Pesticide residues in organic apples: Determining the amounts of thiabendazole, diphenylamine, and myclobutanil in organic apples

Ying Tan[1], Bobby Sidhu[2], Kevin Soulsbury[3], Bob Bower[4]

1 Lead Author, B. Tech Student, School of Health Sciences, British Columbia Institute of Technology, Burnaby, BC
2 Supervisor, School of Health Sciences, British Columbia Institute of Technology, Burnaby, BC
3 Contributor, School of Computing and Academic Studies, British Columbia Institute of Technology, Burnaby, BC
4 Contributor, School of Computing and Academic Studies, British Columbia Institute of Technology, Burnaby, BC

ABSTRACT

Objectives: The popularity of organic diets continues to increase even without sound evidence that these diets are healthier than conventional diets. As its popularity increases, organic foods become more readily available and accessible to the public, and genuinity comes into question as farmers and retailers find ways to profit from this trend. Although organic produce will never be completely free of pesticides, they are expected to have considerably lower amounts. In recent years, pesticide residues in organic apples have been found to be at levels higher than normal background levels, indicating intentional application by farmers. Thiabendazole, diphenylamine, and myclobutanil are some of the more common synthetic pesticides that have been found in organic apples; therefore, the following study tested whether or not the levels of thiabendazole, diphenylamine, and myclobutanil in organic apples were below the acceptable organic standards of 5% of their respective Maximum Residue Limits (MRLs).

Methods: A modified QuEChERS method involving juicing and extraction was used to recover pesticides from the organic apples. Two additional samples were spiked with 1 ppm of each pesticide as controls to determine if the method was able to detect the pesticides. One sample was spiked before the juicing step, and the other sample was spiked after the juicing step. Samples were then analyzed using gas chromatography.

Results: Thiabendazole, diphenylamine, and myclobutanil were not detected in all 30 organic apple samples. Furthermore, these pesticides were only detected in the one of the spiked samples – the sample which was spiked after it was juiced.

Conclusion: Organic apples grown in BC meet the organic standard of containing pesticide residue levels below 5% of the MRL, at least for thiabendazole, diphenylamine, and myclobutanil. However, since pesticides were not detected in the sample which was spiked before juicing, the methodology of this study may require modification. One possible reason for this finding is that pesticides may be concentrated in the pulp that is separated during juicing, therefore suggesting that juicing apples may be a good practice for reducing the consumption of pesticides.

Key words: Pesticides; pesticide residues, thiabendazole, diphenylamine, myclobutanil, organic apples, organic

INTRODUCTION

Pesticide residues are in the environment since they have been used to treat produce for so long – they are present in the soil, in the water, and in the air, so it is expected that organic produce will contain small amounts of pesticide residues. However, when pesticide residues are found in organic produce at high levels, there is a need for investigation into the processes of organic farming. Pesticide residues are being found on organic produce post-harvest, at levels which could be implicative that organic farmers have applied synthetic pesticides intentionally.

Apples have been named the produce containing the highest amounts of pesticide residues by the Environmental Working Group (EWG), a non-profit organization in the United States (EWG, 2014). The EWG as well as The David Suzuki Foundation in Canada have used information regarding the most highly pesticide-contaminated produce to promote converting from eating non-organic produce to eating organic produce, at least for the items that contain higher levels of pesticide residues (EWG, 2014; David Suzuki Foundation, n.d.). Organic diets are constantly increasing in popularity, but Canadian consumer perceptions and knowledge about pesticides in relation to organic produce do not necessarily match with reality (Campbell et al., 2013). According to Campbell et al. (2013), 82.9% of Canadians who participated in their study correctly believe that there is no synthetic pesticide use on organic produce and only 35.2% of participants correctly believe that there are less pesticide residues in organic produce. Although synthetic pesticides are not permitted in organic farming, the residue levels will not be zero due to pesticide residuals existing in the environment; however, pesticide residues should be significantly less in organic produce than in non-organic produce. Since only 35.2% of Canadian consumers believe that there are less pesticide residues in organic produce, this means that for the same survey question, the remaining 64.8% of participants either believe there are no pesticide residues in organic produce, believe there are more pesticide residues in organic produce, or simply do not know. The perceptions represented in this study illustrate the need to ensure that organic farmers are not intentionally using pesticides on their crops.

LITERATURE REVIEW

Pesticides of Interest

In Canada, recent attention has been given to the use of several post-harvest pesticides, including the fungicide thiabendazole and the growth regulator and antioxidant diphenylamine (Levasseur & Kubinec, 2014; Mortillaro, 2014). Research in the U.S. is comparable with these concerns and has found that apples have been shown to contain presences of diphenylamine 82.8% of the time, and thiabendazole 81% of the time (PANNA, 2011).

Diphenylamine and thiabendazole were of particular interest to this current study because they are the two most common pesticides found in both conventional and organic apples (PANNA, 2011; USDA National Organic Program & Science and Technology Programs, 2012; CBC News, 2011; Levasseur & Kubinec, 2014). Health Canada (2014) has previously set Maximum Residue Limits (MRLs) for diphenylamine at 5 ppm and thiabendazole at 10 ppm; however, diphenylamine is under re-evaluation by Health Canada due to concerns expressed by the European Union (Health Canada, 2013a).

Myclobutanil, yet another fungicide, is found 8.1% of the time in apples (PANNA, 2011). This fungicide is important to consider because it is commonly used in Canada to target fungal diseases such as apple scab, the most prominent fungal disease of both conventional and organic apple orchards in Canada (AAFC, 2014; Delate et al., 2008; Jonsson et al., 2010). After a re-evaluation of myclobutanil use, Health Canada determined that this pesticide does not pose a significant risk to human health, but kept the MRL at 0.5 ppm, which is considerably lower than the MRLs for thiabendazole and diphenylamine (Health Canada, 2013b; Health Canada, 2014).

Diphenylamine, thiabendazole, and myclobutanil were the focus of this study due to recent findings of their prominence in apples in Canada (PANNA, 2011; USDA National Organic Program & Science and Technology Programs, 2012; CBC News, 2011; Levasseur & Kubinec, 2014), as well as due to recent re-evaluations of their safety (Health Canada, 2013a; Health Canada, 2013b). Discernable levels of these three common pesticides in organic apples would indicate that switching to organic apples in times of uncertainty regarding the use and safety of pesticides would not safeguard the public from these pesticides.

