria spores	Dutch Standard (NEN-ISO-6877, 1994)	Concentration
Magnusson et al (2007)	Sweden	Bulk tank	81	Spores	*Bacillus cereus*	Phase-contrast microscopy and plating on MYP agar	Concentration and Prevalence
Scheldeman et al (2005)	Belgium	Raw milk	18	Spores	Total spores	Milk plate count agar (Oxoid)	Concentration
Moussa-Boudjemaa et al (2004)	Algeria	Milking machine – Raw milk – Bulk tank	530	Spores	*Bacillus cereus*	AFNOR procedure	Prevalence
Hanus et al (2004)	Czech Republic	Bulk tank	70	Cells	*Bacillus licheniformis*, *Bacillus cereus*, Other bacilli and Total bacilli.	Standard ČSN ISO 7932	Concentration
Giffel et al (2002)	Netherlands	Bulk tank	25	Spores	Aerobic spores	PCMA	Concentration
Lukasova et al (2001)	Czech Republic	Raw milk – Bulk Tank	576	Cells	*Bacillus cereus* and Total Bacilli	MYP agar	Concentration and Prevalence
Eneroth et al (2001)	Sweden	Pasteurized milk – Packaged milk	168	Cells	*Bacillus cereus*	Blood agar plate	Concentration
Svensson et al (2000)	Norway/Sweden	Silo – Pasteurized milk	44	Cells	*Bacillus cereus*	Blood agar plate	Concentration and Prevalence
Svensson et al (1999)	Norway/Sweden	Silo – Pasteurized milk – Packaged milk	98	Cells and Spores	*Bacillus cereus*	MYP and blood agar	Concentration
Mayr et al (1999)	Germany	Packaged milk	Not available	Cells	Psychrotrophic *Bacillus* sp. and Mesophilic *Bacillus* sp.	API50CHB system	Concentration
Lin et al (1998)	Canada	Silo – Pasteurized milk – Packaged milk	232	Spores and Cells	*Bacillus cereus*	BHI plates	Concentration and Prevalence

Table 3.2. (Continuation) Summary of the main characteristics of the citations that were included in the data extraction process.

Reference	Country	Production steps covered	Sample number	Cell stage	Spore-forming bacteria class/species	Analytical method	Concentration or prevalence
Boor et al (1998)	United States	Raw milk	855	Spores	Mesophilic aerobic spores	BHI plates	Concentration
Slaghuis et al (1997)	Netherlands	Raw milk – Bulk tank	1318	Spores	Aerobic spores and *Bacillus cereus* spores	Aerobic Spore Count and Voges-Proskauer on Tryptic Soy Agar (TSA)	Concentration and Prevalence
Larsen et al (1997)	Denmark	Silo – Pasteurized milk	830	Spores and Cells	*Bacillus cereus*	Tryptose blood agar	Concentration and Prevalence
Giffel et al (1996)	Netherlands	Transport – Silo – Pasteurized milk – Packaged milk	388	Cells	*Bacillus cereus*	Voges-Proskauer on TSA	Prevalence
Christiansson et al (1996)	Sweden	Raw milk	144	Spores	*Bacillus cereus*	Blood agar plate	Concentration
Giffel et al (1995)	Netherlands	Raw milk	Not available	Cells	*Bacillus cereus*	Voges-Proskauer on TSA	Prevalence
Sutherland, A. D (1994)	Scotland	Milking machine - Bulk tank – Transport – Silo - Pasteurized milk	951	Spores	Aerobic psychrotrophic spores, Aerobic mesophilic spores,	Na+MnSO4	Concentration
Griffiths et al (1990)	Scotland	Bulk tank, Silo, Pasteurized milk	113	Spores	Psychrotrophic spores and *Bacillus* spp spores	Psychrotrophic spore colony count (PSC)	Concentration and Prevalence
Dasgupta, A (1989)	Australia	Bulk tank	Not available	Spores	Anaerobic spores and *C. tyrobutyricum*	RCM and RCM-lactate + LATA	Concentration
McKinnon et al (1983)	United Kingdom	Bulk tank – Transport – Silo – Pasteurized milk	126	Spores	Psychrotrophic spores and Total spores	Total spore count (TSC) and PSC	Concentration
Oterholm, B (1981)	Norway	Bulk tank	15480	Cells	Anaerobic sporeformers	Weinzirl method	Prevalence
Falkowski et al (1978)	Poland	Bulk tank – Pasteurized milk	300	Spores	Thermophilic streptomyces spores	Kosmachev media	Concentration
Saywell et al (1977)	New Zealand	Raw milk – Bulk tank	60	Spores	*C. tyrobutyricum* spores	RCM-L	Prevalence

3.2 Characteristics of the relevant citations and extracted data

Research described in the 31 citations were conducted worldwide, with the majority in Europe (23), North America (3) and Australia and New Zealand (3). The citations were published in a year range of 1977 to 2015. Samples sizes were very variable, from sizes down to 15 samples and up to 15480. The sample size depended largely on the duration

of the studies, which ranged from one week up to two years. In terms of data coverage, the number of citations covering each processing steps were: 2 (7%) on the Milking Machine, 13 (42%) on the Raw Milk, 17 (55%) on the Bulk Tank, 3 (10%) on the transport, 6 (19%) on the Silo, 11 (35%) on the Pasteurized Milk and 7 (23%) on the Packaged Milk.

Overall, concentration data are more abundant compared to prevalence data. As shown in Table 3.3 and Table 3.4, spores concentrations at the standardized steps were reported and synthesized, ranging from 11 to 161. For both prevalence and concentration data, the results vary considerably within and between the processing steps (Tables 3.3 and 3.4). The more extreme cases are spores for concentration data, especially in the Silo, Pasteurized Milk and Packaged Milk.

