

Acaricidal properties of piperazine and its derivatives against house-dust and stored-food mites

Chi-Hoon Lee, Hyung-Wook Kim and Hoi-Seon Lee*

Abstract

BACKGROUND: Piperazine derivatives possess pharmacological properties, yet the acaricidal activity of these compounds has not been investigated. This study was conducted to evaluate the colour alteration and acaricidal activity of piperazine derivatives against *Dermatophagoides* spp. and *Tyrophagus putrescentiae* (Schrank) using filter paper and fumigant methods.

RESULTS: In a fumigant bioassay, 1-phenylpiperazine ($7.83 \mu\text{g cm}^{-2}$) against *D. farinae* (Hughes) was found to be 4.7 times more toxic than DEET ($36.84 \mu\text{g cm}^{-2}$), followed by benzyl benzoate ($9.72 \mu\text{g cm}^{-2}$), piperazine ($11.41 \mu\text{g cm}^{-2}$), 1-ethoxycarbonylpiperazine ($20.14 \mu\text{g cm}^{-2}$) and 1-(2-methoxyphenyl)piperazine ($22.14 \mu\text{g cm}^{-2}$). In a filter paper bioassay, 1-(2-methoxyphenyl)piperazine ($3.65 \mu\text{g cm}^{-2}$) was 5.7 times more toxic than DEET ($20.64 \mu\text{g cm}^{-2}$), followed by 1-ethoxycarbonylpiperazine ($4.02 \mu\text{g cm}^{-2}$), 1-phenylpiperazine ($4.75 \mu\text{g cm}^{-2}$), benzyl benzoate ($7.83 \mu\text{g cm}^{-2}$) and piperazine ($10.59 \mu\text{g cm}^{-2}$). Similar results have been exhibited with piperazine derivatives against *D. pteronyssinus* (Troussart). However, no activity against *T. putrescentiae* was observed for piperazine derivatives, except for piperazine.

CONCLUSIONS: These results indicate that piperazine derivatives may be suitable as vapour-phase acaricide fumigants owing to their high volatility, acaricidal activity and safety. 1-Phenylpiperazine was found to be an excellent mite indicator based on the colour change it induced. Taken together, these findings indicate that piperazine derivatives may be used to replace existing problematical acaricides owing to their activity and ability to act as a mite indicator.

© 2009 Society of Chemical Industry

Keywords: acaricide; colour alteration; *Dermatophagoides farinae*; *D. pteronyssinus*; filter paper bioassay; fumigant bioassay; piperazine; *Tyrophagus putrescentiae*

1 INTRODUCTION

Piperazines comprise a broad class of chemical compounds, many of which possess important pharmacological properties while retaining a core piperazine functional group. According to a report conducted by the European Commission (EC) and the World Health Organisation (WHO), piperazine has a low acute toxicity in mammals; therefore, worldwide, these compounds have been widely used as a drug for many years.^{1,2} Because of this, many piperazine derivatives have been investigated to determine if they possess various pharmacological properties, such as antimicrobial, antifungal, antipsychotic, antimalarial, antiprotozoal, antioxidative and anticancer activities.^{3–7} Currently, some piperazine derivatives are widely used in medical practice as antidepressants, anxiolytics, antibiotics, antiallergics and antihistamines.^{1,8} In addition, the Merck HIV protease inhibitor, Crixivan, is a piperazine derivative, as are many other drugs currently under development.⁴ However, to the best of the authors' knowledge, no compounds of this class have been reported in the literature to have acaricidal activity, or studied in the field of medical entomology.

Diseases caused by parasites, viruses and bacteria are still a major threat to public health, in spite of the recent progress in medical science. In particular, allergy to house-dust mite allergen is one of the most important allergies worldwide, affecting more than 50% of all allergic patients and up to

80% of asthma sufferers.^{9,10} House-dust mites belonging to the Pyroglyphidae family [*Dermatophagoides farinae* (Hughes) and *D. pteronyssinus* (Troussart)] have been recognised as a major cause of allergic diseases, such as asthma, allergic rhinitis, conjunctivitis and atopic dermatitis.^{11,12} In addition, it has been reported that non-pyroglyphid mites belonging to the Acaridae family [*Tyrophagus putrescentiae* (Schrank)] cause occupational allergic disease among bakers, farmers, dairy workers and grain workers.^{13–17} Furthermore, the above storage mites can induce allergic diseases such as acute enteritis, diarrhoea and systemic anaphylaxis when contaminated foods are ingested.^{16,18} *Tyrophagus putrescentiae*, which is a storage mite that is found in dust in both storehouses and residential homes,^{13,17,19} is the third most common house mite following *Dermatophagoides* spp. in Korea.^{17,19,20} In Korean homes, the most common mite is *D. farinae* (64.4% of total dust mites), followed by *D. pteronyssinus* (20.3%)

