

Effects of Bleach Activator, Sodium Alkyl Acyloxybenzene Sulfonate, on House Dust Mites (*Dermatophagoides farinae*)

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Abstract: House dust mites (*Dermatophagoides farinae*) in bedding and clothes are a major allergen. However, house dust mites cannot be killed by general washing conditions under 50°C. Therefore, low-temperature washing conditions must be improved to eliminate house dust mites. Sodium alkyl acyloxybenzene sulfonate (OBS) is a bleach activator that is used to intensify the bleaching effects of some laundry products. The purpose of this study was to evaluate the effect of OBS on the elimination of house dust mites in low-temperature washing conditions. *D. farinae* was soaked in solutions containing different types of OBS for various durations and at various temperatures. The miticidal effects of the various washing conditions were also evaluated for *D. farinae*. Then sodium lauroxybenzene sulfonate (OBS-12) produced the highest *D. farinae* mortality rate among the OBS solutions that were examined and had a stronger miticidal effect than available chlorine under general washing conditions. OBS exhibited miticidal effects under general washing conditions at low temperatures. Since OBS is already used as an additive in some laundry products to increase the bleaching activity, OBS can be easily used to kill house dust mites under general washing conditions.

Key words: alkyl acyloxybenzene sulfonate, bleach, laundry, mite, *Dermatophagoides farinae*

1 INTRODUCTION

The elimination of environmental allergens is an important way of preventing allergenic symptoms¹⁾. House dust mites (*D. farinae* and *D. pteronyssinus*) live in dust, bedding, and clothes; consequently, mite allergens are produced and accumulate in these environments. The elimination of mite allergens is important for asthma sufferers because mite allergens are released into the air²⁾.

Mite allergens can be removed by vacuuming and washing bedding and clothes^{3,4)}. General washing in tap water is better at eliminating mites than dry cleaning with solvents because mite allergens dissolve in water as a result of their hydrophilic properties⁵⁾. However, killing the mites is also important to prevent the continued production of mite

allergens. House dust mites can be killed at washing temperatures greater than 50°C^{4,6)}. Washing temperatures in Europe have historically been over 50°C, which has been useful for killing mites. However, the conventional washing temperature has recently fallen to below 40°C because of attempts to save energy^{7,8)}; indeed, the general washing temperature worldwide is now under 50°C. Consequently, killing house dust mites is difficult under today's general washing conditions.

House dust mites can also be killed in clothes dryers that produce high temperatures^{9,10)}. However, such dryers are not extensively utilized for bedding in the world because a large machine is required, along with a considerable amount of energy and a long drying time. Acaricidal such as

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Accepted November 30, 2009 (received for review October 17, 2009)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/>

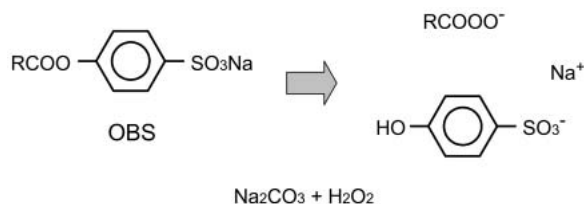


Fig. 1 Generation of Peracid from OBS with Sodium Percarbonate.

benzyl benzoate have been used to prevent mites from accumulating in bedding. However, these repellents are not sufficiently effective. On the other hand, house dust mites can be killed by chlorine bleach in detergent solutions at low temperatures⁶. This application, however is limited to white clothing, such as undergarments, because of the degradation of colors. Thus, general washing conditions at temperatures under 50°C must be improved to kill mites. We investigated practical washing conditions to kill mites at low temperature, and hoped to introduce them to the health care practitioners for improving the quality of the life of patients. Bleach activators are added to powdered detergents or powdered bleaches for fabrics to intensify the bleaching action without degradation of colors¹¹. A general bleach activator, Sodium alkyl acyloxybenzene sulfonate (OBS, C_{n-1}H_{2n-1}COOC₆H₄SO₃Na), generates different lengths of carbonic chains of peracids with sodium percarbonate (Fig. 1)¹². Each of these peracids acts to intensify the bleaching without degradation of colors. OBS also provides a superior bactericidal effect^{13, 14}. The peracids are thought to be responsible for this bactericidal effect, since peracetic acid generated from acetic acid with hydrogen peroxide exerts a bactericidal effect¹⁵.

We hypothesized that the superior bactericidal effect of OBS may mean that it also might have a miticidal effect. However, such an effect has never been reported. In this study, we evaluated the miticidal effects of OBS; here, we report our results and discuss the potential application of OBS as a miticide under general washing conditions.