Health Effects

Health Canada sets Maximum Residue Limits at levels well below the levels that cause harm and states that there is no evidence that eating non-organic produce presents health risks, or even that organic produce is safer to eat (Health Canada, 2013c). However, some studies have suggested that adverse health effects have been linked to dietary pesticide exposures even in “normal” amounts due to chronic exposure and bioaccumulation (Vogt et al., 2012). These studies suggest that many pesticides are endocrine disruptors and have been linked to the disruption of developmental, reproductive, and other hormonally regulated functions within the body (Vogt et al., 2012). In one study, children of mothers who were exposed to pesticides during pregnancy had lower memory skills, stamina, and levels of hand-eye coordination than non-exposed children (Vogt et al., 2012). Another study also found that pesticides have been linked to possible neurologic and neurodevelopmental effects in children with chronic, low-level pesticide exposure, and found that children, especially, are only exposed to pesticides through their diets (Lu et al., 2008). These findings demonstrate that there is a greater concern for pesticide residues being present in a child’s diet than in an adult’s diet since children are still developing and therefore suffer more detrimental effects from exposure.

As previously stated, the pesticides of interest to this current study were thiabendazole, diphenylamine, and myclobutanil. The safety of thiabendazole use was re-evaluated in 2011 and was determined to be safe for continual sale and use at the set MRL (Health Canada, 2011). However, at high doses, thiabendazole has been found to induce growth in cultured human hepatic cells, suggesting thiabendazole enlarges the liver and possibly leads to liver damage and cancer (Price et al., 2008). Along with effects on the liver, thiabendazole has also been reported to be a probable carcinogen that is toxic to human developmental and reproductive systems (PANNA, 2011).

Safety of the use of diphenylamine is currently being re-evaluated in Canada due to it being banned by the European Union (Health Canada, 2013a). According to Global News, the European Union has banned the use of diphenylamine because it is capable of reacting with nitrogen in the air to produce nitrosamines, which are known carcinogens (Mortillaro, 2014). Diphenylamine has also been shown to have a potential effect in genetic alterations of human lymphocyte cells (Santovito et al., 2012).

Lastly, myclobutanil is not thought to be carcinogenic but may act as hormone disruptors and have toxic effects on developmental or reproductive systems as well (PANNA, 2011). At high exposure levels, myclobutanil has toxic effects on the liver, kidneys, adrenal glands, testes, and other organs, as well as adverse effects on pregnant women, such as increased spontaneous abortions (Health Canada, 2013b). Concern regarding pesticide residues in food is valid, and if organic farmers truly avoid all uses of synthetic pesticides, the public should be able to lower their exposures by eating organic if they so choose.

Common Challenges in Organic Apple Orchards

Pests and diseases become more difficult to manage on organic farms (Jonsson, 2006), thereby resulting in two main consequences: reduced fruit yield and shorter storage life. Since no synthetic pesticide use is permitted on these farms, pests from nearby conventional farms can easily invade organic farms (Jonsson, 2006).

Organic apple diseases not related to insects involve mainly fungal diseases such as apple scab and powdery mildew (Delate et al., 2008; Jonsson et al., 2010). In Canada, the most serious disease affecting apples is apple scab; Agriculture and Agri-Food Canada (2014) have developed a reduced-risk strategy specifically for the management of apple scab with fewer pesticides. Apple scab is the number one disease limiting the production of conventional and organic apples and is therefore important to be managed because it poses the greatest economic threat (AAFC 2014; Delate et al., 2008; Jonsson et al., 2010). In organic apple orchards, apple scab is most often treated with combinations of lime, sulfur, and copper (Delate et al., 2008). However, these products are lacking in the efficiency that synthetic fungicides normally possess, and may have unwanted effects as well (Jonsson et al., 2010). Delate et al. (2008) report that sulfur products can decrease the photosynthetic capacity of apple trees, and that copper products can be toxic in high levels and cause russeting in apples, a cosmetic problem that decreases sales.

As mentioned previously, the first overall consequence of challenges with organic apple orchards is reduced fruit yields. A contributing factor may be that the use of sulfur-containing products to control apple scab is not as effective as using synthetic fungicides (Jonsson et al., 2010), and could therefore be a possible factor leading to usage of synthetic pesticides, such as myclobutanil, to treat apple scab. The second major consequence of the challenges presented with organic apple orchards is that apples have shorter storage lives (Delate et al., 2008). This is likely due to mould which develops post-harvest due to fungal spores that survive (AAFC, 2011) because of the lack of post-harvest fungicide applications for organic apples. This could serve as motivation for the usage of synthetic post-harvest fungicides, such as thiabendazole, to extend storage life.

Legislation and Standards

The Organic Products Regulations [SOR/2009-176] (2009) under the Canada Agricultural Products Act [R.S.C. 1985, c. 20 (4th Supp.)] states that agricultural products must be certified to the National Organic Standard before they can be represented as organic. The Regulations lay out the requirements to be met in order for a food item to be labelled as organic, and are enforced by the Canadian Food Inspection Agency (CFIA). Certification Bodies accredited by CFIA are responsible for certifying organic products, and in applying for certification, substances involved in the production and processing of the food item must be disclosed (OPR, 2009). Use of the Canada Organic logo is voluntary, as stated in the Regulations (OPR, 2009). The Organic Products Regulations, along with the Consumer Packaging and Labelling Act and Regulations, also state that labelling cannot be fraudulent or misleading to consumers.

The Canadian General Standards Board (2011a) developed the Canada Organic Standard, Organic Production Systems – General Principles and Management Standards [CAN/CGSB-32.310-2006], which states that during the production or handling of organic products, use of synthetic pesticides is prohibited (Section 1.4.1b). The Board (2011b) also created a list of substances permitted for use: Organic Production Systems – Permitted Substances Lists [CAN/CGSB-32.311-2006]. In British Columbia, the Certified Organic Associations of BC (COABC) adopted the Canada Organic Standard in January of 2009 (COABC, n.d.).

Through the National Chemical Residues Monitoring Program, CFIA tests organic food items for pesticide residues (CFIA, 2014). If high levels of pesticide residues are found, and assessments determine there is a risk, CFIA will issue a Food Recall Warning (CFIA, 2014). If low levels of pesticide residues are found, CFIA will notify the appropriate Certification Body that an investigation has to be made. Although synthetic pesticides are not tolerated in organic products, low levels may be present due to factors like wind drift, run-off, and pesticide contamination during transport (CFIA, 2014). Levels of less than 5% of the MRL for a certain pesticide are generally allowed, provided that follow-up is done by the Certification Body at the next scheduled inspection. Levels higher than 5% of the MRL set by Health Canada result in immediate investigation by the Certification Body, and if the investigation reveals that synthetic pesticides were used intentionally, the organic certification will be suspended or cancelled (CFIA, 2014).

Pesticide Residues in Organic Apples

Organic apples may not be completely free of synthetic pesticide residues since pesticides are already in the environment. The Canada Organic Standards are “designed to assure the least possible residues at the lowest possible levels” (CGSB, 2011a, p. iv). In a review done by

Smith-Spangler et al. (2012), existing data from seven databases between January 1996 and May 2011 were analyzed and it was found that in nine of the studies that involved produce, 7% of all organic produce samples contained detectable amounts of pesticide residues. It was also found that only three studies looked at whether pesticide residues exceeded MRLs. Of those three studies, one did not find any exceedances, another found that MRLs were exceeded in 6% of organic samples and 2% of non-organic samples, and the last study found that MRLs were exceeded in 1% of samples for both organic and non-organic produce (Smith-Spangler et al., 2012). Although this review provides valuable insight, the data analyzed was not reflective of more recent times.

