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Effect of different droplet size on the knockdown efficacy of directly sprayed insecticides

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Abstract

BACKGROUND: Although insecticidal aerosols have been widely accepted for household use, the discharged amount should be maintained at minimum levels because they contain volatile organic compounds. Hence, it would be valuable to develop a technique whereby insecticide droplets adhere efficiently to an insect's body. The present study was undertaken in order to clarify how differences in the mode of adhesion to the insect body influence the knockdown effect.

RESULTS: When the discharged volume of droplets with different diameters was the same, the adhesion volume of larger droplets was twice that of smaller droplets, resulting in a higher insect knockdown. In contrast, when the adhesion volume of the two droplet types was the same, a greater number of smaller droplets than larger droplets adhered, and the smaller droplets caused higher insect knockdown. The knockdown effect of both droplet types was lowered when the mesothoracic spiracles of cockroaches were blocked; however, the effect of larger droplets was lowered to a lesser degree.

CONCLUSION: The results suggest that, the probability of adhesion to the more susceptible regions of an insect's body, i.e., areas surrounding the mesothoracic spiracles, was improved when a greater number of smaller droplets were adhered, resulting in higher knockdown.

Keywords: aerosol; droplet diameter; adhesion mode; knockdown; housefly; cockroach

1 INTRODUCTION

Insecticides used in the agricultural sector are sprayed onto plants. The droplet diameters of these insecticides are preferably 100 µm or more in order to prevent drift.[1] In contrast, insecticides used for controlling public health pests, such as houseflies and mosquitoes, are often sprayed into the air in the form of lower-volume sprays with droplet sizes ranging from 10 to 100 µm in diameter.[2] According to WHO,[3] aerosols have a volume median diameter (VMD) of less than 50 µm, whereas mists have a VMD from 50 to 100 µm. Although insecticidal aerosols have been widely accepted for controlling harmful insects in the domestic environment,[4] they contain volatile organic compounds such as solvents and liquid petroleum gas (LPG). Volatile solvents such as kerosene are commonly used in aerosols because they significantly enhance the activity of insecticides in aerosol formulations.[4–6] Consequently, the use of aerosols is controlled by a regulation of the Ministry of Environment of Japan that deals with volatile organic compounds (VOCs), according to which the amount of both the discharged solvent and the discharged gas should be maintained at minimum levels. Hence, oil-based aerosols that contain volatile solvents have tended to replace water-based aerosols; however, this effect is not great, because only small amounts of solvent are commonly used in water-based aerosols.[7] Therefore, in order to enhance insecticidal efficacy, it would be valuable to develop a technique whereby insecticide droplets adhere efficiently to the insect's body, and thereafter enter rapidly into the body.

The insecticidal effect of sprayed droplets is dependent not only on droplet diameter but also on droplet number and concentration. Ebert *et al.*[8,9] reported that the deposit structure played a major role in toxicant efficacy when a soluble concentrate formulation of insecticide was sprayed on plants, by changing the toxicant concentration of the solution, and the number and diameter of the droplets. According to these authors, droplets that were either too small or too large were not the most efficacious, and an optimum combination of droplet diameter and droplet number produced the best effect when the toxicant concentration was fixed. However, the insecticides tested in this study were not 'aerosols' but 'sprays', as the droplet diameters were greater than 100 µm.[5] The optimal droplet diameter of aerosols used to control

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adult flying insects such as mosquitoes and tsetse flies has been reported to be 10–30 µm.[10,11] The insecticidal effect of aerosol droplets depends on their diameter when the formulation of insecticide in solution is the same; droplets having diameters within a specified optimum range are considered more effective. Tsuda[12] reported that droplets with diameters that are greater than this range fall before they can adhere to the insect body, whereas those with smaller diameters do not adhere easily because their inertia force is small. These authors accordingly demonstrated that the optimal droplet diameter for the knockdown of flying insects is 30 µm. The knockdown effect against flying insects is influenced by the dose of pesticide that adheres to the insect body – it is necessary for a greater number of small droplets to adhere to produce the same knockdown effect as a smaller number of large droplets when the concentration of insecticide in the droplets is the same. For example, the impact of one 30 µm droplet is equivalent to the impact of one thousand 3 µm droplets. Spillman[13] reported that the diameter of spray droplets and the flying velocity of an insect affect the 'catch efficiency' of the droplets.

Many studies have been performed to investigate the relationship between insect susceptibility and the point of application of an insecticide on the insect body. The speed of insecticidal action has been believed to depend on the distance between the point of application and the site of action, i.e. the central nervous system (CNS).[14–19] Accordingly, the response is most rapid when insecticides are applied on the ventral prothorax and sites close to the CNS, and slower when they are applied at points situated further away.[14–19] However, when a pyrethroid aerosol is sprayed, the probability that the amount of the insecticide adhering to a site near the CNS will be sufficient to cause knockdown is assumed to be low, and the quantity of insecticide that penetrates the tissue is also small. Gerolt[16] suggested that insecticides that adhere to the insect body migrate laterally and reach the CNS via the integument around the tracheal system and not via the blood after penetration into the tissues. In addition, mesothoracic spiracles are reported to be one of the most effective entry routes for pyrethroid insecticides that are directly sprayed as aerosols.[20] Therefore, it should be possible to enhance insecticidal efficacy by using a technique that ensures good adhesion of insecticide droplets to an insect's body and rapid entry of the adhered insecticides into the body.

