Original Research Article

Increasing Solubility of Fungicide Chlorothalonil Using Sodium Dodecylbenzenesulfonate

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ABSTRACT

Chlorothalonil is a prominent fungicide and mildewcide that is used on a variety of produce, including field and vegetable crops and turfgrass. Chlorothalonil is also used to increase soil respiration and phosphatase activities. However, this fungicide contains harmful properties and can cause health concerns to humans. Chlorothalonil is especially toxic to aquatic species and environments and can pose a threat towards crucial plants such as peanuts and potatoes. Due to the harmful effects of chlorothalonil on our health and the environment, its complete removal from consumable produce is vital. To assist in quickly and thoroughly removing chlorothalonil from various types of produce, we hypothesized that adjusting the pH of the chlorothalonil would help break down the fungicide. However, with the inconclusive results of our pH testing, we shifted a focus on using the surfactant sodium dodecylbenzene sulfonate (SDBS) to increase solubility in chlorothalonil for easy removal. This study investigates how SDBS concentration influences chlorothalonil solubility as measured by thin-layer chromatography (TLC). The increase in polarity of the chlorothalonil was confirmed with the decrease in R_f values from 0.73 to 0.10 when SDBS increased from 0.00 to 0.400 g, therefore indicating an increase in solubility. The impacts of our research prove the efficacy of the use of SDBS in the removal of the fungicide chlorothalonil from produce that is consumed everyday worldwide.

Keywords: pesticide; fungicide; surfactant; chlorothalonil; sodium dodecylbenzenesulfonate; SDBS

INTRODUCTION

As a commonly used pesticide and fungicide, chlorothalonil creates a large concern regarding the environment and possibilities of human consumption. During 1966, chlorothalonil was first commercially registered as a fungicide for use on turfgrass (1). As its popularity grew, in 1997, chlorothalonil was noted to be the third most used fungicide within the United States (2). However, as more downsides of the usage of chlorothalonil became globally recognized, several countries banned commercial usage of pesticide. For example, in 2017, New Zealand was the first country to ban the manufacturing and importing of chlorothalonil. Although many European and Oceanic countries

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have banned the usage of this fungicide, the U.S. still continues to allow the use of this chemical in agriculture (3, 4).

Chlorothalonil remains widely used in various agricultural sectors worldwide, particularly in the cultivation of fruits, vegetables, and grains. The United States, China, and Brazil are among the largest consumers of chlorothalonil, applying it extensively to crops such as peanuts, potatoes, and wheat to prevent fungal infections. Despite bans of use within several European countries, produce treated with chlorothalonil continues to be exported globally, raising concerns about international exposure. In the U.S., the Environmental Protection Agency (EPA) regulates chlorothalonil, setting maximum residue limits (MRLs) for various crops, while the Food and Drug Administration (FDA) monitors its presence in food products. However, studies have linked chlorothalonil to groundwater contamination, toxic effects on aquatic life, and potential risks to human health, including carcinogenicity and endocrine disruption. The persistence of chlorothalonil in the environment and its potential for bioaccumulation has made it a significant ecological and public health concern.

Chlorothalonil is a dinitrile (two nitrile groups), also known as 2,4,5,6-tetrachloroisophthalonitrile with four chloro groups as substitutes (Figure 1). It is extremely toxic for humans in cases of oral exposure and inhalation, even in amounts as miniscule as 0.09 to 0.54 milligrams per liter. Chlorothalonil can also often cause skin rashes which develop into allergic reactions and is classified as a likely human carcinogen by the International Agency for Research on Cancer (IARC). Prolonged exposure to chlorothalonil can lead to nosebleeds, skin irritation, and potential kidney damage (5). In addition, chlorothalonil has also been shown to be highly toxic in aquatic species, which are the most vulnerable, especially in the early stages of aquatic species development (6). With an increasing focus on wildlife preservation, chlorothalonil poses a threat to the health and safety of many of these species in addition to humans.

