The presence of pesticide residue in farmer market produce: Thiabendazole residues in various produce sold at local farmers' markets

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ABSTRACT

Background: Thiabendazole is a pesticide that is mainly used after harvesting and directly applied to produce such as citrus fruits, apples, pears, bananas, mangos, corn, carrots and potatoes in the form of a spray or dip. The most common and most likely route of exposure to pesticide for the average person is through their diet. Studies have shown that the health risk of regular consumption of pesticide residue through produce is linked with disruption to various functions in the body, such as reproductive, developmental and hormone irregularity. The following study tests whether fruits and vegetables sold at farmers markets contain Thiabendazole and if they are below the acceptable Maximum Residue Limits (MRLs) set by Health Canada.

Methods: A QuEChERS method and solid phase extraction was used to recover Thiabendazole from various fruits and vegetables. The gas chromatography was used to analyze all samples and a calibration curve was produced to identify the concentration of Thiabendazole.

Results: Thiabendazole was detected in all of the citrus fruit samples, but was below detectable limits for all other fruits and vegetables. All Thiabendazole levels were below the Maximum Residue Level allowed by Health Canada.

Conclusion: The various fruits and vegetables analyzed are all below the MRL, with only the citrus fruits having detectable concentrations. However, since the citrus fruits were imported, further studies are required on different pesticide compounds to determine if locally grown produce meet the MRL for other pesticide compounds.

Key words: Pesticides; pesticide residues, Thiabendazole, fruits and vegetables, farmers markets

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INTRODUCTION

Thiabendazole is a fungicide that is currently being used in North America to inhibit the growth of fungi that causes mold, blight, rotting and stains to our farmed fruits and vegetables (Environmental Protection Agency (EPA), 2002). This fungicide is classified as a pesticide residue that is monitored by Health Canada (Health Canada, 2016). Canadian Food Inspection Agency (CFIA) is the governing body that monitors for pesticide residue on fruits and vegetables before they are commercially sold (CFIA, n.d). Produce purchased from local grocery stores are all regulated under CFIA, but produce being sold at the farmer markets operate under no such jurisdiction and only have a guideline to assist in their operations. Thus, there is potential for the public to be exposed to pesticide residuals exceeding the limit set out by Health Canada. Furthermore, current trends of eating healthy and maintaining a healthy lifestyle results in more people purchasing their fruits and vegetables at their local farmers' markets. With the increasing trend and lack of monitoring, this study will determine if pesticide residuals, specifically Thiabendazole, on fruits and vegetables exceed the limit set by Health Canada and if a governing body should be in place to monitor for pesticide residuals in farmer's markets.

LITERATURE REVIEW

Thiabendazole

Chemicals are present in the environment in many forms whether it may be naturally occurring or synthetically made. The use of pesticide in agriculture to grow produce is one attributing factor to the presence of chemicals in the environment. Research done by the Environmental Working Group in the US, analyzed data by the Department of Agriculture and found that close to 70% of the produce being sold in the US contain pesticide residues (Environmental Working Group (EWG), 2019). Thiabendazole is the focus of the study as it is just one particular pesticide residue out of hundreds listed by Health Canada. Thiabendazole is mainly used after harvesting and directly applied to produce such as citrus fruits, apples, pears, bananas, mangos, corn, carrots and potatoes in the form of a spray or dip (EPA, 2002). Research has been done on the correlation of a dipping treatment and residue uptake for grapefruits. It was determined that after fruits have been dipped and gone through a simulated marketing period there were still significant presence of Thiabendazole left on the fruit (Schirra, 2000). We see that the application of Thiabendazole occurs post-harvest and applicable to different produce products, thus Thiabendazole is potentially hazard to public health if not regulated correctly. Currently, the Maximum Residue Limits (MRLs) for

Thiabendazole various depending on the produce, as citrus fruits have a MRL value of 10ppm, bananas have an MRL value of 0.4ppm and corn have MRL of 0.1ppm (Health Canada, 2016).

