

Effectiveness of laundry washing agents and conditions in the removal of cat and dust mite allergen from bedding dust

Euan R. Tovey, PhD, David J. Taylor, BSc(Hons), Teresa Z. Mitakakis, PhD, and Sandra D. De Lucca, BSc(Hons) *Sydney, Australia*

Background: There is limited information about the removal of allergens by laundry washing.

Objective: The purpose of this investigation was to determine the dynamics of the removal of mite allergen (Der p 1) and cat allergen (Fel d 1) from bed dust during simulated laundry processes.

Methods: Three studies were performed. The first compared combinations of 4 laundry agents (water alone, soap, detergent with enzymes, and detergent without enzymes), 4 temperatures (15°, 25°, 45°, and 60°C), and 3 extraction times (5, 20, and 60 minutes). The second study examined allergen extraction by 11 common brands of detergents at 25° and 45°C for 5 minutes. The third study compared 4 detergents containing enzymes before and after the denaturation of their enzymes. To measure the quantity of allergens extracted, each study used an ELISA assay as well as a more sensitive but semiquantitative Halogen immunoassay to detect any allergens remaining after the simulated laundry extraction.

Results: Study 1 showed that detergents extracted more of both Fel d 1 and Der p 1 than either soap or water alone and that almost all allergens were extracted within 5 minutes at 25°. However, washing at 60°C extracted slightly more Fel d 1 and denatured Der p 1, resulting in lower residual amounts of both allergens. Study 2 showed that all of the commercial detergents performed similarly. Study 3 showed that the presence of enzymes in detergent formulations did not produce a significant effect on the extraction of allergens.

Conclusion: Using detergent solutions at 25° for at least 5 minutes was sufficient to extract most mite and cat allergen from dust of bedding. (*J Allergy Clin Immunol* 2001;108:369-74.)

Key words: Allergen, dust mite, Der p 1, cat, Fel d 1, bedding, halogen

Several of the important allergens that are commonly associated with asthma are found indoors. These include house dust mite, cat, dog, cockroach, and mouse allergens. In addition, some allergens that originate outdoors, such as pollen and some fungal spores, can also accumulate indoors and provide high levels of exposure.¹

Abbreviations used

BCIP/NBT: 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium
PBST: Phosphate-buffered saline/0.5% Tween 20
NaCl: Sodium chloride solution

Avoidance of exposure to allergenic triggers is widely advocated as an important component of the management of allergic asthma, perennial rhinitis, and eczema.²⁻⁴ Laundry washing provides the most effective method of removing allergens from washable items such as bedding and clothing; however, the dynamics of such removal have not previously been explored. The only commonly published recommendation is to use a washing temperature above 55°C, a temperature at which mites do not survive.⁵ Data on the solubility of mite and cat allergens from dust is inconsistent. Mite allergens were originally described as being fully extracted from both culture material and dust within minutes.⁶ Most dust extraction protocols use extraction times of several hours, and Siebers et al⁷ showed that the buffer chosen and the extraction temperature could each make a 2-fold difference in the quantity of mite allergen extracted from dust. This suggests that considerable allergen might remain when less favorable extraction conditions are used.

The few published laundry studies measured the reduction in the concentration of mite allergen in dust. This varied from 10- to 20-fold when researchers performed the laundry^{5,8,9} to 10-fold when subjects in an avoidance trial performed the laundry.¹⁰

The removal of cat allergen has not been extensively studied. Avner et al¹¹ found that the majority of cat allergen eluted within 1 minute and that both the extraction rate and the effect of buffers differed widely.

In light of the lack of data on laundry agents and the discrepancies in the literature about allergen extraction, we have examined the extraction of the 2 major domestic aeroallergens associated with asthma, perennial rhinitis, and eczema in a simulated laundry system.

METHODS

Source of dust

A 100-g pool of fine, mixed bed dust was prepared by collecting dust from beds through use of a handheld vacuum cleaner (4017ID, Makita Electric Works Ltd, Japan) and sieving it 3 times through a 400-µm sieve. Baseline allergen concentrations were measured

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Reprint requests: Euan R. Tovey, PhD, Institute of Respiratory Medicine, Allergen Unit, Room 461, Blackburn Building D06, The University of Sydney, NSW, Australia 2006.