To address the toxicity of this chemical, we decided to consider its pH and how it could affect our ability to remove the fungicide from produce efficiently. Chlorothalonil is stable in acidic and moderate alkaline solutions. However, at pH levels 9 and above, slow hydrolysis occurs, with a half-life of 38.1 days. However, from pH 6.6 to 8.6, chlorothalonil has been found to be degraded by less than 20% using free chlorine, monochloramine, chlorine dioxide, permanganate, ozone, hydrogen peroxide, and ultraviolet light (7).

Furthermore, we hypothesized that using a surfactant would be more effective in the removal of chlorothalonil in produce compared to manipulating pH. For example, using low pH chemicals such as sulfuric acid would degrade chlorothalonil, but would damage the produce we consume. Therefore, we chose sodium dodecylbenzenesulfonate (SDBS) to reduce surface tension and enhance solubilization, making them effective in washing solutions (Figure 2). SDBS is an anionic surfactant that is known for increasing solubility of hydrophobic molecules and commonly used in many detergents, shampoos, and more (8). In addition, it is easily accessible, cost-effective, and commonly studied for general surfactant behavior. Currently, most research surrounding chlorothalonil solubility is related to the use of solvents, resulting in a lack of studies focused on the effects of surfactants such as SDBS (9).

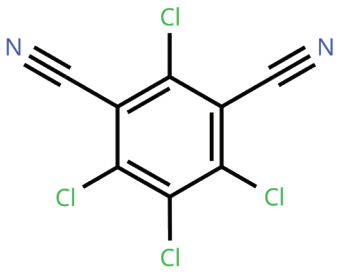

Figure 1. *Structure of chlorothalonil. This figure shows the skeletal structure of the fungicide chlorothalonil. The molecule is a dinitrile made up of a benzene ring with four substituted chloro groups, notated as 2,4,5,6-tetrachloroisophthalonitrile.*

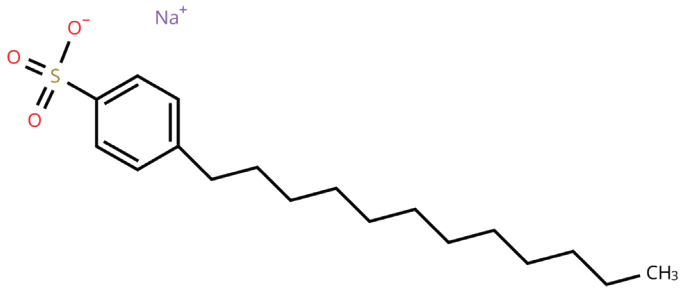

Figure 2. *Structure of sodium dodecylbenzenesulfonate (SDBS). This figure shows the skeletal structure of the anionic surfactant sodium dodecylbenzenesulfonate. This molecule is composed of a benzene ring with a sulfonate group and a long dodecyl chain.*

For the experimental procedure, SDBS was tested on benzonitrile, a chemical consisting of similar properties and structure to chlorothalonil (Figure 3). Through the use of SDBS, we were successfully able to improve pesticide removal efficiency by disrupting the interactions between the pesticide and the produce surface by increasing chlorothalonil solubility. Afterwards, TLC was used to confirm the effects of SDBS on chlorothalonil polarity and solubility with decreasing retention factor (R_f) values with increasing amounts of SDBS, indicating increased solubility of chlorothalonil. Thus, results supported the initial hypothesis which stated that utilizing a surfactant would reduce the R_f value of chlorothalonil, indicating improved solubility, allowing for easy removal of the toxic chemicals consumed on a daily basis.