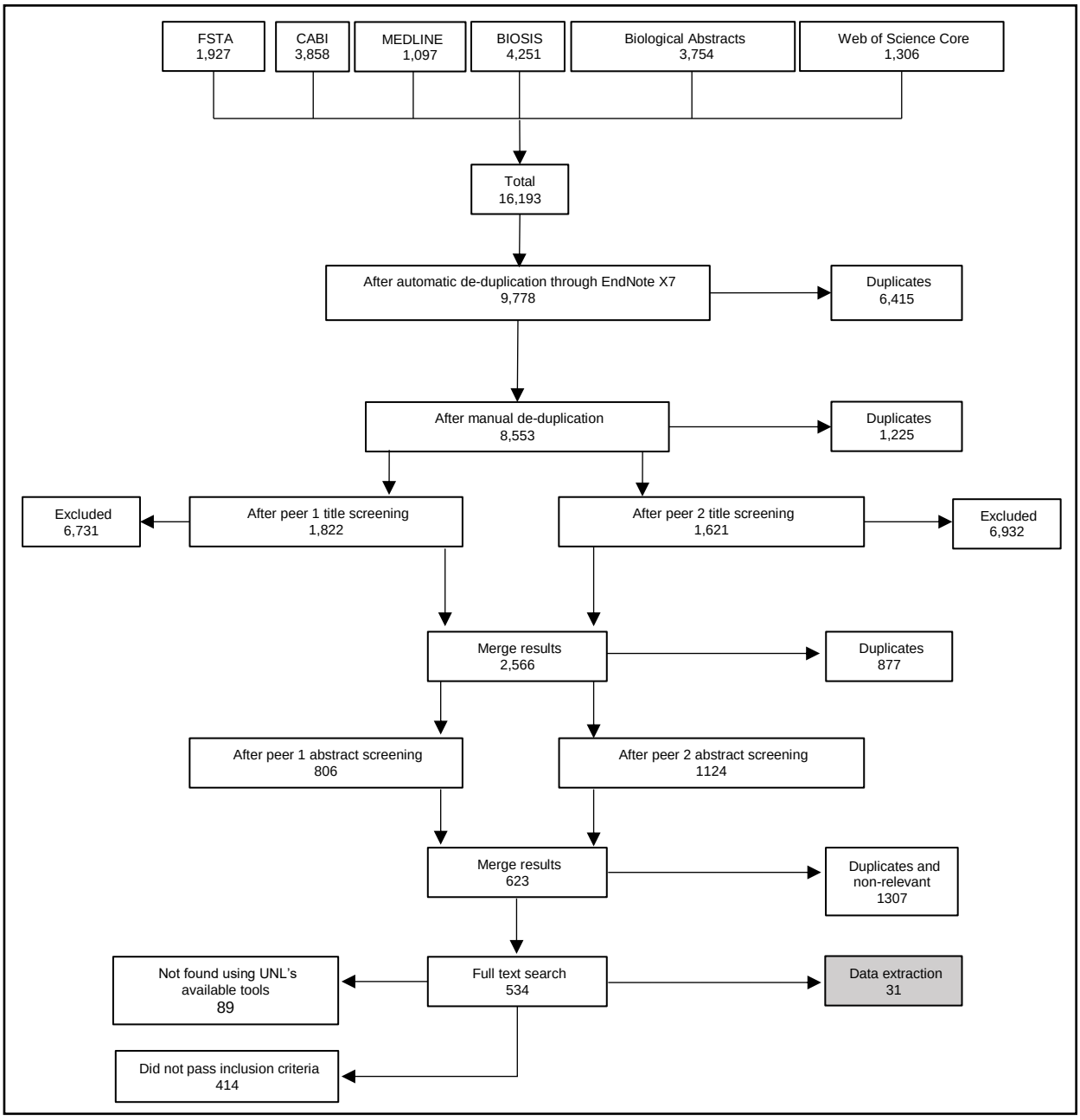

Figure 3.3. Process flow of studies being retrieved, screened, appraised, selected, data-extracted in this systematic review and meta-analysis

Table 3.3 Summary statistics of concentration data by Standardized Supply Chain (log CFU/ml)

Supply Chain Step	Number of data points		Minimum		1st Quantile		Median		Mean		3rd Quantile		Maximum	
	Spores	**Cells**	**Spores**	**Cells**	**Spores**	**Cells**	**Spores**	**Cells**	**Spores**	**Cells**	**Spores**	**Cells**	**Spores**	**Cells**
Milking machine	64	NA	-2.3	NA	-0.1	NA	0.55	NA	0.58	NA	1.58	NA	6.11	NA
Raw milk	26	3	-1.39	1.4	1.49	1.82	1.73	2.24	1.59	2.16	1.9	2.54	3.74	2.84
Bulk tank	161	10	-2.3	0.91	-0.1	0.99	0.39	1.21	0.53	1.54	1.23	2.06	6.23	2.76
Transport	65	NA	-2.3	NA	1.03	NA	2.01	NA	1.88	NA	2.45	NA	7	NA
Silo	92	6	-2.3	-2	-0.16	-1.93	1.38	-1.58	1.5	-0.66	2.38	0.74	7	1.7
Pasteurized milk	89	60	-2.3	1	0.67	1	1.98	1	1.86	1.97	2.38	2.62	7	5.7
Packaged milk	11	64	-1.4	-1.3	-1.35	1	-1.22	1.5	0.41	1.92	-0.04	2.54	6.74	6.7

Table 3.4. Summary statistics of prevalence data by Standardized Supply Chain (% of positives)

Supply Chain Step	Number of data points		Minimum		1st Quantile		Median		Mean		3rd Quantile		Maximum	
	Spores	Cells	Spores	Cells	Spores	Cells	Spores	Cells	Spores	Cells	Spores	Cells	Spores	Cells
Milking machine	1	NA	26.32	NA	26.32	NA	26.32	NA	26.32	NA	26.32	NA	26.32	NA
Raw milk	13	8	0	0	10	18.25	15	23.5	23.13	23.54	40	31.25	53	40
Bulk tank	11	13	3	12	12.09	25	20	34	33.43	36.36	59	50	100	57
Transport	NA	1	NA	35	NA	35	NA	35	NA	35	NA	35	NA	35
Silo	4	5	80	7	81.5	10	83.5	25.22	85	22.44	87	35	93	35
Pasteurized milk	4	7	76	55	82.75	56	89.5	61	87.25	61.86	94	67	94	71
Packaged milk	2	1	90	71	91.5	71	93	71	93	71	94.5	71	96	71

3.3 Concentration of spore-forming bacteria along the milk supply chain

Concentration data was the most abundant in the selected studies with 582 data points extracted from the publications. Figure 3.4 shows the distribution of concentration for both the vegetative cell stage and spores. Table 3.5 shows a summary of the pooled concentration estimates in each step.

Table 3.5. Concentration pooled estimates for each processing step, ND = No data available

Step	Cells (log cfu/ml)	Spores (log cfu/ml)
Milking machine	ND	0.58
Raw milk	2.34	1.34
Bulk Tank	2.35	0.43
Transport	ND	1.67
Silo	0.06	1.59
Pasteurized milk	2.00	2.44
Packaged milk	2.65	3.30

As shown in Figure 3.4, the overall trend of weighted average keeps relatively stable for concentration of both cells and spores. The concentration of spores remains stable between 0-2.5 logs until milk is packaged, where we can see an increase in dispersed data. The great heterogeneity of concentration data of spore-forming bacteria at the step of Packaged Milk can be due to the fact that the studies that reported these data points are very different in the study design, season, location and methods of estimating the concentrations. For example, in the study from Lin and colleagues (Lin et al, 1998), enrichment at 80˚C for 14 days was conducted before counting, whereas Bartoszewicz

and colleagues (Bartoszewicz et al, 2008) enriched the sample only for 48 hours at 25˚C. Differences in methodologies are one of the major issues to overcome when pooling the data together and providing meaningful critical review of the results.

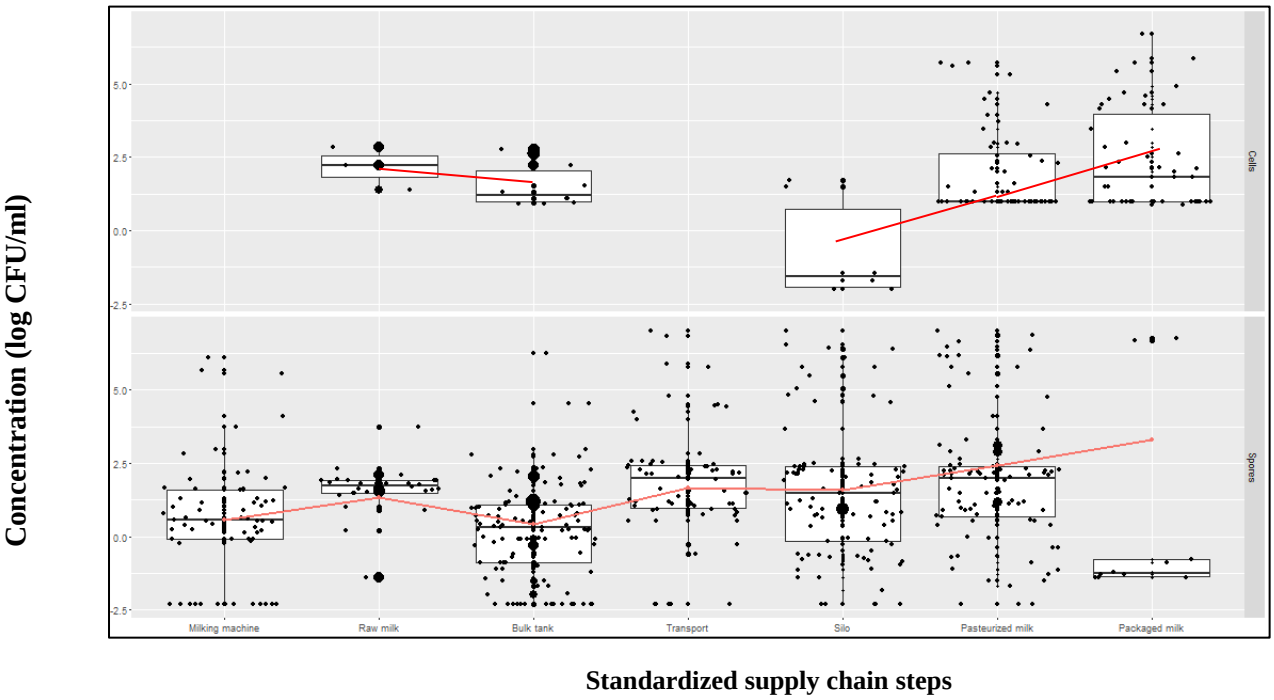

Figure 3.4. Stacked box plot for the concentration of spore-forming bacteria throughout the milk processing chain. The top chart gives information for vegetative cells and the chart below for spores. The red line represents the weighted mean. The dot size corresponds to the sample size associated to a particular dataset. The spread of the dots corresponds to "jittering" to avoid excessive overlapping and improve visualization.