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after extraction (5 mg/mL in 1% BSA in phosphate-buffered saline/0.5% Tween 20 [PBST]) overnight at room temperature followed by centrifugation. Mite allergen (Der p 1) and cat allergen (Fel d 1) in the supernatant were measured through use of monoclonal-based ELISAs,^{12,13} and residual Der p 1, Fel d 1, and particles binding IgE from the pellet were detected through use of a Halogen assay,¹⁴ as described below.

Washing protocol

Temperatures (15°, 25°, 45° and 60°C) and extraction times (5, 20, and 60 minutes) were chosen to reflect the ranges of hot and cold cycles of washing machines used worldwide.

Concentrations of laundry agents were used according to the manufacturers' recommendations; we assumed a standard washing machine volume of 70 L of water. To simulate washing, 50 mg of bed dust was suspended in 10 mL of each of the prewarmed laundry solutions in a gently shaking water bath. At each extraction period, a 1-mL sample was removed and immediately centrifuged at 5000 rpm for 10 minutes. The supernatant was then transferred to an Eppendorf tube containing an equal volume of 1% BSA in PBST and stored at -20°C until required. The BSA was added to stabilize the allergen from possible enzymic degradation and to reduce any interference of the detergent with the assays (data not shown).

Extracted cat and dust mite allergens were measured through use of double mAb ELISA assays (Indoor Biotechnologies, Charlottesville, Va) according to protocols provided on the supplier's web site. The allergen standards used were provided with the kits.

After "laundering," the pellet was vortexed in 600 µL of water, from which a 100-µL aliquot was diluted a further 10-fold in water and then filtered onto a 0.22-µm nitrocellulose membrane with a diameter of 25 mm. The particles were evenly distributed across the membrane. This procedure took less than 2 minutes. This membrane was air-dried, laminated with an adhesive film (Inhalix, Sydney, Australia), and stored at room temperature (24°C) until analyzed for residual cat, mite, and multiple IgE-binding allergens by halogen assay.

The protocol for the Halogen assay has been described elsewhere.¹⁴ Briefly, stored laminated membranes were wetted and then incubated overnight at 22°C in borate buffer (pH 8.2) to extract the allergen from the particles onto the membrane. Fel d 1 was detected with monoclonal 6F9 and Der p 1 with monoclonal 5H8 (Indoor Biotechnologies) followed by antimouse alkaline phosphatase conjugate (Sigma, St Louis, Mo). Samples were then developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT; Sigma). For detection of allergens with IgE, neat serum from a pool (RAST 5+ to mite [*Dermatophagoides pteronyssinus*] and RAST 2+ to cat, dog, and cockroach allergens; Pharmacia CAP immunoassay, Uppsala, Sweden) was used as the primary antibody. IgE bound to the eluted allergen was detected with biotinylated anti-IgE (1.5 hours; KPL, Gaithersburg, Md) followed by extrAvidin Alkaline Phosphatase (1.5 hours) and developed with BCIP/NBT. Particles showing halos of Fel d 1, Der p 1, and IgE were counted; each value was expressed as a percentage of particles with halos obtained from dust that had not been laundered ("baseline").

Study 1

The first study consisted of 2 parts. The repeatability of the allergen measures was initially established in a small study, inasmuch as it was impractical to examine all 48 combinations of conditions for all of the extracted and residual allergens using multiple replicates. Thus 95% CIs were determined by performing 10 replicates of a single combination of time (5 minutes) and temperature (25°C) with a representative of each type of laundry agent. The agents were soap (2.1 g/L Lux, Lever Rexona, North Rocks, Australia), detergent (3.52 g/L Fab, Colgate Palmolive, New York, NY), detergent with enzyme (3.18 g/L Drive, Lever Rexona), and water alone. These

laundered extracts were analyzed for Der p 1 and Fel d 1 by ELISA, and, as described above, the residual allergens in the pellets were detected through use of antibodies specific for Der p 1 and Fel d 1 and with IgE from a serum pool.

In the second part of study 1, the degree of confidence in the performance of single replicates having been established, combinations of temperature (15°, 25°, 45°, and 60°C) and incubation time (5, 20, and 60 minutes) were examined with these 4 laundry agents and tested for both extracted and residual allergens.