* Correspondence to: Hoi-Seon Lee, College of Agriculture and Life Sciences, Chonbuk National University, Chonju 561-756, South Korea.
E-mail: hoiseon@chonbuk.ac.kr

Department of Agricultural Chemistry and Institute of Agricultural Science and Technology, College of Agriculture and Life Sciences, Chonbuk National University, Chonju 561-756, South Korea

and *T. putrescentiae* (6.4%).^{17,19,20} However, *T. putrescentiae* is the most influential mite in damp housing conditions when there is a high relative humidity (>73% RH).^{18–20} Accordingly, over the last few decades, various attempts to control these mite populations have been made. Attempts to control dust mite populations have primarily involved the use of chemical methods such as filter paper and fumigant methods in comparison with benzyl benzoate and DEET.²¹ However, the indiscreet use of these chemicals inevitably leads to resistance and can potentially harm the natural environmental non-target species and human health.^{21–23} Additionally, allergic reactions to dust mites are also induced by their faecal material and their eggs.²⁴ Therefore, there is an urgent need to develop safer, environmentally friendly and efficient alternatives for controlling dust mites in an indoor environment.

The authors have previously reported that plumbagin derived from the root of *Diospyros kaki* L. possesses a potent mite indicator against *Dermatophagoides* spp.²⁵ Therefore, this study was conducted to evaluate the colour alteration and acaricidal activity of piperazine derivatives against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae* in order to identify a suitable mite indicator. The acaricidal activity of the above compounds was examined using filter paper and fumigant methods. In addition, this work included a laboratory study to examine structure–activity

relationships among nine piperazine derivatives, as well as their toxicity against three dust mite species. The biological properties of these compounds were then compared with those of benzyl benzoate and DEET.

2 MATERIALS AND METHODS

2.1 Chemicals

Benzyl benzoate and DEET were purchased from Aldrich (Milwaukee, WI). 1-(4-Bromobenzyl)piperazine, 1-(3-chlorophenyl)piperazine, *trans*-1-cinnamylpiperazine, 1-ethoxycarbonylpiperazine, 1-(4-hydroxyphenyl)piperazine, 1-(2-methoxyphenyl)piperazine, 2-methylpiperazine, 1-phenylpiperazine and piperazine were supplied by Sigma (St Louis, MO). The structures of these piperazine derivatives are shown in Fig. 1. All other chemicals were of analytical grade and available commercially.

2.2 Target mites

Cultures of three mite species were maintained in the laboratory for 7 years without exposure to any known acaricides. These mites were maintained on fry feed no. 1 + dried yeast (1 + 1 by weight) and reared in plastic containers (15 × 12 × 6 cm) containing 30 g of a sterilised diet. The rearing cages were kept in an incubator at