However, the relationships between droplet diameter and adhesion amount and between knockdown efficacy and site of insecticide adhesion on insect bodies for aerosols have not been sufficiently studied. The purpose of the present study was to determine how differences in the droplet diameter of an insecticidal aerosol influence the adhesion amount of an insecticide and its knockdown effect. A further aim was to clarify how differences in the mode of adhesion to the insect body influence the knockdown effect, i.e. which is more effective, the adhesion of a large number of smaller droplets or the adhesion of a small number of larger droplets when the total adhered droplet volume is the same.

2 EXPERIMENTAL METHODS

2.1 Insects

Colonies of 1–3-month-old adult female German cockroaches (*Blattella germanica* L.) and 3–5-day-old adult female houseflies (*Musca domestica* L.) were used. These colonies were obtained from the Institute of Medical Science, University of Tokyo, Japan. The German cockroaches were fed pellet food, whereas the houseflies were fed powdered food (in the larval stage) (NMF; Oriental Yeast Co., Ltd, Tokyo, Japan) and granulated sugar (in the adult stage) in the laboratory of the Research and Development Division, Fumakilla Limited, at room temperature (27±2 °C) under a 14 : 10 h light : dark photoperiod.

2.2 Aerosols tested

Two types of aerosol, each having a different droplet diameter, were manufactured in the laboratory. Combination A (small-droplet aerosol) contained d-T80-tetramethrin (0.45 g, Lot No. 81 303, purity 95.2%; Sumitomo Chemical Co., Ltd, Tokyo, Japan) and d-T80-resmethrin (0.06 g, Lot No. 050 301, purity 95.4%; Sumitomo Chemical Co., Ltd) as active ingredients in 300 ml of total quantities; the liquid (*n*-paraffin)/gas (LPG, 4.0 kg cm^{-2} G) ratio was 45 : 255, and the diameter of the spraying button orifice was 0.45 mm. Combination B (large-droplet aerosol) contained the same amount of active ingredients as combination A; the liquid (*n*-paraffin)/gas (LPG, 1.5 kg cm^{-2} G) ratio was 45 : 255, and the diameter of the spraying button orifice was 1.3 mm.

2.3 Measurement of droplet diameter

The average droplet diameter was measured using a droplet size distribution analyser equipped with a laser light-scattering system (LDSA-1400A; Tonichi Computer Applications Co., Ltd, Tokyo, Japan). This apparatus was set in front of a glass cylinder to prevent the beam hitting the cylinder (Fig. 1), and the aerosol was sprayed across the laser beam from a distance of 150 cm for 5 s. The end of the glass cylinder to which the analyser was attached was turned towards a ventilator, with air flow from the end where the aerosol was sprayed to the end to which the analyser was attached. The average droplet diameter (µm) was expressed as the mean of the frequency distribution of all diameters.

2.4 Efficacy test for aerosols having different droplet diameters

The glass cylinder used for the efficacy tests was the same as that used for the measurement of droplet diameter (Fig. 2). The holes of a glass ring (diameter 9 cm height 6 cm) were covered with 1.2 mm mesh nylon nets (thread diameter 0.15 mm), and eight insects were introduced into the enclosed area created by these nets. The ring was placed such that one of the covered surfaces faced the source of the spray. In the experiment using German cockroaches, the test aerosol (0.5–3 g, of which 1.2–7.5 mg was d-T80-tetramethrin) was sprayed at the glass ring from a distance of 110 cm, whereas in the experiment using houseflies the test aerosol (0.25–1 g, of which 0.6–2.5 mg was d-T80-tetramethrin) was sprayed at the glass ring from a distance of 150 cm. The end of the glass cylinder into which test insects were placed was connected to an airflow and ventilated using a flow velocity of 0.7 m s^{-1}. The spray time was adjusted to match the prescribed spray amount. The number of knocked-down insects was counted at 3 min after spraying, and the KT_{50} (time required to achieve 50% knockdown) was calculated using Bliss' probit method.[21] This test was repeated 3 times. The regression lines between the discharge amount of d-T80-tetramethrin (mg) and KT_{50} (s) for different aerosol droplet diameters were analysed using parallel line analysis, and the potency ratio was estimated. The difference in applicator-to-target distance between cockroaches and houseflies was decided upon on the basis of the susceptibility of each insect. As houseflies are more susceptible than cockroaches, if the test on houseflies is conducted under the same conditions as those used for cockroaches, KT_{50} is too short to observe because the amount

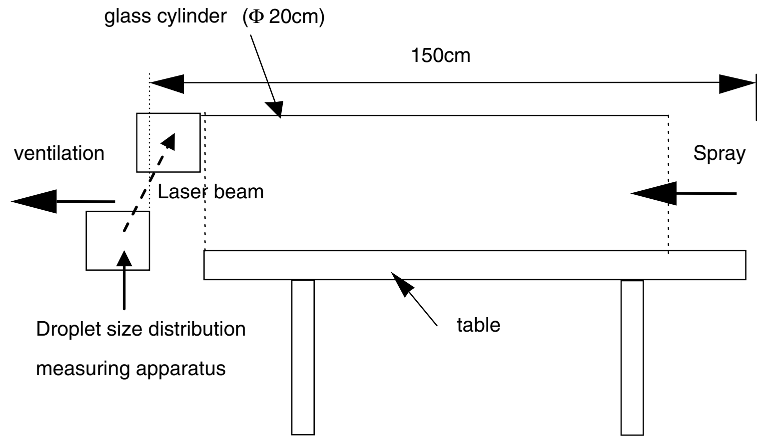

Figure 1. Image of the direct spray apparatus used for measurement of droplet diameter.