After pasteurization, spores stay somewhat stable but cells increase dramatically. This is intuitive as it is commonly known that the vegetative cells do not survive a pasteurization process, but spores will germinate as a result of a thermal shock. Nonetheless, there are only eleven data points contributing to the Silo stage in cell concentration, as opposed to

60 and 64 for pasteurized and packaged milk (Table 3.3), which in turn have more consistent datasets.

Raw milk and Bulk tank counts of cells are within the same range of 1.5-2.5 logs but with no data available in the Milking machine and the Transport which are the previous and following steps, respectively. These data fit well with the previously described concentration ranges in Pasteurized and Packaged milk.

As aforementioned, concentration across steps remain stable, which is either because contamination entry points are limited to the farm mostly, such as teat contamination (McKinnon, 1982), or because modern control procedures are moderately effective or both. More data is needed in the packaged milk step particularly to study the fate of these spores.

3.4 Prevalence of spore-forming bacteria along the milk supply chain

Prevalence data were scarce compared to concentration, with 70 data points, especially in certain processing steps such as Milking Machine and Transport, where one data point or less was available. It is noteworthy that a significant portion of studies were focused in the Bulk Tank, both for spores and cells, with pooled sample sizes of 15492 and 848. As shown in Figure 3.5, prevalence data have more data gaps which makes the analysis more difficult to conduct, but it is shown that prevalence of spore-forming bacteria is increasing as milk moves from the farm to the processing Plant.

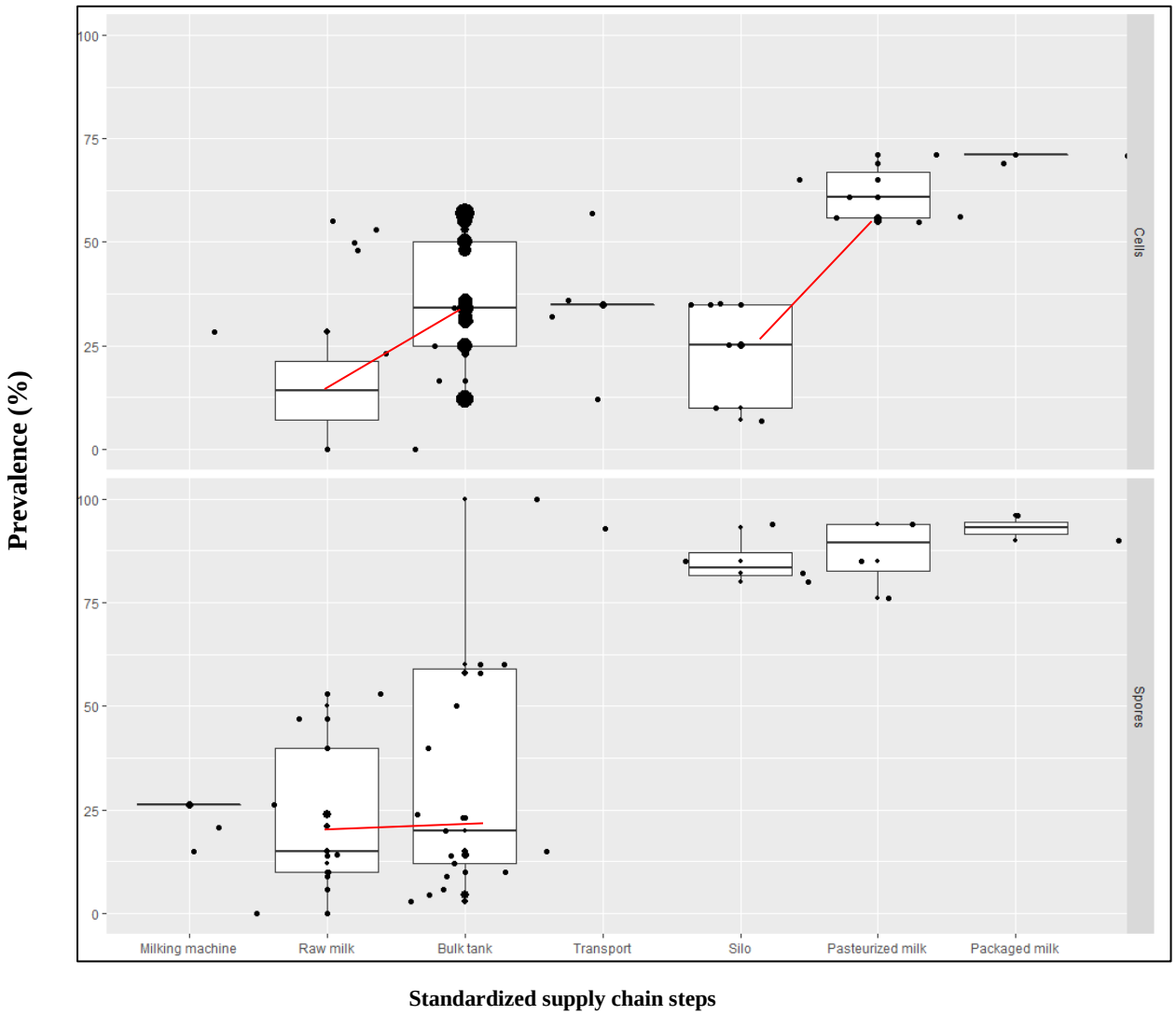

Figure 3.5. Stacked box plot for prevalence of spore-forming bacteria throughout the milk processing chain. The top chart gives information for vegetative cells and the chart below for spores. Red lines show the pooled estimates from random effects analyses. The spread of the dots correspond to "jittering" to avoid excessive overlapping and improve visualization. When there are no red lines but data points available, there is no sample size available to provide an estimate.

Figures 3.6a-3.6d show individual study trends for both concentration and prevalence. While trying to detect individual trends that would be otherwise hidden in the summary charts on Figure 3.4 and Figure 3.5, we found out that before Transport, the individual study trend indicates a stable prevalence and concentration, and in some cases a slight reduction in prevalence. After the Transport, the individual study trends seems to be stable but with a moderate increase. Although the trend is not dramatically increasing, it is certainly shedding light on where the industry should focus their efforts to control the growth and proliferation of spore-forming bacteria. As seen in Figure 3.5, within the Processing Plant (after Transport) there are significant chances that spores and cells may eventually rise, so even if concentration and prevalence might seem to be under control, the results of the present Systematic Review suggest that the focus should be set before and after pasteurization.