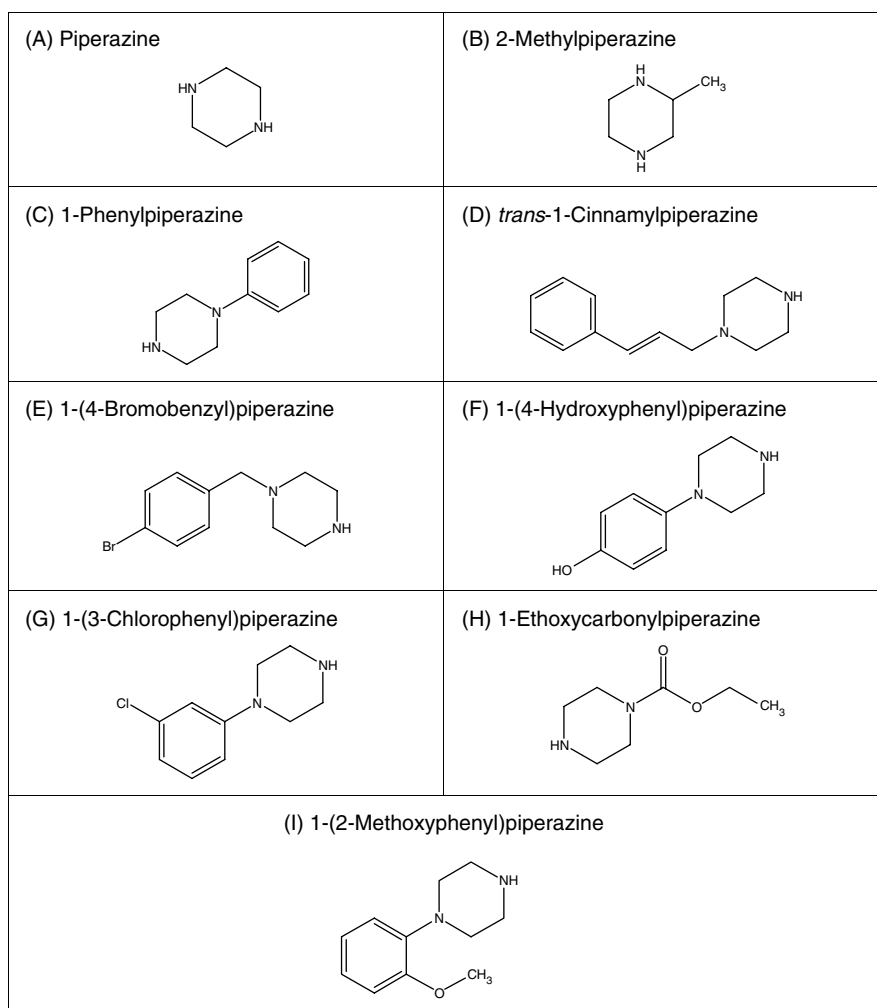


Figure 1. Structures of piperazine derivatives.

$25 \pm 1^\circ\text{C}$ and 75% relative humidity in darkness, within a plastic tray ($18 \times 18 \times 17$ cm) that was maintained with a saturated solution of potassium chloride to prevent the mites from escaping, while providing a high relative humidity. The fry feed was purchased from Korea Special Feed Meal Co. Ltd, Chonju, Korea, and consisted of protein (49%), lipid (4.0%), cellulose (3%), Ca (1.9%) and P (2.0%).

2.3 Bioassay

A fumigant bioassay was employed to evaluate the effects of the compounds against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae*, using colourless microtubes (1.5 mL capacity) (Sarstedt Co., Germany). The most important factor in the preliminary screening for acaricidal activity against test mites may be the starting dose. This is because the amount required to give a dosage of $80 \mu\text{g cm}^{-2}$ is soluble in acetone and allows detection of minor active compounds. Therefore, appropriate amounts (to give 80, 40, 20, 10 and $5 \mu\text{g cm}^{-2}$) of each material were dissolved in 20 μL of acetone and then applied to a paper disc (8 mm diameter) (Advantec Co., Japan), while the control disc received 20 μL of acetone alone. The acetone was then removed in a freeze dryer for 1 h, after which the paper disc was placed in the top of the microtube. Twenty mites (7–10 days old) were separately placed in each microtube and covered with a lid.

A filter paper bioassay was used to evaluate the acaricidal activities of piperazine derivatives on *D. farinae*, *D. pteronyssinus* and *T. putrescentiae*. This was conducted using a slightly modified version of the method described by Jang *et al.*²⁶ Briefly, various amounts (to give 80, 40, 20, 10 and $5 \mu\text{g cm}^{-2}$) of each material

were dissolved in 40 μL of acetone and then applied to white cotton fabric discs (5 cm diameter, 55 μm thick) (Whatman Co., UK), with the control fabric discs being treated with 40 μL of acetone alone. The discs were dried in a fume hood for 2 min, after which each piece was placed in the bottom of a petri dish (5 cm diameter $\times$ 0.8 cm deep). It was previously determined that 2 min of drying time was sufficient to prevent solvent toxicity from occurring (data not shown). Twenty mite adults (7–10 days old) were separately placed in each petri dish, after which the lid was sealed. The treated and control mites were held at $25 \pm 1^\circ\text{C}$ and 75% relative humidity in the dark for 24 h. The mortality of each group was then determined under a binocular light microscope (20 $\times$). Mites were considered to be dead if their appendages did not move when prodded with a pin. All treatments were replicated 3 times, and the LD_{50} values were calculated by probit analysis.²⁷

2.4 Colour alteration and observation of *Dermatophagoides farinae*

Mites were observed using a video microscope [ICS-305B video microscope system (20 $\times$) Sometch[®], Seoul, Korea] for the following parameters: walking/active, slow movement, no movement/dead and colour alteration. The colour alterations of *D. farinae* with piperazine derivatives were determined using a fumigant bioassay as described by Lee and Lee.²⁸ The colour alteration of *D. farinae* before (A) and after (B) treatment was observed using a light microscope (100 $\times$) (Fig. 2) and then compared with the colour alteration that occurred in response to treatment with a commercial acaricide such as benzyl benzoate (C).