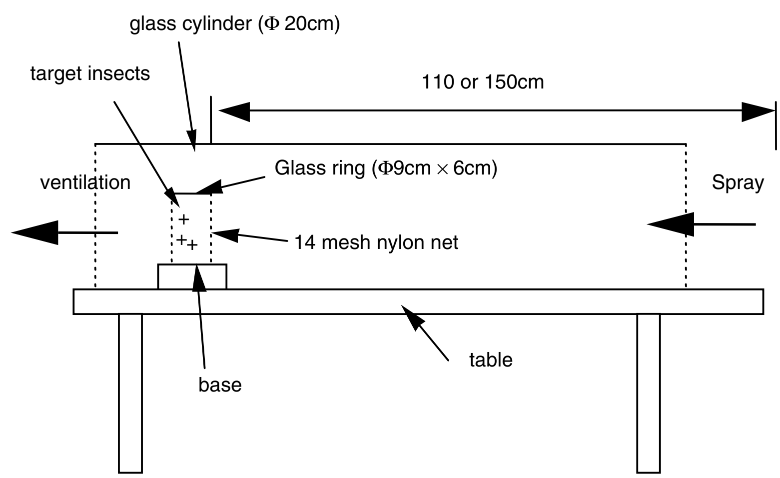

Figure 2. Image of the direct spray apparatus used for performing the aerosol direct spray test.

of adhered insecticide is too large for houseflies. Alternatively, if the test on cockroaches is conducted under the same conditions as those used for houseflies, KT_{50} is not obtained because the amount of adhered insecticide is too small to produce an effect in cockroaches.

2.5 Efficacy against cockroaches after blocking the spiracles

2.5.1 Efficacy of aerosols after blocking the spiracles of cockroaches

Adult female German cockroaches were anaesthetised with diethyl ether and their wings were fixed by clips. A droplet of lacquer was then applied to the mesothoracic spiracles on one side of the cockroaches by using a stainless steel pin under a stereomicroscope. The wing clips were subsequently removed from these cockroaches, and eight of them were introduced into a glass ring covered with 1.5 mm mesh nylon nets, as mentioned above. After the cockroaches had recovered from the anaesthesia, a direct spray test was performed as described previously, using aerosols with different droplet diameters, and the KT_{50} values were calculated. The discharge amount of the aerosol with the smaller droplet diameter (14.4 µm) was adjusted to 2 g, and the discharge amount of the aerosol with the larger droplet diameter (33.4 µm) was adjusted to 1 g, to ensure that the amount of pyrethroid adhering to the cockroaches was the same for both aerosols. This was because the adhesion rate of the larger droplets was twice that of the smaller droplets, as mentioned below. To examine the effect of blocking spiracles, the KT_{50} values observed in this test were compared with those observed for cockroaches whose spiracles were not blocked.

2.5.2 Influence of blocking spiracles on the susceptibility of cockroaches

In order to elucidate the negative effect of spiracle blocking on knockdown efficacy, two series of cockroach groups were prepared: one group comprised cockroaches in which the mesothoracic spiracles were blocked on one side; the other group comprised cockroaches in which the mesothoracic spiracles were blocked on both sides, using the same method as described in Section 2.5.1. In both groups, 52.6 nL of aerosol solution was topically applied to the ventral thorax using a microinjector without anaesthesia. The insects were then released into a polyethylene cup to observe knockdown following the procedure of Sugiura *et al.*[20] The KT_{50} values of these groups were compared with that of the control, and the spiracle blocking effect was examined.

2.6 Measurement of the amount of pyrethroid adhering to the insects and nylon nets

To measure the amount of pyrethroid that adhered to the insects, three German cockroaches or five houseflies were pinned (using

4 cm long stainless steel pins) onto polystyrene foam chips (2 × 4 × 1 cm) and then placed at the bottom of the above-mentioned glass ring within the glass cylinder of the spray apparatus (Fig. 2). The insects were then sprayed with the test aerosol using the above-mentioned method. Subsequently, the insects were immersed in hexane solution (approximately 10 mL), and the solution was ultrasonicated for 30 min to extract the insecticide. The amount of d-T80-tetramethrin extracted was analysed using a gas chromatograph–mass spectrophotometer with di-*n*-amyl phthalate as the internal standard. The following are the details of the equipment and conditions employed for the gas chromatography–mass spectrometry (GC-MS) analysis: model GCMS-QP2010 (Shimadzu Corporation, Kyoto, Japan); CBP1 (Shimadzu Corporation) fused-silica capillary GC column (25 m × 0.32 mm ID, df = 0.5 µm); initial column temperature 150 °C (held for 1 min), rise rate 10 °C min^{-1} (for 15 min), final column temperature 300 °C (held for 2 min); carrier gas helium; constant linear velocity mode 72.1 cm s^{-1}; injection temperature 250 °C; injection method splitless (1 min); injection volume 1 µL; interface temperature 250 °C; ion source temperature 200 °C; electron-impact mode of ionisation; single ion mass (SIM) monitoring ions d-T80-tetramethrin (*m/z* 164) and di-*n*-amyl phthalate (*m/z* 149); SIM sampling interval 0.2 s. This test was repeated 3 times in German cockroaches and 6–9 times in houseflies.

