INVESTIGATING THE IMPACTS OF MYCOTOXIN REGULATIONS ON HUMAN HEALTH AND TRADE

by

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ABSTRACT

The overall goal of this research was to investigate the impacts of mycotoxin regulations on human health and trade. Mycotoxins are toxic and carcinogenic secondary metabolites produced by a variety of fungi that infect food crops around the world. Specifically this research focuses on potential health and trade impacts of regulations on the mycotoxins aflatoxin and ochratoxin A (OTA). Aflatoxin is produced primarily by the fungi *Aspergillus flavus* and *A. parasiticus* and is one of the most potent liver carcinogens known. OTA, produced by the fungi *A. ochraceus, A. carbonarius, A. niger, and Penicilium verrucosum* has been associated with various human nephropathies and has been shown to be a potent renal carcinogen in animals.

This project makes significant contributions to public health through 1) increasing the understanding of how mycotoxin regulations guide trade and how trade patterns influence exposure to aflatoxin and 2) determining if OTA exposure is associated with adverse health effects.

First, in light of Health Canada's recently proposed maximum limits for OTA in a variety of commodities, OTA was evaluated to determine its effects on human health and the economy. A human health risk assessment revealed, with one exception, there appears to be no statistically significant evidence for human health risks associated with OTA exposure. Furthermore, implementation of the proposed OTA MLs in Canada could cause economic losses to Canadian food producers in the hundreds of millions of dollars annually.

Second, using pistachios as a case commodity model, trade patterns from the US and Iran to various countries worldwide were analyzed to determine if countries with similar aflatoxin regulations trade more with each other than countries with dissimilar standards. If countries without aflatoxin regulations are importing increased amounts of foods with high levels of aflatoxin they will be at risk for increased associated adverse health effects. A variety of metrics and social network models were used to confirm that over the past 15 years the US increasingly exported pistachios to countries with stricter aflatoxin standards, while Iran exported to countries with more lenient or without regulations. The US pistachio crop has had consistently lower levels of aflatoxin than the Iranian crop over this same time period. Attempts to determine the causality of the relationship between trade patterns and regulations were made; however, due to conflicting results, no conclusions could be made.

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1.0 INTRODUCTION TO THE MYCOTOXINS OF INTEREST

1.1 AFLATOXINS

Aflatoxins are toxic and carcinogenic secondary metabolites primarily produced by the fungi *Aspergillus flavus* and *A. parasiticus*. These fungi infect a variety of food crops such as maize, peanuts, tree nuts and cottonseed in tropical and subtropical regions of the world (1). In these regions, maize and peanuts are dietary staples and often subject to poor storage conditions which favor fungal infection and mycotoxin contamination (2, 3). High temperatures and drought stress are major factors contributing to high aflatoxin concentrations in maize (4). In addition to *A. flavus* and *A. parasiticus,* the related fungi *A. niger* and *A. nomius* can also produce aflatoxin in a variety of crops.

Aflatoxins were first discovered around 1960 when they were considered etiologic factors in turkey X disease, which caused the deaths of hundreds of thousands of turkeys, ducklings and pheasants in the United Kingdom from consumption of contaminated peanut meal (5, 6). An extensive investigation revealed an association between toxicity and the presence of *A. flavus* in peanut meal (7). The four major metabolites of aflatoxin are AFB1, AFB2, AFG1 and AFG2. Metabolites were given names based on their ability to produce blue or green-yellow fluorescence when placed under UV light, with AFB1 and AFB2 producing blue fluorescence and AFG1 and AFG2 producing green. AFB1 is the most abundant, making up nearly 50% of

total aflatoxins, and is responsible for the majority of associated toxic and carcinogenic effects in humans and animals (6, 8, 9). Other metabolites include AFM1 and AFM2, the hydroxylation products of AFB1 and AFB2, which are found in milk and milk products (8).

Classified by the International Agency for Research on Cancer (IARC) as a Group 1 known human carcinogen, aflatoxins are among the most potent liver carcinogens known (3, 9). Furthermore, individuals co-exposed to aflatoxin and the hepatitis B virus (HBV) are at 20-30 times greater risk of developing hepatocellular carcinoma (HCC) than individuals exposed to aflatoxin or HBV alone (10). It is currently estimated that 5 billion people worldwide are exposed to dietary aflatoxins (2). In sub-Saharan Africa and Asia, where aflatoxin exposure is the highest, HBV prevalence is also high and populations are at increased risk of developing HCC. Other health effects associated with aflatoxin exposure include immunosuppression, childhood stunting and acute aflatoxicosis (6, 8, 11, 12).

1.1.1 Aflatoxin Biomarkers of Exposure and Effect

Dietary consumption has been well documented as the main route of exposure to aflatoxins (6, 13, 14); however, studies have also shown that inhalation of aflatoxin is possible(15, 16). For most species, the LD_{50}, the dose that kills 50% of tested animals, ranges from 0.5 to 10 mg/kg bw. The US Food and Drug Administration (FDA) currently regulate the levels of aflatoxin in food and feed at 20 µg/kg and 0.5 µg/kg for AFM1 in milk. It is important to point out that the current FDA action levels were based on the minimum detectable amount of aflatoxin under UV light and not any specific toxicological data.

Biomarkers are often used as indicators of exposure and refer to the measurement of a specific agent of interest measured in the body which represents the presence and magnitude of

current and/or past exposures (6). Aflatoxin biomarkers allow for the measurement of both internal and biologically active dose of aflatoxin exposure when measuring dietary intake is not feasible (6).

Biomarkers used for measures of internal dose include urinary measures of AFM1, AFB1-mercapturic acid and serum aflatoxin-albumin adducts (8, 17, 18); AFB1-N7-Guanine serves as a urinary biomarker of the biologically effective dose (6). Whereas serum biomarkers of aflatoxin represent past exposure over the past 2-3 months, urinary biomarkers reflect more recent exposure to aflatoxin. Various studies have correlated the levels of urinary aflatoxin biomarkers (AFM1 and AFB1-N7-Guanine), as well as serum biomarkers (aflatoxin-albumin adducts) with dietary aflatoxin intake and have shown dose-response relationships (18-20). Potentially one of the most useful biomarkers recently discovered, a TP53 249^{ser} mutation is common in HCC patients and may represent early neoplastic exposure and/or chronic exposure to aflatoxin (8, 21-23).

1.1.2 Associated Human Health Effects

Various human health effects have been associated with exposure to aflatoxins. Chronic exposure to aflatoxin has been well documented to cause HCC, especially when co-exposed to HBV (3, 23-27). Acute exposure to high levels of aflatoxin is associated with aflatoxicosis (28-30). Relatively less evidence exists linking aflatoxin exposures to childhood stunting (11, 31, 32) and immunosuppression (33-36).

1.1.2.1 Hepatocellular Carcinoma (HCC)

Liver cancer is the ninth leading cause of cancer deaths in the US and third leading cause of cancer deaths globally (37-39). It is estimated that each year there are nearly 750,000 liver cancer cases and over 600,000 deaths from liver cancer with majority of these cases occurring in China, south-east Asia and sub-Saharan Africa (37, 40, 41). The estimated number of liver cancer cases and deaths varies greatly between more developed and less developed regions. In more developed regions, it is estimated that there were nearly 82,000 new liver cancer cases in 2008 and 75,400 deaths. In comparison, less developed regions saw over 440,000 new liver cancer cases and over 402,000 deaths (37).

Hepatocellular carcinoma risk factors include, but are not limited to, alcoholism, HBV, hepatitis C virus (HBC), aflatoxin exposure, smoking, arsenic, diabetes and obesity (42). Worldwide, HBV infects greater than 350 million individuals, while HCV chronically infects nearly 170 million individuals (8). In developed countries, it is estimated that 23% of HCC cases are attributed to HBV and 20% attributed to HCV (41). Developing countries attribute 59% and 33% to HBV and HCV respectively (41). Heavy alcohol consumption, tobacco use and diabetes have been linked to HCC; these risk factors may amplify the effects of HBV and HCV, but also may cause HCC in the absence of the hepatitis virus (39).

The role of the interaction between aflatoxin and HBV in causing HCC has been well studied. The risk of liver cancer in individuals exposed to both HBV and aflatoxin is 20-30 times greater than exposure to only one of the risk factors (10). There may also be synergistic effects of aflatoxin and HCV in causing HCC, however, the quantitative relationship is unclear (43-45).

Multiple proposed models and mechanisms currently exist by which aflatoxin and HBV cause liver cancer in humans. Animal models have shown that HBV transgenic mice expressing various HBV antigens in the liver showed more than HCC than non-transgenic littermates when exposed to AFB1 (8, 46). Other studies have shown that mice with *TP53*ser246 mutations and functional TP53 (8) had the highest incidences of liver cancer and mice expressing the HBx gene exhibited double the number of induced GC to TA transversions compared to wild-type mice when exposed to aflatoxin (47). Also, it has been suggested that aflatoxins may suppress DNA repair mechanisms which act in limiting the development of liver cancer from HBV infection (3, 48) or that HBV may modify the detoxification process of aflatoxin(49); however, aflatoxin itself may interfere with the suppression of cancer(3). Aflatoxin-induced DNA adducts may be fixed as mutations in response to chronic liver injury and regenerative hyperplasia caused by oxidative stress and inflammation (50) generated by HBV (8). Finally, it has been proposed that aflatoxin could affect susceptibility to infection by HBV or replication of HBV based on evidence in ducklings where AFB1 increased indicators of HBV replication (8, 51).

Figure 1-1 summarizes the key steps in the development of HCC from exposure to aflatoxin and infection with HBV (6). The metabolic pathway of AFB1 after dietary ingestion is the first step in afaltoxin's mode of toxicity. The liver is the target organ for aflatoxin and it is here that AFB1 is metabolized to the reactive AFB1-8,9-epoxide (AFBO) by the CYP450 enzyme system (52, 53). AFBO exists in both the endo and exo forms, however, it is the exo form which binds to DNA to form the more mutagenic AFB1-N7-Guanine adducts or serum albumin adducts (52, 54). Other metabolites of AFB1 include aflatoxin Q1 (AFQ1) and aflatoxin P1 (AFP1), which are less toxic than AFB1 (55), and AFM1 which is considered equally toxic, but less mutagenic (56).

Aflatoxin's toxicity can be reduced through conjugation, which makes the toxin more hydrophilic and able to be excreted in bile (57). Conjugation occurs mainly by certain glutathione S-transferases (GSTs) which produces AFB1-glutathione, AFB1-glucaronide, and AFB1-sulfate. AFB1-glutathione is the major conjugate and is the principal detoxification pathway essential to prevention and reduction of AFB1-induced carcinogenicity (57).

The formation of AFB1-N7-Guanine adducts is responsible for aflatoxin's carcinogenic effects due to the guanine to thymine base transversions (58). The double bond at C-8 of the AFB1-8,9-epoxide allows intercalation with the N7-Guanine base in DNA and form the most mutagenic lesions (53, 59). One underlying mechanism of HCC development and progression appears to be caused by a mutation in the p53 tumor suppressor gene (60). Under normal circumstances, p53 acts as a cancer suppressor gene by inducting apoptosis in damaged cells, however, when mutated, uncontrolled cell division and proliferation occurs. This ultimately leads to tumor formation.

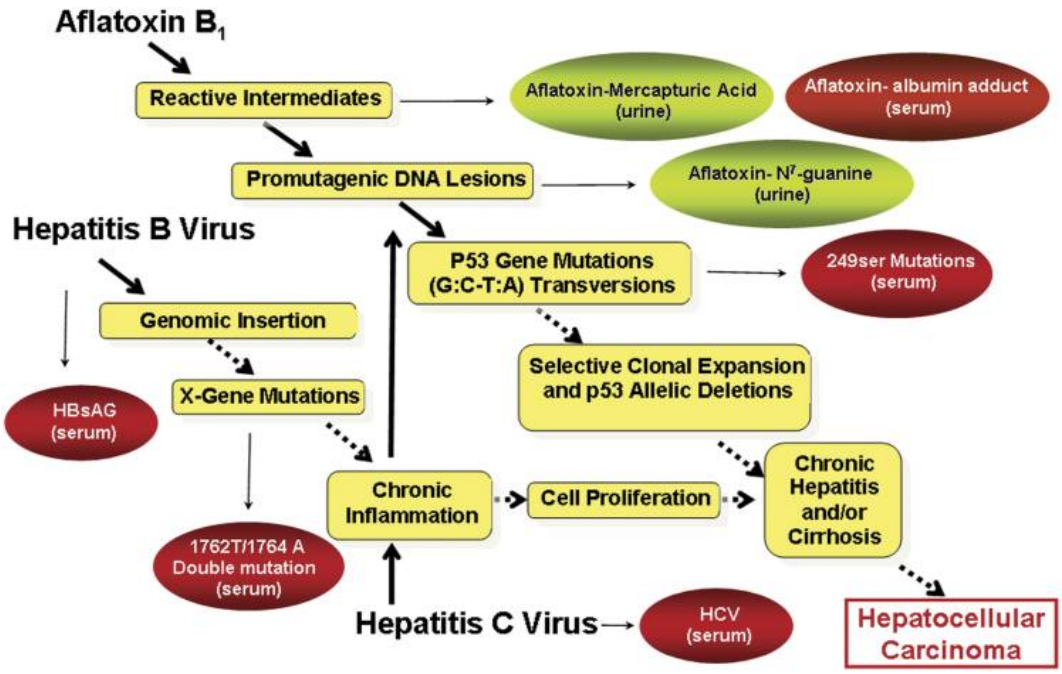

Figure 1-1: Summary of the synergistic effect of aflatoxin and HBV in causing HCC (6).
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1.1.2.2 Aflatoxicosis

Aflatoxicosis, caused by acute exposure to high levels of aflatoxins in the diet, is characterized by jaundice, ascites, vomiting, lethargy, abdominal pain, hepatitis, hemorrhagic necrosis of the liver, edema, bile duct proliferation and death (3, 61). In most cases, aflatoxicosis is associated with high daily consumption of contaminated maize near 300-500 grams/day (8). Fatality occurs when consuming levels of aflatoxins at 5000 µg/kg or above; however, the non-lethal, adverse health effects occur at levels exceeding 1000 µg/kg. The duration of exposure necessary to cause death is estimated to be 7-21 days at levels exceeding 20 µg/kg bw/day in adults (8). While adults are typically more tolerant to aflatoxin exposures, children have the greatest risk of fatal aflatoxin exposures (62).

Major aflatoxicosis outbreaks have occurred over the past 50 years with the largest occurring in India in 1974. This outbreak resulted in 397 recognized cases and 106 deaths from consuming contaminated maize with aflatoxin levels ranging from 0.25 to 15 mg/kg (29). In 1981 another outbreak occurred in Kenya resulting in twenty hospitalizations and a case fatality rate of 60% (30). Most recently, in 2004-2005 in eastern Kenya, 317 cases were reported with 125 deaths (28). It was determined that poorly harvested maize was stored in favorable conditions for aflatoxin contamination. Average measured levels of AFB1 were 50 µg/kg, nearly 220 times the regulation limit set for aflatoxin in food in Kenya (63).

1.1.2.3 Stunting

Multiple studies in animals (3) and humans (11, 31, 64) have suggested a possible link between aflatoxin exposure and childhood stunting (65) . Infants may be first exposed to AFM1, the hydroxylated metabolite of aflatoxin, in mother's breast milk if the mother consumed a diet

contaminated with aflatoxin. As a child grows and is weaned onto solid foods, the child will be exposed to aflatoxin from the household diet (66).

An inverse association between aflatoxin exposure and growth was found in children aged 1-5 in a cross-sectional study carried out in Benin and Togo (11). This association was extended to take into account *in utero* exposure in a sample of Gambian children and an association was found between aflatoxin exposure and impaired growth (32). A longitudinal study in West Africa showed a strong, negative correlation between aflatoxin-albumin adducts and height over an 8-month period (31). Overall, aflatoxin exposure has been correlated with low birthweights and heights (67-69), as well as decreased height and weight at one year of age (32).

The mechanisms by which aflatoxins lead to stunted growth in infants and children are not yet completely understood. A variety of proposed mechanisms exist. These include: 1) aflatoxins causing less efficient food conversion and decreased protein synthesis (67); 2) compromised intestinal integrity through altered barrier function (66), which may have negative effects on micronutrient absorption--specifically vitamins A and E (70); or 3) immune system effects which makes intestinal epithelium vulnerable to bacteria or viruses, increases local inflammation and impairs nutrient absorption (34, 66).

1.1.2.4 Immunosuppression

Aflatoxins have been associated with adverse effects on the immune system dating back to the 1960's and 1970's when aflatoxin exposure was associated with salmonellosis outbreaks causing Turkey X syndrome and an outbreak in swine in the southeastern United States (71). While most of the immunomodulatory effects have been considered in animal and cell culture studies (3, 71, 72), limited human evidence does exist (31, 32, 34, 70, 73).

Human studies have shown reduced phagocytic activity in human peripheral blood monocytes *in vitro* when exposed to aflatoxin (74). One study in The Gambia found significantly higher mean aflatoxin-albumin adducts in children with malaria compared to controls (73), while another correlated aflatoxin-albumin levels with lower salivary IgA in children (34). In Ghana, higher aflatoxin-albumin adducts were associated with alterations in different lymphocyte subgroups compared to controls (35, 36). It is possible that aflatoxin-induced immunosuppression is due to the inhibition of DNA, RNA and protein syntheses or through modulation of cytokines (33, 75).

1.2 OCHRATOXIN A

Ochratoxin A (OTA) is a foodborne mycotoxin produced by a wide variety of fungi including *Aspergillus ochraceus, A. carbonarius, A. niger* and *Penicillium verrucosum*. Due to the wide variety of fungi which produce the toxin, OTA contaminates multiple agricultural commodities ranging from cereal grains to dried fruits and wines and coffee. The fungi each grow under a different set of conditions; however, contamination typically occurs in moderate and subtropical regions at temperatures from 20°C-37°C. Poor agricultural practices such as improper drying and storage of foods favor fungal growth and OTA contamination (76). Ordinary food processing measures fail to reduce OTA levels due to OTA's chemical stability.

The kidneys are the main target organ for OTA (77) and is one of the most potent renal carcinogens known to date (78, 79). IARC has classified OTA as a Group 2B, possible human carcinogen based on carcinogenicity in animal studies (80, 81). Based on its carcinogenic effects, OTA has been associated with various human nephropathies including Balkan endemic

nephropathy (BEN) (82-86) and chronic interstitial nephropathy (87-89). Other OTA-associated adverse effects include teratogenic effects (90), immunosuppression (91), inhibition of macromolecular synthesis, increased lipid peroxidation, and inhibition of mitochondrial respiration (79, 92).

The purposes of examining the human health effects of OTA were threefold in this study. First, OTA was evaluated using the four-step risk assessment process to gain insight about population health effects due to dietary OTA exposure. This research comes in part due to the recently proposed OTA maximum levels (MLs) suggested by Health Canada; however, the studies used to set this MLs were based on animal and cell culture assays. Second, the estimated cost of implementing an OTA standard in Canada was calculated for both domestic losses and losses to countries exporting crops to Canada. Third, OTA was considered as one of the major etiologic factors in BEN. For this analysis, all of the etiologic factors involved with BEN were considered and compared using the Bradford Hill Criteria (BHC), a qualitative evaluation tool.

1.2.1 OTA Exposure and Toxicity

The major source of OTA exposure comes through ingestion of OTA-contaminated foods. While cereal grains are the staple foods contaminated by OTA; grapes, raisins, wine, coffee, beer, corn, and soy are also often found to contain OTA (90). The amount of OTA in food is dependent on a host of factors including location, season and the amount of time kept in storage amongst other factors (93).