A more recent pilot study that lasted from October 2010 to February 2011 tested 571 samples of organic produce for pesticide residues and found that 57% had no detectable levels of pesticide residues, 39% had levels less than 5% of the MRL, and only 4% had levels exceeding 5% of the MRL (USDA National Organic Program & Science and Technology Programs, 2012). Diphenylamine and thiabendazole were two of the most prevalent pesticides in organic apples at levels greater than 0.01 ppm (USDA National Organic Program & Science and Technology Programs, 2012). One sample contained thiabendazole residues greater than 5% of the MRL, which is a USDA Organic violation. Diphenylamine and thiabendazole are both are applied post-harvest to manage apple scald or mould, respectively. According to the USDA National Organic Program and Science and Technology Programs (2012), their presence at levels greater than 0.01 ppm could indicate inadequate separation and cleaning during storage and packing, or intentional usage, especially in the case of the sample containing thiabendazole above 5% of the MRL.

CBC News (2011) also reported that in organic apples from 2009-2010, CFIA reports show that 24% of 178 organic apples contained pesticide residues, with thiabendazole being the most commonly found pesticide at an average level of 0.03 ppm. In January of 2014, CBC News (Levasseur & Kubinec, 2014) reported again on CFIA reports ranging from September 2011 to September 2013. The CFIA data showed that 45% of organic apples tested had detectable amounts of pesticide residue, with the average level of thiabendazole being 0.02 ppm (Levasseur & Kubinec, 2014). Pesticide residue levels in organic apples are normally found in amounts at less than 0.01 ppm (USDA National Organic Program and Science and Technology Programs, 2012). Therefore, since the average residues for both pesticides were reported to be above 0.01 ppm, this implies that residues are not just coming from background environmental factors such as pesticide drift.

Public Health Significance

The topic of pesticide residues in organic apples is significant to public health because one of the main reasons the public buys organic is the assumption that no synthetic pesticides are used on organic produce and that pesticide levels are much lower in organic than in conventional produce (Delate et al., 2008; Campbell et al., 2013). The continual growth of the organic market leads to increasing availability of organic produce – not just in farmers' markets and local food stores, but especially in mainstream supermarkets as well (Wang et al., 2010). This means that many people have the option of purchasing organic produce wherever they do their regular grocery shopping. It is important to ensure that there is truly no use of synthetic pesticides in organic farming, since consumers are spending more on organic produce to reduce their exposure to pesticides and especially since some studies have shown possible links between pesticides on food and adverse health effects, especially in children (Vogt et al., 2012; Lu et al., 2008; Price et al., 2008; Santovito et al., 2012; PANNA, 2011).

Although there is ongoing debate about the health effects of pesticide residues in food, and MRLs are set well below levels of health concerns, consumers have the right to choose to reduce pesticide exposures for themselves and for their children, and therefore need to be protected from organic farmers who may intentionally be applying pesticides on their crops. The public consciously chooses to buy organic to lower their intake of pesticide residues, so the integrity of the production and labelling of organic produce is vital. Many families also choose to buy organic because pesticide residues in food, although not necessarily harmful to the average adult body, have a greater effect on children since they are still developing and are consuming more relative to their bodyweight (Vogt et al., 2012). In fact, it was found that the major source of pesticide exposure in children is through dietary intake, and by switching from diets containing conventionally grown produce to diets containing organically grown produce, the pesticide levels in children were dramatically reduced (Lu et al., 2008). With such robust promotion of organic diets, it is important that the public is not being misled by false organic claims and assumptions.

STUDY PURPOSE

The purpose of this study was to test organic apples for pesticide residues, namely thiabendazole, diphenylamine, and myclobutanil, to determine if residues were below or above the 5% MRL for each pesticide. This study was conducted in order to indicate possible intentional post-harvest use of synthetic pesticides on organic apples. Evidence of these pesticides in the tested organic apples could be an indication of intentional application of synthetic pesticides. This is especially the case if residues are greater than 5% of the MRL of each pesticide – the limit for organic produce that warrants immediate investigation by the CFIA.

MATERIALS & METHODS

Materials

Refer to Table 1 for a summary of materials used to perform this study.

Table 1: Materials Used

Equipment	Description
Organic Spartan, Red Delicious, and Gala Apples	Apples were purchased from a local organic store.
Jack LaLanne Juicer	The juicer used was a centrifugal juice extractor, which works by spinning a metal grate-like blade against a mesh filter and separating the juice from the flesh using centrifugal force.
Acetonitrile	Acetonitrile was used as an extraction solvent because it has been shown to be well suited for the extraction of pesticides with a wide range of polarities (Mastovska & Lehotay, 2004).
Anhydrous Magnesium Sulfate (MgSO4), Sodium Acetate, and Primary Secondary Amine (PSA)	Anhydrous MgSO4 and sodium acetate were used after the extraction phase to further partition water from the samples. PSA and MgSO4 were used in the clean-up phase to further improve recovery.
Internal Standard (triphenylphosphate)	An internal standard was added for better recovery of select pesticides.
Thiabendazole	Used to create standard (for chromatograph comparison) and to spike control samples.
Diphenylamine	Used to create standard (for chromatograph comparison) and to spike control samples.
Myclobutanil	Used to create standard (for chromatograph comparison) and to spike control samples.
Weight Scale	The scale used for weighing samples of 10g was provided by the Chemistry Department of BCIT.
Sample Tubes/Vials, Pipettes, Syringes & Beakers	The tubes/vials and beakers used for sampling were provided by the Chemistry Department of BCIT.
Agilent Technologies 7693 Autosampler	The autosampler was used to inject samples into the gas chromatograph for analysis.
Agilent Technologies 7890A GC Systems	The gas chromatograph (GC) was used to separate chemicals within the samples injected by the autosampler, based on their volatilities. The sample is

	vaporized and travels through the 30m column in the GC which is heated to temperatures ranging from 40 – 320°C in order to separate the chemicals by time and temperature (EHSC, 2014).
Agilent Technologies 5975C inert MSD with Triple-Axis Detector	The Mass Selective Detector (MSD) was used to identify and quantify the chemicals separated by the GC according to their chemical structures (Agilent Technologies, 2011 & EHSC, 2014).
Centrifuge	Used for rotating substances at high speeds using centrifugal force to separate substances into their different phases based on sedimentation.
Microsoft Excel 2010	Excel was used for data entry.