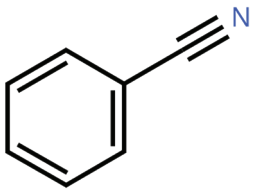

Figure 3. *Structure of benzonitrile. This figure shows the skeletal structure of benzonitrile, a nitrile group attached to a benzene ring. Benzonitrile was used as a substitute for chlorothalonil due to its similar structure and properties.*

METHODS AND MATERIALS

pH Testing on Acetonitrile

Our initial method was to test for pH and temperature conditions in which acetonitrile would degrade. We used 97% acetonitrile instead of chlorothalonil because of its similar properties, but lower toxicity. We boiled solutions of 50 mL H_2O, 5 mL acetonitrile, and different amounts of 85% phosphoric acid to obtain solutions with pHs varying from 7 to 1. We used a YINMIK pH meter to test the different pHs of the solutions. These solutions were heated to 40, 60, and 80ºC using a hot plate and temperature rod. Throughout this process, we utilized a magnetic stir bar to ensure constant stirring. Throughout the experiment, we looked for signs of acetonitrile degradation by the scent of acetic acid.

During the initial experiment, we focused on varying levels of pH and temperature as a way to break down the acetonitrile, which under the same conditions would also break down chlorothalonil because of the similarities in structure. However, during the process of our experiment, we did not see any signs of acetonitrile degradation in the form of acetic acid, as a result, we decided to try another known method of increasing solubility of chemicals through the use of a surfactant.

Finding the TLC Condition

To set up the next two Thin Layer Chromatography (TLC) experiments, we had to find a suitable TLC condition. To do this, we adjusted ratios of our solvent, aiming to find an R_f value close to 0.5. To use as a control for the TLC, we made solution A by diluting 0.1mL diluted benzonitrile (concentration of 30% in ethyl acetate) in 5 mL ethyl acetate. For the solvent, we started with a ratio of 7 mL hexane and 1 mL ethyl acetate and continued to adjust the ratio by observing the results.

First, we spotted solution A onto the Lichen Cottage glass backed TLC silica plates (2.5 × 7.5cm) using ALWSCI Technology glass 0.5 mm × 100 mm capillary tubes. We then placed it into a beaker with the solvent at the bottom and covered it with a larger beaker to prevent evaporation. We waited for the solvent to rise up to around ¾ of the TLC plate. After taking it out and letting it completely dry, we used a TLC UV lamp at 254 nm to measure how much spot A traveled up. We marked the distance spot A traveled and the distance the solvent traveled using a pencil. To find the R_f value, we measured how far up spot A rose and divided it by the distance the solvent traveled. We repeated this process with different ratios of the hexane and ethyl acetate, testing a ratio from 5 mL hexane to 3 mL ethyl acetate to 7.5 mL hexane to 0.5 mL ethyl acetate until we got the R_f value the closest we could to 0.5.

TLC of SDBS Testing on Benzonitrile

We used TLC as a method to determine the amount of surfactants needed to successfully separate benzonitrile. On our TLCs we decided to use two different solutions: solution A, our control, consisting of 0.25 mL of diluted benzonitrile and 5 mL of ethyl acetate, and solution B, which was a mixture of 10 mL water, 0.25 mL of diluted benzonitrile, and varying amounts of our surfactant, sodium dodecylbenzene sulfonate (SDBS). We tested different amounts ranging from 0.25 mL to 3 mL of SDBS. Solution A stayed constant so that it could be used as a control to verify the effects of SDBS on the benzonitrile. Variations of solution B were used to determine the amount of SDBS solution required to result in two separate spots appearing from the initial spot B to indicate a split.

We used a fume hood as an area to spot the TLC

with capillaries to protect against the toxic gases from benzonitrile. Once both solutions were spotted, we checked the TLC plate with a UV light to ensure proper spot size and placement. We then proceeded to place the TLC inside a 500 mL beaker with 3 mL of the previously made 0.5 mL ethyl acetate to 7.5 mL hexane solution to act as the nonpolar mobile phase. Once placed into the beaker, we waited until the mobile phase traveled up to around ¾ of the TLC plate. Then we took out the TLC, let it dry for 10 to 20 seconds ensuring all the solution had evaporated, then analyzed the TLC under a TLC UV lamp at 254 nm to then mark how far the spots traveled and to check for any drag or splitting of solution B. Lastly, we also used a ruler to measure the R_f value of spot A to make sure that the TLC was done correctly.