OTA is present in the foods of many countries around the world. In 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated 85% of sampled commodities including wheat, rye, barley, oats, dried vegetables, olives and milk came from

Europe (94). Outside of Europe OTA has been found in a variety of commodities in the US, Brazil, Canada, Dubai, Japan and throughout Africa (94, 95). JECFA currently estimates OTA exposures from cereals to range from 8-17 ng/kg bw/week based on European data (96).

OTA is absorbed mainly in the gastrointestinal tract and distributed via blood to the kidneys (96). OTA metabolism occurs in the liver and is carried out by several CYP450 isoforms. These isoforms include CYP-1A2, 2B6, 2C9, 2D6 and 2A6 (97). The major metabolites are shown in Figure 1-2. Excretion occurs in urine and feces, however, OTA may be reabsorbed from the intestine, then reenter enterohepatic circulation (98), or be reabsorbed in the kidney proximal and distal tubules (77). OTA has a low acute toxicity and large LD_{50} values exist for different species. Oral LD_{50} values range from 0.2mg/kg in dogs, 1mg/kg in pigs, 3.3mg/kg in chickens, 20-30mg/kg in rats and 46-58mg/kg in mice (94). Intraperitoneal LD_{50}'s are more sensitive with values of 13mg/kg for rats and 22-40mg/kg for mice (94). OTA has a long elimination half-life of up to 35 days in human serum (99), but is much shorter in other species such as pigs (3-5 days), mice (1-1.5 days) and rats (2-5 days) (96). Based on its excretion in urine and ability to bind to serum proteins, various biomarkers exist for measuring exposure to OTA. These will be further detailed below.

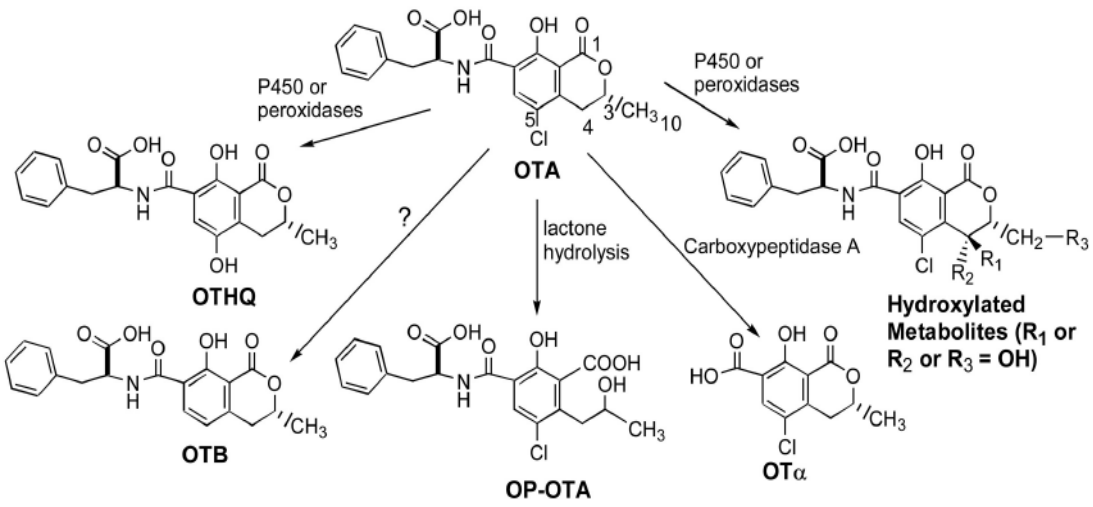

Figure 1-2: Major OTA metabolites (97).
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A lowest observable adverse effect level (LOAEL) in pigs was estimated to be 8 µg/kg in pigs (100, 101). This LOAEL value was used as the basis for multiple regulatory advisory committees' determination of safe OTA intake levels. JECFA used this LOAEL in combination with an uncertainty factor of 500 to set the first provisional tolerable weekly intake (PTWI) at 112 ng OTA per kg bw/week in 1991 and when reevaluating the PWTI at its 44th meeting and relaxing the PTWI to 100 ng OTA per kg bw/week. In a Health Canada risk assessment of OTA, Kuiper-Goodman et al. (90) chose to use a benchmark dose of 10% (BD_{10}) rather than a LOAEL or NOAEL for risk assessment studies. Applying the same uncertainty factor of 500 to the BD_{10} of 1.56 µg/kg bw/day calculated from the Krogh (100) study resulted in a tolerable daily intake (TDI) of 3 ng/kg bw/day (90), much stricter than previous assessments.

1.2.2 OTA Biomarkers of Exposure

Measuring internal dose of OTA exposure is made possible through the use of biomarkers. In the case of OTA, it is possible to measure both human serum and urine to evaluate exposure. It is important to note that neither serum OTA nor urinary OTA have been validated as biomarkers of OTA; however, they have been used in multiple epidemiological studies to estimate exposure.

1.2.2.1 Serum OTA Biomarkers

First detected in 1977 (102), serum OTA has been the most studied approach to biomonitoring OTA exposure (77). A long elimination half-life of 35 days (99) coupled with binding to plasma proteins, renal reabsorption and its enterohepatic circulation (98, 103) allow serum to be measured as a biomarker of OTA exposure over the previous weeks (77). OTA in blood has been used extensively since 1982 when OTA was first considered as an etiologic factor in BEN (77). Since then, serum OTA has been measured around the world in multiple populations including: Romania (104), Spain (105), the Czech Republic (106, 107), Turkey (108), Italy (109), Egypt (89), Algeria (110), and Tunisia (87). In these studies higher serum or plasma OTA levels have been correlated with kidney and urinary disorders when compared to healthy controls, however, the associations may not be causal (93).

Measuring serum OTA has drawbacks. First, collecting blood samples is an invasive process that involves hiring trained personnel and elevated costs (77). Second, high intra-subject variation exists and levels of serum OTA have been shown to vary tenfold in one subject when tested over a ten-year period (111). High variation was also shown in repeatedly tested subjects

over one year in Italy(112), Germany (113), Switzerland(99) , Czech Republic (114), Japan (115), and Bulgaria (116).

1.2.2.2 Urinary OTA Biomarkers

New technologies have made measuring the low amounts of OTA found in human urine possible (77). Relatively easy and non-invasive collection processes are two advantages of using urinary measures over serum biomarkers. Multiple studies have measured urinary OTA as a biomarker of OTA exposure in humans around the world. The study populations were in Italy (109, 117), Hungary (81), Egypt (89), the UK (118) and Portugal (119) as well as in BEN-endemic areas (116). Two French siblings with renal failure had the highest measured urinary OTA levels at 367 ng/ml and 1801 ng/ml (120). Urinary OTA biomarkers also show stronger correlation with dietary intake (118) and are more representative of recent OTA intake when compared with serum biomarkers (83, 116).

1.2.3 OTA Associated Health Effects

The major adverse health effects associated with exposure to OTA relate to the kidney. OTA is one of the most potent renal carcinogens known in animals (90) and has been associated with various nephropathies and urothelial tract tumors(96). OTA has also been considered a teratogen (121-127) and may also be mutagenic (128-130) based on animal and cell culture studies.

1.2.3.1 Potential Mechanisms

Various hypotheses exist attempting to detail the carcinogenic mode of action by OTA. JECFA (96) has summarized both the mechanisms that would account for tumor formation and those that may only contribute to tumor formation. The proposed carcinogenic mechanisms include: generation of tumors secondary to chronic renal toxicity, disruption of cell signaling pathways and cell division, alteration of calcium homeostasis, mitochondrial dysfunction leading to oxidative stress and inhibition of phenylalanine-tRNAPhe synthetase so that amino-acylation and peptide elongation are stopped (96, 131).

Divergent results exist when considering genotoxic modes of action. OTA has been shown to be moderately genotoxic in *in vitro* and *in vivo* mammalian systems in some studies (132); however, other studies have shown more potent effects in mammalian cells (130, 133). Further complicating this issue is the contradictory results regarding DNA adduction. ^{32}P-post-labelling results (97, 134) from multiple tissues have detected both dose and time-dependent DNA adducts in several species (90); however, other studies were unable to detect DNA binding (135, 136), and JECFA has concluded that no genotoxic mode of action or covalent binding to DNA has been confirmed (96).

Non-genotoxic modes of action have also been proposed based on a number of animal and cell culture assay studies. OTA exposure has been associated with both *in vivo* and *in vitro* alterations in oxidative stress pathways, changes in gene expression and cell signaling, increased apoptosis, increased cell proliferation, and disruption of mitosis (96). Further research is needed to fully characterize the proposed mechanisms by which OTA may cause adverse health effects in humans at biologically relevant doses.

1.3 MYCOTOXIN REGULATIONS

The overall theme of this dissertation was to determine how mycotoxin regulations affect human health and trade patterns of contaminated commodities.

The Food and Agriculture Organization (FAO) has published two reports summarizing the status of mycotoxin regulations around the world (137, 138). As of 2003, over 120 countries had mycotoxin regulations for food and feed (137), which is an increase in total nations of 30% compared to 1995 (138). Overall, 87% of the world's population is covered by mycotoxin regulations. This is an increase of 10% compared to 1995 due to slight increases in coverage in Latin America and Europe combined with significant increases in Africa and Asia (137). In addition to the increases in number of countries regulating mycotoxins, the number of commodities regulated has increased between 1995 and 2003 while tolerance limits have remained the same or became stricter (137). Figure 1-3 summarizes all the countries with and without regulations for various mycotoxins (137).

Aflatoxins are regulated in every country that has any mycotoxin regulation(s). This includes regulations for AFB1 and/or total aflatoxins (AFB1+AFB2+AFG1+AFG2). 61 countries currently regulate for AFB1, whereas 76 countries regulate for total aflatoxins in food. Other regulations exist for other mycotoxins, including OTA. Figure 1-4 summarizes the ranges and medians of limits for total aflatoxins in different regions between 1995 and 2003 (137). The largest change in trends is apparent in Europe and Asia where limits became much stricter.

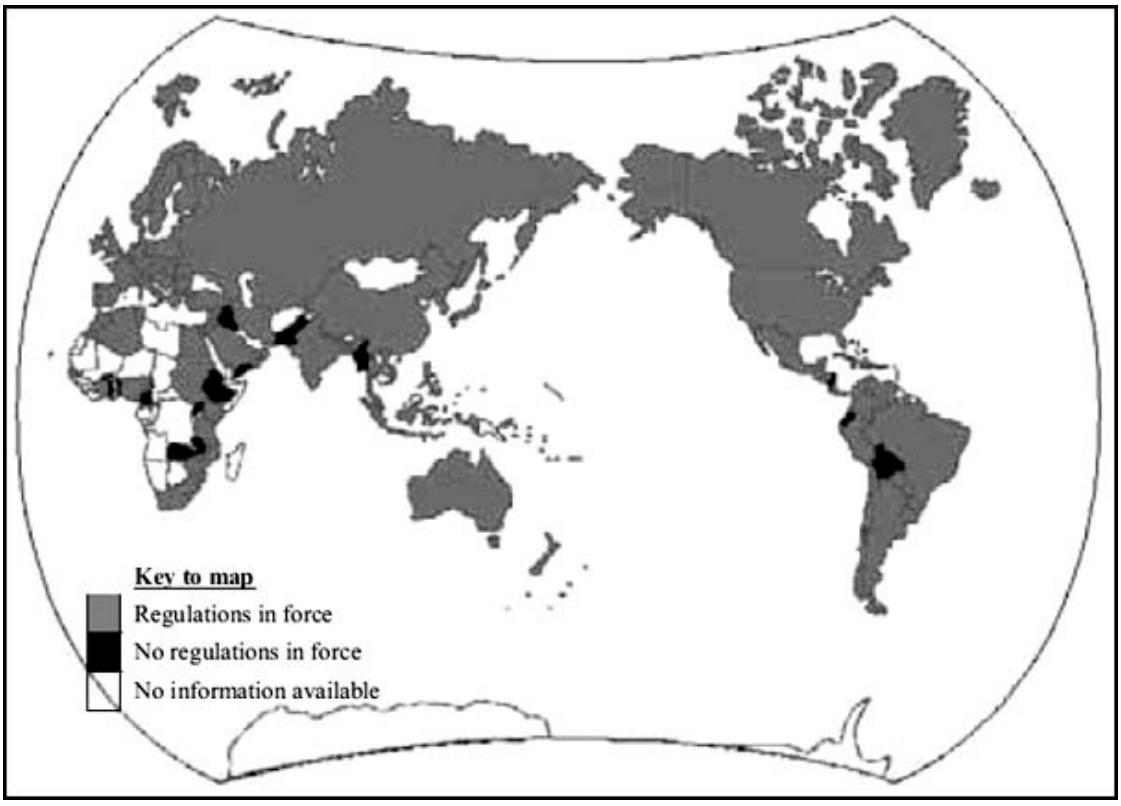

Figure 1-3: Summary of countries with and without mycotoxin regulations in 2003 (137).
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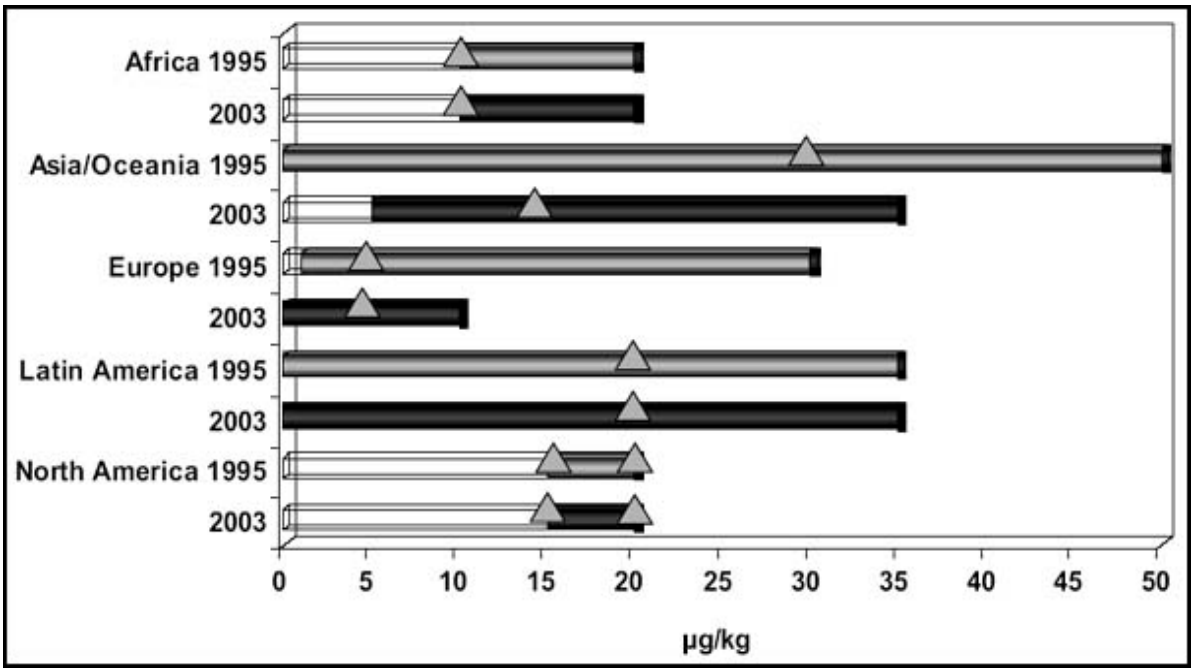

Figure 1-4: Range and median total aflatoxin limits for different world regions (137).
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The number of countries regulating OTA has also increased between 1995 and 2003, specifically for cereal grains and cereal products. Some regulations, like those in the EU, are different depending on if the cereal is raw or processed. As of 2003, 37 countries had OTA regulations in cereal or cereal products ranging from 3-50 ng/g. In this study, it is important to consider Health Canada's proposed OTA regulations in relation to Canada's current levels of OTA in foods. Health Canada's proposed OTA regulations (139) are summarized and compared to current OTA regulations in the EU below (140) (Table 1-1).

Health Canada has proposed matching maximum limits for a variety of commodities already in force in the EU. These regulations cover raw and unprocessed cereal grains, direct consumer grains and cereal products, breakfast cereals, fruit juices, dried fruits, and baby foods. Health Canada has proposed a regulation for derived cereal products (wheat bran) at 7 ng/g for which the EU does not currently regulate. In addition to the products previously listed, the EU also regulates for coffee (roasted beans, instant coffee and grounds), wine/wine products, various spices and licorice (root and extract). As the products are unlikely to be produced in Canada, Health Canada has not proposed any maximum levels regulating those commodities.

Table 1-1: Proposed Health Canada and European Union Maximum OTA Limits.

Product	Proposed Health Canada maximum limits (ng/g OTA)	European Union maximum levels (ng/g OTA)
Raw cereal grains / unprocessed cereals	5	5
Direct consumer grains (i.e. rice, oats, pearled barley)	3	3
Derived cereal products (flour)	3	3
Derived cereal products (wheat bran)	7	N/A
Breakfast cereals	3	3
Grape juice (and as ingredients in other beverages) and related products	2	2
Dried vine fruit (currants, raisins, sultanas)	10	10
Baby foods and processed cereal-based foods for infants and young children	0.5	0.5
Dietary foods for special medicinal purposes intended for infants	0.5	0.5
Roasted coffee beans and ground roasted coffee	N/A	5
Soluble (instant) coffee	N/A	10
Wine	N/A	2
Aromatized wine, wine-based drinks, and wine-product cocktails	N/A	2
Spices	N/A	15
Licorice, licorice root	N/A	20
Licorice extract	N/A	80

*N/A: Not available – OTA not regulated in respective commodity.

2.0 RESEARCH ON OCHRATOXIN A

2.1 OCHRATOXIN A AND HUMAN HEALTH RISK: A REVIEW OF THE EVIDENCE

The data presented in this chapter is published in

Bui-Klimke TR, Wu F (2013). "Ochratoxin A and human health risk: A review of the evidence." *Critical Reviews in Food Science and Nutrition*, in press.

2.1.1 Abstract

Ochratoxin A (OTA) is a mycotoxin produced by several fungal species including *Aspergillus ochraceus, A. carbonarius, A. niger* and *Penicillium verrucosum*. OTA causes nephrotoxicity and renal tumors in a variety of animal species; however, human health effects are less well-characterized. Various studies have linked OTA exposure with the human diseases Balkan endemic nephropathy (BEN) and chronic interstitial nephropathy (CIN), as well as other renal diseases. This study reviews the epidemiological literature on OTA exposure and adverse health effects in different populations worldwide, and assesses the potential human health risks of OTA exposure. Epidemiological studies identified in a systematic review were used to

calculate unadjusted odds ratios for OTA associated with various health endpoints. With one exception, there appears to be no statistically significant evidence for human health risks associated with OTA exposure. One Egyptian study showed a significantly higher risk of nephritic syndrome in those with very high urinary OTA levels compared with relatively unexposed individuals; however, other potential risk factors were not controlled for in the study. Larger cohort or case-control studies are needed in the future to better establish potential OTA-related human health effects, and further duplicate-diet studies are needed to further develop biomarkers of OTA exposure in humans.

2.1.2 Introduction

Ochratoxin A, as previously reviewed, is a naturally occurring foodborne mycotoxin found in a wide variety of agricultural commodities worldwide, ranging from cereal grains to dried fruits to wine and coffee. Table 2-1 summarizes the fungi which are known to produce OTA, their optimal growing temperature, optimal water activity, and the commodities which are affected by each particular fungi. Contamination generally occurs as a result of poor storage of commodities and suboptimal agricultural practices during the drying of foods (76). OTA is a chemically stable compound; hence, ordinary food processing measures fail to substantially reduce its presence in foods and beverages.

Table 2-1: Ochratoxin-producing fungi, optimal growth conditions, and commodities affected.