Methodology

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Method is a simplified and effective approach to analyzing pesticide residues in fruits and vegetables, among other foods (United Chemical Technologies, n.d.). Ultimately, homogenized samples are extracted and partitioned first, using an extraction solvent and a salt. The aliquot is then extracted further and cleaned using a dispersive solid phase extraction (dSPE) technique to increase the recovery of pesticide residues during analysis (Restek, 2012). This study was done by using a modified QuEChERS Method, as seen in Figure 1. The following will further describe the steps used in this study in more detail.

Sample Preparation and Extraction

Whole apples were juiced one at a time, and each apple served as a different sample. Subsequently, each sample was weighed to be 10g of juice. 10mL of acetonitrile was added to each sample as a solvent for extraction. After adding the acetonitrile, the samples were shaken by hand for one minute.

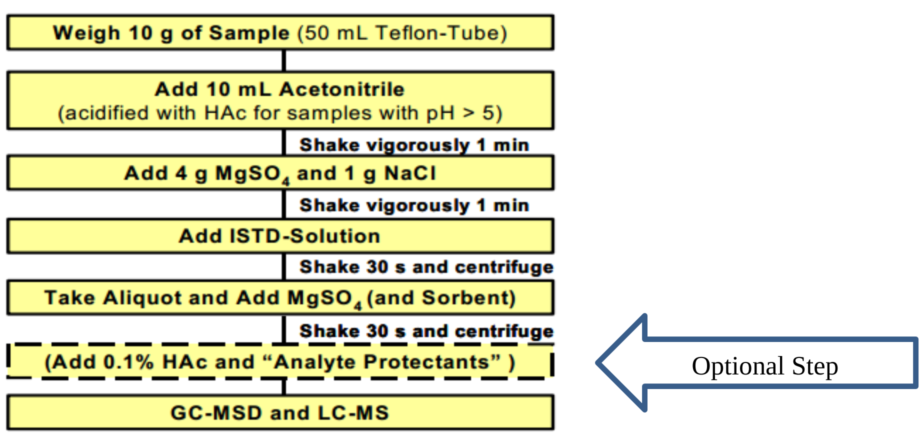
Sample Drying and Buffering

The addition of magnesium sulfate and sodium acetate to each sample was done to further partition the extractions and to stabilize the pH of the samples at 5–5.5 (Restek, 2012 & United Chemical Technologies, n.d.). Each sample was then shaken for one minute. 10µL of the internal standard triphenylphosphate was then added to the samples and the samples were shaken again for 30 seconds.

Phase Separation

Phase separation involved centrifuging each sample for five minutes in order to separate the solid material. From this point, the aliquot of the samples underwent a cleanup process to increase recovery results (Restek, 2012).

Figure 1: QuEChERS – Mini-Multiresidue-Method



(Anastassiades et al., 2003, Retrieved from http://cvuas.xn--untersuchungsmter-bw-nzb.de/pdf/poster_implementation_quechers_eprw.pdf.

Clean-up / Dispersive Solid Phase Extraction (dSPE)

The aliquot from each centrifuged sample was added to vials containing primary secondary amine (PSA) and anhydrous magnesium sulfate to further partition and clean-up the samples (Restek, 2012; Zhao et al., 2012). Each vial was then shaken for 30 seconds and centrifuged again for two minutes. After centrifuging, the liquid portion of each sample, called the pure extract (Anastassiades et al., 2003), was transferred to a vial compatible with the autosampler for analysis by the gas chromatograph.

GC/MS Analysis

Samples were analyzed by the gas chromatograph and results were compared to chromatographs for standards containing 1 ppm of each pesticide for interpretation.

Spiked Samples

As a test to determine whether or not the pesticides could be detected by the selected methodology, two samples were spiked with 1 ppm of each pesticide. The first spiked sample was prepared by injecting each pesticide into the whole apple before it was juiced. The second spiked sample was prepared by adding the pesticides into the pre-juiced sample. After spiking, both samples were further prepared by following the steps used for the regular, non-spiked apple samples.

Reliability and Validity of Measures

To ensure reliability of the results in this study, the same instruments were used for all samples and were done so in a consistent manner by the same user. At least 30 samples were measured to increase reliability as well as power of the study, and decrease the probability of Type II (Beta) errors from occurring (Heacock & Sidhu, 2014).

To ensure validity of the results in this study, the author ensured instruments were calibrated according to established protocols, and adhered to instructions in manuals and Standard Operating Procedures (Heacock & Sidhu, 2014).

To ensure validity of the study design, internal validity was increased by ensuring that appropriate methods, standards, and instruments were used to collect data in order to allow the study conclusions to be as accurate as possible (Heacock & Sidhu, 2014). External validity was increased by having at least 30 samples analyzed and by confining extrapolation of results to populations within British Columbia.

Calibration of Instruments

The weighing scale was calibrated by weighing a known standard, and the weight of the vial was tared each time a sample was measured. The Agilent Technologies instruments were previously factory calibrated.

Inclusion and Exclusion Criteria

For the purpose of this study, only organic Spartan, Red Delicious, and Gala apples grown in British Columbia were included. Non-organic apples and other organic apple varieties were excluded from this study.

Ethical Considerations

Ethical considerations were not applicable to this study, as this study was not performed on humans and was not a survey.

Pilot Studies

A pilot study was conducted on December 15 & 16, 2014 using the same procedures as described earlier, but without the addition of the internal standard. For the purposes of the pilot study, 8 samples were prepared and measured, thereby allowing the author to ensure reliability and validity of this study before commencing the actual experiment. The pilot study allowed the author to test for any possible errors, become more familiar with instrument used in this study, as well as become familiar with sample preparation methods. During the pilot study, it was determined that addition of the internal standard was needed for a better chance of detecting pesticide residues, since without doing so, the methods used were not able to detect any. Additionally, the pilot study led to the determination that a few samples needed to be spiked with thiabendazole, diphenylamine, and myclobutanil. This was required in order to verify that the pesticides were not being detected because they were truly absent from the samples, and not because of any flaws in the methodology.

RESULTS

This study analyzed a sample of 30 organic apples to determine whether or not their levels of thiabendazole, diphenylamine, and myclobutanil, were within their respective prescribed limits of 5% of the MRL for organic produce (CFIA, 2014). The MRL for thiabendazole is 10 ppm, for diphenylamine is 5 ppm, and for myclobutanil is 0.5 ppm, which means the 5% MRLs are 0.5 ppm, 0.25 ppm, and 0.025 ppm, respectively (Health Canada, 2014).

Spiked Samples

Results for the two spiked samples can be seen in Table 2 below. Chromatographs of the spiked samples were compared to chromatographs of the standards, and can be seen in Figures 2, 3, and 4 below. For the sample which was spiked prior to the juicing step ("Spiked 1"), thiabendazole, diphenylamine, and myclobutanil were not detected. Interestingly, the pesticides were detected in the sample which was spiked after the juicing step ("Spiked 2"), as depicted by the matching peaks for each pesticide in figures 2 and 4.