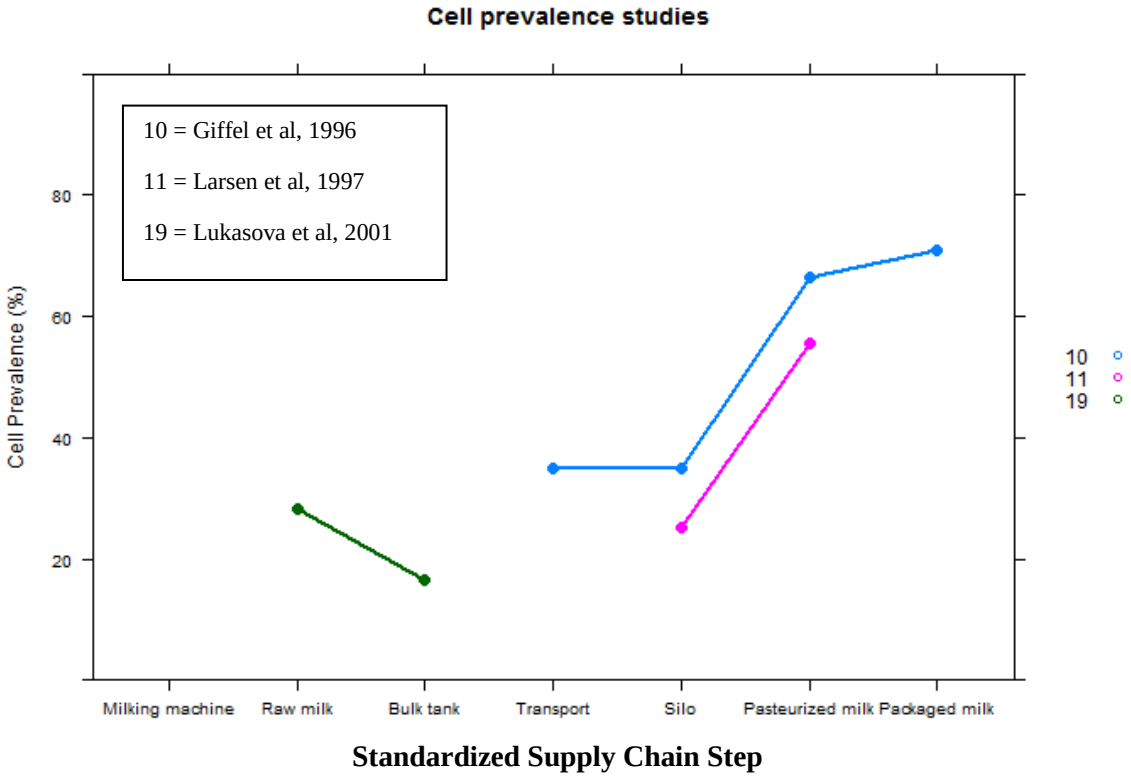

Figure 3.6a. Plot of the trends for individual studies reporting prevalence in cells.

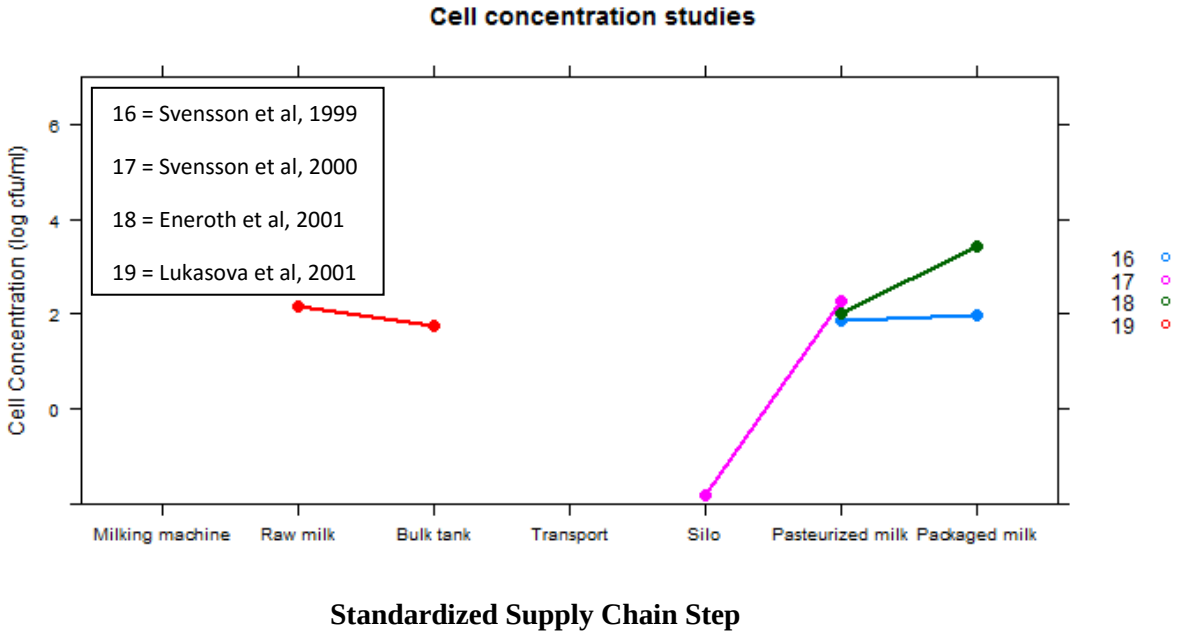

Figure 3.6b. Plot of the trends for individual studies reporting concentrations in cells.

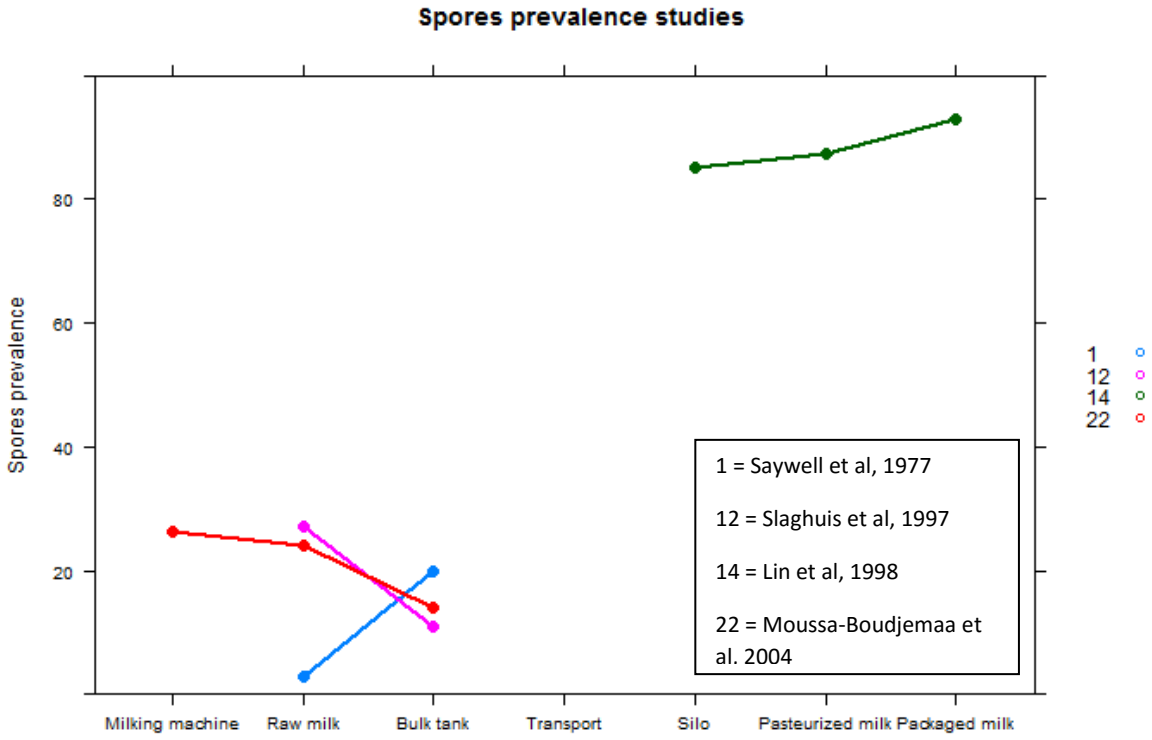

Figure 3.6c. Plot of the trends for individual studies reporting spore prevalence.