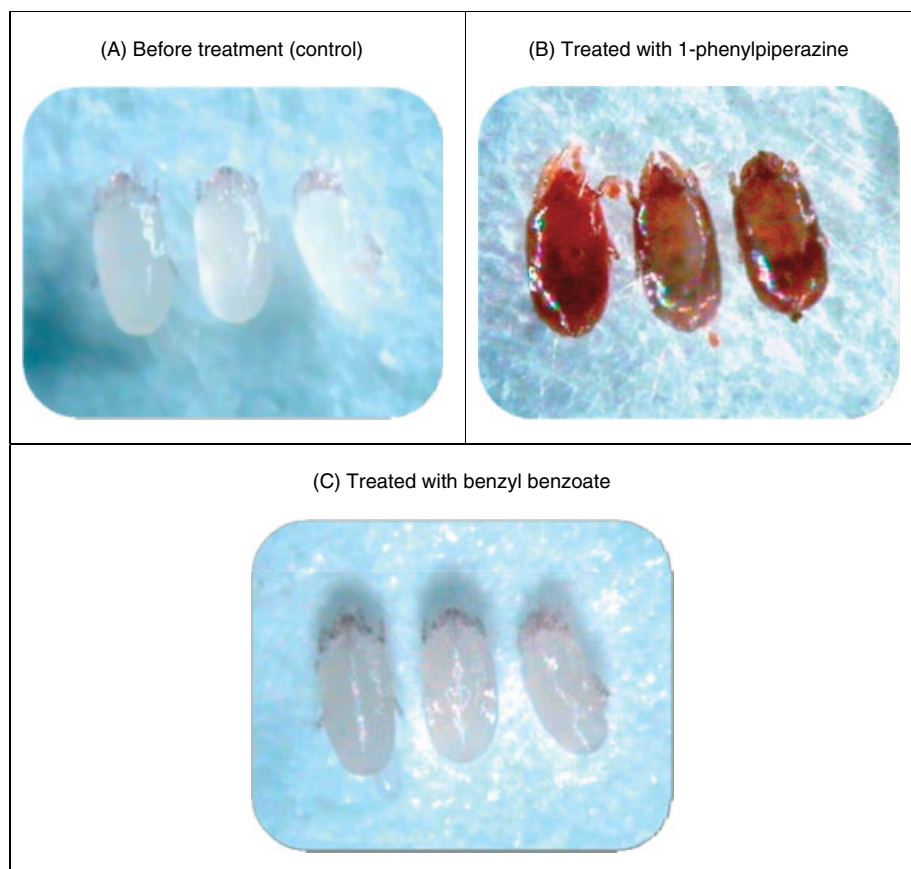


Figure 2. Colour alteration of dust mites by 1-phenylpiperazine.

Table 1. Acaricidal activity of piperazine derivatives against *Dermatophagoides farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae*, using a fumigant bioassay^a

Compound	Mite species	LD ₅₀ (µg cm ⁻²)	95% confidence limits	RT ^b
Piperazine	<i>D. farinae</i>	11.41	11.19–11.65	3.2
	<i>D. pteronyssinus</i>	9.73	9.43–9.92	2.1
	<i>T. putrescentiae</i>	20.82	20.67–21.14	1.5
2-Methylpiperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-Phenylpiperazine	<i>D. farinae</i>	7.83	7.64–7.93	4.7
	<i>D. pteronyssinus</i>	4.86	4.69–4.95	4.1
	<i>T. putrescentiae</i>	–	–	–
<i>trans</i> -1-Cinnamylpiperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-(4-Bromobenzyl)piperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-(4-Hydroxyphenyl)piperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-(3-Chlorophenyl)piperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-Ethoxycarbonylpiperazine	<i>D. farinae</i>	20.14	20.06–20.34	1.8
	<i>D. pteronyssinus</i>	17.47	17.32–17.61	1.1
	<i>T. putrescentiae</i>	–	–	–
1-(2-Methoxyphenyl)piperazine	<i>D. farinae</i>	22.14	22.03–22.44	1.7
	<i>D. pteronyssinus</i>	18.47	18.35–18.61	1.1
	<i>T. putrescentiae</i>	–	–	–
Benzyl benzoate	<i>D. farinae</i>	9.72	9.59–9.85	3.8
	<i>D. pteronyssinus</i>	7.54	7.43–7.62	2.6
	<i>T. putrescentiae</i>	16.38	16.27–16.54	2.3
DEET	<i>D. farinae</i>	36.84	36.68–36.91	1.0
	<i>D. pteronyssinus</i>	19.79	31.62–31.82	1.0
	<i>T. putrescentiae</i>	30.33	30.27–30.41	1.0

^a Exposed for 24 h.^b Relative toxicity = LD₅₀ value of DEET/LD₅₀ value of test chemical.