During this process, we noticed that mixtures of solution B failed to appear on the TLC, so adjustments to the amount of SDBS in solution B were changed. Our second trial for the TLC was the same as the first, but we increased the ratio of SDBS to water in solution B, as it was too diluted in the previous experiment. We mixed together 4 mL water, 1 mL benzonitrile, and varying amounts of SDBS from 0.1 to 0.4 g. The rest of the TLC spotting was done the same as the first experiment.

TLC of SDBS Testing on Chlorothalonil

The same TLC experiment was performed as above, replacing benzonitrile with 1% chlorothalonil diluted in ethyl acetate.

RESULTS

Acetonitrile pH Testing

To understand the effects of pH on chlorothalonil, a substitute acetonitrile was used in place of chlorothalonil for safety measures. Similar to chlorothalonil, acetonitrile contains nitrile groups, containing the majority of the toxicity. Using different values of pH from 1 to 7 as well as temperatures of 40, 60, and 80ºC, we tried to break down acetonitrile and water into acetic acid. We wafted the resulting chemical compounds to distinguish odors to identify acetic acid. However, none of the pH levels or temperatures were able to produce an odor similar to acetic acid.

Finding the TLC Condition

For testing the TLC condition, different ratios of hexane to ethyl acetate were used for the TLC solvent. The goal was to achieve a retention factor (R_f) value of 0.5 using a spot mixture of diluted benzonitrile. First, we started off with 7 mL of hexane and 1 mL of ethyl acetate to create a 7:1 ratio. Through this solvent ratio, we achieved an average R_f value of 0.543 after three trials. Next, we used 6 mL of hexane and 2 mL of ethyl acetate to therefore create a ratio of 6:2. The ratio then achieved an average R_f value of 0.787. We continue this by then using 5 mL of hexane and 3 mL of ethyl acetate, which creates an average R_f value of 0.833. For 7.5 mL of hexane and 0.5 mL of ethyl acetate, the average R_f value stood out to be 0.488. Lastly, with 7.25 mL of hexane and 0.75 mL of ethyl acetate, the average R_f value was 0.554 (Table 1). Looking at the relationship between the ratio and the R_f value, we can conclude that the R_f values increase as the amount of hexane increases. After interpreting our data, we noticed that the experiment with the ratio of 7.5:0.5 had the closest to the R_f value of 0.5.

Table 1. Effect of hexane to ethyl acetate ratios on R_f value of benzonitrile. *TLC was performed using a benzonitrile spot and varying solvent systems were used to identify a ratio yielding an average R_f value (across three trials) close to 0.5. The ratios tested included 6:2, 5:3, 6:2, 7:1, 7.25:0.75, and 7.5:0.5 hexane to ethyl acetate.*

Amount of Hexane (mL)	Amount of Ethyl Acetate (mL)	Average R_f Value
5	3	0.833 ± 0.0245
6	2	0.787 ± 0.0236
7	1	0.543 ± 0.163
7.25	0.75	0.554 ± 0.166
7.5	0.5	0.488 ± 0.146

TLC of SDBS Testing on Benzonitrile

For the benzonitrile TLC, we analyzed and compared the movement of the benzonitrile in two different solutions—one diluted with ethyl acetate (as a constant) and another with our surfactant SDBS—using TLC. By calculating the R_f values for each spot, we were able to evaluate how the surfactant affected the movement and interaction of the benzonitrile in the solvent system.

First, we started with 0.1 g of the surfactant SDBS, which resulted in an average R_f value of 0.272, with a control R_f value of 0.118. Next, we used 0.12 g of the SDBS, which produced an average R_f value of 0.150, with a control R_f value of 0.168. We continued to increase the surfactant to 0.15 g, which yielded an average R_f value of 0.135, and a control R_f value of 0.127. For the 0.2 g of

SDBS, the average R_f value was 0.150 with a control R_f value of 0.057. Lastly, 0.4 g of SDBS gave us an average R_f value of 0.145 with a control R_f value of 0.091 (Table 2). The data obtained from the experiment showed that the surfactant, SDBS, and R_f values had a negative correlation. As the surfactant amount increased, the average R_f value decreased. This shows that the higher surfactant concentration reduced the movement of the benzonitrile on the TLC plate (Figure 4).