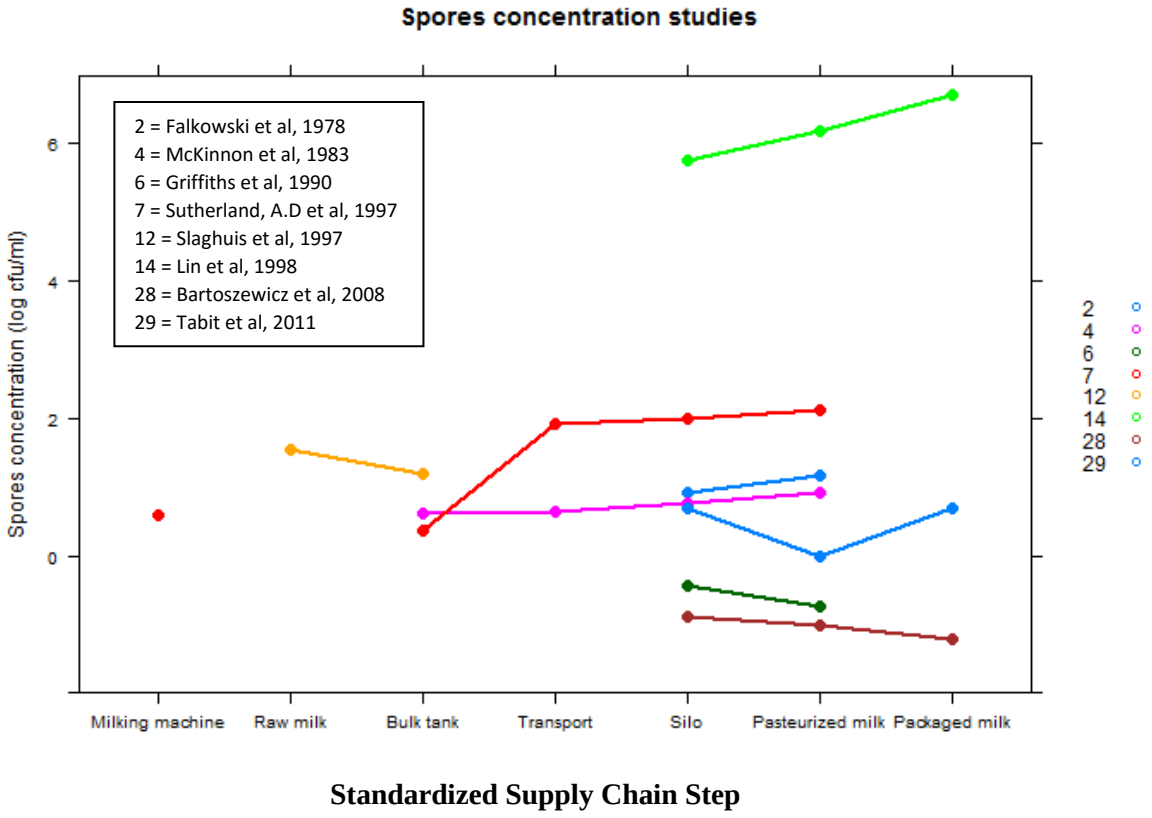

Figure 3.6d. Plot of the trends for individual studies reporting spore concentration.

In Figure 3.6d, the data reported from the Silo-Pasteurized Milk-Packaged Milk steps is variable and shows different trends. Lin et al (1998) results indicate a high spore concentration of about 6 logs cfu/ml and continuously increasing along the supply chain. On the other hand, Falkowski et al (1978), Tabit et (2011), Bartoszewicz et al (2008) and Griffiths et al (1990) indicate a considerable lower concentration, of about 0.5 logs and that is decreasing. The variability of this data has multiple reasons: detection method, season and location of the study among others.

3.4.1 *Meta-analysis for prevalence*

Random effects meta-analysis was conducted to estimate pooled prevalence through the use of the meta() package in R. Figure 3.7 and Figure 3.8 shows several forests plots for the prevalence data. For meta-analysis, data points without sample size reported were discarded. Between study variance (tau squared) was always significant (P-value<0.1) so random effects estimates where used, except in the cell prevalence in the Silo. To estimate pooled prevalence estimates, sample size is needed and very often it was not provided in the studies. Nonetheless, Table 3.6 shows the estimated prevalence when sample size is available. Modern meta-analyses procedures takes into account within and between study variability, so these estimates are much more powerful than normal average estimates. For the last three steps, although there are enough data to provide a pooled estimate, sample sizes are missing. In all cases but the Silo prevalence, Heterogeneity was estimated to be extremely high, so we conducted meta-regression analysis to look for the sources and propose a regression model that accounts for the most heterogeneity possible.

Table 3.6. Prevalence estimates pooled by random effect meta-analysis model for each supply chain step, ND = No data available

Step	Cells (%)	95% Confidence Interval	Spores (%)	95% Confidence Interval
Milking machine	ND	ND	ND	ND
Raw milk	14	2-63	23	16-32
Bulk Tank	36	28-45	23	11-42
Transport	ND	ND	ND	ND
Silo	33	21-49	ND	ND
Pasteurized milk	58	54-62	ND	ND
Packaged milk	ND	ND	ND	ND

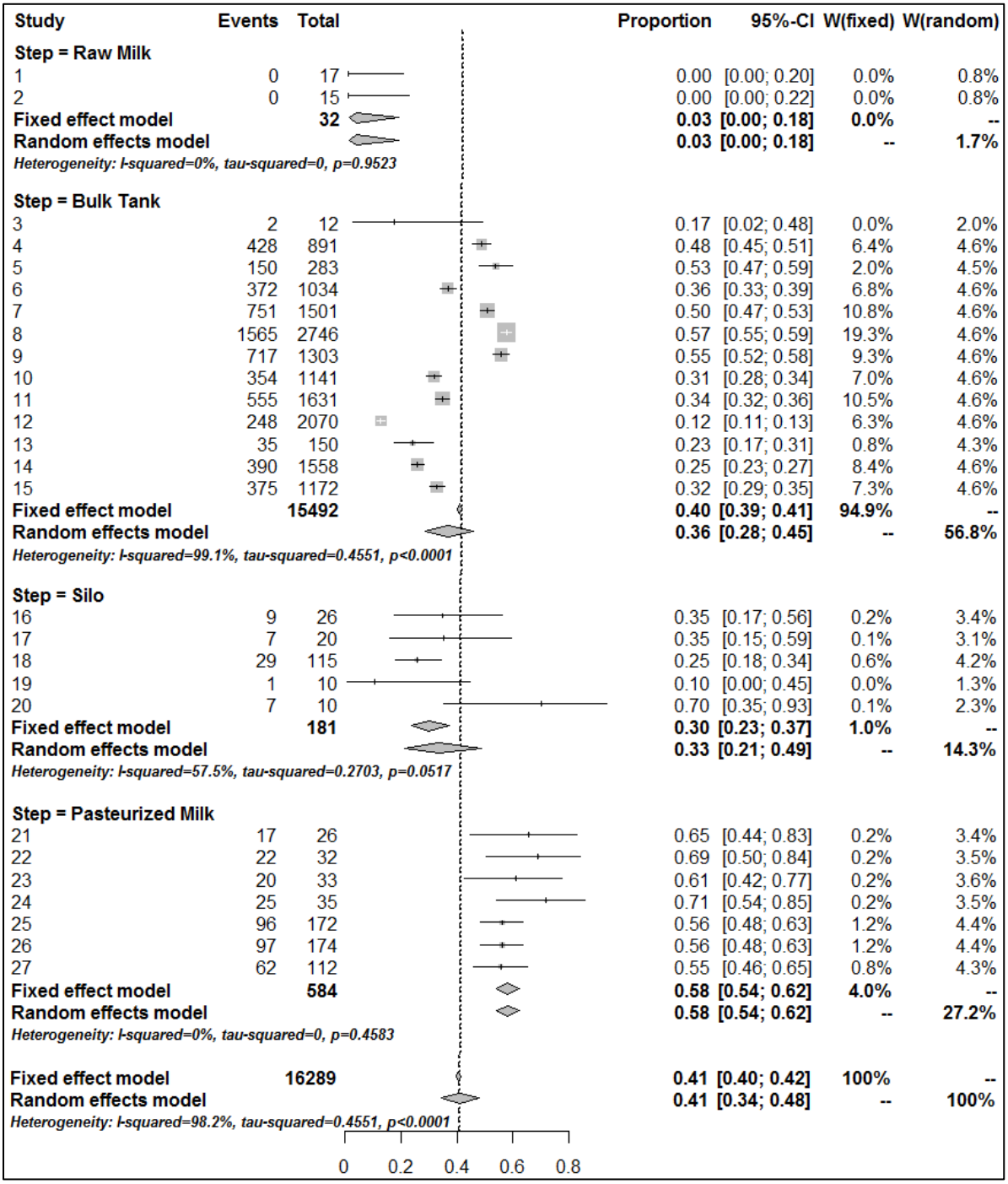

Figure 3.7 Forest plot of reported cell prevalence. Study refers to "Study" definition in section 2.5. Studies can come from the same Citation or different. The vertical dashed lines represent the estimates for the Fixed and Random effects models.