Regular, Non-Spiked Samples

Results from analysis of all 30 apple (juiced) samples are shown in Table 2 below. Thiabendazole, diphenylamine, and myclobutanil were not detected in all 30 samples, as seen in Figure 5.

Table 2: Summary of Results

Apple Sample	Thiabendazole [5% MRL=0.5 ppm]	Diphenylamine [5% MRL=0.25 ppm]	Myclobutanil [5% MRL=0.025 ppm]
Spiked 1	Undetected	undetected	Undetected
Spiked 2	0.235 ppm	3.048 ppm	1.315 ppm
1	Undetected	undetected	Undetected
2	Undetected	undetected	Undetected
3	Undetected	undetected	Undetected
4	Undetected	undetected	Undetected
5	Undetected	undetected	Undetected
6	undetected	undetected	Undetected
7	undetected	undetected	Undetected
8	undetected	undetected	Undetected
9	undetected	undetected	Undetected
10	undetected	undetected	Undetected
11	undetected	undetected	Undetected
12	undetected	undetected	Undetected
13	undetected	undetected	Undetected
14	undetected	undetected	Undetected
15	undetected	undetected	Undetected
16	undetected	undetected	Undetected
17	undetected	undetected	Undetected
18	undetected	undetected	Undetected

19	undetected	undetected	Undetected
20	undetected	undetected	Undetected
21	undetected	undetected	Undetected
22	undetected	undetected	Undetected
23	undetected	undetected	Undetected
24	undetected	undetected	Undetected
25	undetected	undetected	Undetected
26	undetected	undetected	Undetected
27	undetected	undetected	Undetected
28	undetected	undetected	Undetected
29	undetected	undetected	Undetected
30	undetected	undetected	Undetected

Figure 2: Chromatograph of Pesticide Standards

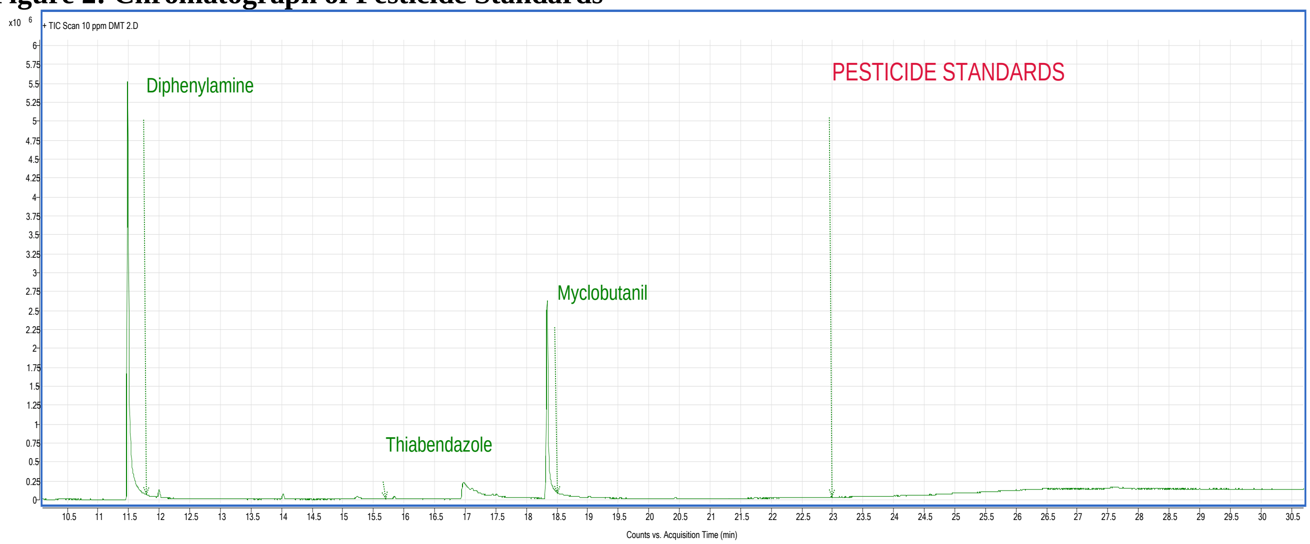


Figure 3: Chromatograph of Spiked Sample before Juicing (Spiked 1)

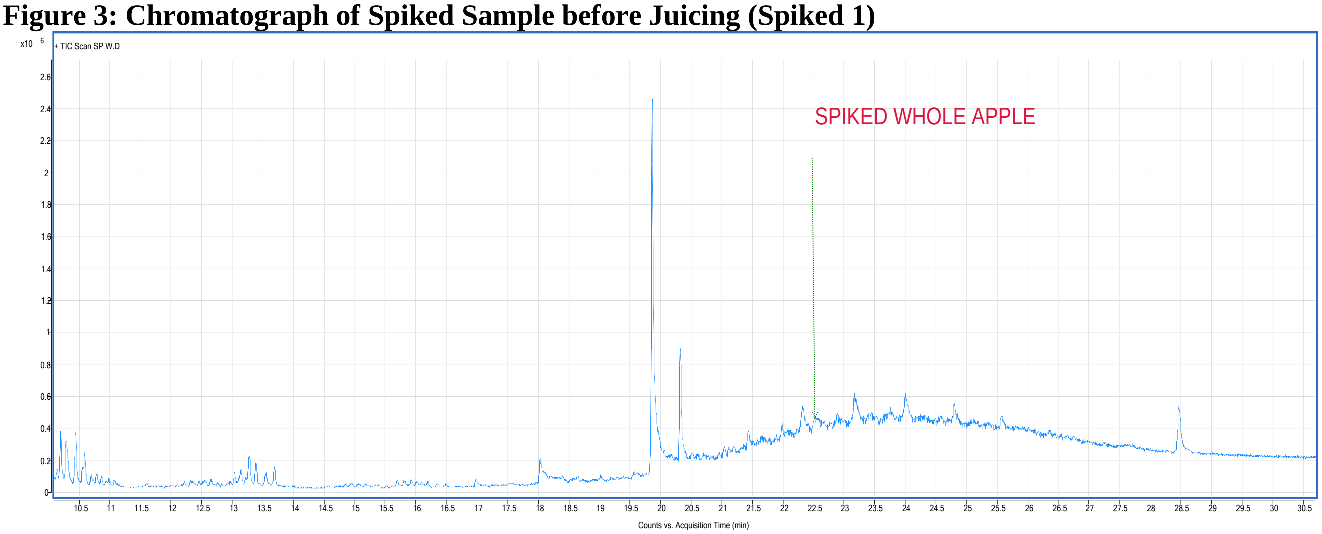


Figure 4: Chromatograph of Spiked Sample after Juicing (Spiked 2)