2.5 Statistical analysis

The percentage mortality was determined and transformed to arcsine square root values, and then evaluated by analysis of variance (ANOVA). The treatment means were compared and separated by Scheffe's test, with $P < 0.05$ being considered statistically significant.²⁷ The acaricidal activity was considered to be significantly different when the 95% confidence limits (CLs) of the LD₅₀ values failed to overlap. The relative toxicity (RT) was determined on the basis of the ratio of DEET LD₅₀/test compound LD₅₀, as previously described.^{25,26,28}

3 RESULTS AND DISCUSSION

The acaricidal activities of piperazine derivatives against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae* were evaluated by comparing the LD₅₀ values estimated by the filter paper and fumigant methods (Tables 1 and 2). Significant differences were

observed in the acaricidal activity against the different mites. The commonly used benzyl benzoate and DEET served as standards of comparison for acaricidal tests, and control mortality was not observed (data not shown). According to the LD₅₀ values estimated by the fumigant method, 1-phenylpiperazine (7.83 µg cm⁻²) was the most toxic compound against *D. farinae* and was 4.7 times more toxic than DEET (36.84 µg cm⁻²), followed by benzyl benzoate (9.72 µg cm⁻²), piperazine (11.41 µg cm⁻²), 1-ethoxycarbonylpiperazine (20.14 µg cm⁻²) and 1-(2-methoxyphenyl)piperazine (22.14 µg cm⁻²) (Table 1). Against *D. pteronyssinus*, 1-phenylpiperazine (4.86 µg cm⁻²) was 4.1 times more toxic than DEET (19.79 µg cm⁻²), followed by benzyl benzoate (7.54 µg cm⁻²), piperazine (9.73 µg cm⁻²), 1-ethoxycarbonylpiperazine (17.47 µg cm⁻²) and 1-(2-methoxyphenyl)piperazine (18.47 µg cm⁻²). Against *T. putrescentiae*, moderate toxicity was observed when benzyl benzoate (16.38 µg cm⁻²), piperazine (20.82 µg cm⁻²) and

Table 2. Acaricidal activity of piperazine derivatives against *Dermatophagoides farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae*, as determined by a filter paper bioassay^a

Compound	Mite species	LD ₅₀ (μg cm ⁻²)	95% confidence limits	RT ^b
Piperazine	<i>D. farinae</i>	10.59	10.34–10.73	1.9
	<i>D. pteronyssinus</i>	8.57	8.42–8.69	1.5
	<i>T. putrescentiae</i>	19.41	19.24–19.66	1.0
2-Methylpiperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-Phenylpiperazine	<i>D. farinae</i>	4.75	4.63–4.94	4.3
	<i>D. pteronyssinus</i>	2.57	2.48–2.75	4.9
	<i>T. putrescentiae</i>	–	–	–
<i>trans</i> -1-Cinnamylpiperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-(4-Bromobenzyl)piperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-(4-Hydroxyphenyl)piperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-(3-Chlorophenyl)piperazine	<i>D. farinae</i>	–	–	–
	<i>D. pteronyssinus</i>	–	–	–
	<i>T. putrescentiae</i>	–	–	–
1-Ethoxycarbonylpiperazine	<i>D. farinae</i>	4.02	3.93–4.24	5.1
	<i>D. pteronyssinus</i>	3.65	3.48–3.79	3.4
	<i>T. putrescentiae</i>	–	–	–
1-(2-Methoxyphenyl)piperazine	<i>D. farinae</i>	3.65	3.44–3.82	5.7
	<i>D. pteronyssinus</i>	3.32	3.23–3.45	3.8
	<i>T. putrescentiae</i>	–	–	–
Benzyl benzoate	<i>D. farinae</i>	7.83	7.63–7.93	2.6
	<i>D. pteronyssinus</i>	5.96	5.86–6.04	2.1
	<i>T. putrescentiae</i>	11.06	10.89–11.24	1.7
DEET	<i>D. farinae</i>	20.64	20.54–20.87	1.0
	<i>D. pteronyssinus</i>	12.48	12.27–12.55	1.0
	<i>T. putrescentiae</i>	18.86	18.72–18.94	1.0

^a Exposed for 24 h.