Table 2. TLC of benzonitrile with varying amounts of SDBS. *This table records the average R_f values of a control solution with 4 mL of water and 1 mL of benzonitrile and an experimental solution with varying amounts of SDBS in grams. The second set of experimentation is displayed below, indicating generally lower average R_f values (across three trials) with increasing amounts of SDBS. The first set of experimentation was excluded as all R_f values for the experimental group were unobservable.*

Amount of SDBS (g)	R_f Value: Control	Average R_f Value: Surfactant + Benzonitrile
0.1	0.118	0.272 ± 0.00816
0.12	0.168	0.150 ± 0.00450
0.15	0.127	0.135 ± 0.00405
0.2	0.057	0.150 ± 0.00450
0.3	0.143	0.116 ± 0.00348
0.4	0.091	0.145 ± 0.00435

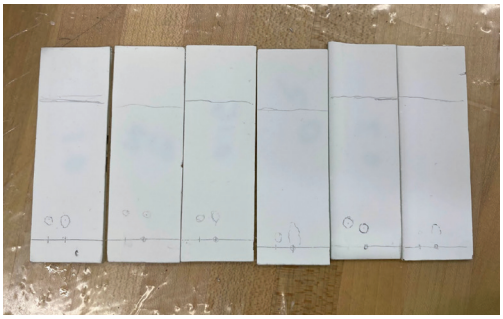

Figure 4. *Benzonitrile thin layer chromatography (TLC) data. This image shows different TLC analyses with the left spot being the control with 4 mL of water and 1 mL of benzonitrile and the right being an experimental solution with the same solution mixed with SDBS. The pencil markings demonstrate where the spot traveled after placing the TLC plate in the solvent. Afterwards, R_f values were calculated. The image shows TLC plates in order of smallest to largest amount of SDBS (0.10g, 0.12g, 0.15g, 0.20g, 0.30g, and 0.40g).*

TLC of SDBS Testing on Chlorothalonil

For the chlorothalonil TLC experiment, we looked for how the increase in SDBS surfactant affected the movement of chlorothalonil on the TLC plate. We observed the average R_f values. Starting with no surfactant, the chlorothalonil presented the highest R_f value of 0.73, showing a significant movement up the TLC plate. When 0.15 g of SDBS was added, the average R_f value decreased to 0.45, this showed a decrease in mobility (Table 3). With a continued increase of SDBS to 0.40 g, the R_f value dropped significantly to 0.10. These results indicate an inverse relationship between the SDBS concentration and the movement of chlorothalonil. When the concentration of SDBS increases, the movement of chlorothalonil is significantly limited. This shows that the SDBS influences chlorothalonil's interaction. This possibly could be because of the increased solubility in the solvent with the addition of the surfactant (Figure 5).

Table 3. TLC of chlorothalonil with varying amounts of SDBS. *The table below suggests increasing amounts of SDBS (0.40g) were more effective in increasing solubility. Adding more SDBS resulted in lower average R_f values (across three trials), making it more polar.*

Amount of SDBS (g)	Average R_f Value: Surfactant + Chlorothalonil
0.00	0.73 ± 0.0219
0.15	0.45 ± 0.0135
0.40	0.10 ± 0.00300