2 EXPERIMENTAL

2.1 House dust mite

D. farinae was obtained from Dr. Matsumoto (Tokyo Women's Medical University)¹⁶; the mites were fed mite food in an atmosphere of 25°C and 70% relative humidity.

2.2 Reagents

Mite food was made from equal weights of crushed fodder (Oriental Yeast Corporation, Tokyo, Japan) and dried yeast (Asahi Breweries Corporation, Tokyo, Japan). Sodium lauroxybenzene sulfonate (OBS-12) was obtained from the condensation of lauroyl chloride with hydroxy benzene

sulfonic acid in pyridine and was purified using repeated recrystallization, according to a commonly used procedure¹⁷. Other OBS solutions were similarly obtained from the condensation of various carboxylic acid chlorides with different length of carbonic chains and hydroxyl benzene sulfonic acid. Sodium percarbonate was obtained from Mitsubishi Gas Chemical Corporation (Tokyo, Japan). Non-ionic surfactant (alkylpoly [oxy-ethylene] ethers, EO; p=15, C13) and sodium linear alkyl benzene sulfonate were obtained from Lion Corporation (Tokyo, Japan). Zeolite was obtained from Mizusawa Industrial Chemical Corporation (Tokyo, Japan). Other chemicals were obtained from commercial sources. The concentration of the available chlorine from sodium hypochlorite was confirmed using a residual chlorine analyzer (Residual chlorine checker DPD; Shibata Corporation, Tokyo, Japan).

2.3 Evaluation of miticidal effect

Sodium percarbonate and each OBS in methanol were added and diluted to distilled water and were left to stand for 10 min at each temperature to generate peracids; a non-ionic surfactant was then added at a concentration of 100 µg/mL. Approximately 30 adult mites were observed with stereomicroscope (SZ61; Olympus Corporation, Tokyo, Japan) and picked up with use of brush-pen, then soaked in 50 mL of each solution for each time and at each temperature; the mites were then filtrated out using a paper filter (No. 131; Advantec Corporation, Tokyo, Japan). The surviving mites were maintained on the paper filter in an atmosphere of 25°C and 70% relative humidity for approximately 24 h. The mites on each paper filter were then observed using a stereomicroscope and the numbers of living (a) and dead mites (b) were counted. The mortality rate (%) was then calculated using the following equation:

$$\text{Mortality (\%)} = \frac{\text{number of (b)}}{\text{number of total (a + b)}} \times 100$$

2.4 Evaluation of washing conditions

Approximately 30 adult mites were confined in a cage made of polyester cloth (50 mm square, 50 deniers-75 deniers), and three cages were enclosed in a cushion made of polyester (width, 30 cm; depth, 30 cm; height, 15 cm; weight, 150 g; Iwasaki Sanshou Corporation, Kyoto, Japan), as strict condition. Tap water was warmed to 40°C and then added to a top-loading washing machine (CW-C30A1-H; Mitsubishi Electric Corporation, Tokyo, Japan). A detergent solution containing 333 µg/mL of sodium linear alkyl benzene sulfonate, 333 µg/mL of sodium carbonate, and 333 µg/mL of zeolite was added to 30 L of the warmed tap water and the bleaching agents (500 µg/mL of sodium percarbonate and 0-50 µg/mL of OBS-12) were added. The cushion was presoaked for 1 hour, washed for 10 min at 36°C, and rinsed twice with 30 L of tap water at 25°C (Fig.

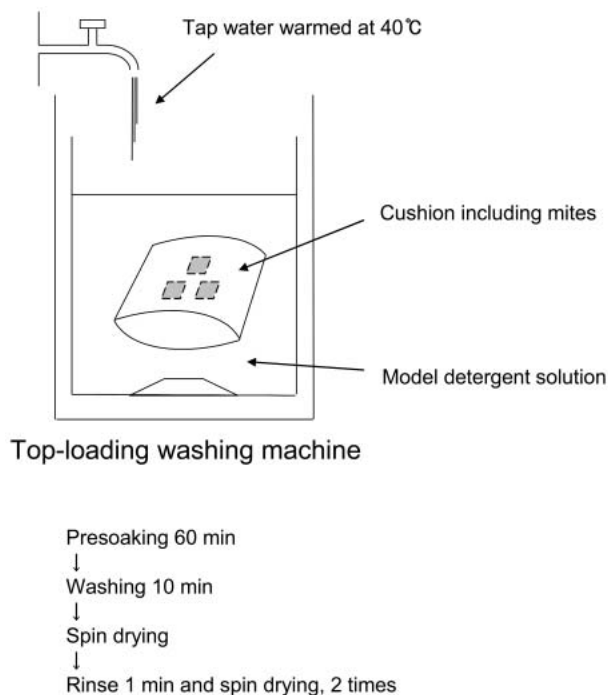


Fig. 2 Outline of Washing Process.