Health Implications

With Health Canada and Canadian Food Inspection Agency being the major federal bodies that monitor, inspect and regulate pesticide residue in produce, it is safe to say that the presence of pesticide residue has been controlled to safe levels. Health Canada is the government body that created a "Maximum Residue Limits" which limits levels of pesticide residue to a level well below a presentable health risk. Thus, there is no evidence that the consumption of produce will result in a hazard (Tan, 2015; Health Canada, 2019). Furthermore, carcinogenic properties, acute or chronic, by the consumption of produce in our diets should not be of concern (EPA, 2002). The model produced from the EPA suggests that the level of exposure of all pesticides regulated under the maximum residue limits are below the levels of concern (EPA, 2002). However, some studies have suggested that the high exposure to pesticide residue has short-term and long-term adverse health effects (EPA, 2002; Hu, 2015). All human beings are exposed to some sort of pesticide, but the degree of exposure is dependent on the environmental setting, type of pesticide and the individuals risk factors (Kim, 2016). Thus, the type of health impact is correlated to various factors that the individual may encounter (Kim, 2016). Some health effects of short-term exposure to pesticide found disruptions to the blood count, functions to the liver and renal (Hu, 2015). More importantly a short-term exposure to pesticide residue can also affect the nerves. The long-term exposure to pesticide is exclusively related to dysfunction in the nerve, more specifically the sensory nerves (Hu, 2015).

The most common and most likely route of exposure to pesticide for the average person is through their diet (Tan, 2015). Studies have shown that the health risk of regular consumption of pesticide residue through produce is linked with disruption to various functions in the body, such as reproductive, developmental and hormone irregularity (Tan, 2015). New studies have found a strong link between the ingestion of fruits and vegetables containing pesticide residue and the reduction in the chance of pregnancy as well as loss of pregnancy (Chiu, 2018). Another study has shown that children exposed to low levels of pesticide will have possible alterations to their neurological development, where most of the exposure in children are through dietary means (Tan, 2015). These studies indicate that the exposure of low pesticide residue through the individual's fruit and vegetable diets could cause adverse health effects for the immunocompromised groups such as children and pregnant women. As for the general public,

exposure to low pesticide residue is not of a major concern as studies have only focused on the immunocompromised and occupational exposure (University of Washington, 2013).

Knowing that pesticide residues are of health concern, we can focus on the study at hand where the pesticide, Thiabendazole, is of interest. According to the Environmental Protection Agency in the US, Thiabendazole has low toxicity properties especially with dermal contact (EPA, 2002). Thiabendazole is also classified as a carcinogen, where high concentration can cause disruption to thyroid hormone balance (EPA, 2002). The imbalance of thyroid hormone can cause thyroid induced tumors, which is why Thiabendazole is classified as a carcinogen. Therefore, the liver would be the target organ for Thiabendazole due to the presence of thyroids at a high level. This is a major health concern as the exposure to high amounts of Thiabendazole will cause liver damage. One study done by Marilyne Seide on rats indicated that Thiabendazole can cause cell death to the hepatocytes (Seide, 2016). Similar studies have tested Thiabendazole on human hepatic cells which showed that Thiabendazole caused enlargement to the liver, where high possibility for damage to the liver is present (Tan, 2015). Currently, the Environmental Working Group (EWG) has determined that further research should be conducted to reassess the health risk of Thiabendazole (EWG, 2019). The EWG found that the EPA is not properly addressing the issue of Thiabendazole being a potential risk for cancer and a considerable risk to children. The EWG recommends that the EPA use the "Key Characteristics of Carcinogens" as well as the "Hallmarks of Cancer frameworks" to perform an evaluation of Thiabendazole regarding its cancer risk (EWG, 2019). The other recommendation is to adjust the safety factor to consider the increased health risks that children will face with the exposure to Thiabendazole (EWG, 2019).