The capture efficiency of the different droplet diameters was estimated by dividing the amount of adhered d-T80-tetramethrin recovered by the amount of discharged d-T80-tetramethrin calculated from the amount of discharged aerosol solution. The calculated capture efficiency was used to estimate the adhered d-T80-tetramethrin in other discharge amounts of the aerosols tested. The regression lines between the estimated amount of d-T80-tetramethrin (mg) that adhered to the insects and the KT_{50} (s) that was obtained by the efficacy test for different aerosol droplet diameters (described in Section 2.4) were analysed using parallel line analysis, and the potency ratio was estimated.

The capture efficiency of the netting was also measured in the experiment using German cockroaches. In order to measure the amount of d-T80-tetramethrin adhered to the nylon nets, the nets covering glass rings were placed at the bottom of the glass cylinder of the direct spray apparatus (Fig. 2) in the absence of cockroaches, and these were sprayed (2 g, of which 5 mg was d-T80-tetramethrin) from a distance of 110 cm, as described previously, using combination A (small-droplet aerosol). Subsequently, the nylon nets were immersed in approximately 50 mL of hexane and ultrasonicated for 30 min to extract the adhered insecticide. The amount of d-T80-tetramethrin was analysed using a gas chromatograph–mass spectrophotometer, as described previously. This test was repeated 5 times.

2.7 Measurement of the amount of the pyrethroid re-adhering to the insects and the influence of residual insecticide on efficacy

In order to measure the amount of pyrethroid re-adhered to the insect body from the sprayed glass cylinder and nylon nets, the insects were placed at the bottom of the glass cylinder of the direct spray apparatus (Fig. 2), which had been sprayed (2 g, of which 5 mg was d-T80-tetramethrin) from a distance of 110 cm, as described previously, using combination A (small-droplet aerosol). Subsequently, eight German cockroaches were brought into contact with the cylinder and nets. At 30 s after contact initiation, the cockroaches were immersed in approximately 10 mL of hexane and ultrasonicated for 30 min to extract the adhered insecticide. The insecticide was then concentrated to 1 mL with acetone by solid-phase extraction (Bond Elut-Fl, 50 mg 1 mL, 100 PK; Varian, Inc.). The amount of d-T80-tetramethrin was analysed using a gas chromatograph–mass spectrophotometer, as described in Section 2.6. This test was repeated 3 times.

To evaluate the influence of residual insecticide on the glass cylinder and nylon nets on the cockroaches, the cylinder and nets were sprayed without the cockroaches, as mentioned above. Subsequently, eight German cockroaches were brought into contact with the cylinder and nets, and the number of knocked-down cockroaches was counted for 5 min after contact and the KT_{50} value was calculated. This test was repeated 10 times.

3 RESULTS

3.1 Relationship between droplet diameter and amount of pyrethroid adhered

The mean droplet diameter of the two types of aerosol and the amount of the aerosols adhering to houseflies (discharge amount 0.5 g, i.e. 1.3 mg of d-T80-tetramethrin) and German cockroaches (discharge amount 2 g, i.e. 5 mg of d-T80-tetramethrin) are shown in Table 1. The adhesion amount of d-T80-tetramethrin droplets with a large diameter (33.4 µm) was significantly greater than that of droplets with a small diameter in both houseflies ($P < 0.05$; *t*-test) and German cockroaches ($P < 0.01$; *t*-test).

The adhesion efficiency was defined as the amount of aerosol adhered onto insects per unit amount of d-T80-tetramethrin discharged. Compared with the adhesion efficiency of the aerosol droplets with a small diameter, that of the droplets with a large

Table 1. Relationship between mean droplet size and adhesion amount of d-T80-tetramethrin

Testing insect	Testing arosol[a]	Mean droplet diameter (D50) (µm)	Discharge amount of d-T80-tetramethrin (µg) (±SD)	Adhesion amount of d-T80-tetramethrin (µg individual)				Adhesion efficiency[c] (ppm)
				Mean	*t*-test[b]	95% CL	99% CL	
Housefly	A	14.4	1263 (±40)	0.044	*	0.035 ∼ 0.053	0.031 ∼ 0.058	35.0
	B	33.4	1238 (±43)	0.089		0.064 ∼ 0.114	0.050 ∼ 0.128	72.1
German cockroach	A	14.4	5032 (±84)	0.559	**	0.402 ∼ 0.715	0.313 ∼ 0.804	111.0
	B	33.4	5047 (±107)	1.342		1.244 ∼ 1.439	1.189 ∼ 1.494	265.8

[a] A: LPG 4.0 kg/cm^{2}G; B: LPG 1.5 kg/cm^{2}G.
[b] *: There is a significant difference between A and B ($p < 0.05$), **: There is a significant difference between A and B ($p < 0.01$).
[c] (Mean adhesion amount/Mean discharge amount) ×10^6.