2). Then, the cages were removed from the cushion, and the mites were extracted with 100 µg/mL of nonionic surfactant, then filtrated using a paper filter.

3 RESULTS

3.1 Miticidal effect of OBS

Each OBS was evaluated in a solution containing 1000 µg/mL of sodium percarbonate. The solutions were left to stand for 10 min to ensure the generation of peracids prior to being used to soak the mites; the solutions were maintained at 40°C to avoid killing the mites from heating. The nonionic surfactant was added to the solution to prevent the adsorption of the mites to the surface of the bottle. Each solution containing 20 µg/mL of OBS showed a higher mortality rate than the solution without OBS after 1 h at 40°C. Therefore, OBS-12 produced the highest mortality rate of 98% among the various OBS solutions that were examined. Increasing the length of the carbonic chain from 6 to 12 at the acyl group of each OBS resulted in an increased mortality, but increasing the chain from 12 to 14 resulted in a decreased mortality (Fig. 3).

The mortality rate for mites in sodium hypochlorite, which produces available chlorine, was evaluated using 100 µg/mL and 1000 µg/mL of available chlorine with 100 µg/mL of nonionic surfactant to cover the range of concentrations used in general washing conditions, since the con-

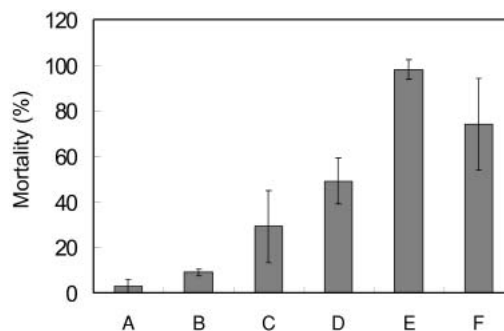


Fig. 3 The Miticidal Effect of Each OBS.

D. farinae was soaked in a solution containing 20 µg/mL of each OBS, 1000 µg/mL of sodium percarbonate, and 100 µg/mL of nonionic surfactant for 1 h at 40°C.

A: without OBS, B: sodium hexoyloxybenzene sulfonate (OBS-6), C: sodium octoyloxybenzene sulfonate (OBS-8), D: sodium decanoyloxybenzene sulfonate (OBS-10), E: OBS-12, F: OBS-14. Mortality (%) was calculated from the number of dead mites / total mites × 100.

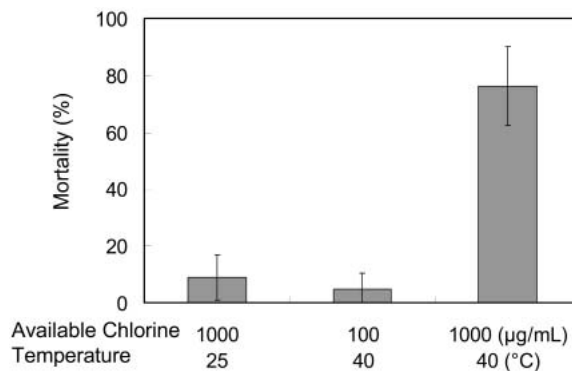


Fig. 4 Miticidal Effect of Available Chlorine.

D. farinae was soaked in a solution containing 100 µg/mL and 1000 µg/mL of available chlorine with 100 µg/mL of nonionic surfactant for 1 h at 25-40°C.

Mortality (%) was calculated from the number of dead mites / total mites × 100.

centration of available chlorine under such conditions was assumed to be a few hundred µg/mL. As a result, the mortality rate was less than 10% for 1000 µg/mL of available chlorine at 25°C and 100 µg/mL of available chlorine at 40°C. In contrast, the mortality rate was 76% for 1000 µg/mL of available chlorine at 40°C (Fig. 4).

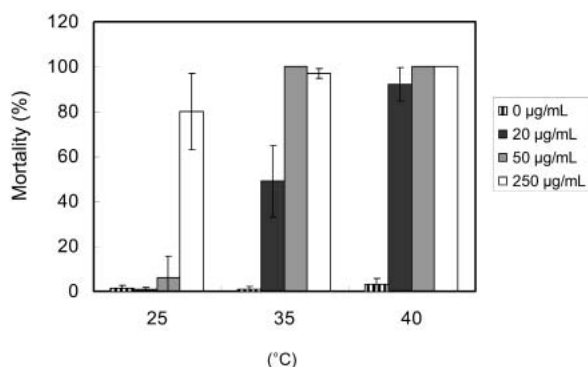


Fig. 5 Miticidal Effect at Each Concentration of OBS-12.