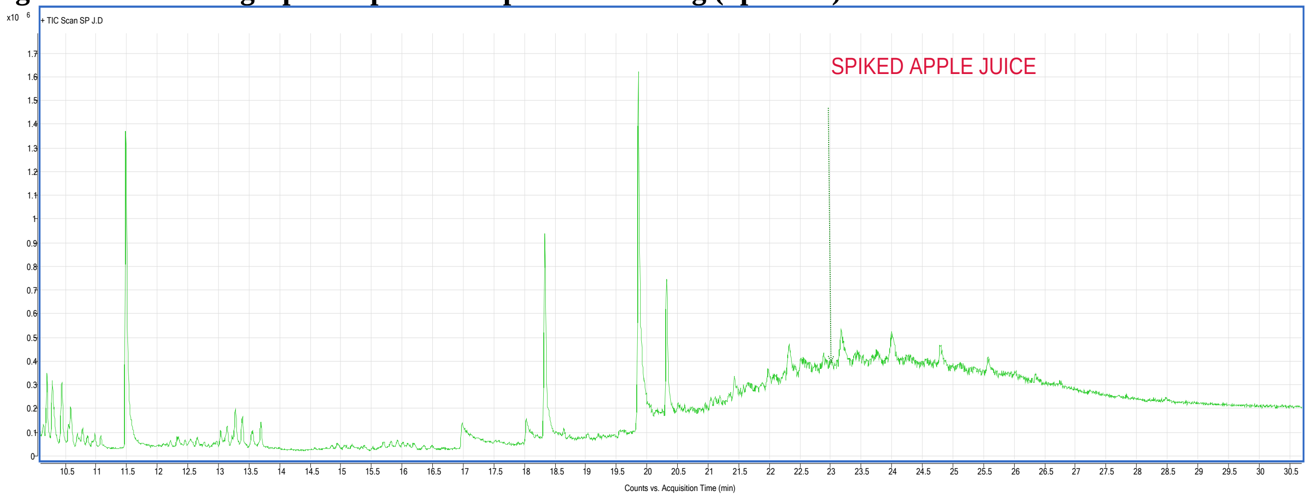
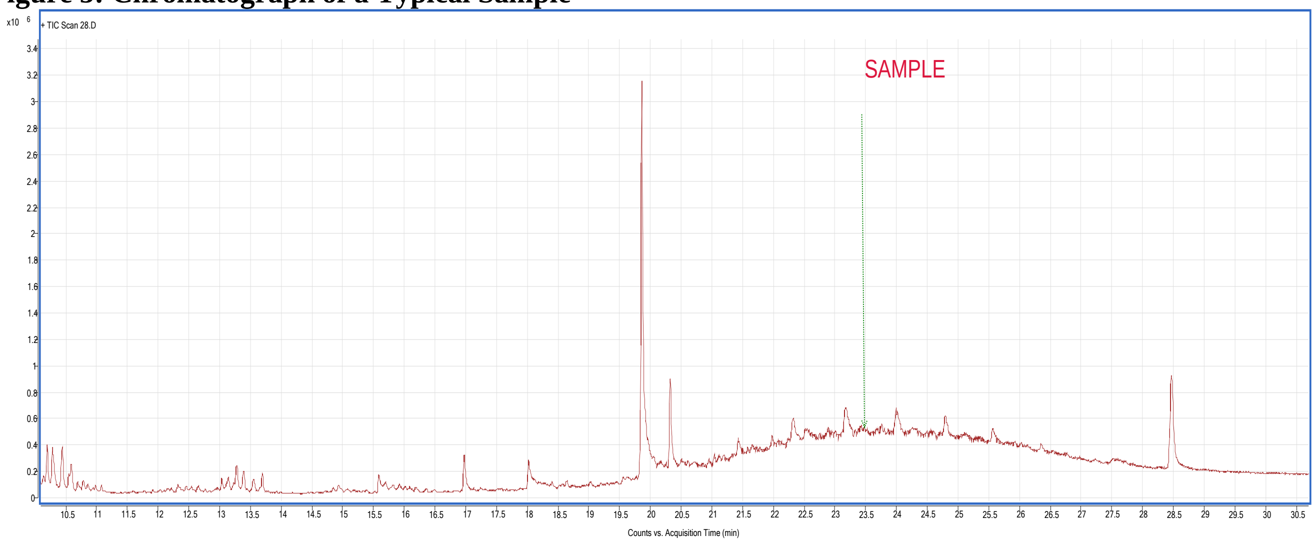


Figure 5: Chromatograph of a Typical Sample



DISCUSSION

This study was conducted to test pesticide residue levels in organic apples to see if they were at levels below 5% of the Maximum Residue Limit (MRL) set by Health Canada for each pesticide. The results of this study suggest there is compliance by organic farmers with existing legislation in meeting the 5% MRLs for thiabendazole, diphenylamine, and myclobutanil. In fact, not only do residue levels meet the organic standards, they exceed them; the results indicate an absence of synthetic pesticides on organic apples grown in British Columbia. This also suggests there is no deliberate addition of synthetic pesticides to organic apple orchards in order to overcome the challenges associated with organic farming. Furthermore, this study indicates that the public can switch to organic apples to reduce their oral exposure to pesticides, especially if the apples are being juiced for consumption. This last suggestion is based on the results for the "Spiked 1" and "Spiked 2" samples. The "Spiked 1" sample was spiked with pesticides before the sample was juiced, and in Figure 3 above, the results show an absence of the thiabendazole, diphenylamine, and myclobutanil peaks. The "Spiked 2" sample was spiked after it was juiced, and the results show a presence of the thiabendazole, diphenylamine, and myclobutanil peaks in Figure 4 above. Since the added pesticides were not detected in the "Spiked 1" sample but were detected in the "Spiked 2" sample, it is reasonable to deduce that the pesticides were removed from the juice in the juicing step.

The findings of this study are not consistent with previous studies claiming that about half of the tested organic apples have detectable levels of pesticide residues (USDA National Organic Program & Science and Technology Programs, 2012; Smith-Spangler et al., 2012; Levasseur & Kubinec, 2014). In this current study, thiabendazole, diphenylamine, and myclobutanil could have been undetected in the organic apple samples for a number of reasons. Firstly, the amounts of each pesticide in the samples may have been too low to be detected by the gas chromatograph, or the capillary column used with the GC may have not been optimal for the pesticides involved in this study. The sensitivity and level of detection of the gas chromatograph depends on how well the chemicals within a sample are separated, and this in turn depends on properties of the column (Restek, 2013). Different column properties are optimal for the separation and therefore detection of different chemicals. Properties such as the length of the column, the material of the coating, the inner diameter, the film thickness, and the stationary phase greatly affect the separation of chemicals (Restek, 2013). Secondly, pesticides in the original samples may have remained in the pulp which was separated from the juice by the juicer and not analyzed. If the pulp had been analyzed and did contain detectable amounts of thiabendazole, diphenylamine, and myclobutanil, those findings would indicate that juicing is in fact a method to decrease dietary intakes of pesticide residues. On the contrary, if the pulp had been analyzed and no pesticides were detectable, the conclusion that juicing reduces pesticide levels would not be made. Lastly, thiabendazole, diphenylamine, and myclobutanil may have truly been absent from the organic apples.