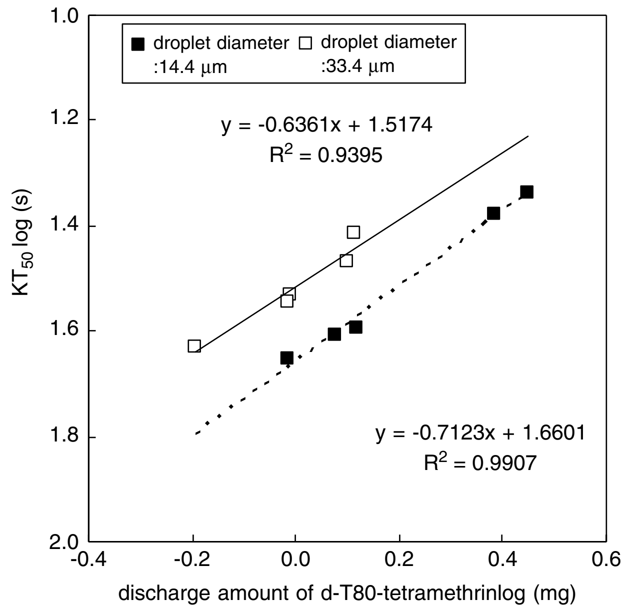

Figure 3. Relationship between discharge amount of d-T80-tetramethrin (mg) and KT_{50} (s) against housefly in each droplet diameter aerosol.

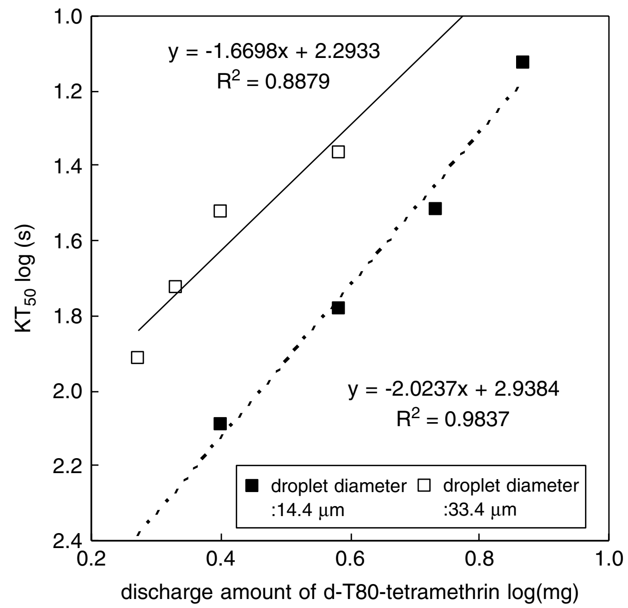

Figure 4. Relationship between discharge amount of d-T80-tetramethrin (mg) and KT_{50} (s) against German cockroach in each droplet diameter aerosol.

diameter was 2.1 times and 2.4 times higher in the houseflies and the German cockroaches respectively.

The amount of aerosol solution that adhered to the nylon nets was 12 781 ± 866 nL (127.81 ± 8.66 µg of d-T80-tetramethrin; mean ± SD). This amount corresponds to 2.5% of the discharged amount of the small-droplet aerosol. The adhesion rate of the large-droplet aerosol to the nylon nets was estimated to be 6%, based on an assumption of the amount of adhesion to the insects.

3.2 Relationship between the discharge amount of pyrethroid with different droplet diameters and their efficacy

Figures 3 and 4 show the relationship between the discharge amounts of the d-T80-tetramethrins with different droplet diameters and their knockdown efficacies (KT_{50}) against houseflies and German cockroaches. When the discharge amount was the same, the knockdown efficacy of the d-T80-tetramethrin droplets with the larger diameter was greater than that of the droplets with the smaller diameter, although the intercepting rate of the net was higher for the droplets with the larger diameter. Tables 2 to 5 show ANOVA for two regression lines between the discharge amounts of d-T80-tetramethrins (mg) with different droplet diameters and their KT_{50} (s) against houseflies and German cockroaches. In both insects, the parallelism of the two regression lines was not rejected ($P > 0.05$) and the regression with a common slope was significant ($P \leq 0.001$). Compared with the knockdown effect of the aerosol droplets with the smaller diameter, that of the droplets with the larger diameter was 1.59 and 1.78 times greater in houseflies and German cockroaches, respectively, when the discharge amount of d-T80-tetramethrin was the same.

