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Survey of Carbendazim and Organophosphate Residues Among Organic and Non-Organic Produce

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ABSTRACT

Pesticides are chemicals used to control pests and diseases in crops, however, excessive or improper application can lead to the presence of harmful contaminants in food. Organophosphates have been utilized in various crops such as fruits, vegetables, cereals and field crops and present health risks to farmworkers and consumers if not used properly. Chronic exposure to low levels has been associated with long-term health effects and increased risk of certain types of cancers. Carbendazim is a systemic fungicide that belongs to the benzimidazole chemical group and is widely used in agriculture to control fungal diseases in crops including fruits, vegetables and ornamental plants. While carbendazim has been widely used in agriculture for several decades, there have been concerns regarding its potential health and environmental effects. In this study, we used commercially available ELISA kits to detect and quantify the levels of these two commonly used pesticides in a panel of organic and non-organic fruits and vegetables. We detected the presence of organophosphate in broccoli and, with the exception of non-organic oranges and apples, carbendazim levels in all samples tested were below 0.5 parts per billion.

Introduction

Carbendazim (methyl benzimidazol-2-ylcarbamate) is a widely used broad spectrum fungicide effective on a wide range of horticultural crops. Benzimidazole fungicides are known by various trade names including Bavistin, Topsin and Carbendazim-50. The mechanism of action of this class of pesticides involves inhibition of polymerization of monomeric tubulin (Zhou et al., 2016). Biochemical assays such as competitive inhibition of [14C] carbendazim binding by colchicine and nocodazole supported the notion that the carbendazim binding target protein was fungal tubulin, thus inhibiting the formation of microtubules and as a result disrupting their normal growth and development (Davidse, 1986). This intricate disruption ultimately leads to the death of the fungal pathogen and is quite effective against many types of agricultural fungal pathogens.

Carbendazim has been associated with certain health issues and the extent of the risks may vary depending on the level and duration of exposure. It's noteworthy that while carbendazim has been widely used in the past, its use has become more restricted due to concerns about its potential toxicity and adverse effects on both human health and the environment. Regulatory authorities have imposed limitations on its use such as maximum residue limits on treated agricultural crops. Animal studies have suggested that carbendazim may have reproductive toxicity such as effects on fertility, fetal development and reproductive organs (Nakai et al., 1992; Kadalmani et al., 2002, Yu et al., 2009). Abolaji et al. have also demonstrated that carbendazim can induce renal, hepatic and oxidative damage in female rats (Ma et al., 2023). Furthermore, carbendazim has been indicated to exert adverse effects on aquatic organisms (van den Brandhof & Montforts, 2010). There have been concerns regarding the potential carcinogenic effects of carbendazim in humans and the relevance of these findings is still being investigated (Bentley et al., 2000). The International Agency for Research on Cancer (IARC) currently classifies carbendazim as "not classifiable as to its carcinogenicity to humans," implying that there is insufficient evidence to suggest causal effect. However, regulations and safety measures are in place to minimize potential risks associated with pesticide use and application. These safeguards include establishing maximum residue limits on treated crops and providing guidelines for its safe use. The United

States Food and Drug Administration has established maximum residue limits (MRLs) for carbendazim on various food commodities. MRLs are the maximum allowable concentrations of pesticide residues that are considered safe for consumption. These limits help ensure that the use of pesticides does not pose significant health risks to consumers. These MRLs can be subject to change, as regulatory authorities continually review and update them based on new scientific information and risk assessments. The European Union has also established MRLs for carbendazim in various agricultural products.

Another class of commonly used pesticides includes organophosphates used to manage a variety of pests, including but not limited to insects, and are also applied to agricultural crops. Organophosphates mechanism of action involves inhibiting the activity of acetylcholinesterase (AChE) (Lee et al., 2016). AChE is responsible for breaking down acetylcholine in the nervous system (Lenina et al., 2020). Inhibition of AChE results in an excessive buildup of acetylcholine, leading to overstimulation of the nervous system (Tsai et al., 2021). Prolonged or excessive exposure to organophosphates can result in neurotoxic effects, including cognitive impairment, memory problems, developmental disorders in children, and long-term neurological damage (Blanc-Lapierre et al., 2013). Some organophosphates have been associated with adverse developmental and endocrine disruption (Patisaul et al., 2021). Disruptions in hormone regulation could potentially affect reproductive health, growth, metabolism and other hormone-dependent processes. Prenatal exposure to these compounds has been linked to developmental delays, neurobehavioral abnormalities, reproductive functions and potential impacts on fetal growth (Engel et al., 2011). Some organophosphates have been classified as possible or probable human carcinogens by international agencies (Lerro et al., 2015). Certain studies have reported associations between organophosphate exposure and increased risks of specific cancers, such as non-Hodgkin lymphoma and leukemia (Koutros et al., 2019).