OTA-producing species	Optimal temperature range (Min-Max) °C	Water activity	Commodities affected
A. ochraceus	24-31 (8-37)	0.95-0.99	Smoked and salted dried fish, dried beans, biltong, soya beans, chickpeas, rapeseed, pepper, dried fruit, and sesame seeds, nuts, cereals rice, barley, maize, wheat, flour, and bran, coffee beans
A. carbonarius	32-25 (N/A-40)	0.82	Grapes and grape products, including table grapes, wines, and dried vine fruits
A. niger	35-37 (6-47)	0.77	Nuts, apples, pears, peaches, citrus, grapes, figs, strawberries, mangoes, tomatoes, melons, onions, garlic, and yams
P. verrucosum	20 (0-30)	0.80	Cereal crops; cheese, meat products

Source: (94)

Ochratoxin A has become an important topic in recent years, as Health Canada has proposed maximum limits (MLs) for OTA in a variety of foods and drinks that could have consequences for the marketability of these commodities in Canada, and could also affect nations that attempt to export food to Canada (141). Yet, little is known about population health impacts of dietary OTA exposure. Thus far, risk assessments on OTA, including those that have guided Health Canada's recently proposed MLs, have largely been based on animal and cell culture assay studies, with relatively less focus on human studies. The goal of this study was to systematically review the epidemiological literature linking OTA exposure with adverse health effects in diverse human populations worldwide. In a discussion of risk assessments conducted on OTA in the past, we compare the state of known data with what is still missing in terms of assessing human health effects. We collected available human studies linking OTA exposure with a variety of health outcomes, and selected those studies that met predefined criteria for

inclusion in the review. Odds ratios were estimated for these different studies where the data permitted these calculations. The current state of exposure assessment for OTA is discussed as well. We characterize the risk of OTA to human populations and describe limitations in the available data.

2.1.3 Background: Risk Assessment of Ochratoxin A

Risk assessment, the process of estimating the magnitude and the probability of a harmful effect to populations from certain agents or activities, consists of four main steps: hazard identification, hazard characterization or dose-response assessment, exposure assessment, and risk characterization (142). The four steps involved in the estimation of risk are outlined below (143).

Hazard identification, determining whether exposure to an agent can increase the incidence of a particular health condition, has been carried out for OTA in assessments conducted by multiple institutions; including the International Agency for Research on Cancer (IARC), Health Canada, the Joint Food and Agriculture Organization / World Health Organization Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA) (80, 141, 144, 145). Ochratoxin is identified as a renal carcinogen to particular animal species (79) and can cause nephrotoxic, teratogenic, and immunosuppressive effects in multiple animal species (79, 146).

For humans, however, hazard identification has been more difficult. Several adverse human health effects, including the kidney diseases Balkan Endemic Nephropathy (BEN) and chronic interstitial nephropathy (CIN), have been associated with exposure to OTA; but these associations have thus far been less conclusive than those for OTA-associated adverse effects in

laboratory animal studies. The hallmark features of BEN include a familial but not inherited pattern of disease, initial manifestation after living in an endemic village for 15 years or more, and an association with upper urothelial tract cancer (147). However, aristolochic acid (AA), a toxin produced in *Aristolochia* weeds commonly found in Balkan grain fields, has emerged as the most likely causative agent of BEN; as aristolactam-DNA adducts have been found in the renal cortex of BEN patients but not in patients with other chronic renal diseases (26). CIN does not appear to have the familial pattern of BEN, and may be acute or chronic with cases presenting anywhere from a few days up to 5 months. The etiology of CIN has been postulated to include infections, toxins such as OTA, or reactions to medications (148).

Hazard characterization or *dose-response assessment* describes the relationship between different levels of exposure to a substance and associated incidence of disease in a population of animals or humans. Dose-response data from animal studies of a particular toxin are used to extrapolate an acceptable daily or weekly exposure to humans, below which no adverse effects are expected. This step usually involves a critical review of toxicological studies to set appropriate exposure metrics (90), such as tolerable daily or weekly intake or negligible cancer risk intake. In the case of OTA, diverse regulatory and advisory bodies have assessed dose-response data on OTA and have set exposure metrics for tolerable exposure to OTA in humans. These are summarized in Table 2-2.

Table 2-2: Summary of calculated tolerable human intakes of ochratoxin A (OTA) by international organization.

Organization	Tolerable intake metric*	Limit	Year (Reference)
European Food Safety Authority (EFSA)	PTWI	120 ng/kg bw/week	2006 (145)
Health Canada	PTDI	3 ng/kg bw/day	2010 (90)
Health Canada	NCRI	4 ng/kg bw/day	2004 (149); 2010 (90)
Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2007	PTWI	100 ng/kg bw/week	2007 (96)
Nordic Expert Group on Food Safety	TDI	5 ng/kg bw/day	1991 (150)
Scientific Committee of Food (SCF) of the European Union	PTDI	5 ng/kg bw/day	1998 Reviewed in (145)

*TDI - tolerable daily intake; PTDI - provisional tolerable daily intake; PTWI - provisional tolerable weekly intake; NCRI - *negligible cancer risk intake*

Various dose-response studies in animals were the basis for advisory groups' determinations of safe weekly or daily OTA intakes for humans. JECFA first evaluated OTA at its 37th meeting (144), setting a provisional tolerable weekly intake (PTWI) at 112 ng OTA per kg bodyweight (bw) per week based on a dose-response study of renal function deterioration in pigs, for which the lowest observed adverse effect level (LOAEL) was 8 µg/kg bw/day (100, 101). A combined uncertainty factor (UF) of 500 was applied in the calculation. JECFA reevaluated OTA at its 44th meeting, taking into account new toxicological data. The PTWI was confirmed, but rounded down to 100 ng/kg bw/week. The most recent assessment of OTA at the 68th meeting in 2008 resulted in retaining the PTWI previously found. JECFA currently estimates OTA exposure from cereals, based on European data, to be about 8-17 ng/kg bw/week: well below the PTWI.

The European Food Safety Authority (EFSA) derived a PTWI for ochratoxin A of 120 ng/kg bw/week, based on the 8 µg/kg bw/day LOAEL used in the JECFA evaluation (145). An uncertainty factor of 450, rather than 500 used in JECFA, was applied to the LOAEL. This composite uncertainty factor was based on an intra-species factor of 10, interspecies factor of 15, and a factor of 3 for use of a LOAEL instead of a no observed adverse effect level (NOAEL). The interspecies factor of 15 was based on the longer OTA half-life in humans and monkeys rather than pigs as determined by Hagelberg et al. (151).

A Health Canada risk assessment team (90) chose to reevaluate EFSA's PTWI for ochratoxin A, positing that the use of LOAEL rather than a NOAEL was not appropriate given the small number of animals per group, and the fact that 4 out of 9 pigs in the lowest dose group showed functional kidney changes. Rather than use a NOAEL or LOAEL, a benchmark dose corresponding to a response of 10% above background (BD_{10}) was derived. Uncertainty factors of 10 for intra-species variability, 25 for interspecies variability, and 2 for use of a sub-chronic rather than chronic study were combined in a composite uncertainty factor of 500. Applying this composite uncertainty factor to the BD_{10} of 1.56 µg/kg bw/day resulted in a TDI of 3.0 ng/kg bw/day after rounding (90), which in practice is considerably stricter than the JECFA or EFSA tolerable limits.

Additionally, Health Canada derived a negligible cancer risk intake (NCRI) for OTA: the exposure associated with an increased cancer risk of 1:100,000 and equivalent in units to the TDI. The tumorigenic dose at which 5% of the animals are likely to have tumors (TD_{05}) was used to derive the NCRI for OTA(78). This dose was determined to be 19.6 µg/kg bw/day. The TD_{05} was then divided by 5000, the linear extrapolation to zero exposure, resulting in a NCRI of 4 ng/kg bw/day (28).

Exposure assessment involves estimating the intensity, frequency, and duration of human exposures to toxic substances. Ochratoxin exposure is a function of the concentration of ochratoxin in foodstuffs, as well as the amount of these foodstuffs that are consumed in different populations. Depending on location, seasons, and amount of time food is kept in storage, both the amount and contamination levels of food may vary greatly even for the same population or individual (93). Estimating human OTA exposure may be done using food surveys combined with OTA surveillance in commodities, or biomarkers of exposure.

By determining ranges of OTA contamination of foods, OTA exposure can be estimated from the known intake levels of the given commodities (152). However, the accuracy of this estimation is limited due to the large variability in OTA content in commodities, as well as variation in dietary habits (81). Surveillance data on OTA concentrations in different regions of the world is limited. JECFA provides extensive data on OTA exposure concentrations in commodities(94); however, 85% of sampled commodities including wheat, rye, barley, oats, dried vegetables, olives, and milk came from Europe. However, it was noted that OTA occurs in coffee in countries including Brazil, Canada, Dubai, Europe, Japan, and the USA(94). In Denmark, Norway, and the UK, OTA was found in oat samples. In Germany, high OTA levels were found in unprocessed cereals, rye and buckwheat, with levels ranging from 95.6-125 ug/kg. In Africa, OTA was found in wheat, barley, cereals, dried vegetables and olives. Specifically, Maaroufi et al. (95) found high OTA levels, with a maximum of 33,000 ug/kg in wheat, barley, mixed cereals, dried vegetables, and olives in a Tunisian population. In Nigeria, Ghana, and Burkina Faso, OTA was detected in sorghum, maize, and millet.

A more accurate method to estimate exposure, when possible, is through measuring human biomarkers of OTA exposure, as reviewed in Duarte et al.(77). Although neither serum

OTA nor urinary OTA has been validated, they have been used in multiple studies to estimate OTA exposure. OTA is found in serum due to its long elimination half-life of about 35 days(99), and is excreted in urine as both unchanged OTA and its derivatives.

OTA in serum was first detected in 1977 and has been one of the most widely used biomonitoring approaches for human OTA exposure. Renal absorption, enterohepatic circulation and binding to plasma proteins results in a long half-life for OTA in the body, allowing it to be detected in human blood (98, 103). Epidemiological studies conducted in multiple countries, including Romania (104), Spain (105), the Czech Republic (106, 107), Turkey (108), Italy (109), Egypt (89), Algeria (110), and Tunisia (87, 153), have associated higher serum or plasma OTA levels in patients with kidney and urinary disorders compared to healthy controls, although the associations may not be causal (93). Although many studies have used serum OTA as a human biomarker of OTA exposure, considerable intra-subject variation has been noted. Levels of serum OTA have been noted to vary up to tenfold in one subject when tested over a ten-year period (111) and in repeatedly tested subjects over one year in Tuscany (112). Furthermore, studies in Germany (113), Switzerland(99), Czech Republic (114), Japan (115), and Bulgaria (116) all showed high intra-subject variability in human subjects tested over time. This variability is likely due to the decreases in plasma concentrations based on the half-life of OTA (93).

Urinary OTA, another potential biomarker of OTA exposure, is often found in very low amounts compared to those in blood; however, new technologies have increase the sensitivity and accuracy of detection(77). Pascale & Visconti (117) detected OTA in 37 out of 55 healthy individuals in Italy with levels ranging from 0.012-0.046 ng/ml. In Hungary, Fazekas et al. (81) detected OTA in 54 out of 88 samples from healthy individuals at levels of 0.006-0.065 mg/ml.

Patients with end stage renal disease or nephritic syndrome in Egypt had significantly higher levels of urinary OTA than two reference groups (89). The highest incidence of detectable OTA exposures in urine, 100%, were found by Petkova-Bocharova et al. (116) in a BEN-endemic region and in non-endemic regions including Portugal (119) and Italy (109). The highest recorded levels of OTA in urine, 367 ng/ml and 1801 ng/ml, were found in two French siblings with renal failure (120). Petkova-Bocharova et al. (116) and Castegnaro et al. (83) applied similar methodology as Gilbert et al. (118) when studying 16 human participants (83). Increases of OTA intake resulted in an increase of OTA elimination a week after ingestion, not immediately (83). Table 2-3 summarizes urinary OTA levels in populations from different world regions. The table includes OTA levels from Duarte et al. (77) and more recent studies.

Table 2-3: OTA occurrence in human urine.

Country	Year collected	Number of positive samples (%) for OTA	Range of urinary OTA levels (ng/ml)	Sampled population	Reference
Bulgaria	1984-1990	44/127 (35)	0.005-0.604	Healthy humans in Balkan Endemic Nephropathy (BEN) areas, Non-BEN areas and BEN patients	(154)
Bulgaria	2003	16/16 (100)	0.016-0.860	Patients from BEN areas	(116)
Bulgaria	Not Available	61/152 (40)	n.d.-0.03	BEN and Urothelial Tract Tumor (UTT) patients	(155)
Croatia	2000	24/63 (38)	0.005-0.086	BEN and Non-BEN areas	(156)
Croatia	2005	9/63 (14)	0.005-0.015	BEN and Non-BEN areas	(156)
Egypt	1998	19/122 (16)	0-8.19	Healthy controls, kidney donors, patients with End	(89)

Table 2-3 Continued

				Stage Renal Disease (ESRD), transplant recipients, nephritic syndrome patients, and UTT patients	
Germany	2008	13/13 (100)	0.02-0.13	Healthy volunteers	(157)
Hungary	2003	54/88 (61)	0.006-0.065	Healthy volunteers	(81)
Italy	2001	25/41 (61)	0.012-0.140	Healthy individuals and karyomegalic interstitial nephritis patients	(117)
Italy	Not Available	10/10 (100)	0.02-0.25	Healthy volunteers	(158)
Korea	Not Available	12/12 (100)	0.012-0.093	Healthy volunteers	(159)
Portugal	2007	174/198 (88)	n.d.-0.071	Healthy volunteers	(160, 161)
Portugal	2004	42/60 (70)	n.d.-0.105	Healthy volunteers	(162)
Portugal	2005	13/30 (43)	n.d.-0.208	Healthy volunteers	(163)
Sierra Leone	1992-93	63/434 (25)	0.06-148	Healthy child volunteers	(164)
Spain	2005	25/31 (81)	n.d.-0.124	Healthy volunteers	(163)
Spain	2011	9/72 (13)	0.057-0.562	Healthy volunteers	(165)
Spain	2011	3/27 (11)	n.d.-<1.5	Healthy volunteers	(166)
United Kingdom	2001	46/50 (92)	<0.01-0.058	Healthy volunteers	(118)

A 2001 study on both serum and urinary biomarkers of OTA exposure revealed a stronger correlation between dietary OTA intake and urinary OTA than serum OTA. Gilbert et al. (118) examined OTA levels in urine and plasma as a function of dietary OTA intake in 50 subjects in the United Kingdom. The volunteers kept a daily food diary and provided blood samples once per week, urine samples daily, and duplicate diet samples daily, for one month. Baseline samples were taken at the beginning of the study. OTA was detected in all but four urine samples, with levels ranging from <0.01-0.058 ng/ml. OTA was detected in all plasma

samples with baseline sample levels ranging from 0.15-2.17 ng/ml and composited plasma samples ranging from 0.4-3.11 ng/ml. A statistically significant correlation between urine OTA levels ($R^2 = 0.52$) and dietary OTA consumption was found. However, the authors caution that this relationship is too weak to be used in a predictive manner (118). In plasma, no significant correlation was found between the two ($R^2 = 0.29$). For the purpose of establishing a valid biomarker of OTA exposure in the future, urinary OTA appears a stronger candidate.

Risk characterization integrates the dose-response and exposure assessments to determine the probability of an adverse effect to human populations by an agent. To estimate risk associated with OTA exposure and various health effects, it was not possible to estimate a population attributable risk due to a lack of available epidemiological data. Instead, a systematic review was performed in this study, and unadjusted odds ratios (ORs) were calculated for various health effects associated with OTA exposure based on data from existing studies.

2.1.4 Systematic Review

The purpose of this systematic review was to attempt to reconcile the human and animal study results on OTA toxicity, or lack thereof. A literature search was performed on PubMed until October 4th, 2011. Search terms used without restriction included combinations of: (ochratoxin A), (human), (population), (disease), (urinary ochratoxin), (urinary OTA), and (urinary biomarker). Additionally, we searched reference lists from retrieved articles and searched ochratoxin review papers for any additional epidemiological studies on adverse effects associated with OTA exposure that may not have been retrieved in the initial search.

Eligibility criteria for inclusion in the review were as follows: (1) epidemiological studies; (2) case-control or cohort study design; (3) ochratoxin A as the exposure of interest; (4)

OTA exposure measured either in terms of dietary intake or urinary OTA levels; and (5) relative risk (RR) or odds ratio (OR) estimates with 95% confidence intervals (CIs) reported, or data to calculate these. Studies using serum OTA as a marker for exposure were excluded because of the poor correlation between dietary OTA intake and serum OTA as measured in (33).

Data on the following were extracted from each study: authors, publication year, study design and sample size, study location, study period, participants' gender and age, range of ochratoxin exposure, health effect under investigation, and data necessary to calculate ORs for each health effect if the OR was not already calculated. From these data, we calculated unadjusted ORs and 95% confidence intervals for each ochratoxin-related health effect examined in each of the studies (several studies examined more than one adverse effect).

2.1.5 Results

The step-by-step process of our literature search is presented in Figure 2-1 (Figure format (167)). From 2431 results, only those studies that met the criteria listed above were included. Fifteen studies were selected based on information in the title and abstract, and seven more were added based on reference lists in those selected studies. A full-text review of all 22 articles resulted in 19 being excluded because they did not measure urinary OTA or dietary OTA, or did not include both diseased and healthy individuals. Three studies contained the relevant information needed to calculate unadjusted ORs for different OTA health effects.

Table 2-4 provides an overview of the three eligible studies. Based on the data needed to calculate ORs, three studies were included. Due to the lack of similar health endpoints across the different studies, data could not be combined for meta-analysis. The three eligible studies included two in the Balkans (Croatia and Bulgaria) and one in Egypt. All studies measured

urinary OTA levels and associated these levels with several different adverse health effects. Each study also had at least one corresponding control group. Domijan et al. (156) compared individuals in a BEN-endemic village to those in a non-BEN-endemic village, whereas Nikolov et al. (154) and Wafa et al. (89) study used healthy human controls. Epidemiological studies were not included in the review if they did not examine both cases (i.e., those with confirmed disease) and controls. For example, Petkova-Bocharova et al. (116) and Castegnaro et al. (83) examined OTA in serum and urine in human subjects living in BEN-endemic vs. non-BEN-endemic villages, but all subjects involved in the study were healthy.