^b Relative toxicity = LD₅₀ value of DEET/LD₅₀ value of test chemical.

DEET (30.33 μg cm⁻²) were used. Based on the relative toxicity, the acaricidal activity of piperazine was only slightly lower than that of benzyl benzoate against the three species of mites, but was 1.5–3.2 times more toxic than DEET. According to the LD₅₀ values estimated by the filter paper method (Table 2), 1-(2-methoxyphenyl)piperazine (3.65 μg cm⁻²) was the most toxic compound against *D. farinae* and was approximately 5.7 times more toxic than DEET (20.64 μg cm⁻²), followed by 1-ethoxycarbonylpiperazine (4.02 μg cm⁻²), 1-phenylpiperazine (4.75 μg cm⁻²), benzyl benzoate (7.83 μg cm⁻²) and piperazine (10.59 μg cm⁻²). Against *T. putrescentiae*, moderate toxicity was observed when benzyl benzoate (11.06 μg cm⁻²), DEET (18.86 μg cm⁻²) and piperazine (19.41 μg cm⁻²) were used. However, little or no activity was observed by the filter paper and fumigant methods when the remaining compounds [1-(4-bromobenzyl)piperazine,

1-(3-chlorophenyl)piperazine, *trans*-1-cinnamylpiperazine, 1-(4-hydroxyphenyl)piperazine and 2-methylpiperazine] were tested against the three mite species.

Table 3 shows the relative toxicities of 1-ethoxycarbonylpiperazine, benzyl benzoate, DEET, 1-(2-methoxyphenyl)piperazine and 1-phenylpiperazine in the filter paper and fumigant methods. Piperazine exhibited a similar activity in both methods. However, the filter paper bioassay with 1-ethoxycarbonylpiperazine and 1-(2-methoxyphenyl)piperazine was approximately 5.0–6.1 times more toxic than the fumigant bioassay against *D. farinae*, and approximately 4.8–5.6 times more toxic than the fumigant bioassay against *D. pteronyssinus*. Conversely, the filter paper bioassay with benzyl benzoate, DEET and 1-phenylpiperazine was slightly (1.2–1.9 times) more toxic than the fumigant bioassay against *Dermatophagoides* spp. Moreover, the acaricidal activities of piperazine derivatives [piperazine, 1-phenylpiperazine,

Table 3. Comparison of toxicities of piperazine derivatives against *Dermatophagoides* spp. by filter paper and fumigant methods

Compound	Bioassay	LD ₅₀ (µg cm ⁻²) ^a	
		<i>D. farinae</i>	<i>D. pteronyssinus</i>
Piperazine	Filter paper	10.59	8.57
	Fumigant	11.41	9.73
	Filter paper/fumigant ^b	1.1	1.1
1-Phenylpiperazine	Filter paper	4.75	2.57
	Fumigant	7.83	4.86
	Filter paper/fumigant	1.7	1.9
1-Ethoxycarbonylpiperazine	Filter paper	4.02	3.65
	Fumigant	20.14	17.47
	Filter paper/fumigant	5.0	4.8
1-(2-Methoxyphenyl)piperazine	Filter paper	3.65	3.32
	Fumigant	22.14	18.47
	Filter paper/fumigant	6.1	5.6
Benzyl benzoate	Filter paper	7.83	5.96
	Fumigant	9.72	7.54
	Filter paper/fumigant	1.2	1.3
DEET	Filter paper	20.64	12.48
	Fumigant	36.84	19.79
	Filterpaper/fumigant	1.8	1.6

^a Exposed for 24 h.^b Relative toxicity = LD₅₀ value of filter paper bioassay / LD₅₀ value of fumigant bioassay.

1-ethoxycarbonylpiperazine and 1-(2-methoxyphenyl)piperazine] by the filter paper and fumigant methods were generally higher than those of commercial acaricides such as DEET against the three mite species. However, little or no activity by the filter paper and fumigant methods was observed when the other compounds [1-(4-bromobenzyl)piperazine, 1-(3-chlorophenyl)piperazine, *trans*-1-cinnamylpiperazine, 1-(4-hydroxyphenyl)piperazine, 2-methylpiperazine] were evaluated. In addition, these results indicated that the piperazine derivatives were more effective in the filter paper bioassay than in the fumigant bioassay.