D. farinae was soaked in a solution containing 0-250 µg/mL of OBS-12, 1000 µg/mL of sodium percarbonate, and 100 µg/mL of nonionic surfactant for 1 h at 25-40°C.

Mortality (%) was calculated from the number of dead mites / total mites $\times$ 100.

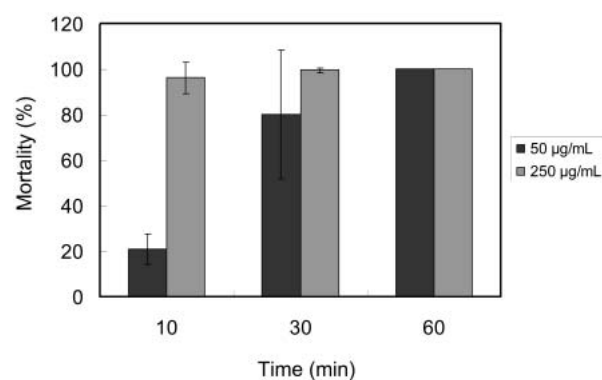


Fig. 6 Miticidal Effect of OBS-12 for Each Soaking Time.

D. farinae was soaked in a solution containing 50-250 µg/mL of OBS-12, 1000 µg/mL of sodium percarbonate, and 100 µg/mL of nonionic surfactant for 10-60 min at 40°C.

Mortality (%) was calculated from the number of dead mites / total mites $\times$ 100.

3.2 Effect of OBS-12 at each concentration and temperature

The miticidal effect of OBS-12 was evaluated at general washing temperatures. At 40°C, the mortality rates were very high for each concentration. At 35°C, the mortality rate was 49% for 20 µg/mL of OBS-12 and the mortality rates were relatively high for 50 and 250 µg/mL of OBS-12. At 25°C, the mortality rates were very low for 20 and 50 µg/mL of OBS-12, but the mortality rate reached 80% for 250 µg/mL of OBS-12 (Fig. 5). Thus, while the miticidal effect of OBS-12 decreased at lower temperatures, it still had a definite effect when applied at a concentration of 250 µg/mL at 25°C.

3.3 Effect of OBS-12 for each soaking time

The miticidal effect was evaluated for each soaking time (10 min, 30 min, and 60 min) with 50-250 µg/mL of OBS-12 at 40°C. The resulting mortality rates were 97% for 250 µg/mL of OBS-12 and 21% for 50 µg/mL after 10 min of soaking, 99% for 250 µg/mL and 80% for 50 µg/mL after 30 min, and 100% for each concentration after 60 min (Fig. 6).

3.4 Effect of OBS-12 on washing conditions

The miticidal effect of OBS-12 was evaluated for general washing conditions based on common washing practices in Japan and US, where bleach activator is often dissolved at a concentration of tens µg/mL of detergent solution. A cushion that was also used as a pillow was selected. The temperature of the detergent solution was decreased during the pre-soaking time from 40°C to 36°C. OBS-12 produced a miticidal effect under general washing conditions,

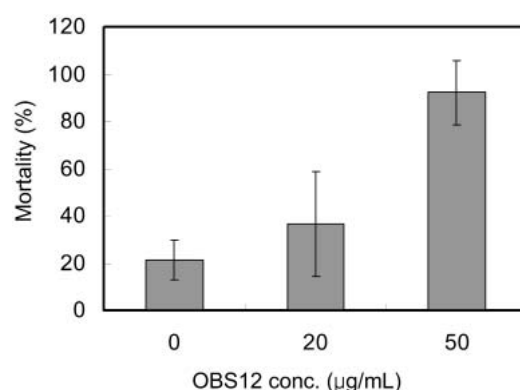


Fig. 7 Miticidal Effect of OBS-12 under Conventional Washing Conditions.

D. farinae was confined within a cushion and presoaked in detergent solution containing 0-50 µg/mL of OBS-12, 333 µg/mL of sodium linear alkyl benzene sulfonate, 333 µg/mL of sodium carbonate, 333 µg/mL of zeolite, and 500 µg/mL of sodium percarbonate for 1 h at from 40°C to 36°C and then washed for 10 min and rinsed twice in a top-loading washing machine. Mortality (%) was calculated from the number of dead mites / total mites $\times$ 100.

with a mortality rate of 37% at a concentration of 20 µg/mL and of 92% at a concentration of 50 µg/mL (Fig. 7).