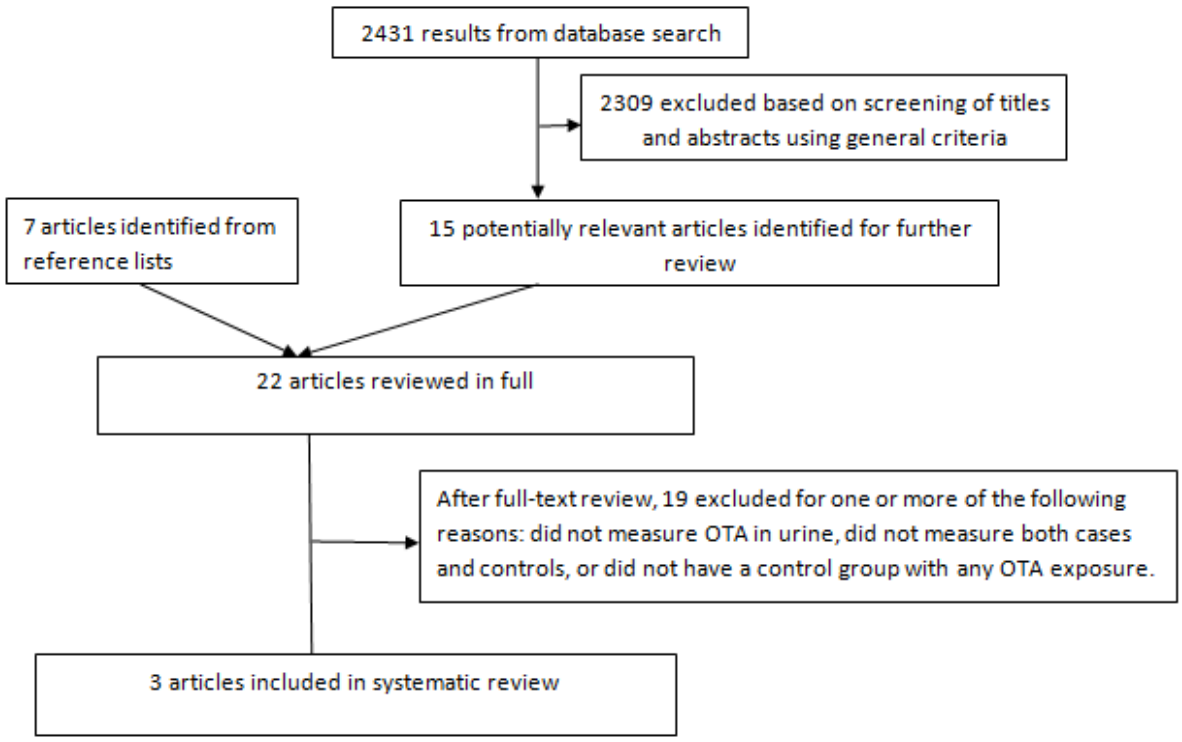

Figure 2-1: Selection of studies for inclusion in systematic review of adverse health effects associated with ochratoxin A (OTA) exposure as measured by urinary OTA.

Table 2-4: Eligible studies and Unadjusted Odds Ratios (ORs).

Location	Population characteristics	Proportion positive for urinary OTA (%)	Mean urinary OTA (ng/ml) (Range)	OR (95% CI)	Reference
Egypt	End Stage Renal Disease (ESRD) patients w/ treatment	4/11 (36.4%)	1.85 (nd*-6.70)	2.74 (0.402-18.69)	(89)[a]
	ESRD dialysis patients	1/11 (9.1%)	0.36 (nd-4.0)	1.23 (0.115-13.14)	
	ESRD Totals	5/22 (22.7%)	Not Available	1.94 (0.357-10.52)	
	Renal transplant recipients	2/15 (13.3%)	0.12 (nd-1.36)	1.89 (0.28-12.65)	
	Nephritic Syndrome patients	8/15 (53.3%)	3.09 (nd-8.19)	10.79 (2.28-50.91)	
	Patients with urothelial tract tumors (UTT)	1/15 (6.7%)	0.36 (nd-4.64)	0.88 (0.085-9.14)	
	Potential kidney donors	2/15 (13.3%)	0.26 (nd-3.42)	Not Available	
	Controls	3/40 (7.5%)	0.01 (nd-0.31)	Not Available	
Bulgaria	BEN/UTT patients	14/36 (38.9%)	Not Available (0.005-0.604)	1.29 (0.58-2.87)	(154)[b]
	Healthy persons from BEN families	12/25 (48%)	Not Available (nd-0.033)	Not Available	
	Healthy persons from non-BEN families in BEN villages	14/32 (43.8%)	Not Available (nd-0.043)	Not Available	
	Healthy persons from non-BEN villages in BEN area	4/31 (12.9%)	Not Available (nd-0.041)	Not Available	

Table 2-4 Continued

	Healthy persons from non-BEN area	0/3 (0%)	Not Available	Not Available	
Croatia 2000	Endemic BEN village	19/45 (43%)	0.007 (nd-0.086)	1.90 (0.58-6.23)	$(156)_c$
	Healthy control village	5/18 (28%)	0.003 (nd-0.02)	Not Available	
Croatia 2005	Endemic BEN village	8/45 (18%)	0.001 (nd-0.015)	3.68 (0.43-31.78)	$(156)_c$
	Healthy control village	1/18 (6%)	0.005 (0-0.01)	Not Available	
	Combined BEN Villages	27/90 (30%)	Not Available	2.14 (0.80-5.74)	

*nd: non-detect
**Mean urinary OTA levels were not provided for all sampled groups and were therefore labeled as 'Not Available'. Odds ratios labeled 'Not Available' were for control groups, hence, no odds ratio could be calculated.
Methods
a: HPLC confirmed by hydrolysis
b: OTA determination methods unknown
c: HPLC confirmed using internal standard

The calculated unadjusted odds ratios are summarized by study and health effect in Table 2-4. The table includes the three studies used to calculate unadjusted ORs organized by location. Information on each disease assessed, the proportion of subjects with each disease who had detectable urinary OTA, the levels (mean and range) of measured urinary OTA, and unadjusted odds ratio with 95% confidence interval are included in the table. The highest level of measured urinary OTA was in nephritic syndrome patients in Egypt, followed by patients being treated for ESRD in Egypt. Patients mean levels of urinary OTA were 3.09±3.4 ng/ml and 1.85±2.8 ng/ml respectively. Unadjusted ORs ranged from 0.88-10.79 for all adverse health endpoints. While these ORs were unadjusted, demographics including age, sex, socio-economic status, and other lifestyle factors, were similar across both cases and controls in each accepted study.

No statistically significant associations between OTA exposure and any human disease were found in the Bulgarian or Croatian study populations. Only one adverse health effect, nephritic syndrome, was found to have a statistically significant association with OTA exposure, in the Egyptian study population (44). The OR of OTA-related nephritic syndrome in this population was 10.79 (95% CI: 2.28-50.91). However, it is worth noting that the sample size of this particular study group was 15: relatively small. Figure 2-2 summarizes ORs and 95% confidence intervals for each of the adverse health outcomes associated with OTA exposure as measured by urinary OTA in the three study populations.

Urinary OTA levels obtained by Wafa et al. (89) were compared to those obtained by Gilbert et al. (118) and the urinary OTA studies summarized in Table 2-3. Upon comparison, the levels of OTA found in urine in the Gilbert study ranged from non-detectable to 0.06 ng/ml: much lower than the 3.09 ng/ml mean urinary OTA level found in the nephritic syndrome patients in Wafa et al.(89). Urinary OTA levels in humans from several different world regions, summarized in Table 2-3, range from <0.01-148 ng/ml. The extremely high end of this range comes from a study in Sierra Leone (72). When this study and the Egyptian study are excluded, the urinary OTA levels measured in different world regions ranges from non-detectable to 0.860 ng/ml: much lower than the levels found in the study populations in Egypt and Sierra Leone.

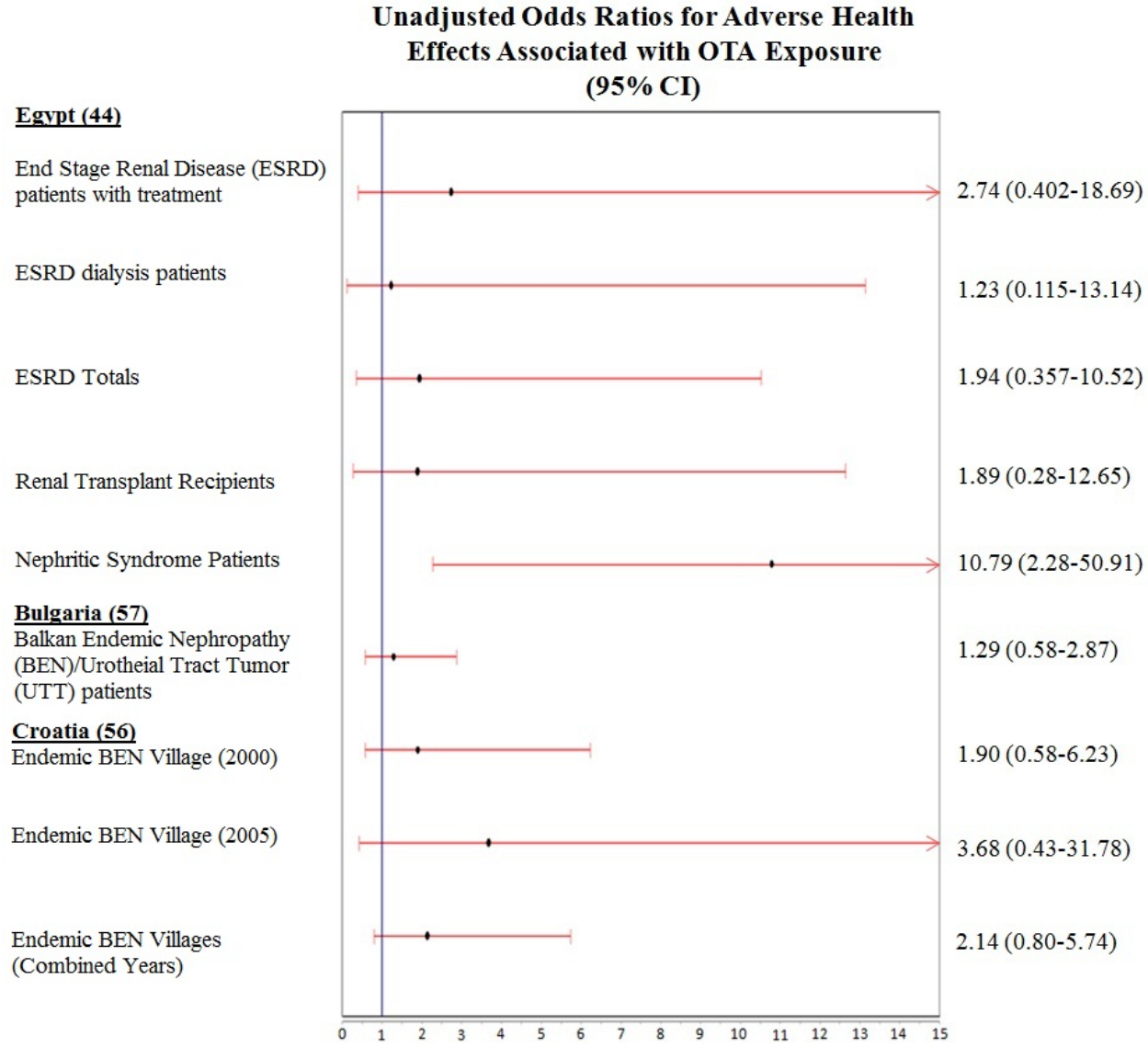

Figure 2-2: Unadjusted odds ratios and 95% confidence intervals for various health endpoints of OTA exposure identified through a systematic review.

2.1.6 Conclusions

The review of the epidemiological data suggests that, with one exception, there appears to be limited statistically significant evidence for human health risks associated with OTA exposure. The one statistically significant association concerns an increased risk of nephritic

syndrome at very high exposures to OTA, based on case-control studies assessing multiple potential adverse health effects in an Egyptian population (44). However, the sample size of this studied population was very small, and the urinary OTA levels associated with nephritic syndrome were much higher than urinary OTA levels measured in multiple other world regions, with the exception of Sierra Leone.

Nephritic syndrome, also known as glomerulonephritis, is a disorder of the glomeruli characterized by body tissue swelling (edema), high blood pressure, and the presence of red blood cells in the urine (168). The cause of nephritic syndrome is multifactorial, and the term "nephritic syndrome" itself describes a condition with multiple symptoms. As no other risk factors were controlled for in Wafa et al. (89), it is possible that OTA is not the only etiologic factor in all the cases of nephritic syndrome in this study population. Moreover, the OTA exposures measured in Wafa et al. (89) were over three orders of magnitude higher than the highest exposures measured in Gilbert et al. (118) and the vast majority of other urinary biomarker studies. Populations in which OTA exposures are extremely high (such as those studied in Egypt and Sierra Leone) may experience a significantly increased risk of nephritic syndrome. However, because this extremely high level of OTA exposure is not expected in most other parts of the world as evidenced by urinary OTA levels collected in multiple other world regions, the risk of OTA-related nephritic syndrome on a global scale is not expected to be significant. In relation to Health Canada's recently proposed MLs in a variety of commodities, it appears that these regulations would not significantly improve human health due to limited evidence linking OTA exposure to adverse health effects in diverse populations.

Finally, when looking a bladder cancer, an adverse health endpoint often associated with OTA exposure, both incidence and mortality rates remain relatively low compared to other

countries. Bladder cancer incidence, for both sexes in Canada, sits at 6.8/100k, while mortality is much lower at 2.5/100k individuals (169). This incidence rate is outside of the top fifty rankings worldwide and is third in the Americas. Canada falls behind the US, which does not regulate OTA, and Uruguay, which has a very lenient regulation of 50 ng/g in rice, barley, beans, coffee and corn. In relation to other incidence rates for cancers in Canada, bladder cancer is twelfth on the list behind prostate, breast, colorectum, lung, corpus uteri, non-Hodgkin lumphoma, skin melanoma, thyroid, leukemia, kidney and ovarian cancers.

Several limitations exist with our analysis of epidemiological studies on OTA. The main limitation is lack of validated markers of exposure in human populations. While multiple studies examined urinary and serum OTA levels in humans in different regions of the world, none of the studies for which odds ratios were calculated measured actual OTA exposure in the diet. Ideally, the urinary OTA biomarker of exposure should be further studied to determine if it accurately measures internal dose of OTA and/or dietary exposure. This may be done by investigating repeated associations of serum or urinary OTA with dietary intake, with reasonable statistical significance, in other populations worldwide. Another limitation of our analysis concerns the small number of studies assessed, and the relatively small sample population sizes in these few case-control studies. It was not feasible to conduct a meta-analysis of OTA-related health disease, because each study assessed measured a different health endpoint. Finally, it was not possible to calculate adjusted odds ratios, because the studies did not provide sufficient data on potential confounders; instead, unadjusted odds ratios were calculated.

For the purposes of establishing appropriate regulatory policies regarding human exposure to ochratoxin A, it is critical to gain a better understanding of OTA's impacts on human health. To improve our understanding of possible effects of OTA exposure on human health,

two types of further studies would be useful. First, larger cohort or case-control studies in different parts of the world, which control for sociodemographic and other potential risk factors, are needed to better establish potential OTA-related human health effects. Second, further duplicate-diet studies are needed to validate biomarkers of OTA exposure in humans. Ideally, these studies would be replicated in different parts of the world; and, similar to (56), would assess OTA intake and biomarker levels over at least one month to account for the long serum half-life and renal elimination of OTA. Such studies would allow for improved exposure assessment, as well as improved correlation with human diseases and conditions, to better inform human health risk assessment of OTA.

2.2 ADDITIONAL RESEARCH REGARDING OCHRATOXIN A

In addition to analyzing available human health data and evaluating adverse health effects associated with OTA exposure, one further project investigated the economic impacts of Canadian OTA regulations and the likelihood of individual's exposure to OTA in Canada. First, Drs. Felicia Wu and Kyra Naumoff-Shields estimated the potential economic impacts to implementing OTA standards in Canada using a combination of studies to estimate OTA levels in Canada and extrapolate the amount of crops which would be rejected under Health Canada's proposed maximum limits. Second, as part of this group project, my research involved estimating the amount of OTA that would need to be consumed in order to develop any adverse health effect in Canada. A brief summary of the overall study along with the dietary consumption calculation are detailed below.

2.2.1 Potential Economic and Health Impacts of Ochratoxin A Standards

Wu F, Bui-Klimke TR, Shields KN. "Potential Economic and Health Impacts of Ochratoxin A Standards." (submitted for publication).

2.2.1.1 Introduction

As previously discussed, OTA has been shown to cause adverse renal effects in animal species; however, a risk assessment on human health effects showed only nephritic syndrome was associated with extremely high OTA exposures. Until recently, most nations did not have regulations for OTA in food and beverages. Implementation of regulations is likely to cause significant economic impacts which should be considered alongside potential health benefits.

2.2.1.2 Methods

The potential economic impacts to Canadian food producers was estimated using data from reported proportion of foodstuffs exceeding OTA MLs (90), and market data from farm cash receipts from Statistics Canada (170). For nations exporting to Canada, it was assumed that the proportion of rejected commodities was the same as those that would be rejected within Canada. This proportion data was used in combination with the Canadian Importer's Database by Industry Canada (171) and the United States Department of Agriculture (USDA) Foreign Agricultural Service's Global Agricultural Trade System (GATS) Database (172) to estimate foreign economic losses. Finally, the estimated level of OTA intake necessary to cause adverse health effects in humans was estimated using a combination of studies. This includes the previous risk assessment on human health and OTA exposure (173), the duplicate diet study by

Gilbert et al. (118), and the estimated level of OTA in certain Canadian commodities (90). This calculation was then compared to the actual per capita consumption for select commodities.

2.2.1.3 Results

Results indicate that if Health Canada's proposed MLs are enforced, losses to Canadian food producers could exceed $260 million Canadian dollars (CD) annually based on the proportion of products expected to exceed the MLs (Table 2-5). The greatest losses would occur to producers of wheat, oats and barley. Also included in Table 3-1 is information regarding the mean level of OTA in selected commodities, percent of samples that would exceed proposed MLs and the total annual value of Canadian commodities in 2011.

In the previous human health risk assessment on OTA, it was determined that only nephritic syndrome in an Egyptian population was significantly associated with OTA exposure (173). The mean urinary level of OTA of these patients was 3.09 ng/ml (89). Dietary OTA exposure corresponding to a urinary OTA level of 3.09 ng/ml was estimated using Gilbert et al.'s (118) duplicate diet study. Based on linearizing the data from (118) and extrapolating to OTA exposures in (89), the amount of OTA consumed necessary to achieve a urinary OTA level of 3.09 ng/ml and subsequently be at increased risk for developing nephritic syndrome is about 25,749 ng OTA per day. The risk that Canadians would ever reach these levels of OTA exposure is extremely small, regardless of the presence of absence of a ML. Even without an OTA ML established, a Canadian would need to consume over 29,000 g of wheat per day to achieve the exposures associated with nephritic syndrome in (89). However, the average Canadian adult was estimated to consume 53 g wheat per day (174).

2.2.1.4 Conclusion

Implementation of the proposed OTA MLs in Canada could cause economic losses to Canadian food producers in the hundreds of millions annually. The countervailing health benefits, however, of such OTA standards are unclear. Unless OTA levels significantly increase in Canadian commodities, it is highly unlikely that Canadians would consume the necessary amounts of contaminated foods necessary to cause adverse health effects. It is important that policymakers consider both the economic impacts and potential human health benefits when setting regulatory standards.

Table 2-5: Potential economic losses for Canadian domestic market under implementation of Health Canada OTA MLs.

Commodity Assessed	Mean (ng OTA/g)*	% Samples > ML	Total Annual Value of Canadian Commodities, 2011 (CD)**	Year	Potential Economic Loss (CD)
All wheat excl. durum	0.98	2%	$4,300,081,000	2011	$86,001,620
Durum wheat	1.05	6%	$816,410,000	2011	$48,984,600
Oats	2.24*	15%	$524,241,000	2011	$78,636,150
Barley	0.46	5%	$631,073,000	2011	$31,553,650
Ready-to-serve breakfast cereal foods	0.196	1%	$1,197,437,000	2008	$11,974,370
Other breakfast cereal foods, including infant cereal	0.23	9%	$37,411,000	2009	$3,366,990
TOTAL					**$260,517,380**

*Kuiper-Goodman et al. (90) and **Statistics Canada(170). (2011 Farm cash receipts)

3.0 AFLATOXIN REGULATIONS AND GLOBAL PISTACHIO TRADE: INSIGHTS FROM A SOCIAL NETWORK ANALYSIS

Bui-Klimke TR, Guclu H, Kensler TW, Yuan J-M, Wu F. "Aflatoxin Regulations and Global Pistachio Trade: Insights from a Social Network Analysis." (submitted for publication).