Impact on Public Health

Organic diets continue to rise in popularity, especially since the Environmental Working Group produced the list of the most highly pesticide-contaminated fruits and vegetables (EWG, 2014). On this list, apples were named one of the top fruits to avoid eating unless they were organically grown. Members of the public are actively trying to reduce their dietary intakes of pesticide residues by switching to organic foods, even when some studies have shown unacceptable levels of pesticides in organic produce (USDA National Organic Program & Science and Technology Programs, 2012; Smith-Spangler et al., 2012; Levasseur & Kubinec, 2014). Additionally, some members of the public may be unaware that there are acceptable levels of pesticide residues for organic produce, and that the levels are not necessarily zero. This becomes important especially for parents raising their children on organic diets, since children are more susceptible to the negative health effects associated with pesticide exposures. These health effects include developmental disruptions especially in the memory and motor functions (Lu et al., 2008; Vogt et al., 2012). Furthermore, in populations of all ages, high dosage exposures to pesticides in food can produce damaging toxic effects and/or cancer in vital organs such the liver, kidneys, and adrenal glands (PANNA, 2011; Health Canada, 2013b). The same effects can also be produced in lymphocyte cells, thereby affecting the body's ability to fight off infections (Santovito et al., 2012).

The public may also be vulnerable to instances of false advertising regarding organic claims, which is why this study, and others like it, are relevant to public health. Organic apple samples from this study had undetectable levels of pesticide residues, but further research is still necessary, as will be discussed in the next few sections.

LIMITATIONS

Due to restrictions on time and resources available for conducting this study, a relatively small and unvaried sample size was tested.

Under a less restrictive budget and time frame, a wider range of organic apple varieties from different sources could have been analyzed. With more time and resources, the capillary column of the gas chromatograph could have been optimized for this study's chemicals of interest. In addition, due again to time restrictions, the pulp of the juiced apples could not be analyzed for pesticide residues and only the juice was analyzed. Because the pulp was not analyzed, it was not possible to determine whether or not the pesticides were truly confined to the pulp. Subsequently, it could not be determined if juicing is in actuality an effective method for removing pesticide residues from fruit.

RECOMMENDATIONS

Based on this research, dietary exposures to pesticides can be reduced by switching to organic diets, especially switching from conventional to organic apples. Moreover, based on the findings in this study, it is recommended that organic apples be juiced rather than consumed whole in order to further reduce dietary exposures to pesticides to negligible levels. Although these findings could not be confirmed in this study, there is a high possibility that the act of juicing was responsible for removing pesticides, as seen in the case with the first spiked apple sample.

Since the organic market is continuously expanding, more research is required in order for Environmental Health Officers (EHOs) to work with Health Canada and CFIA to be able to provide accurate information to the public. Conflicting information on pesticides in organic foods is still apparent in the literature. While pesticide residues in organic apples based on this study meet Health Canada's standards, a more comprehensive study is needed in order for results to be appropriately extrapolated. Further research can also offer the best practices for further reduction of dietary intakes of pesticides. For instance, research on processing methods, such as juicing, fermenting, boiling, or canning, can be conducted to determine their effectiveness in eliminating pesticide residues. This would enable EHOs to be better equipped to advise the public as well as operators of natural food stores (which are a growing trend) on ways pesticides can be further removed from diets.

FUTURE STUDIES

Suggestions for future studies are as follows:

- Test a larger number of samples and organic apple varieties from multiple sources
- Test the pulp that results from juicing to determine if juicing truly reduces pesticide residues
- Comminute the samples to undergo the same QuEChERS method rather than juicing the samples
- Test apples that have been processed in different ways to see which methods are most effective at reducing pesticide residues
- Test organic apples for pesticides other than thiabendazole, diphenylamine, and myclobutanil

CONCLUSIONS

The findings in this study suggest there is an absence of pesticide residues in organic apples grown in British Columbia. Further, these findings propose that dietary exposures to pesticides can be reduced by switching from conventionally grown to organically grown apples, and that exposures can be reduced especially by juicing the organic apples. These findings reflect positively on organic farmers and organic apple orchards in BC and translate positively to the public as well, since members of the public who purchase certified organic apples to reduce their exposures to pesticides can continue to do so justifiably.

The juice of organic apples was analyzed for specific pesticide residues using a modified QuEChERS method, and none of these pesticides were detected in the samples. Since only one of the spiked samples had detectable levels of the pesticides, either the methods used in this study require additional modifications, the residues in the organic apples were too low to be detected, juicing effectively reduces pesticide residues, or the organic apples had no pesticide residues to begin with. It is important to note that only three apple varieties were used in this study, which may not be representative of all or most apple varieties in British Columbia. With the additional questions arising from this study, it is apparent that further research needs to be conducted.

ACKNOWLEDGEMENTS

The author would like to thank several supporters of this research study, namely Fred Shaw, for supplying the materials required to conduct this study; Bobby Sidhu, for his valuable feedback and guidance throughout the course of this study; and Kevin Soulsbury and Bob Bower, for their time, support, suggestions, guidance, and supervision, for providing the equipment and instruments necessary for conducting this study, and for granting access to the chemistry laboratories.

COMPETING INTEREST

The authors declare that they have no competing interests.

REFERENCES

Agilent Technologies. (2011). *Agilent 5975C series GC/MSD: Performance, productivity, and confidence.* Retrieved from http://www.chem.agilent.com/Library/brochures/5990-7641EN.pdf.

Agriculture and Agri-Food Canada. (2011). *Apple crop profile for Canada, 2009*. Retrieved from http://publications.gc.ca/collections/collection_2011/agr/A118-10-7-2011-eng.pdf.

Agriculture and Agri-Food Canada. (2014). *Reduced-risk strategy for apple scab management*. Retrieved from http://www.agr.gc.ca/eng/?id=1299079705240.

Anastassiades, M. Scherbaum, E., & Mack, D. (2003). *Implementation of the QuEChERS method for the routine analysis of pesticide residues – validation and experiences*. CVUA Stuttgart. Retrieved from http://cvuas.xn--untersuchungsmter-bw-nzb.de/pdf/poster_implementation_quechers_eprw.pdf.)

Campbell, B. L., Mhlanga, S., & Lesschaeve, I. (2013). Perception versus reality: Canadian consumer views of local and organic. *Canadian Journal of Agricultural Economics, 61*(4), 531-558.