Study 2

The efficiency of 11 detergents marketed in the United States to remove allergen was compared to that of commonly used allergen-extracting buffers and water. Four of the detergents contained added enzymes: Wisk with Colorhold bleach (Lever Brothers, New York, NY) and Gain with bleach, Tide, and Cheer with bleach (Procter & Gamble, Cincinnati, Ohio). The 7 detergents without enzymes were Arm and Hammer (Church & Dwight, Princeton, NJ), All Free Clear and All with Colorsafe bleach (Lever Brothers), Sun with bleach (Huish City Products, Salt Lake City, Utah), Ultra Power Clean (Cap City Products, Kearny, NJ), Ajax (Colgate Palmolive), and Purex (Dial Corporation, Scottsdale, Ariz). Buffers tested were PBS, borate buffer, and sodium chloride solution (NaCl). Bed dust was extracted at 2 temperatures (25° and 45°C) for 5 minutes. Both the extracted and the residual Der p 1 and Fel d 1 allergens were measured for each laundry agent at each temperature. All sampling was conducted in duplicate.

The pH of each laundry solution was measured to test for possible associations between pH and both extraction and residual allergen data.

Study 3

To test whether enzymes in detergents increase the amount of allergen extracted—or, alternatively, destroy the allergens—we compared the 4 detergents with active enzymes from study 2 with the same detergents after their enzymes had been heat-inactivated. Because the characteristics of enzymes present in detergents are covered under product patents, we have assumed from the limited information available from industry sources that they are protease and lipase enzymes derived from the *Bacillus* genus. A portion of each was boiled for 5 minutes to inactivate the enzymes, as suggested by an industry source. Bed dust was then washed with the heat-inactivated and active enzyme solutions for 5 minutes at 25° or 45°C, according to the methods used in study 2. Samples were assayed as described above for extracted and residual Fel d 1 and Der p 1.

Statistical analysis

Statistical analyses were conducted through use of Analyse-It for Microsoft Excel (Analyse-It Software Ltd, Leeds, United Kingdom). Data were evaluated through use of Wilcoxon signed rank tests for nonparametric distributions, with $P < .05$ determined to be significantly different. Correlations were conducted through use of Spearman rank analysis.

RESULTS

The pooled dust contained 94.25 ± 0.7 µg Der p 1 allergen per gram of dust and 166.78 ± 8.6 µg Fel d 1 allergen per gram of dust, as estimated by the ELISA assay. Positive Halogen-binding particles at baseline were 339.5 ± 47.3 Der p 1 particles, 371.8 ± 56.5 Fel d 1 particles, and 479.5 ± 80.0 allergenic particles, on the basis of binding with pooled serum IgE.

Study 1

The 95% CIs for repeated measures of extracted and residual allergen washed for 5 minutes at 25°C were narrow (Tables I and II). Temperature affected the removal of both cat and dust mite allergen (Fig 1). Values for detergent with enzymes were similar to those for detergent alone and thus are not illustrated. The amount of Der p 1 extracted by all agents, including water, was significantly higher at 25°C than at 15°C ($P = .0005$) and was significantly lower at 60°C (25° vs 60°C, $P = .0068$; 45° vs 60°C, $P = .0068$), the exception being extraction with water.

During the wash cycle, extraction of cat allergen showed a pattern similar to that of extraction of Der p 1 allergen, the exception being washes at 60°C (Fig 1). Allergen extracted by all washing products was significantly higher at 25°C than at 15°C ($P = .0087$) and significantly lower than at 45°C ($P = .0017$). At 60°C, cat allergen was more stable than mite allergen, and the amount did not significantly decrease from that extracted at 15°C ($P = .1608$).

Incubation time did not significantly affect the quantity of Der p 1 extracted for any temperature ($P > .05$); 86.2% to 103.6% of the Der p 1 allergen extracted after 60 minutes was extracted in the first 5 minutes (excluding samples at 60°C). Cat allergen extraction was slightly increased between 5 and 60 minutes ($P = .049$), showing that 76.8% to 94.7% of the allergen extracted after 60 minutes was extracted after 5 minutes. The quantity of allergen extracted after 20 minutes was not significantly different from that of either extraction time. Overall, after 5 minutes no significant additional cat allergen was extracted.

With the 3 laundry agents and water combined, the percentage of residual dust mite, cat, and pooled serum IgE-binding allergenic particles significantly decreased with increasing temperature (all $P < .01$ except IgE 15° vs 25°C; $P = .677$; Fig 1). A 6-fold reduction in Der p 1 allergen was detected at 60°C in comparison with 15°C with detergent, and an 11-fold reduction was produced by the detergent with enzymes.

Residual Fel d 1 allergen followed a pattern similar to that seen with Der p 1 residual allergen, except that less residual allergen remained at higher temperatures. This pattern was mirrored by multiple allergens detected by IgE.