3.3 Relationship between adhesion amount of pyrethroid with different droplet diameters and their efficacy

Figures 5 and 6 show the relationship between the adhesion amounts of d-T80-tetramethrins with different diameters and their knockdown efficacies (KT_{50}) against houseflies and German cockroaches. The knockdown efficacy of d-T80-tetramethrin droplets with the larger diameter was lower than that of the droplets with the smaller diameter. Tables 6 to 9 show ANOVA for two regression lines between adhesion amounts of d-T80-tetramethrin (mg) with different droplet diameters and their KT_{50} (s) against houseflies and German cockroaches. In both insects, the parallelism of the two regression lines was not rejected ($P > 0.05$) and the regression with a common slope was significant ($P \leq 0.001$). The knockdown effect of aerosol droplets with the larger diameter was 0.658 and 0.741 that of the droplets with the smaller diameter in houseflies and German cockroaches, respectively, when the adhesion amount of d-T80-tetramethrin to the insects was the same.

3.4 Influence of the re-adhered insecticide on efficacy

The amount of aerosol solution that was re-adhered to the cockroaches was 6.9 ± 2.7 nL (0.069 ± 0.027 µg of d-T80-tetramethrin; mean ± SD) during the 30 s contact with the glass cylinder and nylon nets. This amount corresponds to 10.2% of the amount of aerosol solution that adhered directly to each insect [67.6 ± 14.9 nL (0.676 ± 0.149 µg of d-T80-tetramethrin; mean ± SD)].

The KT_{50} value in the direct spray test when the discharge amount was 2 g (5 mg of d-T80-tetramethrin) was 26.4 s, whereas in the residual contact test, in which the glass cylinder and nylon nets were sprayed under the same conditions, the KT_{50} value obtained was greater than 300 s. Therefore, it is thought that the influence that re-adhered insecticide exerts on the efficacy of direct spray is small.

3.5 Knockdown effect of directly sprayed aerosols on cockroaches with blocked mesothoracic spiracles

The KT_{50} values for the cockroaches in which mesothoracic spiracles were blocked on one side and those for the cockroaches in which the spiracles were not blocked were not significantly different (41.9 and 42.1 s respectively, $P < 0.05$; *t*-test) when aerosol solution was applied topically.[20] Therefore, it was assumed

Table 2. ANOVA for two regression lines between discharge amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against housefly with different droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Common Linearity	1.074E−01	1	1.074E−01	2.726E+02	***	3.148E−06	5.987E+00
Disparallelism	2.577E−04	1	2.577E−04	6.541E−01	NS	4.495E−01	5.987E+00
Residual	2.364E−03	6	3.940E−04				
Total	1.100E−01	8					

[a]*** Commom linearity is significant (P <= 0.001), NS Disparallelism of two regression lines is not significant (P > 0.05).

Table 3. ANOVA for regression lines with common slope between discharge amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against housefly with different droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Regression	1.074E−01	1	1.074E−01	2.868E+02	***	6.14E−07	5.591E+00
Residual	2.622E−03	7	3.746E−04				
Total	1.100E−01	8					

[a]*** Regression with the common slope is significant (P <= 0.001).
Regression lines with common slope are as follows
$y = -0.6917x + 1.6558$ (mean particle diameter : 14.4µm)
$y = -0.6917x + 1.5174$ (mean particle diameter : 33.4µm)
Potency Ratio (Pr) 1.59.

Table 4. ANOVA for two regression lines between discharge amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against German cockroach with different droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Common Linearity	6.417E−01	1	6.417E−01	9.372E+01	***	6.371E−04	7.709E+00
Disparallelism	4.702E−03	1	4.702E−03	6.868E−01	NS	4.538E−01	7.709E+00
Residual	2.739E−02	4	6.847E−03				
Total	6.738E−01	6					

[a]*** Commom linearity is significant (P <= 0.001), NS Disparallelism of two regression lines is not significant (P > 0.05).

Table 5. ANOVA for regression lines with common slope between discharge amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against German cockroach in each droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Regression	6.417E−01	1	6.417E−01	9.998E+01	***	1.710E−04	6.608E+00
Residual	3.209E−02	5	6.418E−03				
Total	6.738E−01	6					

[a]*** Regression with the common slope is significant (P <= 0.001).
Regression lines with common slope are as follows
$y = -1.9136x + 2.8671$ (mean particle diameter : 14.4µm)
$y = -1.9136x + 2.3900$ (mean particle diameter : 33.4µm)
Potency Ratio (Pr) 1.78.

Table 6. ANOVA for two regression lines between adhesion amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against housefly with different droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Common Linearity	1.074E−01	1	1.074E−01	2.726E+02	***	3.148E−06	5.987E+00
Disparallelism	2.577E−04	1	2.577E−04	6.541E−01	NS	4.495E−01	5.987E+00
Residual	2.364E−03	6	3.940E−04				
Total	1.100E−01	8					

[a]*** Commom linearity is significant (P <= 0.001), NS Disparallelism of two regression lines is not significant (P > 0.05).