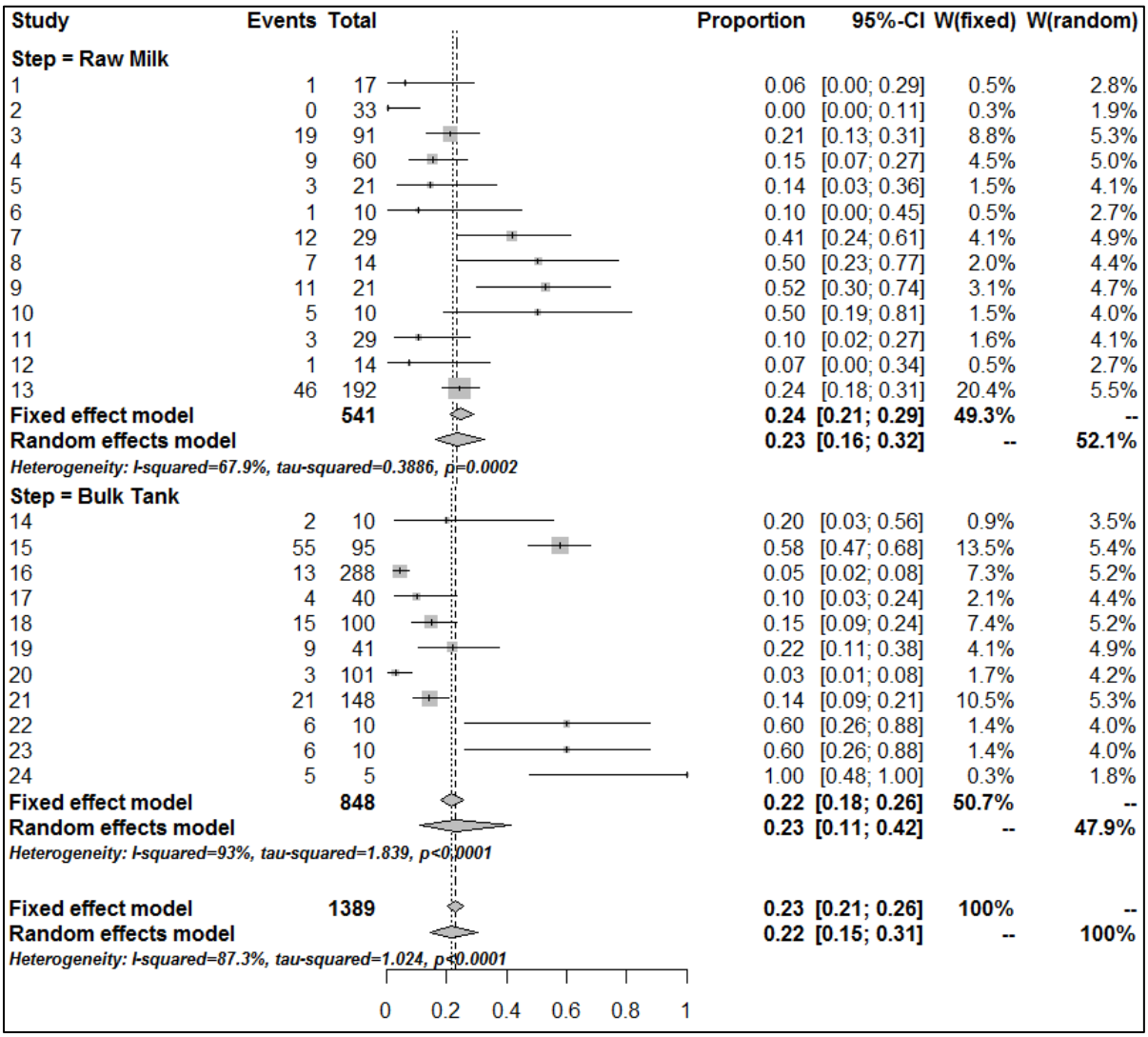

Figure 3.8. Forest plot of reported spore prevalence. Study refers to "Study" definition in section 2.5. Studies can come from the same Citation or different. The vertical dashed lines represent the estimates for the Fixed and Random effects models.

3.4.2 Meta-regression analysis

Sources of heterogeneity can be detected through the use of this approach. The variables identified in this study were: Location of the study, year of publication (clustered in a 10

year range), season when the study was conducted, type of bacteria detected, the step in the processing chain where the sample was taken and the detection method used.

For both cells and spores, the final model was Season, Step and Year of publication. In cells, the meta-regression model explained 65.71% of heterogeneity, while in the spores was 35.11%.

Seasonality has been reported as a critical factor in the variation of spore-forming bacteria populations (Sutherland et al, 1994), whether it is increasing or decreasing, the general consensus is that the season is a major force driving spore-forming bacteria population along the milk chain. The step where the sample was taken is also relevant as very different characteristics are present in different steps. Finally, Year of publication is also critical as sampling plans and detection methods are being updated and perfected along the years, generating significantly different results.

All these three moderators were expected to be relevant in the meta-regression analysis but the detection method was a variable that would not be deemed as explaining heterogeneity. This could be based on the fact that it is closely linked to the publication year.

3.4.3 Significance for regulators and evidence-based policies.

Systematic Review is readily usable by Governments as it is a structured process and it is recognized world-wide as a powerful tool to synthetize data. Although it requires some statistical expertise and is time-consuming, it can be done successfully by looking at

online resources and seizing the opportunities of creating strategic collaborations with the Academia.

The outputs of the Systematic Review act as a source of evidence for policy makers and also feed the Risk Assessment data gaps.

V. CONCLUSIONS

This study is the first systematic review in this field to our knowledge. Holistic understanding of food processing systems is fundamental to provide bias free conclusions and when proposing more focus on certain interventions or processing steps. Although the outputs of a systematic review of this type is not investigating specific interventions or practices in the dairy industry, it does give useful insight for researchers, policy makers and the industry itself about where are the potential issues for controlling spore-forming bacteria and evidently where the current system seems to be working well, in order to refocus resources where needed.

In this particular Systematic Review project, the conclusions in relation to this thesis are:

1. There is a critical need for more research in this topic, especially in the steps where no or very scarce data are available, such as Milking Machine, Raw Milk, Bulk tank milk and Transport for cell concentration and Milking Machine, Raw milk, Transport and Packaged milk for prevalence in both cells and spores. Not only are more data needed, but also data with quantified variability.

2. Prevalence meta-regression analysis indicates that Year of Publication, Season and Step are the moderators explaining 65.71% of heterogeneity in cells and 35.11% in

spores. There is still a significant amount of heterogeneity yet to be explained. We believe that in the first place, more data is needed in the steps where little information is available and also to explore new variables such as Detection Limits, and Sampling Plans. Regarding concentrations, more statistical descriptors are needed in the publications retrieved to provide a pooled estimate for each step.