Comparisons of different washing products showed no significant difference in performance between the detergent and the detergent with enzymes in extracting Der p 1 and Fel d 1 allergen ($P = .4488$ and $P = .1013$, respectively). However, there were significant differences in the proportion of particles with halos in the residual dust. For Der p 1 and Fel d 1, there were significantly ($P = .001$ and $P = .0005$ respectively) fewer allergenic particles in the pellet washed with detergent only than in the pellet washed with detergent plus enzymes. The opposite was true for IgE-stained particles ($P = .0122$). Both detergents extracted significantly more allergen than soap and water ($P \leq .0213$ in all cases). Water extracted the smallest amount of allergen.

TABLE I. CIs (95%) measured by ELISA from 10 replicates of extracted dust mite and cat allergen, calculated in reference to values from untreated dust after washing with different solutions for 5 minutes at 25°C

	Mean $\mu\text{g/g}$ dust	SE	95% CI
Mite			
Water	32.0	0.49	30.9 to 33.1
Soap	79.6	1.12	77.1 to 82.2
Detergent	88.6	0.93	86.5 to 90.7
Enzyme	86.4	0.74	84.7 to 88.1
Cat			
Water	61.3	0.53	60.1 to 62.5
Soap	110.6	1.09	108.1 to 113.1
Detergent	133.1	1.96	128.7 to 137.5
Enzyme	134.2	2.11	129.4 to 138.9

TABLE II. CIs (95%) measured from percentage of residual particles with halos (measured by halogen), calculated in reference to values from untreated dust, after washing with different solutions for 5 minutes at 25°C

	Positive particles (%)	SE	95% CI
Mite			
Water	66.7	1.67	62.9 to 70.5
Soap	33.7	1.00	31.5 to 36.0
Detergent	21.9	0.71	20.3 to 23.5
Enzyme	18.7	0.81	16.9 to 20.6
Cat			
Water	70.3	1.54	66.8 to 73.7
Soap	36.4	1.04	34.1 to 38.8
Detergent	22.2	0.96	20.0 to 24.3
Enzyme	16.3	1.00	14.0 to 18.5
IgE-binding allergenic particles			
Water	81.0	1.42	77.8 to 84.3
Soap	51.8	1.12	49.3 to 54.4
Detergent	25.9	1.04	23.5 to 28.3
Enzyme	20.5	0.90	18.5 to 22.6

Study 2

All 11 US laundry detergents were similar with respect to efficiency at extracting Der p 1 and Fel d 1, independent of enzyme presence (Table III). Water alone resulted in the lowest removal of both Der p 1 and Fel d 1 allergen. Borate buffer performed relatively well—in some cases, better than many of the washing products.

For all of the formulations, the amount of Der p 1 extracted did not increase significantly as the temperature was raised from 25° to 45°C ($P = .5412$). However, a significant difference was detected for Fel d 1 extraction by all products when the temperature was increased from 25° to 45°C ($P = .001$).

For the detergents with (+) and without (–) enzymes, the mean percentages of particles with Der p 1 halos in comparison with controls were 12.5% (+) and 10.7% (–), respectively. Similarly the mean percentages for Fel d 1 were 7.0% (+) and 5.6% (–), respectively. However the mean percentages of particles forming IgE halos were