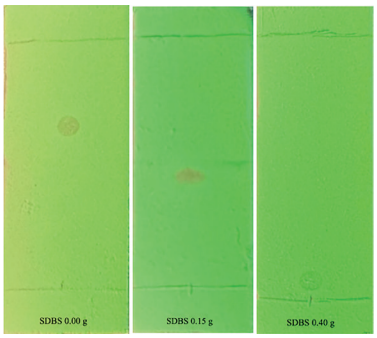

Figure 5. *Chlorothalonil thin layer chromatography (TLC) data. This image shows the TLC analyses conducted on chlorothalonil mixed with varying amounts of SDBS following the same procedures as benzonitrile under a UV lamp. The control TLC analysis with 0.00 grams of SDBS resulted in an R_f value of 0.73, and the other two decreased with larger amounts of SDBS (0.15 g and 0.40 g) to 0.45 and 0.10 respectively.*

DISCUSSION

pH Testing on Acetonitrile

In our experiment with pH testing on acetonitrile, we were unable to notice the proper degradation of acetonitrile. Acetonitrile was used in place of chlorothalonil for the initial experiment due to the similar chemical structure and properties of it to chlorothalonil. Originally, we tested different levels of pH and increased temperatures, but the acetonitrile did not break down, likely due to its nitrile group (C≡N) which is highly stable. Since phosphoric acid is a weak acid, it may not have been strong enough to break down the nitrile group. At an acidic pH of 1, acetonitrile hydrolysis is slow unless strong acids and high temperatures are applied for extended periods. Additionally, a neutral pH of 7, would likely be unreactive with the acetonitrile since water alone cannot effectively break the C≡N bond. Since we used pH values of 1-7, the reaction was likely too slow to cause significant breakdown. Moreover, we used a temperature range of 40–80°C, which was likely not high enough to significantly break down acetonitrile without strong acids, bases, or catalysts. Since the hydrolysis of acetonitrile typically requires very high temperatures (over 150ºC) or stronger acidic or basic conditions, our failure to break down the acetonitrile likely resulted from our ranges of pH and temperatures. We were able to conclude the ineffectiveness of changing pH as a method to degrade chlorothalonil based on the results of testing on acetonitrile, which contains the same, stable, nitrile group as does chlorothalonil. However, using extremely high temperatures and low pHs would be inefficient on large scales, especially for produce, which is why we decided to try another method to remove chlorothalonil from produce. Instead of trying to break down the chlorothalonil, we decided to try and make it more soluble for easier removal.

Finding the TLC Condition

When finding the prime TLC condition, we tested different ratios of hexane to ethyl acetate for the solvent to achieve an R_f value close to 0.5 for a good ratio of polarity to nonpolarity. The spot on the TLC would move depending on the polarity. We tried various ratios by adjusting the amount of each solution and maintained a total volume of 8 mL. The results showed that the ratio of 7 mL hexane and 1 mL ethyl acetate had the closest R_f value so we used that as a basis for further testing. After, we tried a 6:1 ratio, which resulted in a R_f value that was too high, so we tried adding more hexane and less ethyl acetate to make the solution more nonpolar as the TLC we were using was more on the polar side. We tried 7.5:0.5 and the spot moved less, which was what we wanted so we tried 7.25:0.75, but the results showed that the spot moved more than before. We ended up using the 7.5:0.5 ratio because it had the closest R_f value to 0.5.

TLC of SDBS Testing on Benzonitrile

Before testing our surfactant on chlorothalonil, a similarly structured chemical benzonitrile was used instead due to safety concerns and limit in materials. Due to the benzene ring and nitrile group, benzonitrile contains many similarities in both structure and properties as chlorothalonil. During our initial benzonitrile testing, when examining how SDBS changes benzonitrile through the TLC analysis, the results indicated an error in the TLC. We had spotted a TLC plate with solution A, a control of benzonitrile diluted in ethyl acetate, and solution B, which was a mixture of benzonitrile, water, and varying amounts of SDBS. We were testing to see if SDBS could aid in the separation of benzonitrile into its polar and nonpolar components. However, although the control moved up consistently, traveling 1.8, 1.9, and 1.5 cm for the first three trials, the second spot of benzonitrile and SDBS, solution B, did not show up under the UV analyzer. The control spots (solution A) consistently had R_f values of 0.32, Upon repeating the experiment, we noticed that the solution was visible under UV light for only a few seconds before seemingly fading away. We knew that this problem was not due to a chemical reaction, as the SDBS does not react with benzonitrile. Instead, we theorized that the surfactant was not working because it had been too diluted. In our next experiment, we increased the concentration of the surfactant by reducing the amount of water.