Organophosphates can persist in the environment and contaminate water, soil, and can have detrimental effects on non-target organisms, including wildlife and beneficial insects, disrupting ecosystems. Due to their toxicity, the use of certain organophosphate pesticides has been restricted or banned in some countries. Regulatory authorities and health organizations have implemented measures to mitigate the risks associated with organophosphates, including establishing safety standards, setting maximum residue limits on food commodities, and providing guidelines for their safe use. While organophosphates have been widely used in the past, there is a growing emphasis on the development and use of safer alternatives with lower environmental and health impacts. The United States Food and Drug Administration and the European Food Safety Authority have established tolerances for specific organophosphate pesticides on various agricultural products enforced in the United States and the European Union.

Several studies have explored the potential association between organophosphate exposure and cancer risk. The research has mainly focused on occupational exposure among agricultural workers and individuals living in close proximity to agricultural areas where these pesticides are utilized (Pedroso et al., 2021). The findings suggest that certain organophosphates may be associated with an increased risk of certain types of cancer (VoPham et al., 2017). Latifovic et al. have reported a possible link between organophosphate exposure and an increased risk of developing non-Hodgkin lymphoma. The specific mechanisms by which organophosphates could potentially contribute to cancer development are not fully understood. Nonetheless, some studies suggest that organophosphates may disrupt normal cellular processes, interfere with DNA repair mechanisms, or act as endocrine disruptors, increasing the risk of cancerous changes in cells (Rakak et al., 2021; Prathiksha et al., 2023; Mnif et al., 2011; Lerro et al., 2015). The risks associated with organophosphate exposure depend on various factors, such as the specific organophosphate used, the level and duration of exposure, and individual susceptibility. Subsequently, regulatory bodies have established guidelines and regulations to mitigate potential health risks associated with organophosphates, including imposing safety standards for their use and residue limits in food. The level of organophosphate pesticide residues in fruits can vary depending on several factors such as agricultural practices, pesticide usage and compliance with imposed regulations. Interestingly, some fruits have been found to have relatively higher occurrences or levels of organophosphate pesticide residues. It is noteworthy that pesticide residue levels can vary depending on factors such as the country of origin, farming practices and compliance with regulatory standards.

Organic farming is an agricultural practice that highlights the use of natural and environmentally friendly practices while avoiding the use of synthetic chemicals. The organic produce label typically refers to fruits, vegetables and other agricultural products that have been grown and processed using organic farming methods. When a product is labeled as "organic," it indicates that the commodity has been produced in accordance with certain organic standards and regulations set by government bodies or independent certification organizations. These standards generally include guidelines regarding soil fertility, pest and weed management, and the use of pesticides. In this study, we measured the residue contents of carbendazim and organophosphates in a panel of sixteen fruits and vegetables available at local supermarkets.