The structure–activity relationships between piperazine derivatives and toxicity against the three mite species were evaluated by comparing the LD₅₀ values. As shown in Fig. 1 and Table 1, introducing a methyl group into piperazine attenuated the acaricidal activity against the three mite species. Furthermore, introduction of a functional group such as 4-bromobenzyl, 3-chlorophenyl, 1-cinnamyl or 4-hydroxyphenyl resulted in little or no acaricidal activity. However, replacing the 2-methyl group with a 1-phenyl group resulted in 1-phenylpiperazine becoming more active than 2-methylpiperazine against *Dermatophagoides* spp. Introduction of a 1-ethoxycarbonyl group or 2-methoxyphenyl group led to a significant increase in the acaricidal activity of both 1-ethoxycarbonylpiperazine and 1-(2-methoxyphenyl)piperazine. As shown in Table 3, the acaricidal activity of the above compounds was greater in the filter paper bioassay than in the fumigant bioassay.

The mite allergen is also present in mite faecal material and dead mite bodies.²⁴ Because of this, much effort has been focused on developing a mite indicator to allow complete removal of mites from the affected area. Therefore, the colour alteration of piperazine derivatives against *D. farinae* was evaluated using a fumigant bioassay (Fig. 2). The colour alteration of *D. farinae*

before (A) and after treatment with 1-phenylpiperazine (B) was observed using a light microscope (100×). Prior to treatment and when treated with benzyl benzoate, *D. farinae* was colourless and transparent (A, C). Therefore, it is impossible to remove allergens from the house using common acaricides.^{25,28} However, following treatment with 1-phenylpiperazine, the skin of *D. farinae* was found to be dark-red over the entire body. The authors previously demonstrated that plumbagin and juglone were effective at altering the colour of house-dust mites.^{25,28} In this study, treatment with 1-phenylpiperazine was also found to be an excellent indicator that induced a change in the colour of the skin from colourless transparent to dark-red. These results indicate that 1-phenylpiperazine can be a useful aid when removing mite allergens; however, the present results revealed that there was no colour alteration in *D. farinae* in response to treatment with any of the other compounds evaluated here. Furthermore, 1-phenylpiperazine can be used as a mite indicator to allow easy discrimination in carpet, cloth and house dust with the naked eye.

Recently, many acaricides have been replaced with newer, safer agents owing to the toxicity, resistance and environmental damage caused by older agents.^{21–23} The results in this study indicate that piperazine derivatives would make good replacements for existing acaricides. According to the European Commission (2005), the oral LD₅₀ value of piperazine for pigs is 1–5 g kg⁻¹, which indicates that piperazine has a low acute toxicity to mammals.¹ Similar reports indicated that piperazine can be rapidly degraded by photolysis in the atmosphere, with a half-life of only 0.8 h.² Moreover, the piperazine structure is associated with a wide range of biological activity, and piperazine derivatives have been widely used as medical drugs for many years.^{1,2,8} Taken together, these findings suggest that piperazine derivatives would be suitable as fumigants in vapour-phase acaricides owing to their high volatility, fumigant activity and safety. For this reason, further

research should be conducted to evaluate piperazine derivatives [1-ethoxycarbonylpiperazine, 1-(2-methoxyphenyl)piperazine, 1-phenylpiperazine] to determine their impacts on human health and the environment, as well as to develop formulations with increased acaricidal potency and stability.