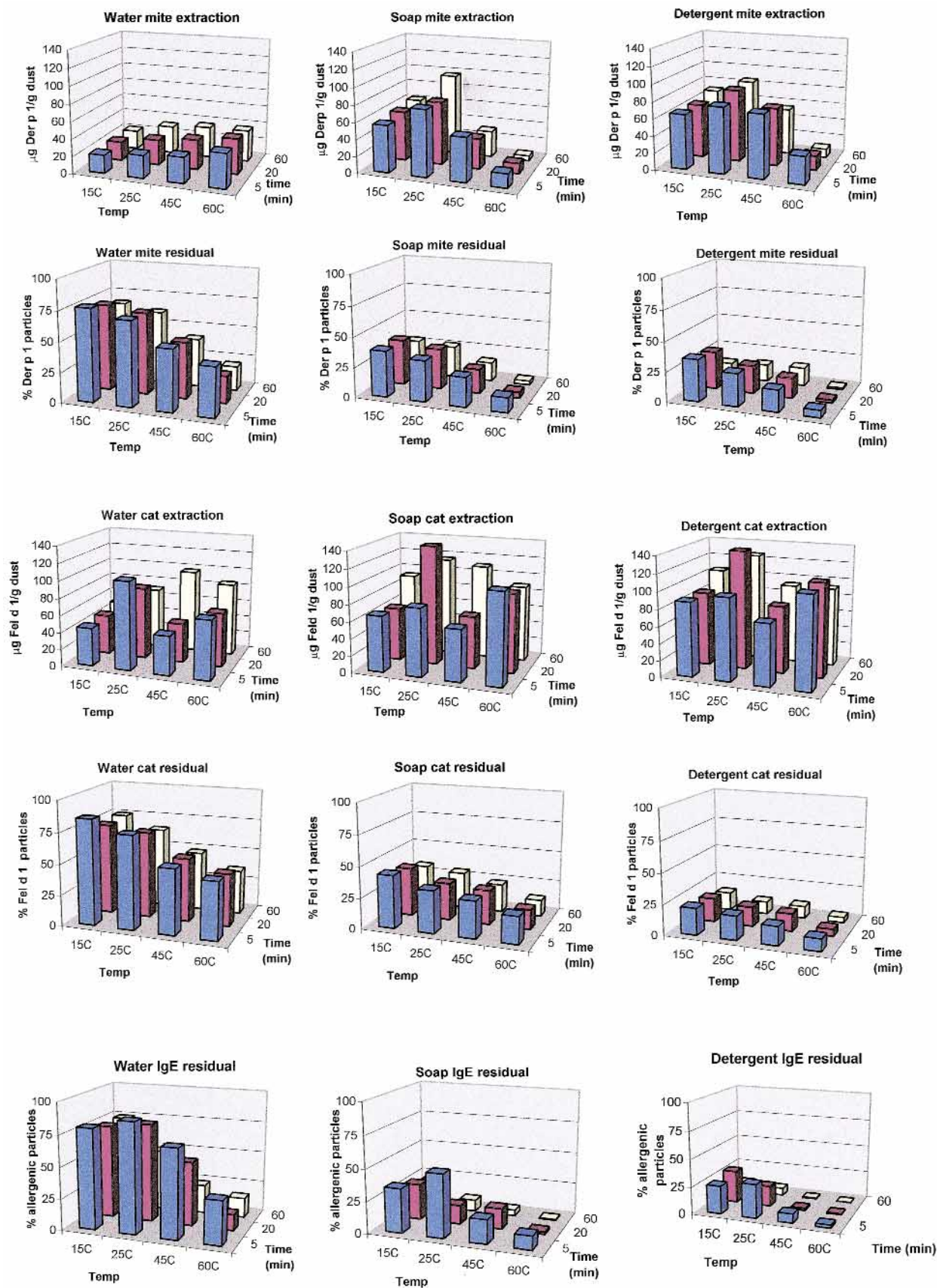


FIG 1. Extracted dust mite and cat allergen (measured by ELISA) and residual dust mite, cat, and IgE-binding allergenic particles (detected by halogen assay) from bed dust after washing with water, soap, and detergent solutions at 4 temperatures for 3 wash durations.

TABLE III. Extracted Der p 1 and Fel d 1 allergen per gram of bed dust by ELISA after washing with different washing agents at 2 temperatures for 5 minutes

Washing solution	25°C		45°C	
	Der p 1(μg/g)	Fel d 1(μg/g)	Der p 1(μg/g)	Fel d 1(μg/g)
Water	26.2	110.4	34.6	86.3
PBS	62.4	103.8	72.3	116.2
NaCl	50.6	105.0	65.6	86.3
Borate	61.6	142.9	78.7	168.4
Detergent 1	72.7	131.5	71.2	137.5
Detergent 2	56.0	107.8	67.4	174.7
Detergent 3	65.7	121.3	68.6	154.4
Detergent 4	67.9	125.7	65.4	167.4
Detergent 5	67.6	128.8	68.1	178.1
Detergent 6	67.1	135.1	62.3	181.9
Detergent 7	70.7	128.7	68.0	157.3
+Enzyme 1	69.5	155.9	68.8	143.3
+Enzyme 2	64.4	150.9	62.3	164.6
+Enzyme 3	65.0	136.2	64.5	143.8
+Enzyme 4	66.2	138.1	67.1	157.8

33.6% (+) and 25.8% (–) for detergents and were greater than for the monoclonal-based assays ($P < .0001$). Most of the extractions with buffers left more residual allergens than did the laundry solutions; they ranked in order as follows: water > PBS = NaCl > borate.

Increasing the wash temperature from 25° to 45°C significantly reduced the amount of residual allergen extracted for all 3 allergen measurements (Der p 1, $P = .0003$; Fel d 1, $P < .0001$; IgE, $P = .0095$).