3.1 ABSTRACT

Aflatoxins, carcinogenic toxins produced by a variety of *Aspergillus* fungi, contaminate maize, peanuts, and tree nuts in many regions of the world. Pistachios are the main contributor to human dietary aflatoxin exposure from tree nuts worldwide. Over 120 countries have regulations for maximum allowable aflatoxin levels in food commodities. We developed social network models to analyze the association between nations' aflatoxin regulations and global trade patterns of pistachios from 1996-2010. The main pistachio producing countries are Iran and the United States (US), which together contribute to nearly 75% of the total global pistachio market. Over this time period, which saw changes in nations' aflatoxin regulations in pistachios, global pistachio trade patterns changed; with the US increasingly exporting to countries with stricter aflatoxin standards. The US pistachio crop has had consistently lower levels of aflatoxin than the Iranian crop over this same time period. As similar trading patterns have also been

documented in maize, public health may be affected if countries without regulations, or with more relaxed regulations, continually import crops with higher aflatoxin levels.

3.2 INTRODUCTION

Aflatoxins are among the most potent naturally occurring liver carcinogens known, and contaminate a variety of food crops around the world. With an estimated 5 billion people exposed to dietary aflatoxins and over 120 countries regulating aflatoxin in food as of 2003, this research investigated the role aflatoxin regulations play on impacting human health. Aflatoxin regulations are in place to protect human health by decreasing dietary exposures to aflatoxin (137, 175). Several of these regulations are summarized in Table 3-1.

While many regulations on maximum allowable aflatoxin levels are put in place to protect human and animal health, they may also have substantial impacts on food trade activities around the world. For example, imposing a harmonized worldwide standard of 20 nanograms of aflatoxin per gram of maize (ng/g) was estimated to result in annual losses of $92 million USD in global maize trade, compared to a standard of 4 ng/g, which would result in over $450 million USD in annual losses (176). Because of the large number of countries that have regulations on allowable mycotoxin levels in imported foodstuffs, there has been recent interest in whether associations exist between regulations and trade. Wu and Guclu (173, 175) recently examined aflatoxin regulations in a network of global maize trade and found nations tend to trade maize with other nations that have identical or very similar aflatoxin standards, even defying geographical distances to engage in such trade.

Table 3-1: Summary of aflatoxin regulations (total aflatoxins) for pistachios in select countries.

Country	Standard for total allowable aflatoxins in 1995(138) (ng/g)	Standard for total allowable aflatoxins in 2003(137) (ng/g)
USA	15	15
Iran	No Regulation	15
European Union (EU)	No Regulation	4 (Changed to 10 in 2009)
Belgium	No Regulation	4 (Changed to 10 in 2009)
Canada	15	15
Germany	4	4 (Changed to 10 in 2009)
Hong Kong	15	15
Japan	20*	20*
Saudi Arabia	No Regulations	No regulations
China	No Regulations	No Regulations
Egypt	No Regulations	No Regulations
The Netherlands	10*	4 (Changed to 10 in 2009)
Russia	5	5

*The standard in Japan and The Netherlands is based on a standard for AFB_1 only. AFB_1 represents about half of the sum of total aflatoxins (AF B_1 + B_2 + G_1+ G_2), thus, the standard was doubled.

Aflatoxin contamination events in pistachios have commonly disrupted trade in the last two decades. Most notably, in 1997, the European Union (EU) banned all pistachio imports from Iran due to aflatoxin levels between 11-400 ng/g. In 2002, the UK called for a reinstatement of the 1997 ban on Iran pistachios due to aflatoxins contaminating over 10% of sampled consignments. Most recently, in 2010, the US instituted a ban on all Iran pistachios. The global pistachio market is dominated by Iran and the United States; nearly 75% of the world's pistachio exports come from Iran (47%) and the US (25%) (177). However, there appears to be a difference in the crop quality between countries with Iran pistachios containing an average of 54 ng/g aflatoxin and majority of US pistachios containing average levels below

the EU standard of 10 ng/g (96). The history of pistachio contamination with aflatoxin combined with the market domination by Iran and the US make it feasible for trade patterns to be analyzed over time to determine if associations exist between pistachio crop quality, exports and global trade.

The goal of this paper is to examine the impact of aflatoxin regulations on trade patterns for pistachios worldwide. Pistachios, like maize, are commonly infected by *Aspergillus spp.* and subsequently contaminated with aflatoxin. Furthermore, pistachios are the main contributor to dietary aflatoxin exposure from tree nuts, accounting for 7-45% of total aflatoxin exposure from all sources (96).

Using social network modeling tools (178), we tracked global trade patterns from the US and Iran each year between the years of 1996 and 2010, inclusive, to determine if aflatoxin regulations in the pistachio-importing nations worldwide appear to play a role in whether nations import primarily from the US or Iran, independent of other political factors. Each model contains information about the volume of trade of pistachios as well as aflatoxin regulation data for each country. Network modeling has provided a useful tool for other public health applications, including prediction models for disease transmission and control (179-181), prediction of obesity and smoking in social groups(182, 183), and modeling global maize trade (173, 175).

We hypothesized, based upon an earlier study examining the impact of EU aflatoxin regulations on US pistachio and almond trade (175, 184), that the nations with the strictest standards would import from countries with the highest quality crop in order to reduce economic losses. If this hypothesis holds true, public health is likely being negatively affected in many ways. As shown in Wu & Guclu (175), countries with similar regulations trade more maize with

each other than countries with dissimilar regulations. If this pattern also exists for pistachios on a global scale, it may also exist for a wide range of commodities. Therefore, countries without regulations may be importing more contaminated commodities from other countries with lenient or no regulations, predisposing individuals in those nations to higher risk of adverse effects associated with food contaminant exposures.

3.3 METHODS

3.3.1 Social Network Modeling

To determine market trends in pistachio trade, a social network model was created for every year from 1996 to 2010, inclusive. Each model depicts the amount of pistachios exported from the US and Iran to each importing nation worldwide. Each nation is represented as an individual node or "actor" in the network models, connected to other nations by lines (edges) if these two nations traded pistachios (one nation exporting to the other). In these specialized directed social network models - one for each year from 1996-2010 - the US and Iran are the two central nodes exporting pistachio to other countries, and the amounts of pistachio exports are represented by the thickness of the line in the network representations. The nodes on the boundary of the graphical network representations signify the countries, which are importing pistachios from either the US or Iran or both (Figure 3-1 through 3-5).

The nodes representing the pistachio-importing nations are color-coded according to the strictness of their aflatoxin regulations; i.e., maximum tolerable level of total aflatoxins

(aflatoxin B1+B2+G1+G2) in pistachios. As aflatoxin regulations and the amount of exports changed over the years, the colors of nodes and line thickness also changed.

The network analysis software Pajek™ (185) and igraph library (186) was used to create the 15 yearly network models. Pistachio export data was compiled using the USDA Foreign Agricultural Service Global Agricultural Trade System (FAS GATS - http://www.fas.usda.gov/gats/default.aspx) and the Iran Pistachio Association (IPA - http://www.iranpistachio.org/). Data on the number of pistachio consignment rejections in the EU was compiled using the EUROPA - Rapid Alert System for Food and Feed (RASFF) - (http://ec.europa.eu/food/food/rapidalert/index_en.htm).

The models were analyzed longitudinally to determine the main importers from the US and Iran; as well as to determine the association, if any, between aflatoxin standards and pistachio trade patterns over the years. The total amount of pistachios exported from the US and Iran was compared year by year to determine the major pistachio exporter in each model. Additionally, the amount of pistachios and aflatoxin regulatory level was compared between years to determine trends in exports. The amounts of pistachio exports from the US and Iran to countries which had changes in aflatoxin tree nut regulations were analyzed to determine if countries with strict standards imported from Iran or the US, or if there were no differences. Likewise, this analysis was done for pistachio-importing nations with relaxed or no aflatoxin standards on pistachios.

3.3.2 Crop Quality Assessment

The relative levels of aflatoxin contamination in the US and Iranian pistachio crops were compared in two ways. First, governmental reports, peer-reviewed publications, and online

agricultural databases were searched for information regarding aflatoxin levels in US and Iranian pistachios. Next, the RASFF database was used to determine the number of rejected consignments being exported from the US and Iran to the EU. The number of rejected consignments from each country was graphed along with the amount of pistachios imported from the US and Iran. As the RASFF database also reports contamination levels measured in rejected food lots, it was possible to calculate average aflatoxin levels in rejected pistachio consignments entering the EU from both the US and Iran.

3.3.3 Market Segregation Analysis

We analyzed market segregation due to competition between USA and Iran in two ways. First, the United States' export share over that of Iran was calculated for the top ten pistachio importers worldwide. The proportion of the US exports was graphed for each of the top ten countries for each year, using the equation:

$$\text{US Export Share Over Iran} = \left\langle \frac{n_{\text{USA}}}{(n_{\text{USA}} + n_{\text{Iran}})} \right\rangle,$$

where n_{USA} represents the amount of pistachios exported from the US to a particular country, n_{Iran} represents the amount of pistachios exported from Iran to the same country, and $\langle \ldots \rangle$ represents an average over the top ten importers. To calculate the US's export share over that of Iran, the amount of pistachios exported from the US to each of the top ten countries was divided by the total amount exported to each country from the US and Iran. A proportion of 1 signifies that 100% of pistachios imported to a particular country came from the US, whereas a proportion closer to 0 signifies that the majority of pistachios were imported from Iran.

Second, in order to take into account each importing country's aflatoxin standard, the weighted average for each country's imports was assessed longitudinally. The inverse of each country's standard (a measure of strictness of the aflatoxin standard) was multiplied by the amount of pistachios imported to obtain a strictness-weighted amount. The inverse of each standard was used so that a stricter standard was associated with a higher score, while nations with no aflatoxin standards for pistachios were given a strictness score of zero. This analysis was used to determine if the US or Iran traded with more countries with stricter aflatoxin standards. Because the US dataset contained more countries than Iran, the datasets were matched, each containing the same 41 pistachio importing countries. The following equation was used:

$$\text{Weighted Market Strictness} = \frac{\sum\left(\frac{1}{\text{AflaStd}} \; x \; n_{country}\right)}{n_{total}}$$

where AflaStd represents the aflatoxin standard of the importing country, $n_{country}$ represents the amount of pistachios imported from the US or Iran, and n_{total} represents the total amount of pistachios exported from both the US and Iran that year.

Finally, to serve as a control in order to determine if segregation was a result of aflatoxin regulations or of political factors unrelated to aflatoxin, we conducted the same analyses using grape exports. Grapes, which are not commonly contaminated by aflatoxin, are not subject to aflatoxin regulations. It was assumed that any sanctions placed on Iran would be followed by all UN nations. Greece is a member of the UN. Therefore, Iran's grape exports were compared to Greece's grape exports (a country with similar amounts of exports) to a variety of countries. Any difference in grape trading patterns between Greece and Iran with other countries could

infer the impact of sanctions – or any type of non-aflatoxin-related barriers – on Iran's exporting business activities.

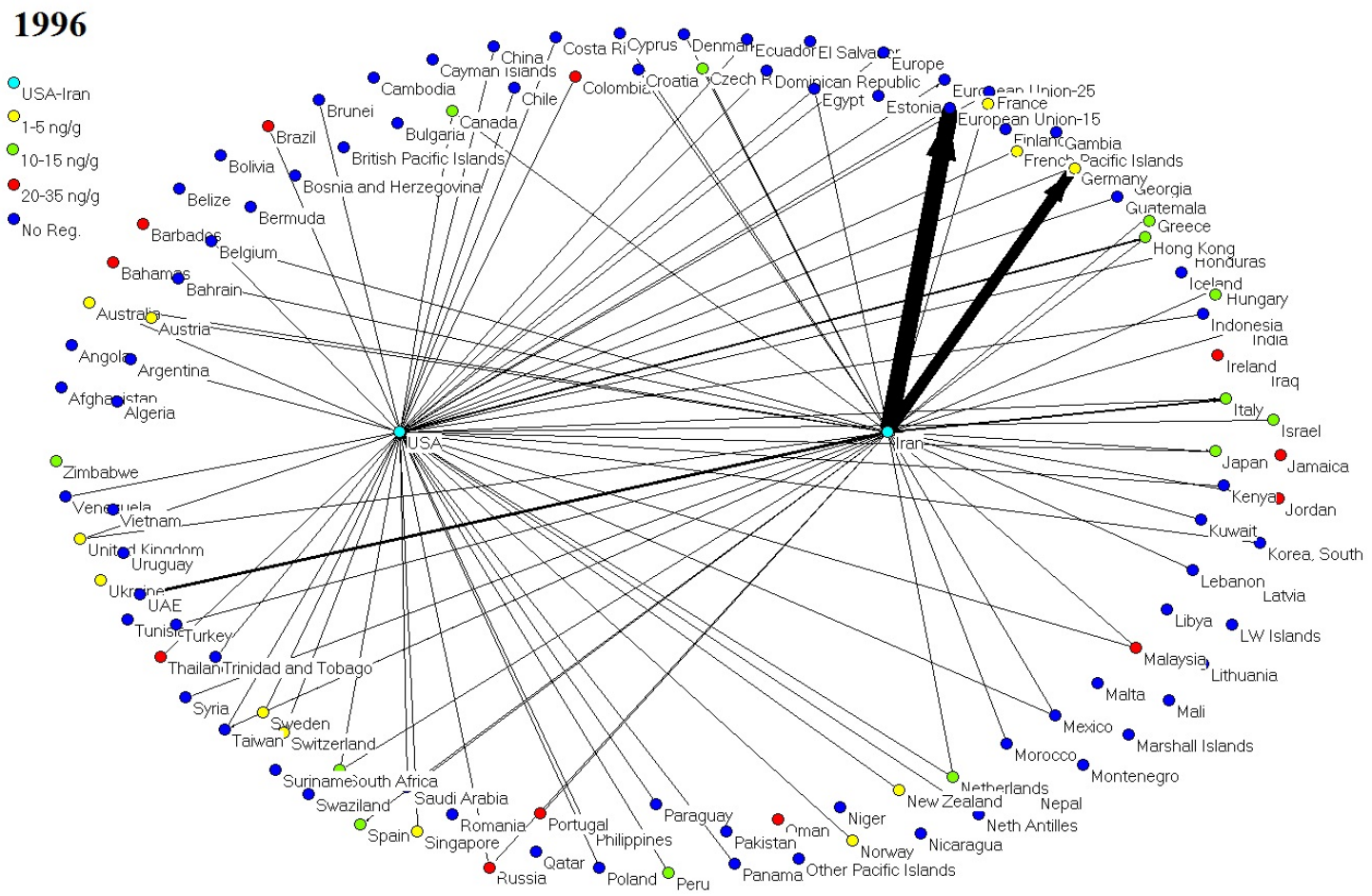

Figure 3-1: Social network model for 1996.

1996 serves as an initial time point for our dataset.

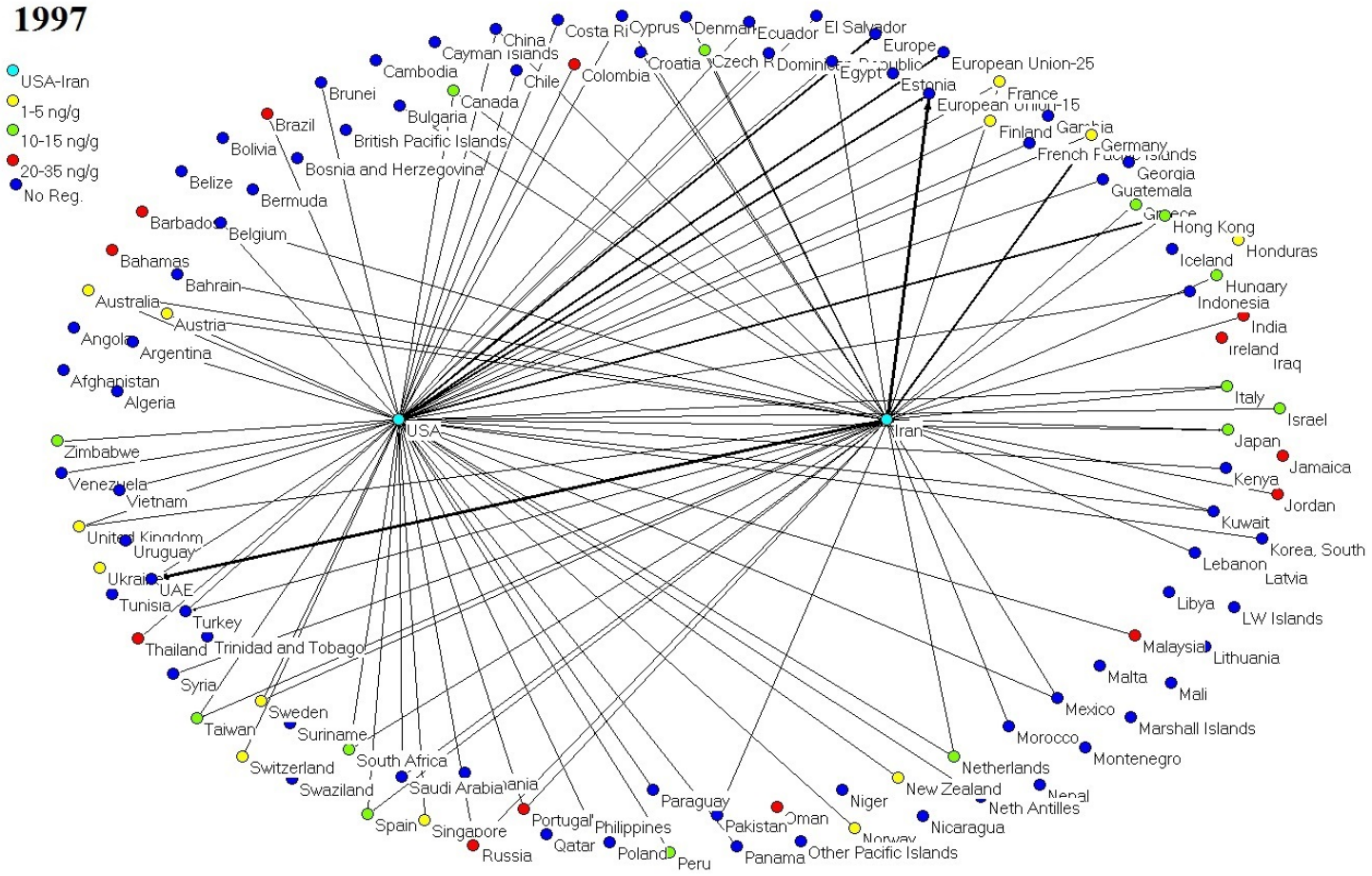

Figure 3-2: Social network model for 1997.

1997 shows a year when a notable aflatoxin outbreak occurred in Iran pistachios.