3. These results are very useful for establishing performance objectives, which provide the dairy industry solid and easy to establish metrics to add another layer of assurance of quality to their products. Performance objective is a term borrowed from food safety sciences, which refers to a specific level that must be met in earlier steps in the food chain to comply with a Food Safety Objective, which in turn consists of the "maximum frequency and/or concentration of a hazard in a food at the time of consumption" (IMCSF, 2006). These metrics can be easily converted to food quality levels that must be met, for example, not to surpass a certain threshold, which was proposed using data from this present study.

4. To fully harness the potential of data synthesis technologies such as SR, it is highly recommended for developing countries to form Government-Academia collaborations. Academics usually have the resources and expertise but lack the data, which Governments can provide by consulting their surveillance or regulation compliance control systems. Governments in turn benefit from acquiring evidence to support their decision making process that was created using high quality, robust and non-biased methods to synthetize data.

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SUMMARY AND FUTURE DIRECTION

In this thesis, two cases studies were performed to understand how can two commonly used research tools such as Risk Assessment and Systematic Review in food safety can feed the decision making process, in developing countries were technology and research itself is as not as developed compared to the United States or the European Union.

To show the application of risk assessment in food safety regulatory decision making procedure, a collaborative project with the Chilean Food Quality and Safety Agency to assess the risks of raspberry production of Chilean farmers was conducted. Regarding the Systematic Review application in agri-food field, it was demonstrated through a case study of evaluating the contamination of spore-forming bacteria along the milk supply chain, which can be extended to address food safety questions of other hazard-food pairs. For example, the systematic review approach can be used to fill up the data gaps and further improve the risk assessment model of the microbial contamination in Chilean raspberry products by reducing the parameter uncertainty involved. On the other hand, Risk Assessment can tell the Agencies which are the production steps that needs improvements and focused allocation of resources or new regulations. It also indicates in a visual and simple way which are the main factors who are driving the risk along a certain process flow. The ability to evaluate scenarios and interventions *in-silico* gives Governments unprecedented opportunities to have a wide arrange of scientifically assessed recommendations and potential interventions to improve whatever process is being assessed, without the need of experimentation, field trials or further data collection.

The focus of this thesis was set in the method investigation and demonstration, and how a non-scientific stakeholder can benefit from the results of these high-end scientific procedures. Both activities delivered simple to understand and sound evidence, although they were performed under strict scientific procedures and state-of-art knowledge. The collaboration between Academia and Government was fundamental in achieving these accomplishments, since it harness the comparative advantages of each party, creating synergies and successfully delivering evidence that is ready to be used for regulation and policy making.

This thesis can serve as the basis of several different projects, for example, turning Risk Analysis and Systematic Reviews procedures presented in this thesis into guidelines for developing countries on how to conduct these processes. For this purpose, it is very important to design it in collaboration with the Government Agencies as they know their limitations and the best way to convey these topics to their target audiences.

This successful experience can be replicated in other developing countries, specifically making Chile a strategical center of training in creating these collaborations and how to bridge the gap between science and policy in developing countries.

ANNEX I

STUDY SURVEY

FARMS

Region: _____

Municipality: _____

Location: _____

Geographical Coordinates (WGS 84) X: _____ Y: _____

0.a) Farm size: _____ ha

0.b) Average production: _____ kg/season

Mark with an x the way you trade your raspberries:

0.c) Collection Center ☐ Sells to intermediary ☐ Direct sale to packing ☐ Local sells ☐

Please answer this questions in the simplest way possible. If you don't have detailed information, please provide a simple estimate.

1. IRRIGATION PRACTICES

1.a) What irrigation type you use?

Drip ☐ Surface ☐ Furrow ☐ Other ☐

Other: _____

1.b) During the growth of the fruits, how often you irrigate?

Daily ☐ _____ per week ☐ Other ☐

Other:_____

1.c) How many times a day?

____ times a day

Other:_____

1.d) How much water you use per irrigation event? (Approximate flow).

_____ per hectare ☐ farm total ☐

1.e) What is the source of the irrigation water?

Well ☐ Dike ☐ Ferris ☐ Deep well ☐ Other ☐

Other: _____

2. PESTICIDE APPLICATIONS

How many times and how often does pesticides had been applied during the flowering and fruit formation during the present season? (If possible, provide a simple description on pesticide application)

2.a.1) Number of applications: _____

2.a.2) Time between applications: ____ days

2.b) What type of water you use for pesticide applications?

Well ☐ Dike ☐ Ferris ☐ Deep well ☐ Potable ☐ Other ☐

Other: _____

2.c) What type of pesticide application system you use?

Pulverize ☐ Knapsack sprayer ☐ Nebulizer ☐ Dredger ☐ Other ☐

Other: _____

Please indicate the type of pesticide and the amount used (pesticide + water) in the farm **per application.**

Pesticide name	**Active Ingredient**	**Liter/Application**
2.d.1)	2.d.2)	2.d.3)

2.e) How much times goes by between the last application and the harvest? (withholding period)

_____ days.

3. SOIL AMENDMENTS PRACTICES

Do you apply any soil amendment procedure?

3.a.1)Yes ☐ No ☐

If yes, what type?
3.a.2) Compost ☐ Sludge ☐ Manure ☐ Other ☐

Other: _____

3.b) When and how often are these procedures applied?

_____

3.c) How much do you apply? (kg per hectare, per farm o any information available)

_____

3.d) How many days goes by between application and harvest?

_____ days

4. HARVEST PRACTICES

4.a) What kind of personal security/hygiene equipment are used in the harvest?
Safety footwear ☐ Gloves ☐ Apron ☐ Mask ☐

4.b) During the current season, have any worker been absent for diseases?

Yes ☐ No ☐

4.c) If yes, for how long? (average)

____ days

4.d) Does any of these diseases had been food poisoning, diarrhea or vomit?

Yes ☐ No ☐

Before the harvest, are the trays meant for the harvest:

Washed?
4.e.1) Yes ☐ 4.e.2) With potable water ☐ Non- potable water ☐
No ☐

Disinfected?
4.e.3) Yes ☐ 4.e.4) Indicate chemical:_____
No ☐

5. ANIMAL PRESENCE IN THE FARM

5.a) Have you detected the presence of animals (mammals or birds) on the farm?
Yes ☐ No ☐

5.b) What type of animals?

Domestic mammals or birds ☐ Wild mammals or birds ☐

5.c) Do these animals come in direct contact with the fruits?

Yes ☐ No ☐

5.d) How often does the latter happen?

Daily ☐ Weekly ☐ Monthly ☐

5.e) Have you seen animal waste in direct contact with the fruits of harvest equipment?
Yes ☐ No ☐

5.f) How often does the latter happen?

Daily ☐ Weekly ☐ Monthly ☐

6. FARM TO PACKING TRANSPORT

6.a) How long does it take from the Collecting Center or Farm to the Processing Plant?

_____ hours ☐ minutes ☐

6.b) At which temperature are the raspberries usually transported?

_____ °C No refrigeration ☐

6.c) The shipment is carried:

Closed wagon ☐ Covered with loom ☐ Covered with raschel mesh ☐
Just tied without mesh or loom ☐ Other ☐

Other: _____

ANNEX II

STUDY SURVEY

1. COLLECTION CENTERS

Region: _____

Municipality: _____

Location: _____

Geographical Coordinates (WGS 84) X: _____ Y: _____

Infrastructure:

Type of ceiling _____

Type of floor _____

Type of walls _____

Closed ☐ Open ☐ (with or without access doors?)

0.a) Is it located alongside a raspberry farm Yes ☐ No ☐

0.b) Is it located in a location with no raspberry farm Yes ☐ No ☐

0.c) Average number of farmers that collects here by season _____

1.a) For the raspberries that come from a farm, how much time in average stays in the Collecting Center?

_____ days ☐ hours ☐

1.b) Is the same tray used in the harvest used in the Collection Center?