The pH values of all laundry solutions were highly alkaline (range, 10.27–10.83), and no correlation was found between pH and amount of either extracted allergen ($r = 0.07$; $P = .6342$) or residual allergen ($r = -0.03$; $P = .8051$).

Study 3

The presence of active enzymes in the detergent was not associated with the extraction of significantly different amounts of allergen in comparison with detergent with inactivated enzymes (Der p 1, $P = .5469$; Fel d 1, $P = .4609$; overall, $P = .8209$). For combined temperature results, mean values for the detergents with enzymes (Der p 1 treated, 66.0 μg/g dust; Der p 1 untreated, 67.3 μg/g dust; Fel d 1 treated, 153 μg/g dust; Fel d 1 untreated, 129 μg/g dust) were no different from those of detergents and detergents with enzymes as found in study 2. Data from Halogen assays of residual dust confirmed no significant difference in extraction of allergen (Der p 1, $P = .8438$; Fel d 1, $P = .1094$; IgE, $P = .3828$; overall, $P = .8334$).

DISCUSSION

This study is the first to systematically examine the dynamics of the most commonly used method of domestic allergen removal. Overall, the results show that the important indoor domestic allergens, Der p 1 from house dust mites and Fel d 1 from cats, are highly soluble even during brief washing and are largely removed within 5

minutes at moderate temperatures. With Der p 1, lower amounts of allergen were recovered when extraction was performed at 60°C for all laundry solutions except water, which is consistent with the thermal lability of Der p 1.¹⁵ This reduction with temperature increase was not observed with Fel d 1, which is heat-stable, and would probably not have been observed if the more thermostable Der p 2 had been measured.¹⁵

The more than 2-fold greater quantity of mite allergen extracted by detergent or buffer solutions in comparison with soap, virtually independent of time, is consistent with the observation of Siebers et al⁷ that the amount of mite allergen that can be extracted is dependent on the buffer used. The solubility of proteins in different concentrations of various buffers has long been used in biochemical analysis to characterize and fractionate proteins. We did not examine whether after water extraction an even greater quantity of Der p 1 could have been further extracted with detergent, which the results suggest was possible. It can also be speculated that the greater quantity of allergen observed with detergent was partly a function of the detergent breaking up soluble aggregates containing Der p 1 allergen in the dust extract.

The use of both ELISA to quantify extracted allergens and Halogen assay to semiquantify allergens in residual dust provided a complementary and sensitive system. However, because the Halogen assay is nonlinear in sensitivity and only semiquantitative, the Halogen results should be viewed only relatively and should not be summed with the ELISA results. The advantage of using confirmatory Halogen assays is that they permit the detection of multiple allergens on the basis of IgE binding through use of pooled atopic sera. In this case, the serum pool was shown by RAST assay to contain specific IgE for mite, cat, dog, and cockroach allergens, so the presence of a combination of one or more of these allergens on the blots of residual dust was determined. Although the pat-

terms of results for the amount of residual allergens detected by monoclonal and IgE antibodies were generally similar, in post-enzyme-plus-detergent washing IgE binding was proportionately greater than monoclonal binding, suggesting that where epitopes on allergens are being degraded, monoclonal binding assays might not always reflect allergenicity. The finding that all laundry detergents were similarly effective is encouraging; there is no need for specific recommendations, there are no grounds for competitive product claims, and it is unnecessary to conduct an extensive study to improve their performance with respect to targeting allergens, given that detergent extraction under mild conditions is highly effective at removing allergen.

The recommended practice of washing at elevated temperatures can be justified only if the objective is to kill live house dust mites, inasmuch as allergen is efficiently removed by less aggressive protocols. The benefit of killing mites is assumed to be a slower resurgence of allergen, but this has not been demonstrated. In another study in which an acaricidal wash was used,¹⁰ we examined the rate of mite allergen resurgence and suggested that even after mite-killing, laundry should be repeated each 6 to 8 weeks, though no explicit period can be justified. It is important that items containing high levels of allergen (clothing, bed covers, etc) be washed regularly; even in beds with mattress encasings, allergen quickly reaccumulates.^{6,16}

In conclusion, the 2 common allergens found in houses were removed quickly, with great efficiency, and at moderate temperatures through normal domestic laundering by all of the detergents tested. Laundry is a simple, inexpensive, and extremely effective method of massively reducing allergen reservoirs.

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