Farmers Market

The definition of farmers' market according to the BC Association of Farmers' Markets (BCAFM) is a market in BC that consists of "vendors who grow, make, bake, raise or wild harvest the products they sell, all of which products must be grown or processed in BC" (BC Association of Farmers' Markets (BCAFM), 2017). Therefore, vendors that sell fruits and vegetables are only restricted to the "Guideline for the Sale of Foods at Temporary Food Markets" from BCCDC, as they categorized farmers market as a temporary food market and the bylaws of the BCAFM. The sale of produce harvested locally without going out of province is a major reason why they are exempt from inspection by CFIA. CFIA only inspect and monitor imports and exports interprovincially and globally (CFIA, 2019). Thus, CFIA would only target commercial grocery stores as their produce may be imported from out of province or from another country.

According to the Farmers Market Coalition, a farmers' market is "a public and recurring assembly of farmers or their representatives selling the food that they produce directly to consumers" (Farmers Market Coalition, n.d). This is another major difference between farmers market and commercial grocery stores. Vendors at a farmers' market are selling directly to their consumers, while commercial grocery stores are intermediaries, where the producers of the fruits and vegetables are selling to their consumers indirectly. Thus, there is more risk involved as any misusage or non-standardized practices of pesticide usage will be directly transmitted to the public without any preventative measures, such as an inspection from CFIA.

BC Centre for Disease Control (BCCDC) offers a "Guideline for the Sale of Foods at Temporary Food Markets" for the vendors to follow (BCCDC, 2012). The guideline defines low risk food and high-risk food, where vendors must be pre-approved for sale of high-risk foods before being sold at the farmers' market (BCCDC, 2012). However, low risk foods, such as non-PHFs, fresh fruits and vegetables, may be sold without contacting the local health authorities (BCCDC, 2012). Moreover, since the produce is only being sold at the local farmers market, CFIA is not involved in monitoring for pesticide residue. They also work very closely with different stakeholders regarding food safe and communicable disease (BC Association of Farmers' Markets (BCAFM), 2017). They have established committees and consultations with regards to FoodSafe, ProcessSafe, MarketSafe and annual meetings with BCCDC (BCAFM, 2017).

Standards, Guidelines and Legislation

The major federal organization that safeguards our food products in Canada is the Canadian Food Inspection Agency. They have established a chemical residue surveillance program that has three main components which monitor, sample and regulate based on the legislation (CFIA, 2019). The first component is monitoring of samples in the supply of food performed by the National Chemical Residue Monitoring Program (NCRMP). The National Chemical Residue Monitoring Program is essential in managing the pesticide residue in the food supply (CFIA, 2019). In the presence of high level of pesticide residue, a risk assessment will be performed to identify the severity of the risk and potentially a food recall may occur (Tan, 2015). Regarding a low level of pesticide residue, CFIA will investigate using "Certification Bodies" such as certified laboratories to determine if the levels are within the allowable amounts set by the MRL (Tan, 2015). The findings of pesticide levels above the allowable amounts set by the MRL will trigger an investigation and potential for suspension or elimination of certification accreditation. These steps are considered the second component of the surveillance program, where direct sampling is performed to determine

chemical contamination (CFIA, 2019). The last component of the surveillance program is to comply to the findings of the sampling process. This can range from further investigations on the samples to eliminating the products in violation of the MRL from the marketplace (CFIA, 2019). The two main regulations that CFIA use to ensure products consumed by Canadians are safe is the Food and Drug Regulation and Safe Food for Canadians. The Food and Drug Regulations are used to ensure safety and protect Canadians by establishing the requirements for manufacturing, packaging, labelling, import and export of food, advertisement and distribution of food for sale (Minister of Justice, 2019). The Safe Food for Canadians lists the monitoring of imported and exported foods and the traceability of the product along with preventative controls being applied (Minister of Justice, 2019).

Furthermore, as stated earlier BC Association of Farmers' Markets have their own policies and by-laws that monitor activities occurring at their temporary markets. According to their own policies, a farmer's market is a market located in BC comprised of vendors who grow, make, bake, raise or wild harvest the products they sell. Products must be grown or processed in BC, prioritize in accordance to criteria established by the board (BCAFM, 2017). This indicates that they have a criterion that restricts the resell of products purchased from commercial sources. In terms of pesticide use this is a potential concern because with the limited governance regarding foods sold at farmers market, there could be misuse or overuse of pesticides while growing produce to be sold at these farmer markets. In other words, there could be practices used by the vendors, who are usually local residential farmers, during their growth and harvest of produce that could potentially result in high pesticide residue onto their products. However, there would be no surveillance steps performed to catch this error, which would ultimately affect the public who do purchase and consume these produces.