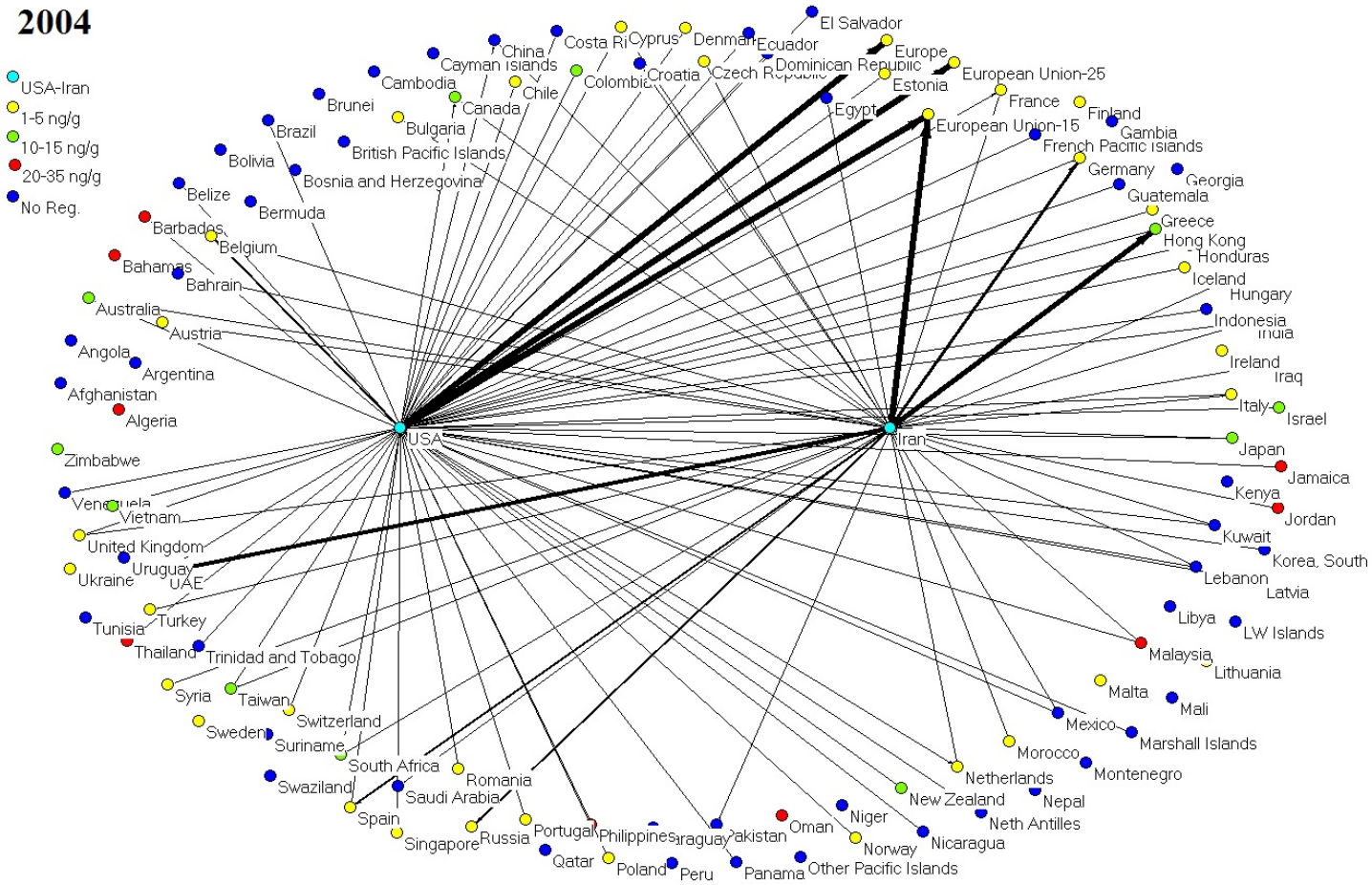

Figure 3-3: Social network model for 2004.

2004 shows the year after the EU implemented a 4 ng/g aflatoxin standard in tree nuts.

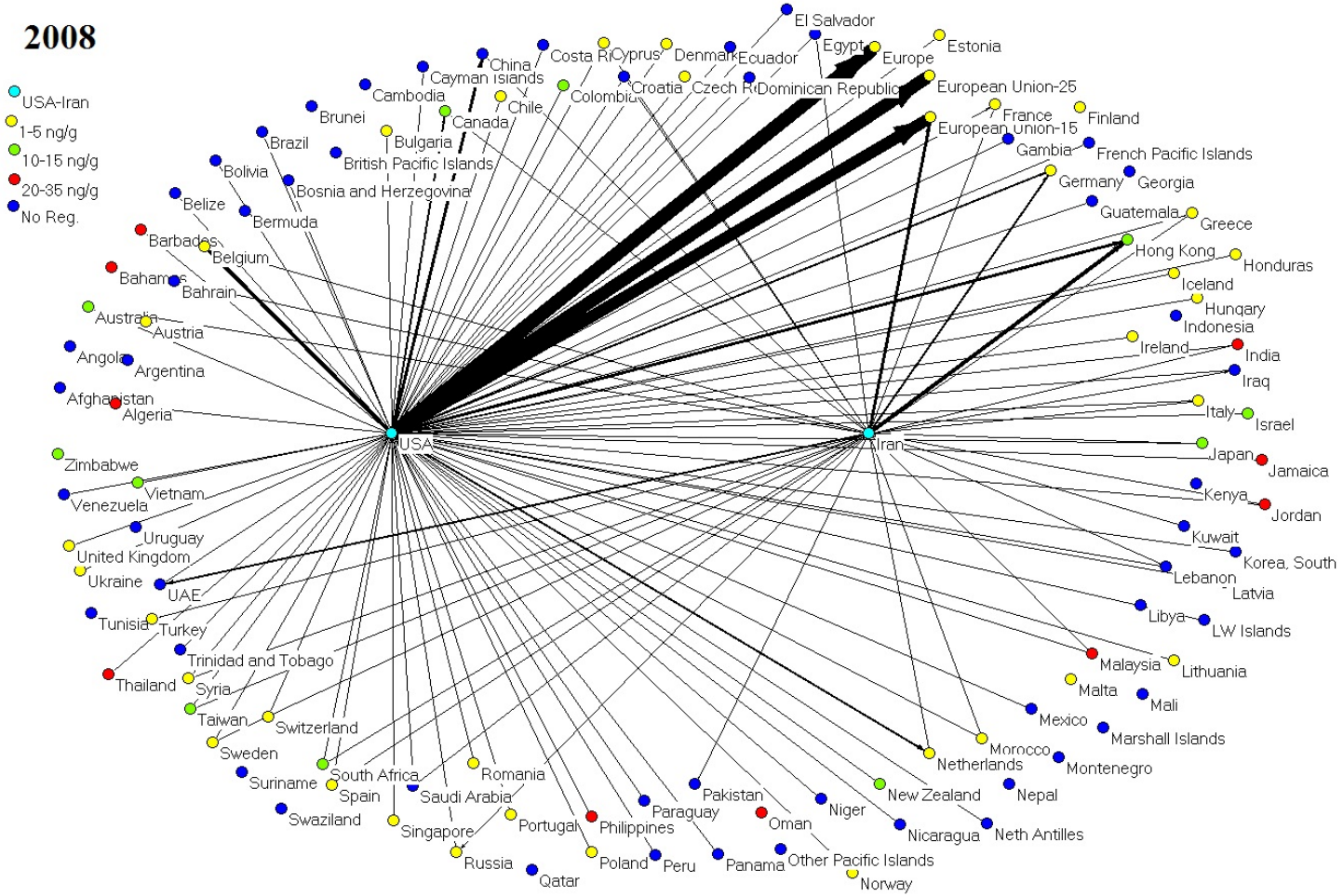

Figure 3-4: Social network model for 2008.

2008 shows the first year the US produced more pistachios than Iran and the final year before the EU relaxed the aflatoxin regulation to 10 ng/g.

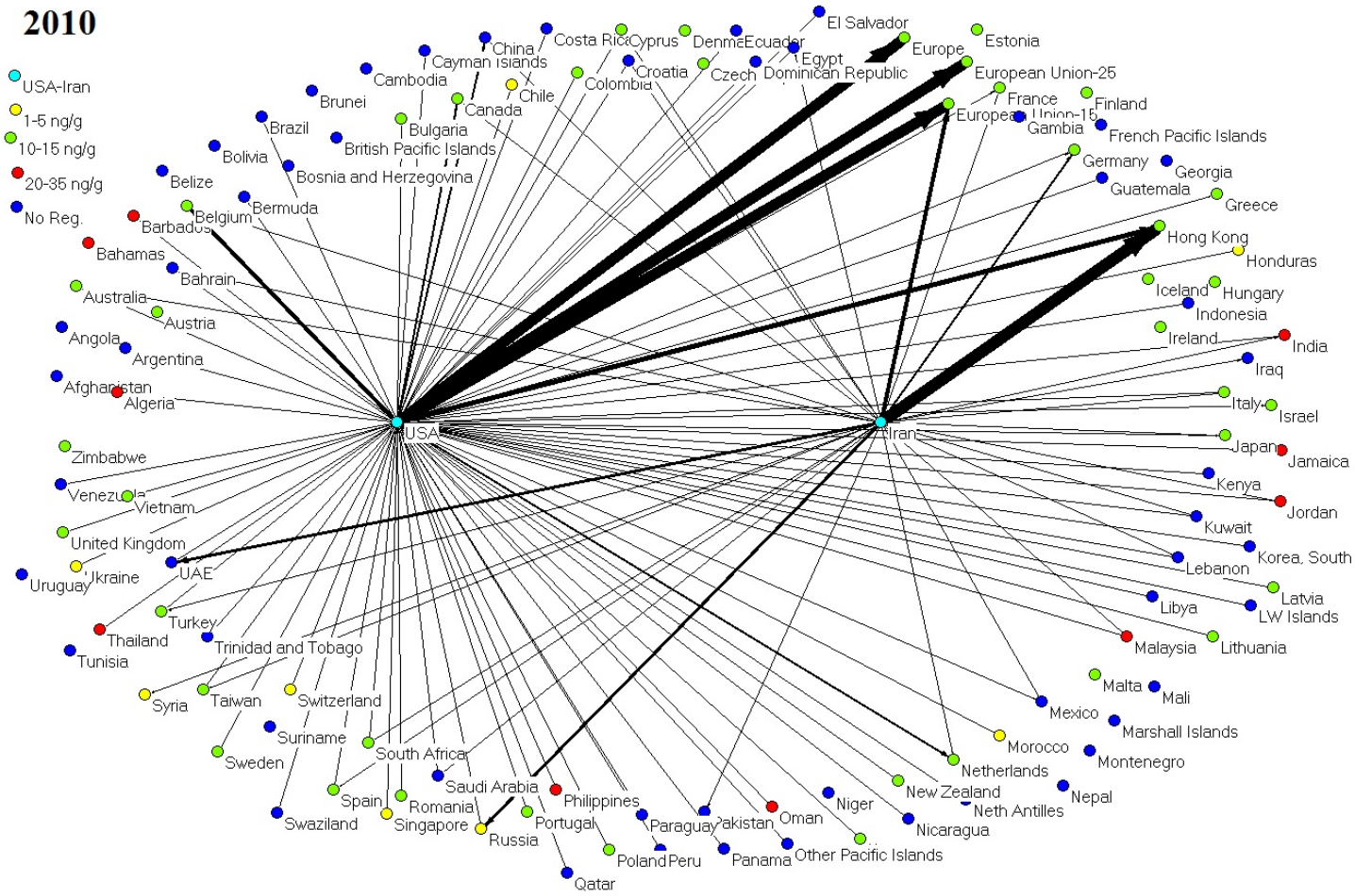

Figure 3-5: Social network model for 2010.

2010 shows the recent trends in pistachio trade. Also, in 2009, the EU relaxed its tree nut aflatoxin standard to10 ng/g.

3.4 RESULTS

Figures 3-1 through 3-5 shows five of the fifteen network models created of pistachio exports from the US and Iran to various nations worldwide. In 1996 (Figure 3-1), Iran was the major exporter of pistachios to all countries, with over 120,000 tons of pistachios exported; compared to only 22,000 tons for the US. The EU-15 was the major importer of pistachios in 1996 with 89,000 tons imported; 96% of which came from Iran. The US' largest exports went to Hong Kong, the EU and Canada; the three countries made up 65% of the United States' exports. As for Iran, 68% of pistachio exports went to the EU while 13% went to the United Arab Emirates (UAE).

In 1996**,** 71 out of 113 pistachio-importing countries did not have aflatoxin regulations for pistachios. Three of the top 5 importers from Iran did not have aflatoxin regulations; this included the EU, which did not have a blanket regulation for all member countries. Hong Kong and Canada, the US' major pistachio importers, each regulated aflatoxin at 15 ng/g (138). Thirteen countries had strict regulations ranging from 1-5 ng/g, fourteen had moderate regulations of 10-15 ng/g, and thirteen had the least strict regulations at 20-34 ng/g (138). Most importantly, no aflatoxin regulation existed for Iran in 1995, while the US regulated total aflatoxins at 15 ng/g (138). This did not change until 2003 when Iran began to regulate total aflatoxins in pistachios at 15 ng/g (138).

Due to high levels of aflatoxin contamination in Iranian pistachios in 1997, Iran's pistachio exports in that year (Figure 3-2) decreased by 46%, whereas the US increased total pistachio exports by 17%. Globally, Iran remained the major exporter of pistachios over the US;

however, the EU imported only 23% of its crop from Iran in 1997, compared with 96% the year before. Yet Iran's major importer in 1997 remained the EU (30% of total Iranian exports), while the UAE imported 28% of Iran's total crop. The US continued to export the majority of its pistachios to Hong Kong (34%), the EU (33%) and Canada (7%). No major changes in aflatoxin regulations occurred between 1996 and 1997.

Between the years 1998 and 2003, Iran remained the top exporter of pistachios globally and to the EU. In the year following the 1997 aflatoxin outbreak, the EU nearly quadrupled its pistachio imports from Iran while also increasing imports from the US. Over the next four years, Iran's major importers remained the EU, UAE, and Hong Kong. For the US, Japan, Hong Kong, and the EU were the major importers with Mexico importing increasing amounts of pistachios starting in 2000.

Over this time, regulations also began to change. By 2003, the EU had imposed a harmonized aflatoxin standard in tree nuts, including pistachios, of 4 ng/g. This regulation went into place for all EU member states, as well as the candidate member states in 2003. Regardless of the aflatoxin regulation in the EU, Iran has remained the top producer and exporter of pistachios globally for most of the years since 1996. In 2003, the total Iranian export amount topped 160,000 tons with over 70% exported to the EU (21%), UAE (36%), and Hong Kong (13%). Just over 35,000 tons of pistachios were exported from the US in 2003, the second highest total to date. The EU imported 65% of the US' pistachio crop, while Hong Kong only imported 2%, choosing Iran as their main supplier. Japan and Canada remained main importers of US crops and China increased its imports from 29 tons in 1996 to over 3100 tons in 2003.

Figure 3-3 shows 2004 exports from Iran and the US a year after the significant changes were made for aflatoxin standards in the EU. From 2003 to 2004, Iran total exports dropped

30%, while the US increased exports about 36%. Shortly after the EU aflatoxin regulations came into place, there was a significant decrease in Iranian pistachio exports to the EU and UAE; however, there were substantial increases in Iranian exports to Russia, Iraq and Hong Kong. Canada and Japan remained major importers of US pistachios in 2004, but were joined by the UK and UAE.

From 2004 to 2008 (Figure 3-4), major changes occurred in pistachio trade with little or no changes occurring in pistachio regulations. For the first time, the US was the major exporter of pistachios with exports reaching over 120,000 tons, almost 40,000 more than Iran. The EU imported nearly 60,000 tons of pistachios from the US, compared to only 13,500 from Iran. The top 5 importers of US pistachios in 2008 were the EU, China, Hong Kong, Mexico and Canada. China and Mexico have no aflatoxin regulations; however, Hong Kong and Canada regulate aflatoxin at 15 ng/g and the EU at 4 ng/g.

In 2009, the EU revised the aflatoxin standard in tree nuts to a more relaxed standard of 10 ng/g. Results of this change are shown in Figure 3-5. As of 2010, Iran has regained the lead in global pistachio exports over the US, with over 160,000 tons exported. Their major importers were Hong Kong (38%), the EU (11%), and the UAE (10%). While the EU was a top importer of Iranian pistachios, the amount of pistachios imported from the US was nearly triple this amount. Major importers of US crops, other than the EU (43%), included Mexico (3%), Japan (3%), China (6%) and Canada (7%).

Overall, these network models and the associated analyses show that the US and Iran have exported to different markets over the past 15 years. Notable changes in trade occurred after the EU instituted stricter aflatoxin standards. The US is trading more with countries with

stricter standards; however, Iran has kept total exports up by exporting to countries with less strict regulations.

Figure 3-6 summarizes the amount of pistachios produced and exported by the US and Iran over the past 15 years. Over this time, Iran has remained the top producer and exporter of pistachios; however, US pistachio production and exports are slowly trending upwards. Iran had noticeable drops in both production and exports in the years 1997-1998 and 2000, largely due to excessively high aflatoxin levels. Over this same time period, the US has continued to increase pistachio exports such that it now roughly matches Iranian pistachio exports, although overall, US production is still much lower.

To determine the relative quality of Iranian and US pistachios in terms of aflatoxin levels, the number of RASFF rejections for aflatoxins exceeding the EU regulation was calculated between 1997 and 2010. No data prior to 1997 were available. The number of RASFF pistachio consignment rejections in the EU were graphed alongside the amount of pistachios imported from the US and Iran in Figure 3-7**.** The amount of pistachios exported from the US to the EU is slowly on the rise, whereas EU imports from Iran have been decreasing. The number of rejections of Iranian pistachios peaked in 2003 with 489 and was followed closely in 2004 with 485. US rejections peaked at 32 in 2009, but have remained under 20 per year for 11 out of the 14 years sampled. Even with Iran instituting a 15 ng/g maximum allowable aflatoxin regulation in 2003, the number and proportion of consignments rejected for excessively high aflatoxins has remained higher than those from the US. Between 2003 and 2005 inclusive, Iran and the US exported similar amounts of pistachios to the EU; yet the number of rejections over the three-year span for excessively high aflatoxin levels was 477 for Iran vs. 9 for the US. As of 2010, the number of Iranian pistachio consignment rejections remained higher than the US, though Iran

exported 35,000 fewer tons than the US to the EU. It appears as though the US crop has remained a more viable option because of lower aflatoxin levels, and the EU has increasingly accepted pistachios from the US.

A 2007 report by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (96) obtained 1849 pistachio samples from Iran to be tested for aflatoxin. The mean level of aflatoxin detected in these samples was 54 ng/g. As of 2007, aflatoxin regulatory limits for pistachios ranged from 4 ng/g to 35 ng/g around the world. JECFA estimates that the proportion of rejected pistachio consignment samples from Iran would range from 40% in countries with MLs at 20 ng/g, to 60% in countries, like those in the EU, with MLs at 4 ng/g. The high mean level of aflatoxin reported by JECFA was likely caused by several samples containing extremely high levels of aflatoxins.

No reports or publications were found in the publicly available literature that estimated aflatoxin levels in pistachios produced in the United States. However, the EU RASFF database has reported aflatoxin levels in rejected consignments since 2003; from which it is possible to infer relative pistachio quality from exporters to the EU, including the US. The mean level of aflatoxin reported in rejected consignments sent from the US to the EU between 2003 and 2011 was found to be 24 ng/g, whereas the mean rejected level in Iranian pistachios was 63 ng/g. The mean total aflatoxin level of US to EU rejected crops is less than half of the Iranian crops.

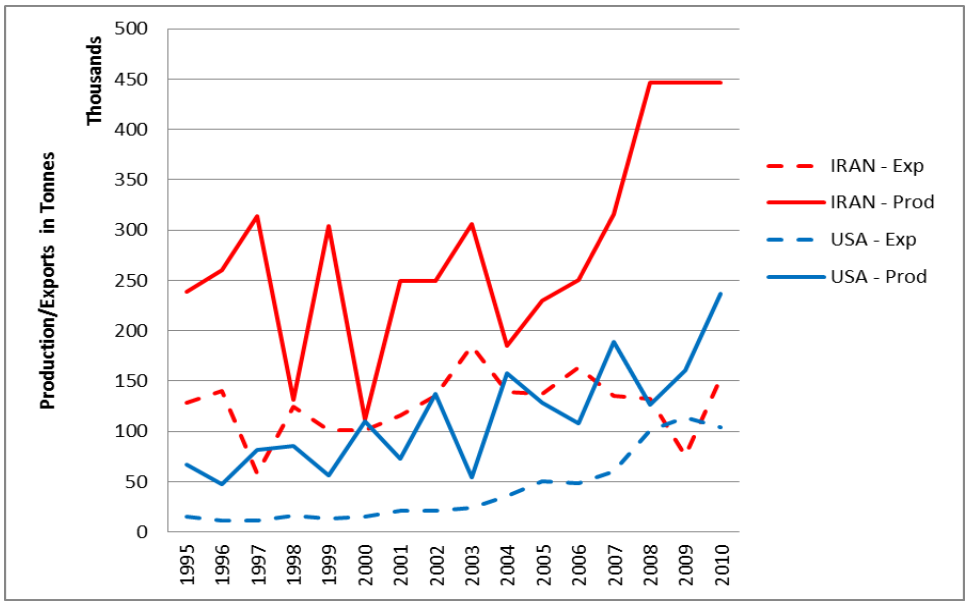

Figure 3-6: Iranian and US pistachio production and exports between 1995 and 2010.