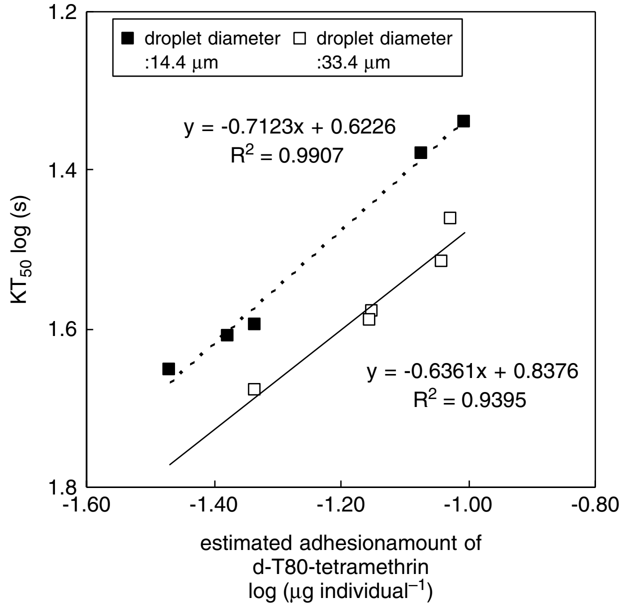

Figure 5. Relationship between adhesion amount of d-T80-tetramethrin (μg individual^{-1}) and KT_{50} (s) against housefly in each droplet diameter aerosol.

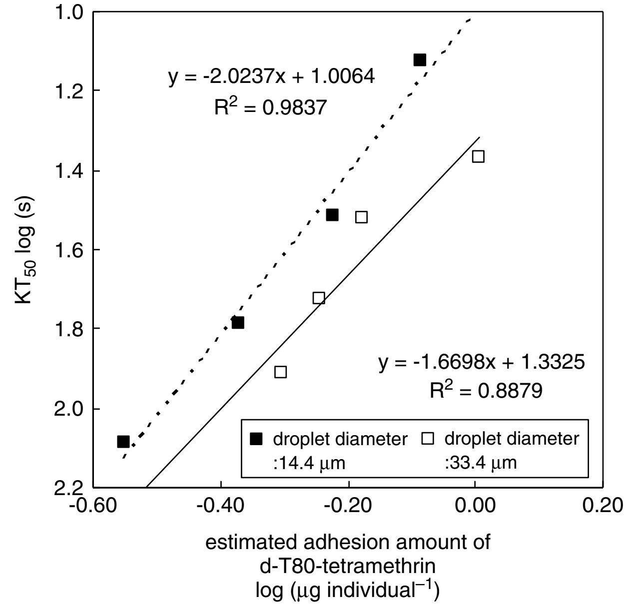

Figure 6. Relationship between adhesion amount of d-T80-tetramethrin (μg individual^{-1}) and KT_{50} (s) against German cockroach in each droplet diameter aerosol.

that conducting aerosol efficacy tests with the mesothoracic spiracles blocked on one side was appropriate, as this did not affect the insecticide susceptibility of cockroaches. In contrast, the KT_{50} values of cockroaches in which the mesothoracic spiracles were blocked on both sides and those of cockroaches in which the spiracles were not blocked were significantly different (29.9 and 42.1 s respectively, $P < 0.05$; *t*-test) when aerosol solution was applied topically.[2] Therefore, it is assumed that conducting aerosol efficacy tests with the mesothoracic spiracles blocked on both sides is not appropriate, as this affects the insecticide susceptibility of cockroaches.

Table 10 shows the relationship between spiracle blocking and the knockdown efficacy of directly sprayed aerosols with different droplet diameters. The KT_{50} values for the cockroaches in which the mesothoracic spiracles were blocked on one side and those for the cockroaches with unblocked spiracles were 46.3 and 26.4 s, respectively, when the aerosols with the smaller droplet diameter were sprayed, and 43.7 and 32.0 s when the aerosols with the larger droplet diameter were sprayed. Thus, for the smaller droplets, the KT_{50} values were 1.8-fold higher for cockroaches with blocked spiracles than for cockroaches with unblocked spiracles. In the case of larger droplets, these values were 1.4-fold higher for the cockroaches with blocked spiracles.

4 DISCUSSION

When the sum of discharged volume of the droplets with the two different diameters was the same, both the adhesion volume and the knockdown efficacy against insects were higher in the case of the larger droplets than in the case of the smaller droplets. The smaller droplets did not adhere sufficiently to the insect body when sprayed directly. It is assumed that they were easily borne away by air currents flowing over the surface of the insect body. In contrast, the larger droplets were borne away from the insect body less easily because their inertia force was greater. The adhesion efficiency of the smaller droplets was lower; accordingly, to achieve the same knockdown efficacy as that of the larger droplets, the sum of discharged volume of the smaller droplets needed to be doubled. Tsuda *et al.*[2] monstrated that the optimal droplet diameter for the knockdown of flying insects is 30 μm. Consistent with this estimate, the present test results indicate that the larger droplets with a diameter of 33.4 μm are more effective than the smaller droplets with a diameter of 14.4 μm.

Table 7. ANOVA for regression lines with common slope between adhesion amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against housefly with different droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Regression	1.074E−01	1	1.074E−01	2.868E+02	***	6.136E−07	5.591E+00
Residual	2.622E−03	7	3.746E−04				
Total	1.100E−01	8					

[a] *** Regression with the common slope is significant (P <= 0.001).
Regression lines with common slope are as follows
$y = -0.6917x + 0.6485$ (mean particle diameter : 14.4μm)
$y = -0.6917x + 0.7442$ (mean particle diameter : 33.4μm)
Potency Ratio (Pr) 0.658.