Since the first experiment did not work, we decided to add less water to SDBS, so the spot would show and travel up the TLC plate. For our experiment, we were looking for a trial with the lowest R_f value, which indicated the spot traveled less, making it more polar and soluble. During our first trial, we used 0.1 grams of SDBS, which gave the R_f value 0.272. Throughout the trials, we increased the amount of SDBS. The R_f value continued to decrease but increased again on our sixth trial. For our fifth trial we used 0.3 grams of SDBS giving us an R_f value of 0.116 and during the sixth trial we used 0.4 grams of SDBS giving us an R_f value of 0.145, making 0.3 g of SDBS our optimal trial.

TLC of SDBS Testing on Chlorothalonil

After testing benzonitrile for safety and accessibility reasons, the same conditions were tested on chlorothalonil. For chlorothalonil, adding more SDBS resulted in lower R_f values, which indicate reduced movement of the solution (chlorothalonil + SDBS) on the TLC plate. When no SDBS was present, the R_f value was 0.73, which indicates higher solubility in the mobile phase and was more nonpolar, with 0.15 g of SDBS, R_f value was decreased to 0.45, showing the compound's mobility was restricted and the compound was more polar. This is likely due to the increased interactions between chlorothalonil and SDBS. The lowest R_f value of 0.10 was shown at 0.40 g of SDBS. This trend suggests that SDBS affects the polarity of the TLC system.

Impacts, Limitations, and Future Directions

The results from our study support that SDBS can be used to effectively aid the removal of chlorothalonil through the adjustment of its solubility. Chlorothalonil is a contact fungicide, which works by forming a protective barrier around produce. By making chlorothalonil more soluble with the help of SDBS, it can more easily be removed from the surface of produce. This development is impactful because past studies have shown that traditional methods such as washing and soaking fruit cannot wholly remove residue and generally "only lead[s] to a certain degree of reduction in residue level." Using SDBS to help remove chlorothalonil can lower the risks related to human exposure to the fungicide, such as irritation and the consumption of a likely carcinogen. The benefits of chlorothalonil removal extend to animals as well, as chlorothalonil has shown to be highly toxic to aquatic species and moderately toxic to mammals.

However, within our experiments, a factor that may have influenced the data result was the dragging issue, which was due to high concentrations. This likely altered the data, making it hard to interpret the correct and accurate trends and relationships. Additionally, limited resources may have also restricted the number of replicates or the range of conditions tested, decreasing the reliability and generalizability of the findings. To improve the data, it would have been better to incorporate more replicates and optimize control conditions to provide a more solid dataset, which would increase the validity of the experiment.

In the future, with access to a greater number of resources and access to labs, we could do additional trials to find an optimal amount of surfactant necessary. In addition, it would be beneficial to address the concern that chlorothalonil has on aquatic species by further researching safe ways to separate and dispose of it to minimize negative consequences on aquatic species and the general environment. In addition, it would also be beneficial to find alternatives to SDBS, as there are possibilities of contaminating the environment in large amounts as well, although not as toxic as chlorothalonil.

With our research, we were able to conclude that SDBS is an effective way to increase solubility of the toxin and fungicide chlorothalonil. Solubility can be improved with more surfactant. These findings are a crucial step towards ensuring the produce we consume are free of harmful chlorothalonil. In the future, further research can be conducted to decrease its negative effect on the environment and increase efficiency in its removal from consumable foods.

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CONFLICT OF INTEREST

The authors Sophia Lee, Anna Han, Miriam Kang, Hailey Choi, Taylor Choi, Jacob Park, David Park, Zoe Lok, and Ayah Atay declare that there are no conflicts of interest regarding the publication of this article.

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