Public Health Significance

Pesticide residue in produce being sold at farmers' markets is a topic of public health significance because there is an increase in the amount of people who purchase from farmers' markets, thus more people are potentially being exposed to products with high levels of pesticide residue (BCAFM, 2017). According to BCAFM's annual reports, there is consistent number of farmers market locations in many communities throughout BC as well as a consistent number of vendors that participate in these markets annually. The increase in the amount of people purchasing locally can be attributed to the changing lifestyle and a focus on eating healthy. Thus, the public has turned to farmers' markets as a source of fruits and vegetables because of the perception that farmers market offers food of higher quality and freshness (Bittman, 2014).

Purpose of Study

The purpose of this study is to find out if there is Thiabendazole residue on various produce sold at the farmers' markets and whether they are within the MRL listed by Health Canada.

METHODS AND MATERIALS

All the materials used in this study were provided by the Chemistry department at BCIT. The GCMS instrument was used as the main analysis instrument to calculate the Thiabendazole concentration in each fruit and vegetable. All other materials used for the extraction to isolate Thiabendazole from fruits and vegetables includes chemical solvents, test tubes, beakers, weighing balance, vortex, and a centrifuge. The standards in this experiment were obtained from SGS Canada Inc. which consists of Thiabendazole-d3 and Thiabendazole chemical compound. Fruit and Vegetable samples used in this experiment were obtained from the Burnaby Farmers Market, while the citrus fruits were obtained from Save-on-Foods.

Method

The standard method used was a quick, easy, cheap, effective, rugged, and safe (QuEChERS) technique partnered with a dispersive solid-phase extraction of the chemical pesticide Thiabendazole. The QuEChERS extraction was done with the use of a chemical solvent and a drying agent (BCIT Chemistry Department, 2019). The chemical solvent that was used is a mix of acetonitrile containing 1% acetic acid, while the drying agent is a mixture of magnesium sulfate and sodium acetate (Table 1). The dispersive solid phase extraction (dSPE) was done using the dSPE tube to extract the pesticide into the acetonitrile solution.

Sample Preparation

All fruits and vegetables used in this experiment was cut into chunks, blended using the NINJA blender and frozen in a freezer until the extraction date. Around 15g of the blended sample was weighed out in a 50ml test tube. The samples in the 50ml test tubes are ready to proceed onto the extraction steps.

Preparation of Standard Solution

Using the chemical standard and chemical materials listed in Table 1, the Thiabendazole standard was prepared and ready for the GC-MS instrument.

QuEChERS Extraction

Adding magnesium sulfate and sodium acetate to all the samples is to separate the solvent from the solute. Each sample was vortexed for five minutes and transferred to a dSPE tube (Tan, 2015).

Dispersive SPE Cleanup

The solute from each sample from the QuEChERS extraction was added to tubes for further partitioning and clean-up of the samples. Each vial was vortexed for thirty seconds and centrifuged for five minutes. The samples were then transferred to a GC vial for analysis with the gas chromatography

Calibration Curve, Internal Standard and Spikes

A calibration curve was created to determine the quantity of thiabendazole in each fruit and vegetable sample. In other words, it is used to determine the concentration of unknown substances based on known concentration of measurements. Spikes were conducted to each category of samples to confirm the right concentration can be detected and the addition of an internal standard is to account for possible loss of solute during the extraction process.

GC/MS Instrumentation

The vials filled with supernatant of fruit and vegetable samples was injected into the Agilent Technologies 7890A (Agilent, 2020). The instrumentation generates chromatographic peaks that will be indicative of the internal standard, standard solution, and potential Thiabendazole peaks for presence of Thiabendazole in the fruit and vegetable samples (Lawal, 2018).