4 DISCUSSION

In this study, each OBS produced a significant miticidal effect. In particular, OBS-12 showed the strongest effect (Fig. 3). The miticidal effects of each OBS tended to be similar to the bactericidal effects of each OBS. Therefore, the peracids generated from each OBS were regarded to have miticidal as well as bactericidal effects. Increasing the length of the carbon chain of the peracids from 6 to 12 was also thought to augment the affinity to and the adsorption by mites, resulting in more effective oxidation in the bodies of the mites. On the other hand, sodium myristoyloxybenzene sulfonate (OBS-14) produced a lower mortality rate than OBS-12, even though its peracid carbon chain is longer than that of OBS-12. OBS-14 is less effective at generating peracids than OBS-12, since OBS-14 is very slightly soluble in water and consequently has a poor reactivity¹³⁾. The small amount of peracid generated from OBS-14 under the experimental conditions was thought to be one of the reasons for the lower mortality than that of OBS-12, not the effectiveness of the peracids.

In this study, OBS-12 produced a miticidal effect when applied at a concentration of 250 µg/mL at the relatively low temperature of 25°C. Thus, OBS-12 is thought to be useful for killing mites at lower temperatures. Available chlorine exhibited a miticidal effect at 40°C but little or no effect at 25°C. Vyszynski-Moher *et al.* also demonstrated a miticidal effect of 3500 µg/mL of chlorine bleach in detergent solution for 1 h at 35°C but not at 15°C⁶⁾. When chlorine bleach with 10% available chlorine was included, the concentration of the available chlorine in the detergent solution reached 350 µg/mL. Our results also suggest that the miticidal effect of available chlorine decreases with decreasing water temperature when applied at similar concentrations. Thus, chlorine bleach appears to have little or no miticidal effect when used at lower temperatures, such as around 20°C.

In this study, 250 µg/mL of OBS-12 showed a significant miticidal effect even when applied for only 10 min at 40°C (Fig. 6). Thus, the miticidal effect of OBS-12 can be presumed to occur during the agitation period of typical washing cycles. On the other hand, 50 µg/mL of OBS-12 also produced the same effects when applied for 60 min. It is apparent that the effect is influenced according to the soaking time and the temperature. Vyszynski-Moher *et al.* also demonstrated a more effective miticidal effect after soaking in detergent solution with chlorine bleach for 4 h, rather than 1 h⁶⁾. Thus, the miticidal effect of chlorine bleach and OBS is likely produced by bleaching reactions. Lower concentrations of OBS-12 are expected to exert a miticidal effect over prolonged periods of time.

Clothes are typically washed in 700-1300 µg/mL detergent solution in Japan and 1000-2000 µg/mL in US with each top-loading washing machine for approx. 10 minutes, and over 2000 µg/mL with front-loading machine for tens

of minutes in Europe. Then they are rinsed twice with tap water which volume is roughly same to detergent solution. Pre-soaking is often performed for from 1 h to overnight. Tap water is warmed for washing cycle at under 40°C by washing machine in US and Europe. On the other hand, it is rarely warmed by washing machine in Japan, because no heater is equipped in a lot of washing machine. Warm bath-water is often used for washing cycle instead. Therefore, the experimental conditions were based on pre-soaking with warmed water in Japan and US. In this study, OBS-12 produced a miticidal effect when applied under the washing conditions. A sufficient mortality rate of house dust mites can be expected when only 50 µg/mL of OBS-12 is used under general washing conditions. In our experiment, the water temperature was decreased from 40°C to 36°C. If the temperature could be kept at 40°C by heating the water in the washing machine, the OBS-12 would likely be more effective. Furthermore, if the cushion could be agitated more, OBS-12 likely has a greater effect, since this would increase the exposure of the mites confined within the cushion to the peracids being generated. Arlian *et al.* were concerned about the transfer of mites from contaminated clothes to others during the washing process, and they were able to suppress the transfer of mites using chlorine bleach¹⁸⁾. OBS would likely also suppress such transfer, since it exerts an even greater miticidal effect than available chlorine.

5 CONCLUSION

In this study, we evaluated the miticidal effects of OBS under experimental washing conditions at low temperatures. House dust mites can be killed using these washing process and laundry products containing OBS.

ACKNOWLEDGEMENT

We thank Mr. Hideharu Shirai of the Institute of Tokyo Environmental Allergy for support with the experiments. This research was supported by the Promotion and Mutual Aid Corporation for Private Schools of Japan, Grant-in-Aid for Matching Fund Subsidy for Private University.

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