Yes ☐ No ☐

1.c) What is the average temperature of the Collection Center?

_____ °C

1.d) What is the storage capacity of the Collection Center? (Indicate the number of trays and average weight of the tray with raspberries)

_____ trays _____ grams ☐ kilograms ☐

1.e) Is there any ventilation system? If yes, which type?

Yes ☐ No ☐

Type: _____

1.f) Have you ever detected the presence of animals (mammals or birds)?
Yes ☐ No ☐

1.g) What kind of animals?

Domestic mammals or birds ☐ Wild mammals or birds ☐ Pests ☐

1.h) Does this animals take direct contact with the fruits?
Yes ☐ No ☐

1.i) How often does the previous happen?

Daily ☐ Weekly ☐ Monthly ☐

1.j) Only answer this if the collected fruit comes from **different** farmers:

The fruit from different farmers is stored in different places?

1.j.1) Yes ☐ No ☐
1.j.2) In pallets ☐ Directly on the ground ☐ Other ☐

1.j.b) Is there a label that identifies the farm source on the trays?

Yes ☐ No ☐

1.j.c) Is there a label that identifies the farm source on the pallets?

Yes ☐ No ☐

2. TRANSPORT FROM COLLECTION CENTER TO PACKING OR PROCESSING CENTER

2.a) How long does it takes the transport from the Collection Center to the Processing Plant or Packing Facility?

_____ minutes ☐ hours ☐

2.b) What it the temperature in this process?

_____ °C No refrigeration ☐

2.c) Describe the transportation process:

Closed wagon ☐ Covered with canvas (or similar) ☐ Covered with raschel mesh ☐ Only tied and no cover ☐ Other ☐

Other: _____

ANNEX III

STUDY SURVEY

EXPORT PACKING

Region: _____

Municipality: _____

Location: _____

Geographical Coordinates (WGS 84) X: _____ Y: _____

1. RASPBERRIES RECEIVING

1.a) The place is:
Open ☐ Closed ☐ Under a ceiling ☐

1.b) The wait time is around (in minutes):
1.b.1) Max ____ 1.b.2) Min _____ 1.b.3) Average ___

1.c) Average temperature in unloading place: _____°C

2. FIRST COLD CHAMBER

2.a) Target temperature is:

_____ °C

2.b) Time needed to reach target temperature:

_____ minutes ☐ hours ☐

2.c) Time that the fruits stays here?

_____ hours ☐ days ☐

3. OPERATIONS

3.a) How many shifts? (even if they work with different fruits)

_____ shifts

3.b) How long does the shifts lasts?

_____ hours

3.c) What is the temperature inside the Packing area? (Temperature records)

3.c.1) Max ____ 3.c.2) Min _____ 3.c.3) Average _____

3.d) Generally, how long does it takes since the fruit exits the cold chamber and goes through the first selection and goes into the freeze chamber?

_____ minutes

4. SANITATION

4.a) Do you conduct any Sanitation procedure?

Yes ☐ No ☐

4.b) How often you conduct these procedures?

Infrastructure/Equipment	**Routine cleaning**	**Deep cleaning**
Steel tabletops	4.1)	4.15)
Conveyor belt	4.2)	4.16)
Calibrators	4.3)	4.17)
Bins	4.4)	4.18)
Boxes transporter truck	4.5)	4.19)
Metal detector	4.6)	4.20)
Hands washing station	4.7)	4.21)
Precooling tunnel	4.8)	4.22)
Static tunnel	4.9)	4.23)
IQF Frost tunnel	4.10)	4.24)
Tray washer	4.11)	4.25)
Wash tub	4.12)	4.26)
Walls and ceiling	4.13)	4.27)
Trash bins	4.14)	4.28)

4.c) Washing
4.c.1) Type of soap used: _____
4.c.2) Dilution used: _____

4.d) Disinfection
4.d.1) Name of chemical used: _____
4.d.2) Concentration used: _____

4.e) Type of personal protection and/or hygiene that workers use.
Safety footwear ☐ Gloves ☐ Apron ☐ Mask ☐ Hat ☐ PVC apron ☐

4.f) How often are the work clothes changed?
_____

4.g) Do workers change clothes when the shift starts/end?
_____

4.h) During the current season: How many workers had shown symptoms related to a possible foodborne illness, such as diarrhea?

_____ workers

4.i) Do you conduct a hands sampling procedure to look for fecal coliforms and pathogens?
(If yes, please describe shortly the procedure, if it is done to all the personnel or only some. Please describe the criteria that selects who is going to be sampled)

Yes ☐ No ☐

4.i.1) If yes, please describe as requested:

_____
_____
_____
_____

5. FREEZING PRACTICES

5.a) Target freezing temperature?

_____ °C

5.b) How much time is needed to reach the target temperature?
_____ minutes ☐ hours ☐

5.c) How long does the fruits stay here?

_____ hours ☐ days ☐

6. ENVIRONMENT

6.a) Is there any other sampling plan in the Process or Packing plants? (surface contact materials and other surfaces for example)

Yes ☐ No ☐

6.a.1) If yes, please describe briefly:

_____

_____

7. TRANSPORT

7.a) Is there any disinfection and/or cleaning procedure applied to the trucks or cold chambers, before loading?

7.a.1) Cleaning Yes ☐ No ☐
7.a.2) Disinfection Yes ☐ No ☐

7.b) Temperature of the loading room.

_____ °C

7.c) Temperature of the cooling truck during transport

_____ °C

7.d) Time taken to destination.

7.d.1) Minimum
_____ hours ☐ days ☐

7.d.2) Average
_____ hours ☐ days ☐

7.d.3) Maximum
_____ hours ☐ days ☐

ANNEX IV: Expert Elicitation data spreadsheet.

Data requested	Type of data	Explanation	Microorganism	PREVALENCE		Distribution	Parameter 1	Value
				Positives	Total			
Example 1	*Distribution*	*Hepatitis A distribution in water used for raspberry irrigation*	*Hepatitis A*	-	-	*Gamma*	*Alfa*	*0.084*
Example 2	*Occurrence*	*Time between last application and harvest*	-	-	-	*Laplace*	*Mean*	*120*
Example 3	*Distribution*	*E. coli concentration for groundwater in Chile*	*E. coli*	-	-	*Pert*	-	-
Example 4	*Prevalence*	*E. coli prevalence in harvester's hands*	*E. coli*	*6*	*40*	-	-	-
Contamination due to soil amendments	Prevalence	Microbiological prevalence in manure	*E. coli* or coliforms					
			Hepatitis A or norovirus					
	Distribution	Microbiological distribution in manure	*E. coli* or coliforms					
			Hepatitis A or norovirus					
	Occurrence	Frequency that manure touches the fruits	*E. coli* or coliforms					
			Hepatitis A or norovirus					
Contamination due to harvest tray contamination	Prevalence	Prevalence in trays	*E. coli* or coliforms					
			Hepatitis A or norovirus					
	Distribution	Distribution in trays	*E. coli* or coliforms					
			Hepatitis A or norovirus					
Contamination due to animal contact	Prevalence	Microbiological prevalence in animal waste	*E. coli* or coliforms					
			Hepatitis A or norovirus					
	Distribution	Microbiological distribution in animal waste	*E. coli* or coliforms					
			Hepatitis A or norovirus					
	Occurrence	Frequency that animal waste touches the fruits	*E. coli* or coliforms					
			Hepatitis A or norovirus					

ANNEX IV: Expert Elicitation data spreadsheet. (continuation)

Parameter 2	Value	Min value	Max value	Mode	Units	Reference
Beta	*0.039*	*-*	*-*	*-*	*PCR-detectable units/Liter*	*M. Bouwknegt et al 2015*
SD	*61.29*	*-*	*-*	*-*	*Days*	*ACHIPIA survey*
-	*-*	*10^2*	*10^7*	*10^3*	*cfu/ml*	*Expert elicitation*
-	*-*	*-*	*-*	*-*	*-*	*Aceituno et al, 2016*