Methods

Sixteen different fruit and vegetable samples of organic and conventional origin were obtained from local supermarkets. 1.0 g of each fruit or vegetable sample was chopped and blended in 5 mL of phosphate buffered saline (PBS) solution. Levels of carbendazim and organophosphate were measured using commercially available ELISA kits purchased from Attogene (Austin, TX). The assays were carried out according to the manufacturer's instructions. For detection of carbendazim, 50µL of standard and unknown samples were added to a 96 well plate. Then, 100µL of the primary antibody was added into appropriate test wells. The samples were gently mixed for 30 seconds and incubated for 30 minutes at room temperature. Following the incubation period, the contents of the wells were aspirated off and the wells were rinsed with 250µL of the 1X wash solution three times. The residual wash solution was removed and 150µL of freshly prepared 1X HRP-conjugated secondary antibody was added to each well. The samples were mixed gently for 30 seconds using a back-and-forth motion. The plate was then covered and incubated for 30 minutes at room temperature. Following incubation, the contents were aspirated off and wells were washed with 250µL of the 1X wash solution three times. Residual solution was removed and 100µL of TMB substrate solution was added to each well, mixed, covered and incubated for 5-15 min at room temperature. Finally, 100µL of the stop solution was added to each well, mixed and the absorbance at 450nm was measured. To determine the amount of pesticide in a sample, a standard curve was prepared using several different known quantities. All standards and samples were carried out in duplicates. For detection of organophosphate in samples, 50µL of each sample were added into microplate wells followed by addition of 5µL of Oxonation Reagent 1 and 10µL of Reaction Buffer to each well containing sample or control. Samples were mixed and incubated for 10 minutes at room temperature. Then 5µL of Oxonation Reagent 2 was added to each well, mixed and incubated for 5 minutes at room temperature. Following the incubation period, 20µl of AchE solution was added into each well, mixed and incubated for 15 minutes at room temperature. Next, 100µL of Reaction Buffer and 20µL Chromogen Solution were added to each well. The reaction was initiated upon addition of 100µL Substrate Solution to each well. The increase in absorbance at 405 nm was measured over a 10-minute interval for each well. All samples were carried out in duplicates.

Results

Colorimetric ELISA test kits were utilized to detect the presence of organophosphates and to measure levels of carbamates in samples. We tested sixteen fruit and vegetable samples and used commercially available ELISA kits to detect and assess the levels of these pesticides. Of the sixteen samples tested, only one contained organophosphate residues. As indicated in Table 1, broccoli appeared to be the only sample that tested positive for the presence of organophosphates. Organophosphate contaminations were detected in both organic and non-organic broccoli samples tested.

As indicated in Figure 1, the levels of carbendazim was significantly higher in organic strawberries (0.31997 ppb) than the non-organic product (0.09823 ppb). The levels of carbendazim were more than four times higher in non-organic blueberries than the levels detected in organic blueberries (0.16195 ppb vs. 0.03718 ppb, respectively). We also tested the levels of this pesticide in a panel of fruits. As indicated in Figure 2, levels of carbendazim were also detected in non-organic oranges (7.79384 ppb) and apples (2.9999 ppb) in comparison to their organic counterparts (0.18199 and 0.26637 ppb, respectively). There were no significant differences in levels of carbendazim tested in organic and non-organic grapes (0.14207 ppb vs. 0.18198 ppb, respectively) and pears (0.16546 ppb vs. 0.14106 ppb, respectively).

With the exception of tomatoes, less than 0.5 ppb of carbendazim levels were detected in the produce samples tested (Figure 3). Surprisingly, organic broccoli appeared to contain more than two times the levels of carbendazim compared to its non-organic counterpart (0.12673 ppb vs. 0.28883 ppb). We didn’t detect any significant differences in carbendazim levels between organic and non-organic samples of Bell Pepper, carrot, cauliflower, green beans, kale, spinach and tomatoes.

Table 1. Levels of organophosphates were measured using a commercially available kit. Sixteen organic and non-organic samples were tested.

Sample		Presence of Organophosphate
Apple	Organic	-
	Inorganic	-
Banana	Organic	-
	Inorganic	-
Bell Pepper	Organic	-
	Inorganic	-
Blueberry	Organic	-
	Inorganic	-
Broccoli	Organic	+
	Inorganic	+
Carrot	Organic	-
	Inorganic	-
Cauliflower	Organic	-
	Inorganic	-
Grapes	Organic	-
	Inorganic	-
Green Bean	Organic	-
	Inorganic	-
Kale	Organic	-
	Inorganic	-
Orange	Organic	-
	Inorganic	-
Pear	Organic	-
	Inorganic	-
Raspberry	Organic	-
	Inorganic	-
Spinach	Organic	-
	Inorganic	-
Strawberry	Organic	-
	Inorganic	-
Tomato	Organic	-
	Inorganic	-