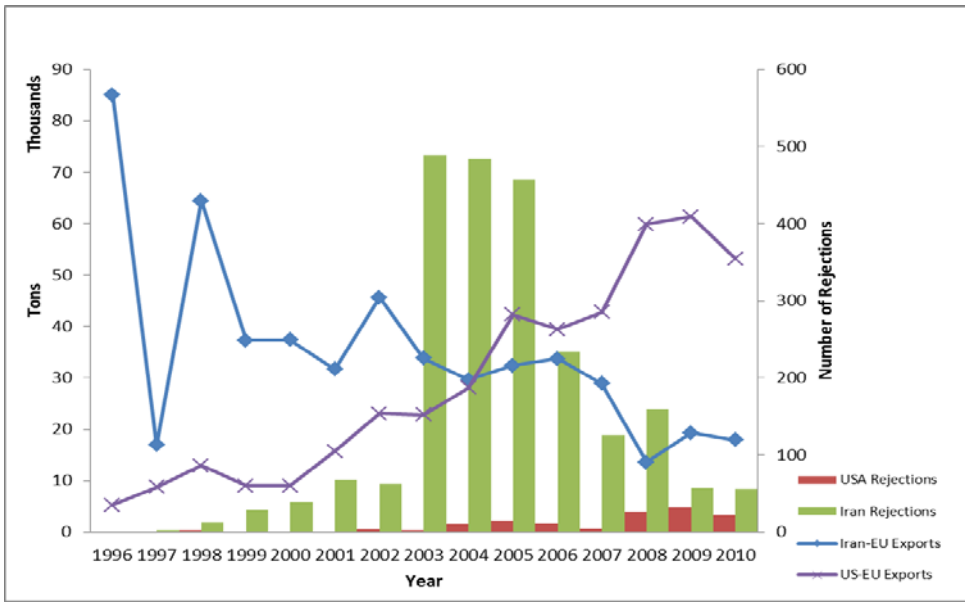

Figure 3-7: EU-specific exports from the US and Iran and number of pistachio consignment rejections.

Figure 3-8 demonstrates the market segregation of global pistachio trade, with nations with stricter aflatoxin standards importing primarily from the US, and nations with more relaxed or nonexistent aflatoxin standards importing primarily from Iran. There appeared to be little or no market segregation in the mid to late 1990s, with most countries importing equally from Iran

and the US. Russia and Germany appeared to import the majority of its pistachios from Iran over the past 15 years, whereas Canada and Belgium imported mainly from the US. In 2003, when the EU set its most stringent limit for aflatoxins in pistachios, the market began to segregate with the Netherlands, Belgium, Canada, China, and Japan importing 70% or more of its crop from the US, while Russia, Egypt, UAE, Hong Kong and Germany imported primarily from Iran. When comparing the number of rejections by the EU (Figure 3-7), the market appeared to respond accordingly by segregating, with more strict countries importing from the US and more lenient countries importing from Iran. Among these top ten pistachio-importing countries, China is the only country to which the US exports without an aflatoxin regulation. The remaining four countries import primarily or exclusively from the US have aflatoxin regulations at 15 ng/g or stricter. On the other hand, Iran exports to Egypt and the UAE, which have no regulations, to Hong Kong which has a regulation on aflatoxin at 15 ng/g, Russia at 10 ng/g, and Germany at 4 ng/g. Figure 3-9 summarizes grape export data used as a control, to bolster the hypothesis that the pistachio market segregation occurred based upon aflatoxin standards rather than other policy factors, including political ones. Unlike the pistachio data where a split in the markets was apparent around 2003, no obvious segregation of markets between Iran and Greece was seen in the same year. It appeared that only a few countries import only from one country, while most countries imported grapes at varying levels from each country over the 15-year period. Grapes, unlike pistachios, are not subject to aflatoxin regulations.

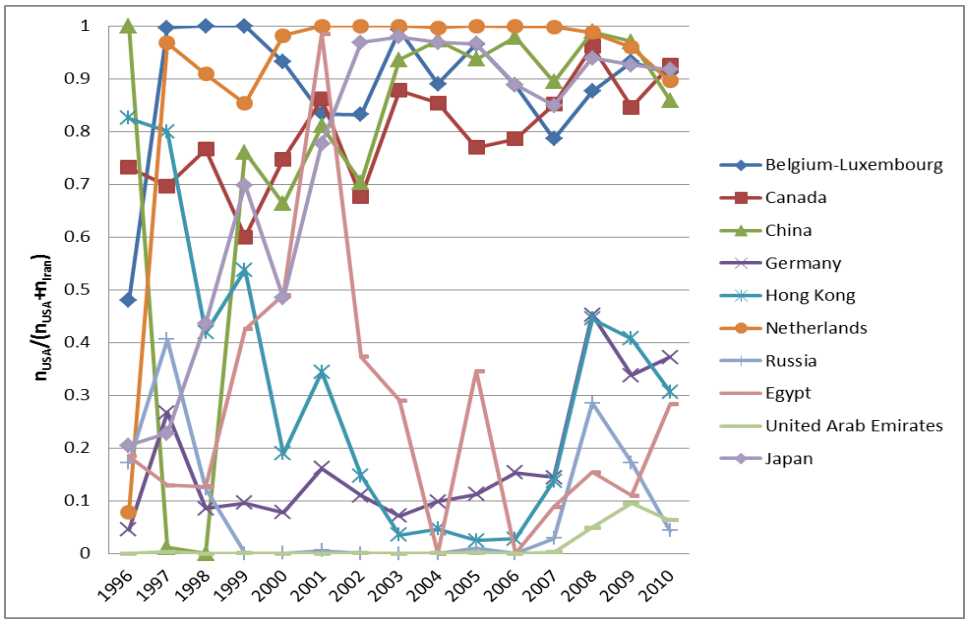

Figure 3-8: Market segregation for the top ten global importers of pistachios, as a function of the ratio of total US exports to total exports from both Iran and the US.

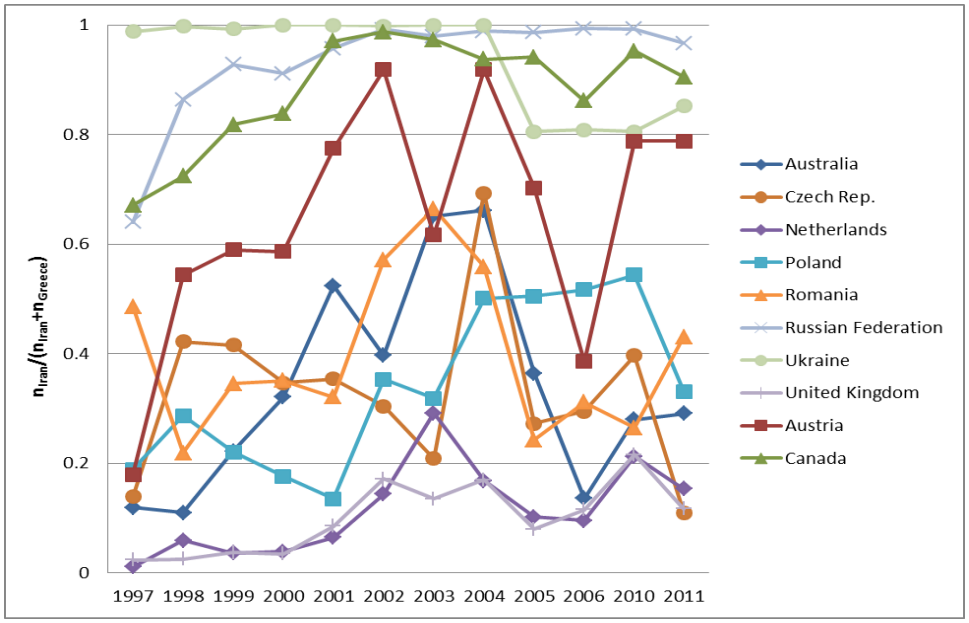

Figure 3-9: Lack of market segregation for grape exports from Iran and Greece.

As a final assessment to determine if differences between pistachio-trade patterns of US and Iran exist when the amount of pistachio exports to each country was weighted with the importing nations' aflatoxin standards (Figure 3-10). There appeared to be no difference in exports until 2003, when the markets began segregating with the US exporting to countries with stricter aflatoxin standards. Iran, continuing to export a larger pistachio crop, was exporting to countries with less strict regulations. After 2009, when the EU relaxed its tree nut standard to 10 ng/g, the market segregation began to diminish, although the US is still the EU's main pistachio source.

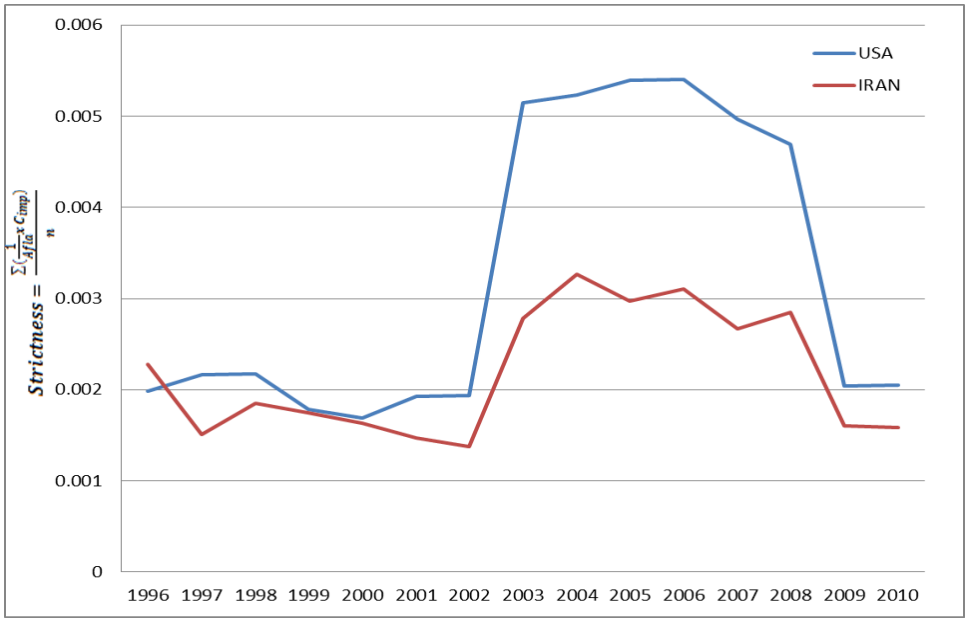

Figure 3-10: Market segregation taking into account the importing countries aflatoxin standard.

3.5 DISCUSSION

The time-series network modeling analyses conducted in this study suggest that the global pistachio market is segregated based on aflatoxin regulations worldwide, with the top exporters, the United States and Iran, exporting to nations with strict standards and relaxed/non-existent standards, respectively. Iran once dominated the global pistachio market; however, in recent years, the US has increased its pistachio exports substantially. Since 2003, the US has been exporting the majority of it pistachios to countries with stricter aflatoxin standards than Iran. Regardless of the amount produced and viability of the crop, Iran continues to trade with countries with relaxed regulation levels, or no aflatoxin regulations at all.

Since the implementation of the European Union's RASFF system for tracking consignment rejections due to aflatoxin, rejections by the EU of Iranian pistachios have greatly exceeded those of US pistachios. Between 2003 and 2005, the US and Iran exported similar amounts of pistachios to the EU, but the number of rejected consignments for excessively high aflatoxin in pistachios was substantially higher for Iran than for the US (477 vs. 9). After 2005, Iran pistachio rejections by the EU decreased, but this is likely due to the decrease in the total amount of Iranian exports to the EU, while US to EU exports were increasing.

Clear evidence of market segregation based on aflatoxin regulations started in about 2003. Prior to 2003, the main pistachio-importing countries varied their pistachio imports between the US and Iran. However, as aflatoxin regulations became stricter in certain nations worldwide, the US became the major exporter to countries with strict standards.

Political factors were considered when analyzing results. Since 2006, the United Nations (UN) has imposed multiple sanctions on Iran (187). In total, six different UN sanctions occurred between 2006 and 2010. Investigating each sanction, no sanctions were placed on food or feed

trade between Iran and members of the UN. The sanctions focused on embargoes on arms and assets, which would likely have little or no impact to the global trade of pistachios (187). Indeed, the grape market (of which Iran is a key exporter) shows no evidence of segregation based on different nations. It is used as a control in this study to demonstrate the potential role of the aflatoxin regulations in contributing to the market segregation seen in global pistachio trade.

Due to segregation in the global market, not only for pistachios but also for maize and other aflatoxin-contaminated commodities (175) many economic and health issues may arise (184). First, strict aflatoxin standards mean that less developed nations will export their best crops to avoid economic losses, but in turn be subject so consuming the highest contaminated crops themselves. Second, due to varying aflatoxin regulations in each country, even the best crops may be rejected resulting in large economic losses. Third, even if a rejected consignment can be returned to the country attempting to export, the cost of demurrage fees is substantial, and vulnerable populations may be exposed to higher levels of aflatoxin, resulting in adverse health effects. In many cases, it is low-income importing nations that have more relaxed or non-existent aflatoxin regulations, predisposing populations who are already at risk of various health effects from inadequate diets to higher levels of risk.

In summary, social network models show that pistachio trade patterns have changed globally over the past 15 years, with aflatoxin regulations likely playing a key role in the changing patterns of trade. Iran once dominated the global market individually, but now must compete with the US to be the world's top exporter of the crop. Aflatoxin regulations play a part in organizing the global trade of the crop, with the US exporting to countries with stricter aflatoxin standards. Whether it is to protect human health or reduce economic losses, countries

are increasingly importing pistachios from the US, especially those countries with strict maximum levels.

4.0 AFLATOXIN REGULATIONS OR MAIZE TRADE PATTERNS: WHICH CAME FIRST? A "CHICKEN-OR-EGG" INVESTIGATION

4.1 RESEARCH OVERVIEW

Previous research on global maize trade has shown that countries with similar regulations tend to trade more maize with each other than countries with dissimilar regulations (175). Three main maize trading clusters exist. The US is the center of one cluster and the countries which make up the EU represent another major maize trading group. The third cluster, which exports maize all over the world, is made up of Argentina, Brazil and China (175). In most circumstances, the top maize trading pairs have total aflatoxin regulations varying no more than 5 ng/g (175).

While it has been shown that nations tend to cluster into maize trading communities that share aflatoxin regulations, no causality has been elucidated. The goal of this project was to build upon the results of Wu and Guclu (175) to determine if maize trading patterns influenced aflatoxin regulations, or *vice versa*. Using a variety of annual maize trade data combined with each countries aflatoxin regulation and maize production, attempts were made to shed light on this "chicken-or-egg" scenario.

4.2 METHODS

4.2.1 Data Collection

Maize trade data were collected from the United Nations Commodity Trade Statistics Database (UN Comtrade, comtrade.un.org) between 1991 and 2010. Data included the amount of maize traded (in tons) for every import-export pair around the world. The mean maize trade was used in cases where countries reported imports differed from their exporting countries reported exports. For example, if Country A reported importing 100,000 tons from Country B, but Country B reported exporting 125,000 to Country A; the mean of the two values was used for analysis.

Aflatoxin regulation data was collected from both the FAO 1995(138) and FAO 2003(137) mycotoxin reports. Relevant databases were searched for any reports of aflatoxin regulations occurring (or changing) before or after the FAO reports were published.

4.2.2 Data Analysis

The top twenty maize trading partners, identified in Wu & Guclu (175), were graphed longitudinally along with aflatoxin regulation data. Changes in trade were examined specifically before and after maize trade regulations changed. This change could have occurred to the maize importer, exporter, or both. This preliminary analysis would indicate whether maize trade guided aflatoxin regulations or if countries became partners after regulations became more similar.

Further analyses focused on maize trade from the top ten exporters over the past 20 years. The amount of maize traded and aflatoxin regulations for the top ten exporters was compared with the top ten importers from each country, as well as ten "middle" importers. A middle importer was defined by sorting all of the importing countries from one major exporter from largest to smallest and taking the median ten countries. It was assumed that the top importers would have more similar regulations than the middle countries compared to the exporting country. The number of countries matching an exporter's aflatoxin regulation was compared with the number of countries which had more lenient, stricter, or no regulations. This comparison was broken up into two time periods, 1991-2002 and 2003-2010 based on the number of countries which changed aflatoxin regulations in 2003.

Other metrics calculated comparing top exporters to top and middle importers included:

1) Comparing the average of the inverse aflatoxin standards between top and middle importers and between 1991-2002 and 2003-2010 groups to the inverse aflatoxin standard of the exporter. This was calculated using the following formula for each of the ten exporting countries considered:

$$\text{Average Inverse} = \left[\frac{\left(\Sigma\left({}^{1}/_{AflaStd}\right)\right)}{(10 \times k)}\right],$$

where *AflaStd* represents the aflatoxin regulation level for each of the ten top or middle importers and *k* represents the number of years sampled in each time period (12 in the case of 1991-2002; 9 in the case of 2003-2011).

2) Calculating the differences in weighted averages using the inverse aflatoxin standards and amount of maize exported for each group of countries.

$$\text{Weighted Strictness} = \frac{\left[\sum_k \left(s_i^k - s_e^k\right) \times m_{i,j}^k\right]}{\sum_k \left(m_{i,j}^k\right)},$$

where *s* represents the inverse of an aflatoxin standard, s_i^k represents the strictness of top or middle importing country in year *k*, s_e^k represents the strictness of exporting country in year *k*, $m^k_{i,j}$ represents the amount of maize traded from country *i* to *j* in year *k*.

3) Calculating a weighted average inverse using the absolute value of the average difference in aflatoxin standards. This used the same formula as the weighted inverse calculation; however, the absolute value of the inverse aflatoxin standards was used. This calculation was used to remove the negative weight associated with some regulation changes between importer and exporter. For example, if Country A had a regulation of 10, Country B had a regulation of 5, and Country C had a regulation of 15; the difference of A and B is 5 and the difference of B and C is -5. Since the comparison is just looking at the difference between importer and exporter, the absolute difference should be considered and a negative weight should be avoided.

In all cases, values near 0 mean there is less difference between exporter and importer. Trends were examined to determine if regulations guided trade to trade guided regulations by comparing across time periods (1991-2002 vs. 2003-2010) and between top and middle

importers. Each formula was used for each of the major exporting countries, however, each country's top importers varied.

4.3 RESULTS

While many trading pairs increased the amount of maize traded over the past 20 years, no noticeable changes occurred after aflatoxin regulations changed or came into place. As of 1991, all of the top twenty exporters had aflatoxin regulations in place and 14/20 importers had aflatoxin regulations. In 2003, all exporting and importing countries had aflatoxin standards. There appeared to be no increases or decrease in maize trade depending on whether countries did or did not have aflatoxin regulations. For example, Figure 4-1 shows the amount of maize traded from the USA to Mexico from 1990-2011. The US regulated aflatoxin in maize at 20 ng/g during this time period, however Mexico did not regulate aflatoxin in maize until 1996 when a regulation was set at 20 ng/g (arrow). No changes in maize trade are apparent before or after regulations were put into place. Algeria (Figure 4-2) instituted a regulation of 20 ng/g in 2004, however, even though it matched the US' standard, showed decreasing imports from the US.