REFERENCES

- 1 Summary risk assessment report. Final report, Piperazine, CAS No. 110-85-0. EINECS No. 203-808-3. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, Sweden (2005).
- 2 *Management of Severe Malnutrition: a Manual for Physicians and Other Senior Health Workers*. WHO, Geneva, Switzerland (1999).
- 3 Tomar V, Bhattacharjee G, Kamaluddin and Kumar A, Synthesis and antimicrobial evaluation of new chalcones containing piperazine or 2,5-dichlorothiophene moiety. *Bioorg Med Chem Lett* **17**:5321–5324 (2007).
- 4 Chaudhary P, Kumar R, Verma AK, Singh D, Yadav V, Chhillar AK, et al, Synthesis and antimicrobial activity of N-alkyl and N-aryl piperazine derivatives. *Bioorg Med Chem Lett* **14**:1819–1826 (2006).
- 5 Quintela JM, Peinador C, González L, Iglesias R, Parama A, Álvarez F, et al, Piperazine N-substituted naphthyridines, pyridothienopyrimidines and pyridothienotriazines: new antiprotozoals active against *Philasterides dicentrachi*. *Eur J Med Chem* **38**:265–275 (2003).
- 6 Kimura M, Masuda T, Yamada K, Kubota N, Kawakatsu N, Mitani M, et al, Novel diphenylalkyl piperazine derivatives with dual calcium antagonistic and antioxidative activities. *Bioorg Med Chem Lett* **12**:1947–1950 (2002).
- 7 Guo CC, Tong RB and Li KL, Chloroalkyl piperazine and nitrogen mustard porphyrins, synthesis and anticancer activity. *Bioorg Med Chem Lett* **12**:2469–2475 (2004).
- 8 Radicheva N, Vydevska M and Mileva K, Effects of piperazine derivatives on the activity of frog skeletal muscle fibers. *Gen Pharmacol* **26**:1431–1439 (1995).
- 9 Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, et al, Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* **34**:597–603 (2004).
- 10 de Blay F, Pauli G, Velten M and Bessot JC, Influence of mite exposure on symptoms of mite-sensitive patients with asthma. *J Allergy Clin Immunol* **93**:136–138 (1994).
- 11 Thomas WR and Smith W, House-dust-mite allergens. *Allergy* **53**:821–832 (1998).
- 12 Sporik R, Chapman MD and Platts-Mills TAE, House dust mite exposure as a cause of asthma. *Clin Exp Allergy* **22**:897–906 (1992).
- 13 van der Heide S, Niemeijer NR, Hovenga H, de Monchy JGR, Dubois AEJ and Kauffman HF, Prevalence of sensitization to the storage mites *Acarus siro*, *Tyrophagus putrescentiae*, and *Lepidoglyphus destructor* in allergic patients with different degrees of sensitization to the house-dust mite *Dermatophagoides pteronyssinus*. *Allergy* **53**:426–430 (1998).
- 14 Iversen M, Korsgaard T, Hallas T and Dahl R, Mite allergy and exposure to storage mites and house dust mites in farmers. *Clin Exp Allergy* **20**:211–219 (1990).
- 15 Vidal C, Chomón B, Pérez-Carral C and González-Quintela A, Sensitization to *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Acarus siro* in patients allergic to house dust mites (*Dermatophagoides* spp.). *J Allergy Clin Immunol* **100**:716–718 (1997).
- 16 Hughes AM, *The Mites of Stored Food and Houses*, Vol. 9, 2nd edition. Technical Bulletin of the Ministry of Agriculture, Fisheries and Food, UK, p. 400 (1976).
- 17 Lee JY, *Tyrophagus putrescentiae*: an important allergen in Daejeon. *J Asthma Allergy Clin Immunol* **22**:703–710 (2002).
- 18 Matsumoto T, Hisano T, Hamaguchi M and Miike T, Systemic anaphylaxis after eating storage-mite-contaminated food. *Int Arch Allergy Immunol* **109**:197–200 (1996).
- 19 Ree HI, Jeon SH, Lee IY, Hong CS and Lee DK, Fauna and geographical distribution of house dust mites in Korea. *Kor J Parasitol* **35**:9–17 (1997).
- 20 Munhbayarlah S, Park JW, Ko SH, Ree HI and Hong CS, Identification of *Tyrophagus putrescentiae* allergens and evaluation of cross-reactivity with *Dermatophagoides pteronyssinus*. *Yonsei Med J* **39**:109–115 (1998).
- 21 Pollart SM, Ward GW and Platts-Mills TAE, House dust sensitivity and environmental control. *Immunol Allergy Clin North Am* **7**:447–461 (1987).
- 22 van Bronswijk JE and Sinha RN, Pyroglyphid mites (Acari) and house dust allergy. *J Allergy* **47**:31–52 (1971).
- 23 Hayes WJ and Laws ER, *Handbook of Pesticide Toxicology*, Vol. 1. Academic Press, San Diego, CA (1991).
- 24 Hong CS, House dust mite and clinic allergy. *J Kor Soc Allergol* **11**:297–308 (1991).
- 25 Lee CH and Lee HS, Acaricidal activity and function of mite indicator using plumbagin and its derivatives isolated from *Diospyros kaki* Thunb. roots (Ebenaceae). *J Microbiol Biotechnol* **18**:314–321 (2008).
- 26 Jang YS, Lee CH, Kim MK, Kim JH, Lee SH and Lee HS, Acaricidal activity of active constituent isolated in *Chamaecyparis obtusa* leaves against *Dermatophagoides* spp. *J Agric Food Chem* **53**:1934–1937 (2005).
- 27 *SAS/STAT User's Guide*, Version 6. SAS Institute, Cary, NC (1990).
- 28 Lee CH and Lee HS, Color alteration and acaricidal activity of juglone isolated from *Caesalpinia sappan* heartwoods against *Dermatophagoides* spp. *J Microbiol Biotechnol* **16**:1591–1596 (2006).