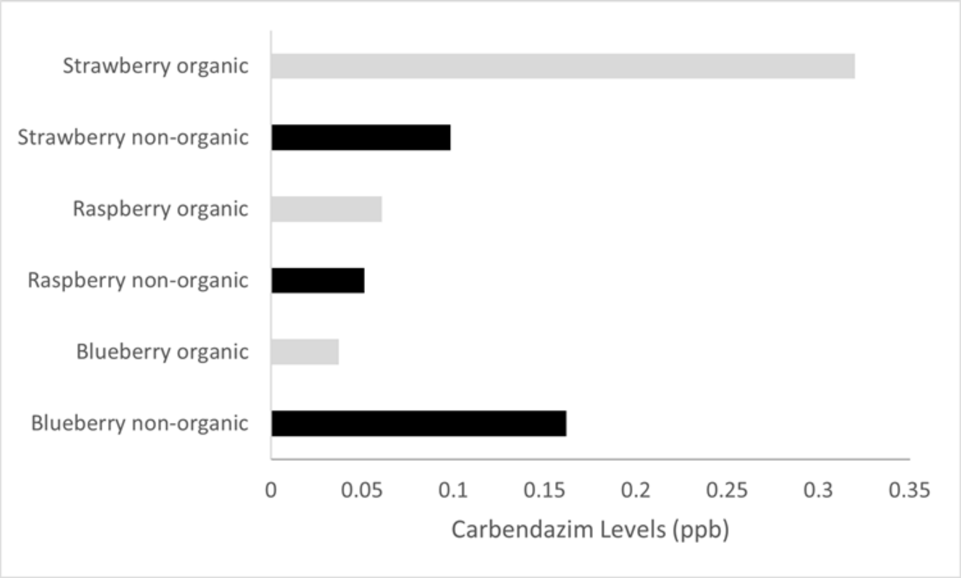

Figure 1. Levels of carbendazim were measured using a commercially available ELISA kit. The levels of carbendazim appeared to be significantly higher in organic strawberries in comparison to non-organic products (0.31997 ppb vs. 0.09823 ppb). Conversely, non-organic blueberries were contaminated with more than four times the amount of carbendazim than its organic counterpart (0.16195 ppb vs. 0.03718 ppb, respectively).

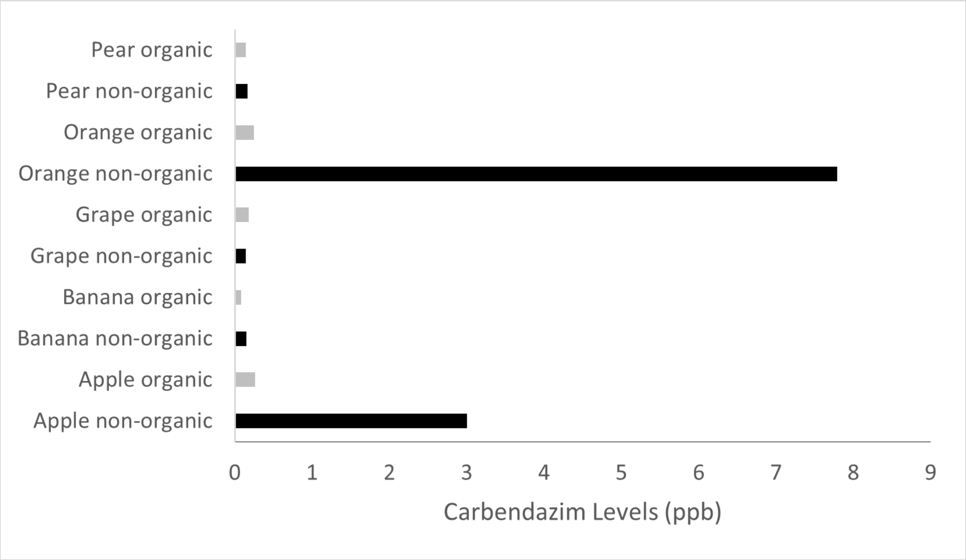

Figure 2. Levels of carbendazim were measured using a commercially available ELISA kit. Significant levels of carbendazim were detected in non-organic oranges (7.79384 ppb vs. 0.24449 ppb) and apples in comparison to their organic counterparts (2.9999 ppb vs. 0.26637 ppb). There were no differences in the levels of carbendazim detected in tested organic and non-organic bananas, grapes and pears.