Calibration

The Gas Chromatography-Mass Spectrometer (GC-MS) is factory calibrated by the manufacturer (Lawal, 2018). The weighing balance is calibrated by weighing known weights and taring each test tube before weighing the fruit and vegetable samples. The taring of each test tube is considered a routine calibration. Furthermore, the standards are used to calibrate the instrument to read the specific concentration needed to extrapolate the standard concentration curve.

Inclusion and Exclusion Criteria

In this study, only fresh fruits and vegetable purchased from a vendor registered with the BC Farmers' Market are used. However, due to citrus fruits being grown outside of BC, they were purchased from local organic markets who have purchased them from the USA. The fruit and vegetables from other distribution channels such as commercial grocery stores and specialty produce stores were excluded.

Ethics

Ethics review or approval is not necessary as the study does not involve human testing or survey conducting. The study is based on analysis of Thiabendazole in fruits and vegetables purchased at a farmers' market. However, it

would be in the ethical realm to report any samples that are above regulations.

RESULTS

The concentration of Thiabendazole obtained from the GC-MS are numerical values with an absolute zero. The concentration of Thiabendazole obtained have the units of µg/g or ppm and it is obtained from the calibration curve from excel shown in the appendix (Excel, 2020). The chromatography of the Thiabendazole peak and internal standard peak is a visual representation of the presence of Thiabendazole in the sample. The instrument detected Thiabendazole concentrations for citrus fruits except for lime, which was unknown. The orange gave an average Thiabendazole concentration of 1.14ppm, lemon had an average of 0.35ppm and grapefruit an average of 1.56ppm. All other fruits and vegetables had undetectable levels of Thiabendazole. NCSS was used to conduct the inferential statistics for the dataset obtained (NCSS, 2020). Through the 2-sample t-test, a P value of 1 was obtained indicating that fruits and vegetables sold in Thiabendazoles is a chemical fungicide that is commonly used in the food industry to prevent mold and blight vegetables and fruits during the transportation of the products. Thus, the presence of Thiabendazole in citrus fruits is because of the dipping treatment process used in the food industry to preserve the fruit. One study by McDonald indicates that the application of farmers' market did not exceed the MRL set by Health Canada of 10ppm. Through the ANOVA test, the P value of 0 was obtained indicating that there is difference in Thiabendazole levels between the different classification of fruits.

DISCUSSION

Based on the results of the study, we conclude that all the fruits and vegetables have Thiabendazole levels that are less than the Maximum Residual Level set by Health Canada of 10ppm. Furthermore, the fruits and vegetables are split into three different categories: citrus fruits, produce grown above ground and produce grown below ground. There is no difference between the produce grown above and below ground as the Thiabendazole levels were all at undetectable levels, however the citrus fruits had Thiabendazole levels that were detectable, thus different from the produce grown above and below ground. It is important to note that the lime used in this study was eliminated from the calculations as it did not generate a good representation as interference in the lime samples created a false positive.
Thiabendazole help reduce the chilling injury and decay of the citrus fruit, grapefruit (McDonald, 1991). Furthermore, other studies have been conducted to access the factors that affect the Thiabendazole uptake and persistence in citrus fruits after the dip treatment. The findings indicated that the presence of Thiabendazole residuals are correlated with the

temperature of the dipping procedure, where a process with lower treatment temperature will result in 2.5 times less deposition on the citrus fruit (Cabras, 1999). Thus, previous research agrees with my findings of residual Thiabendazole in citrus fruit because it is a widely used pesticide for citrus fruits and many factors of the process leaves residual Thiabendazole on citrus fruits.

The results obtained are valid as the method and analysis was done in a professional laboratory setting as well as on an instrument that has been certified and calibrated to generate accurate results. The data extrapolated indicates that there are residual levels of Thiabendazole, but on only on citrus produce. However, this may suggest that there may be other types of pesticides that exist on produce being sold. Thus, this information can be given to local health authorities that inspect these farmers' market to confirm that produce being sold at these locations do not contain residual pesticide levels. Moreover, the data can be given to the BC Association of Farmers' Markets as an indicator that produce being sold has the potential for pesticide residual. Since there are many types of pesticides being used in the agricultural industry, presence of residual pesticides is bound to be found on produce, thus it is the onus on health officials to ensure that vendors are conducting their business to the standards required by the government.