Table 8. ANOVA for two regression lines between adhesion amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against German cockroach with different droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Common Linearity	6.417E−01	1	6.417E−01	9.372E+01	***	6.371E−04	7.709E+00
Disparallelism	4.702E−03	1	4.702E−03	6.868E−01	NS	4.539E−01	7.709E+00
Residual	2.739E−02	4	6.847E−03				
Total	6.738E−01	6					

[a]*** Commom linearity is significant (P <= 0.001), NS Disparallelism of two regression lines is not significant (P > 0.05).

Table 9. ANOVA for regression lines with common slope between adhesion amount of d-T80-tetramethrin (mg) and KT_{50}(sec) against German cockroach with different droplet diameter aerosol

Factor	SS	DF	Ms	F	significance[a]	P-Value	F(0.05)
Regression	6.417E−01	1	6.417E−01	9.998E+01	***	1.710E−04	6.608E+00
Residual	3.209E−02	5	6.418E−03				
Total	6.738E−01	6					

[a]*** Regression with the common slope is significant (P <= 0.001).
Regression lines with common slope are as follows
$y = -1.9136x + 1.0403$ (mean particle diameter : 14.4µm)
$y = -1.9136x + 1.2889$ (mean particle diameter : 33.4µm)
Potency Ratio (Pr) 0.741.

The diameter of the larger droplets is 2.3 times that of smaller the droplets; therefore, the volume of one larger droplet is 12 times greater than that of one smaller droplet, i.e. the number of smaller droplets is 12 times greater than the number of larger droplets when the sum of discharged volume is the same. Nevertheless, the test results indicate that the actual number of adhered smaller droplets is approximately 6 times greater than the number of larger droplets, i.e. the actual adhered volume of the smaller droplets is approximately half that of the larger droplets.

In contrast, when the amount of insecticide adhered to the insects was the same, the knockdown efficacy of the smaller droplets was higher than that of the larger droplets. In this case, a greater number of aerosol droplets with the smaller diameter than with the larger diameter adhere. Therefore, it is considered that there is a greater probability of droplets with the smaller diameter adhering to a more susceptible site. Mesothoracic spiracles are reported to be one of the most effective entry routes for pyrethroid insecticides that are directly sprayed as aerosols.[20] In cockroaches in which the mesothoracic spiracles on one side were blocked, the knockdown efficacy of droplets with both large and small diameters decreased compared with that in cockroaches in which the mesothoracic spiracles were unblocked. This is because the amount of insecticide flowing into the mesothoracic spiracles is assumed to be lower in insects with blocked spiracles than in insects with unblocked spiracles. However, the degree to which the efficacy is decreased by spiracle blocking was greater for the smaller droplets than for the larger droplets. These data suggest that, when the spiracles were not blocked, the droplets with the smaller diameter adhered around the mesothoracic spiracles and entered into the insect body via these structures to a greater extent than the droplets with the larger diameter. Therefore, the amount of insecticide that entered into the insect body via the spiracles decreased to a greater extent for the smaller droplets when the spiracles were blocked. Thus, the knockdown efficacy of directly sprayed insecticides is closely related to the adhesion site on the insect body. The lower knockdown efficacy of larger droplets against cockroaches when the adhesion volumes of large and small droplets containing pyrethroid were the same suggests that

Table 10. Knockdown effect of directly sprayed aerosols on cockroaches with blocked mesothoracic spiracles

Aerosol[a]	Mean droplet diameter (D_{50}) (µm)	Mesothoracic spiracles	Dischargeamount of d-T80-tetramethrin (µg) (± SD)	Estimated adhesion amount of d-T80-tetramethrin[b] (µg individual^{-1})	KT_{50} (s) Mean	KT_{50} (s) 95% CL	KT_{50}ratio[c]
A	14.4	Blocked	4936 (±65)	0.548	46.3	41.0–52.3	1.8
B	14.4	Unblocked	4959 (±15)	0.551	26.4	23.5–29.8	
A	33.4	Blocked	2448 (±107)	0.651	43.7	38.1–50.0	1.4
B	33.4	Unblocked	2464 (±84)	0.655	32.0	27.7–36.9	

[a] A: LPG 4.0 kg cm^{-2} G; B: LPG 1.5 kg cm^{-2} G.
[b] Discharge amount of d-T80-tetramethrin (µg) × adhesion efficiency (Table 1) $\times 10^{-6}$.
[c] Blocked/unblocked.

the probability of adhesion around the mesothoracic spiracles and subsequent penetration of larger droplets via the mesothoracic spiracles is lower.

It is believed that the entry of insecticides via the spiracles is important for insecticide efficacy;[16] however, further studies are necessary to clarify the route by which insecticides reach the target organ from the spiracle, i.e. whether it is through the inner wall of the tracheal system or directly into the CNS. Moreover, it would be valuable to develop a technique whereby many smaller insecticide droplets adhere efficiently to more susceptible regions of an insect's body, i.e. areas surrounding the mesothoracic spiracles,[20] to maintain the discharged amount of volatile organic compounds contained in insecticidal aerosols at minimum levels.

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