The top ten maize exporting countries over the past 20 years are Argentina, Brazil, China, France, Hungary, India, Paraguay, South Africa, Ukraine, and the United States. Table 4-1 summarizes the number of top and middle importers which have aflatoxin regulations matching the exporting nation for the two main time periods analyzed. The top importers have more matching countries compared to the middle importers and the amount of countries matching regulations increases between 1991-2002 and 2003-2011 for both top and middle importers. A similar opposite trend for countries without regulations. 100 countries were considered in each

time period since the analysis took into account ten importers from each of the ten major exporters.

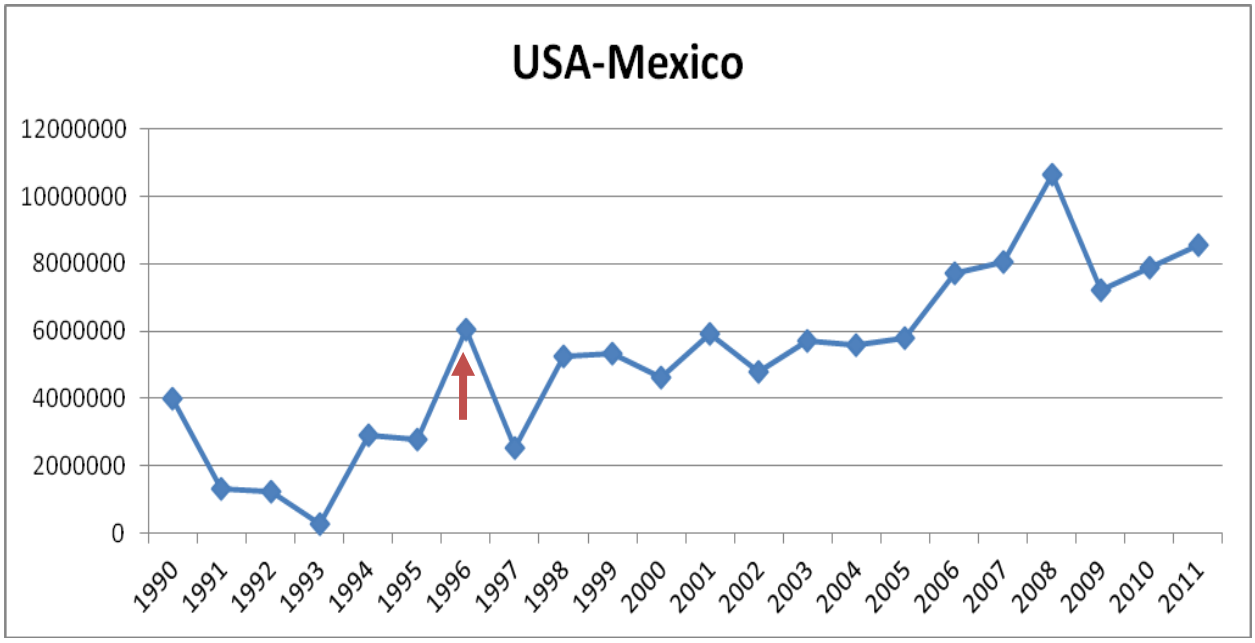

Figure 4-1: USA-Mexico maize trading relationship from 1991-2011.

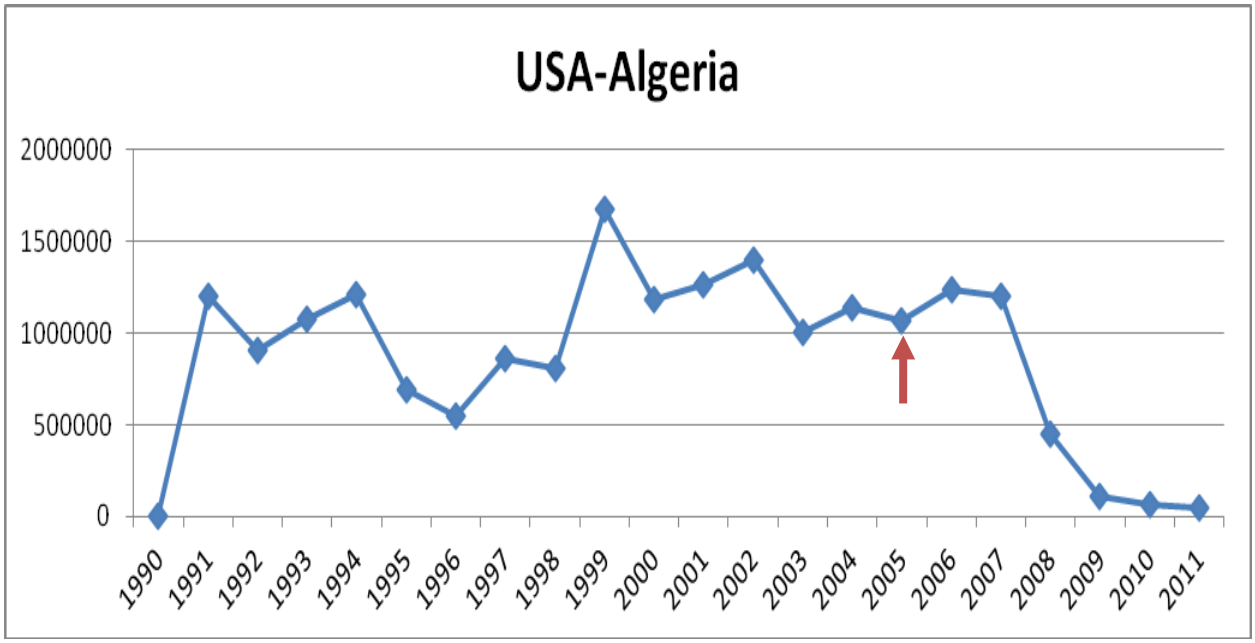

Figure 4-2: USA-Algeria maize trading relationship from 1991-2011.

Table 4-1: Number of Countries Matching Aflatoxin Regulations with the Top Ten Maize Exporting Countries.

Top Ten Importers From Major Exporters				
	Matched	No Regs	Strict	Lenient
1991-2002	18	44	26	12
2003-2011	44	20	22	14
Middle Ten Importers From Major Exporters				
	Matched	No Regs	Strict	Lenient
1991-2002	6	53	31	10
2003-2011	16	32	38	14

Table 4-2 summarizes the average inverse difference, weighted average inverse difference, and absolute weighted inverse difference calculations. For each metric, values closer to 0 represent standards that are less different than the exporter's standard; both larger positive and negative numbers represent average difference in regulations comparing top and middle importers to major exporters. Average inverse of importers showed regulations in the top ten importers were closer to the exporter country's standard in 2003-2011 than 1991-2002. Also, comparing between top and middle importers, 6/10 top importers were closer in 2003-2011 compared to 1991-2002, while only 3/10 were closer for middle importers between the time periods. For most importing countries, the average inverse difference was smaller in 2003-2011 than in 1991-2002, potentially indicating regulations were more similar for top importers that had established trading patterns.

When weighting the average inverse difference with the amount of maize exported by each country the differences are less apparent. Half of the top ten exporters showed weighted average inverse differences closer to 0 in 2003-2011 than in 1991-2002. Only two exporters to middle importing countries indicated smaller differences between the time periods. The top

importers did have increased number of countries which changed regulations between the time periods; however, the number of countries that did show improved change remained at five.

Similar results exist for the absolute average difference calculations. In the 1991-2002 time period, 9/10 of exporting countries have values closer to 0, indicating regulations closer to their top importers. However, when comparing between time periods, only four exporting countries had more similar regulations to their top importers.

4.4 CONCLUSIONS

Conflicting results are present when attempts were made to determine causality in maize trade and aflatoxin regulations; i.e., which came first, the trading partnerships or the aflatoxin regulations? When examining the amount of maize traded between the top twenty maize trading pairs longitudinally along with aflatoxin regulations of each country, there did not appear to be any consistent trends. No significant changes in maize trade occurred before or after aflatoxin regulations became more similar or different. In some trading pairs, one country adopted a more similar regulation to its partnering country, which may be indicative that maize trade guided regulations. However, in some cases, after countries aflatoxin regulations became more similar the amount of trade decreased or showed varying patterns of increased and decreased trade. In other cases, pairs without similar regulations maintained high maize trade levels over the past 20 years.

The most compelling evidence that maize trade may have guided the implementation of aflatoxin regulations comes from comparing the inverse standard of the exporting country to the

average regulations of the top ten and middle ten importers from 1991-2002 and 2003-present. Since many countries, including the European Union, adopted aflatoxin regulations in 2003 it was assumed trading patterns may have changed after their implementation. The average inverse regulations of the top importers were more similar to their respective major importer than the middle importers. Also, the average inverses were more similar for the top importers in 2003-2011 than in 1991-2002. Both indicate that once trading partnerships were established the aflatoxin regulations became more similar. This pattern was also evident in Table 4-1 comparing the number of exporting countries which had top importers with more matching aflatoxin regulations compared with middle importers.

Weighting the difference of aflatoxin regulations between exporters and top/middle importers did not show the same correlation. Mixed results using both the weighted difference and absolute value of the difference did not indicate that countries with established trading patterns assumed more similar regulations when compared between time periods or between top and middle importers. Overall, conflicting data did not provide enough evidence to determine causality as to which occurred first: aflatoxin regulations or trading patterns.

Various limitations existed in the data. The primary issue was a lack of maize trade data prior to 1991. Many aflatoxin regulations came into place prior to 1991 and without maize trade during periods both before and after regulations it was difficult to determine which came first: regulations or trade. Ideally, analysis would have included a time period analyzing major importers from particular countries before regulations were put into place and a time period between the same countries after regulations were implemented. Also, a lack of validated metrics analyzing differences in regulations was a limitation.

Future analyses investigating causality into how aflatoxin regulations impact maize trade, or *vice versa*, should focus on commodities recently regulated for aflatoxin. Any commodity which has been recently regulated would likely have databases documenting trade before and after the regulation was put into place. Social network analysis could be used to compare country trade patterns and clustering, while metrics comparing the similarity of trading partners could be compared to countries trading smaller amounts of the same commodity.

Table 4-2: Summary of metrics comparing aflatoxin regulations between 1991-2002 and 2003-2011 and between top and middle maize importers.

			Top 10 Importers			Middle 10 Importers		
	Exporting Country	Inverse Exporter Regulation	Avg Inverse of Importers	Weighted Inverse Difference	|Weighted Inv| Difference	Avg Inverse Difference	Weighted Inverse Difference	|Weighted Inv| Difference
1991-2002	**USA**	0.05	0.026	0.013	0.013	0.07	-0.025	0.08
	Argentina	0.05	0.036	0.007	0.03	NA	0.005	0.046
	Brazil	0.033	0.035	-0.014	0.023	0.087	0.023	0.038
	China	0.025	0.0069	0.0062	0.022	0.062	-0.007	0.017
	France	0.05	0.086	-0.041	0.075	0.04	-0.004	0.09
	Hungary	0.1	0.083	0.04	0.065	0.081	0.071	0.08
	India	0.0166	0.014	0.0025	0.014	0.04	-0.036	0.037
	Paraguay	NA	0.062	-0.005	0.038	0.059	-0.045	0.045
	South Africa	0.1	0.03	0.058	0.059	0.083	0.04	0.099
	Ukraine	0.1	0.0467	0.058	0.067	0.078	0.058	0.12
2003-2011	**USA**	0.05	0.04	-0.0003	0.0016	0.08	-0.09	0.12
	Argentina	0.05	0.07	-0.022	0.05	0.072	-0.017	0.067
	Brazil	0.05	0.1	-0.046	0.065	0.087	-0.038	0.062
	China	0.025	0.038	-0.024	0.025	0.12	-0.009	0.009
	France	0.25	0.23	0.00000029	0.000000029	0.105	0.075	0.075
	Hungary	0.25	0.235	0.0009	0.0009	0.135	0.081	0.08
	India	0.033	0.026	-0.0026	0.029	0.122	-0.23	0.229
	Paraguay	0.05	0.115	0.019	0.014	0.125	-0.049	0.055
	South Africa	0.1	0.058	0.045	0.048	0.103	0.007	0.085
	Ukraine	0.1	0.095	-0.006	0.068	0.141	-0.042	0.12

5.0 FINAL CONCLUSIONS AND PUBLIC HEALTH SIGNIFCANCE

The overall goal of this research was to investigate the impacts of mycotoxin regulations on human health and trade, specifically focusing on aflatoxin and OTA.

A systematic review of epidemiologic literature associating OTA exposure with adverse health effects in humans revealed limited statistically significant associations. The one significant association concerns an increased risk of nephritic syndrome at very high exposures to OTA; however, the sample size of this population was small and the urinary OTA levels measured were much higher than in multiple other studies. Furthermore, the cause of nephritic syndrome is multifactorial, and it is possible that OTA was not the only etiologic factor in the disease in the particular population studied.

In relation to Health Canada's recent proposal to implement OTA regulations in a variety of commodities, it is critical to gain a better understanding of OTA's impacts to human health. While previous risk assessments were based on animal and cell culture assay studies, this risk assessment was one of the firsts focusing on adverse human health endpoints. Additionally, bladder cancer incidence and mortality rates remain low. Bladder cancer incidence in Canada sits at 6.8/100k, while mortality is much lower at 2.5/100k individuals (169). This incidence rate is outside of the top fifty rankings worldwide and is third in the Americas. Canada falls behind the US, which does not regulate OTA, and Uruguay, which has a very lenient regulation of 50 ng/g in rice, barley, beans, coffee and corn. With limited evidence that OTA is causing adverse

health effects in Canada and low rates of cancers associated with OTA in Canada, it appears the recently proposed MLs would have little benefit to improving human health.

Furthermore, with limited evidence associating OTA with adverse health effects in humans, the economic losses to Canadian associated with implementation of an OTA ML was investigated. Based on the estimated amount of Canadian crops that would be rejected under the new regulations and the current price of each crop, Canadian farmers are at risk of losing $260 million CD annually, while foreign exporters would be at risk of losing up to $18 million CD annually. Using contamination data in from the crops most likely to be affected by OTA, Canadians would need to consume unrealistic amounts of the respective crops in order to be at risk any adverse health effects. At this time, implementation of OTA regulations in Canada appears unlikely to significantly improve human health, while costing both Canadian farmers and foreign exporters millions of dollars annually.

It is important that policymakers consider the balance between economic losses and improvements to human health when implementing new regulations. In the case of OTA, there is no reliable way to estimate health gains associated with the economic losses because of the lack of evidence linking OTA exposure to adverse health effects. The animal and cell studies used to develop Health Canada's MLs, which used OTA doses of three to four orders of magnitude higher than current human exposures (188), cannot be used to link current OTA exposures to future OTA exposures under new MLs. Policymakers must consider both the economic impacts along with the potential improvements to human health. This is especially the case for OTA standards due to a lack of countervailing health effects linking exposure to adverse health impacts and limited OTA exposure in Canada.

Using pistachios as a case commodity to investigate the impacts aflatoxin regulations have on trade a human health revealed that countries with strict regulations tend to trade with other countries with strict regulations, and *vice versa*. Time-series network modeling and various metrics showing market segregation suggested that the pistachio market was once dominated by Iran, however, in recent years has become segregated with the US becoming a major pistachio producer and exporter. Because these trends in trade changed after the 2003 implementation of an aflatoxin regulation in tree nuts, it appears that regulations have varying impacts on global trade and, subsequently, human health.

Results from this study should be considered with trade trends for multiple aflatoxin-contaminated commodities including maize and peanuts. Countries without aflatoxin regulations may be subjected to consuming total crops high in aflatoxin due to implementation of aflatoxin regulations in other countries. For example, farmers in countries attempting to export their crops may be forced to export only the highest quality crop to avoid rejections and consume the most heavily contaminated crops for their own diets. On the other hand, countries without regulations may be forced to import crops of low quality since the exporting country is exporting high quality crops to countries with strict regulations. If these trends exist for multiple commodities, countries without aflatoxin regulations may be subject to diets containing high levels of aflatoxin and be at increased risk for developing aflatoxin-related health effects.

Regulations, which are put in place to product human health, may also have adverse effects to human health in some regions. Regions at greatest risk for adverse health impacts from aflatoxin exposure include sub-Saharan Africa and Asia, both of which have Hepatitis B prevalence of greater than 8% (189). These same regions have the highest incidences and mortality rates from liver cancer. Eastern Asia has the highest incidence (24.0/100k) and

mortality (21.6/100k) followed by Africa with an incidence of 8.3/100k and mortality of 8.4/100k (169). In these "high-risk" regions, many countries are without aflatoxin regulations or have very lenient regulations. Furthermore, many countries in these areas with regulations may not necessarily enforce them. This is the case for subsistence farmers which consume most of their crops or trade them locally where no crop is monitored for contamination. The pistachio case-commodity modeling and market segregation data has shown that some high-risk countries in these areas already import majority of their pistachios from Iran and would have higher aflatoxin in their diets because of this trading trend. If these high-risk areas are also importing multiple other aflatoxin-contaminated commodities of low quality, their total diets would be high in aflatoxin and individuals would be at increased risk for developing liver cancer. It is important that policymakers take this into account and realize that regulations may have unintended negative consequences for public health in regions without regulations.

Overall, mycotoxin regulations have varying effects on human health and economics. A human health risk assessment did not associate any adverse health effects with OTA exposure except for nephritic syndrome. Recently proposed OTA regulations in Canada may have little impact to improving human health while costing Canadian producers and foreign exporters millions of dollars. Aflatoxin regulations appear to have molded trade patterns over the past 15 years, at least for pistachios. Countries without regulations appear to be importing lower quality crops than countries with strict regulations. Both results indicate important aspects of regulations which may have significant impacts to public health that policymakers should consider in future decision-making processes.

APPENDIX

ABBREVIATIONS

AA = Aristolochic acid

AFB1 = Aflatoxin B1

AFB2 = Aflatoxin B2

AFG1 = Aflatoxin G1

AFG2 = Aflatoxin G2

AFM1 = Aflatoxin M1

AFM2 = Aflatoxin M2

AL-DNA = Aristolactam-DNA

BD_{10} = Benchmark dose 10%

BEN = Balkan Endemic Nephropathy

BHC = Bradford Hill Criteria

CD = Canadian dollars

CIN = Chronic interstitial nephropathy

EFSA = European Food Safety Authority

ESRD = End-stage renal disease

EU = European Union

EU RASFF = European Union Rapid Alert System for Food and Feed

FAO = Food and Agricultural Organization

FAS GATS = Foreign Agricultural Service – Global Agricultural Trade System

FDA = Food and Drug Administration

GST = Glutathione S-transferases

HBV = Hepatitis B virus

HCC = Hepatocellular carcinoma

HCV = Hepatitis C virus

HPLC = High-performance liquid chromatography

IARC = International Agency for Research on Cancer

IPA – Iran Pistachio Association

JECFA – Joint FAO/WHO Expert Committee on Food Additives

LD_{50} = Lethal dose 50%

LOAEL = Lowest observable adverse effect level

ML = Maximum limit

NCRI = Negligible cancer risk intake

NOAEL = No observable adverse effect level

OR = Odds ratio

OTA = Ochratoxin A

PAH = Polycyclic aromatic hydrocarbons

PTDI = Provisional tolerable daily intake

PTWI = Provisional tolerable weekly intake

RR = Relative Risk

SCF = Scientific Committee of Food

TDI = Tolerable daily intake

UAE = United Arab Emirates

UF = Uncertainty factor

UK = United Kingdom

UN = United Nations

UN COMTRADE = United Nations Commodity Trade Statistics Database

US = United States

UTT = Urothelial tract tumors

UUC = Upper urothelial carcinomas

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