LIMITATIONS

The major limitation of this study was the choice of pesticide compound as the detectable limit of Thiabendazole was present only in citrus fruits, which are all imported from warmer climate and not associated with produce sold in a Farmers' Market. Thus, in order to improve the study, a different pesticide should be chosen to better present the type of pesticides used for locally grown produce. The choice of instrumentation, time and money were also limitations in the study. The instrument used in this study was a gas chromatograph with a single quad mass spectrometer. However, with the lime sample there were other prominent ions that mimicked the Thiabendazole compound, thus resulting in an over-representation of Thiabendazole for the lime compound. There was not enough sufficient resolution between Thiabendazole and the other prominent ions, therefore the Thiabendazole in lime cannot be determined. The one recommendation to improve the study is the use of another instrument called the LC-MS/MS. This instrument is able to extract the compound, Thiabendazole at a much quicker time and determine the concentration of Thiabendazole and the other prominent ion because of a double mass spectrometer that can split the ions further. Furthermore, the limitation of money results in the availability of only 10 types of produce as the method requires the use of many chemical reagents and materials and the amount of time given for analysis on the instrument were

limited, where only limited amounts of samples could be analyzed.

KNOWLEDGE TRANSLATION

Knowing that there will be a consistent presence of farmers markets, an increase in foot traffic and purchase of produce sold at these markets, it is important to protect the public from exposure to pesticide residue by implementing the same surveillance system that are being used in large scale agriculture industries. Currently the consumption of pesticide residue from produce sold at farmers market is unknown. This is especially concerning as new data show that the risk of low-level pesticide residue is potentially carcinogenic and major risks to high risk groups such as children and pregnant women. Therefore, something must be in place federally to protect the public from potentially hazardous foods sold at farmers' markets. Thus, it is recommended that governments are aware of this gap in the legislation and monitoring system and have methods to combat the gap through policies, guidelines or legislation.
Furthermore, in British Columbia, farmers' markets are operating throughout the province and supported by the BC Association of Farmers' Markets (BCAFM). They are a registered society in BC that develop and promote the farmers' markets across BC. Their focus is to provide education and training to different stakeholders involved in the operation of a farmer market such as managers, vendors, and market boards (BCAFM, 2017). They are a well-established organization that have their own policies and by-laws to monitor activities within the organization. They work very closely with different stakeholders regarding food safe and communicable disease to minimize the hazards caused by food (BCAFM, 2017). Through this knowledge, perhaps the responsibility of pesticide residue could also be placed on this association.
Additionally, education regarding the fruits and vegetables being sold at farmers' markets can be established for the general public. For instance, the knowledge that not all produce sold in these markets are locally grown, as many vendors may have imported them from other sources to sell. Moreover, the produce being sold through these farmers' markets are not monitored by CFIA, thus any misuse of pesticide to grow their produce are not properly monitored and it is on the consumers to be aware.

FUTURE RESEARCH

The following are suggestions for future studies to be conducted:

- Conduct the test using a larger sample size with greater variety in the fruits and vegetables selected.
- Conduct the test using an LC-MS/MS instrumentation to analyze the data and increase or use different pesticide compounds.

- Test other products sold at the farmer markets that are produced from fruits and vegetables

CONCLUSION

Through the various sources identified in this study, Thiabendazole is a pesticide that has the potential to inflict short- and long-term health effects. The study was able to identify presence of Thiabendazole in all the citrus fruits, but Thiabendazole was non-detectable in other produce. Furthermore, the sources suggest that more research is required to determine the concern with pesticide residual and further studies are required on different pesticide compounds to have empirical evidence on the level of residual pesticide on produce being sold at farmers' markets. With the addition of further studies, there is a potential to reassess if there is a need for monitoring and surveillance for produce being sold at farmers' markets.

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COMPETING INTEREST

This author declare that they have no competing interests.

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