Canada Agricultural Products Act: Organic products regulations, SOR/2009-176. (2009). *Canada Gazette Part II,* 143(13). Retrieved from http://gazette.gc.ca/rp-pr/p2/2009/2009-06-24/html/sor-dors176-eng.html.

Canadian Food Inspection Agency. (2014). *Fact sheet: Organic products in Canada*. Retrieved from http://www.inspection.gc.ca/food/organic-products/fact-sheet/eng/1389651477171/1389651725636.

Canadian General Standards Board. (2011a). *Organic production systems – general principles and management standards,* CAN/CGSB-32.310-2006. Retrieved from http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/internet/bio-org/documents/032-0310-2008-eng.pdf.

Canadian General Standards Board. (2011b). *Organic production systems – permitted substances list,* CAN/CGSB-32.311-2006. Retrieved from http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/internet/bio-org/documents/032-0311-2008-eng.pdf.

CBC News. (2011, December 8). Pesticides found in Canadian organic produce. *CBC News*. Retrieved from http://www.cbc.ca/news/canada/manitoba/pesticides-found-in-canadian-organic-produce-1.985584.

Certified Organic Associations of BC. (n.d.). *COABC – Standards*. Retrieved from http://www.certifiedorganic.bc.ca/standards/index.php.

David Suzuki Foundation. (n.d.). *What are the dirty dozen and the clean fifteen*. Retrieved from http://www.davidsuzuki.org/what-you-can-do/queen-of-green/faqs/food/what-are-the-dirty-dozen-and-the-clean-fifteen/.

Delate, K., McKern, A., Turnbull, R., Walker, J. T. S., Volz, R., White, A., Bus, V., Rogers, D., Cole, L., How, N., Guernsey, S., & Johnston, J. (2008). Organic apple systems: constraints and opportunities for producers in local and global markets: introduction to the colloquium. *HortScience, 43*(1), 6-11.

Environmental Health Sciences Center, Oregon State University. (2014). *Unsolved mysteries of human health: GCMS – how does it work*? Retrieved from http://unsolvedmysteries.oregonstate.edu/MS_05

Environmental Working Group. (2014). *EWG's 2014 shopper's guide to pesticides in produce*. Retrieved from http://www.ewg.org/foodnews/.

Heacock, H. & Sidhu, B. (2014). *Module 4: Research Design & Data Collection*. [Lecture Slides]. BCIT ENVH 8400 – Research Methods, Burnaby, BC.

Health Canada. (2011). *Re-evaluation decision RVD2011-02, thiabendazole*. Retrieved from http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_decisions/rvd2011-02/index-eng.php.

Health Canada. (2013a). *Re-evaluation note REV2013-06, special review initiation of 23 active ingredients*. Retrieved from http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_decisions/rev2013-06/index-eng.php.

Health Canada. (2013b). *Re-evaluation decision RVD2013-01, myclobutanil*. Retrieved from http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_decisions/rvd2013-01/index-eng.php.

Health Canada. (2013c). *Pesticides and food*. Retrieved from http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_fact-fiche/pesticide-food-alim/index-eng.php.

Health Canada. (2014). *Maximum residue limits for pesticides*. Retrieved from Health Canada's MRL database at http://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php.

Jonsson, A. (2006). Insects and insect damage in an apple orchard converted from integrated production to organic production. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 56,* 70-80.

Jonsson, A., Nybom, H., & Rumpunen, K. (2010). Fungal disease and fruit quality in an apple orchard converted from integrated production to organic production. *Journal of Sustainable Agriculture, 34,* 15-37.

Levasseur, J. & Kubinec, V. L. (2014, January 8). Pesticide residue found on nearly half of organic produce. *CBC News*. Retrieved from http://www.cbc.ca/news/canada/manitoba/pesticide-residue-found-on-nearly-half-of-organic-produce-1.2487712.

Lu, C., Barr, D. B., Pearson, M. A., & Waller, L. A. (2008). Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. *Environmental Health Perspectives, 116*(4), 537-542.

Mastovska, K. & Lehotay, S. J. (2004). Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues. *Journal of Chromatography A, 1040*, 259-272.

Mortillaro, N. (2014, April 29). Enjoying that apple? European Union thinks it contains carcinogens. *Global News*. Retrieved from http://globalnews.ca/news/1295658/enjoying-that-apple-it-could-be-toxic-european-union-believes/.

Pesticide Action Network North America. (2011-2014). *What's on my food: Apples*. Retrieved from http://www.whatsonmyfood.org/food.jsp?food=AP.

Price, R. J., Scott, M. P., Giddings, A. M., Walters, D. G., Stierum, R. H., Meredith, C., & Lake, B. G. (2008). Effect of butylated hydroxytoluene, curcumin, propyl gallate and thiabendazole on cytochrome P450 forms in cultured human hepatocytes. *Xenobiotica, 38*(6), 574-586.

Restek. (2012). *QuEChERS methodology: AOAC method*. Retrieved from http://www.restek.com/pdfs/805-01-002.pdf.

Restek. (2013). *Guide to GC column selection and optimizing separations*. Retrieved from http://www.restek.com/pdfs/GNBR1724-UNV.pdf.

Santovito, A., Cervella, P., & Delpero, M. (2012). Micronucleus frequency in human lymphocytes after exposure to diphenylamine in vitro [Abstract]. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* *747*(1), 135-137. Retrieved from http://www.sciencedirect.com/science/article/pii/S138357181200188X.

Smith-Spangler, C., Brandeau, M. L., Hunter, G. E., Bavinger, J. C., Pearson, M., Eschbach, P. J., Sundaram, V., Liu, H., Schirmer, P., Stave, C., Olkin, I., & Bravata, D. M. (2012). Are organic foods safer or healthier than conventional alternatives? A systematic review. *Annals of Internal Medicine*, *157*, 348-366.

United Chemical Technologies. (n.d.). *Pesticide residue analysis: QuEChERS informational booklet*. Retrieved from https://www.chromspec.com/pdf/e/uct19.pdf.

USDA National Organic Program & USDA Science and Technology Programs. (2012). *2010-2011 pilot study: Pesticide residue testing of organic produce*. Retrieved from http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5101234.

Vogt, R., Bennett, D., Cassady, D., Frost, J., Ritz, B., & Hertz-Picciotto, I. (2012). Cancer and non-cancer health effects from food contaminant exposures for children and adults in California: A risk assessment. *Environmental Health*, *11*(83), 1-14.

Wang, Q., Sun, J., & Parsons, R. (2010). Consumer preferences and willingness to pay for locally grown organic apples: Evidence from a conjoint study. *HortScience*, *45*(3), 376-381.

Zhao, L., Wylie, P. L., & Stevens, J. (2012). Analysis of pesticide residues in apple using Agilent Bond Elut QuEChERS EN kits by GC/MS. *Agilent Technologies*. Retrieved from http://www.chem.agilent.com/Library/applications/5990-4073EN.pdf.