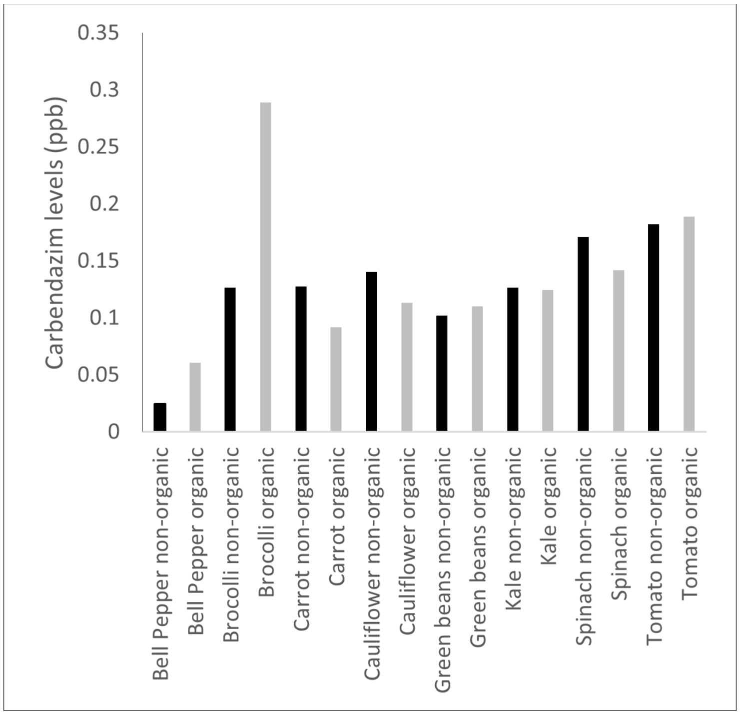

Figure 3. Levels of carbendazim were measured using a commercially available ELISA kit. Carbendazim levels detected in all samples except for broccoli were less than 0.2 ppb. Organic broccoli appeared to contain higher levels of carbendazim compared to its non-organic counterpart (0.28883 ppb vs. 0.12673 ppb, respectively).

Discussion

In this study, we evaluated the presence of organophosphates and assessed the levels of carbendazim fungicides in a panel of sixteen organic and non-organic fruits and vegetables. Organophosphates were only detected in broccoli in both organic and non-organic samples. None of the other samples tested positive for the presence of organophosphates. The levels of carbendazim were the highest in non-organic oranges, apples and tomatoes. Surprisingly, the carbendazim levels were higher in organic strawberries than their non-organic counterparts. We didn't observe significant variations in carbendazim levels in our assays between the remaining organic and non-organic samples evaluated.

Carbendazim is mainly used as a fungicide and is typically approved for use on apples and citrus fruits. Its use on vegetables is further limited and the specific tolerances for carbendazim on vegetables vary depending on the crop and the specific regulations in place. Trace amounts of carbendazim or its metabolites can occasionally be detected in milk or honey due to the use of carbendazim in agricultural practices. The presence of pesticide residues in food products is usually quite low and is regulated to ensure that the levels are well below those considered harmful to human health. Regular monitoring and quality control measures are in place to minimize the presence of pesticide residues in food. Pesticide regulations and tolerable residue levels can vary over time and depend on the country of origin. Carbendazim has been used in agricultural crops in the United States and is a broad-spectrum fungicide utilized to control fungal diseases in various crops. However, the use of carbendazim in the United States has been gradually phased out or restricted for certain crops due to concerns about its potential health and environmental impacts. The Environmental Protection Agency has implemented regulations to limit its use and to ensure the safety of consumers. The MRLs for carbendazim in fruits can vary depending on the specific fruit and the country of origin. Carbendazim is not commonly used on broccoli in the United States and while tomatoes are not typically associated with carbendazim residues, there have been isolated instances of contamination. Monitoring pesticide levels and use in crops allow regulatory bodies to promote the safe and responsible use of pesticides, protect public health, safeguard the environment, and ensure compliance with regulations. It should be noted that different countries often have their own regulations and standards regarding pesticide residues in imported food and monitoring ensures that crops meet the established standards and safeguards.

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