

Stringent environmental control in pregnancy and early life: the long-term effects on mite, cat and dog allergen

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Summary

Background As part of a primary prevention of asthma study, we measured the effect of environmental control measures on Der p 1, Fel d 1 and Can f 1 over a 3.5-year period.

Methods High-risk infants (both parents atopic) without pets, were randomized to the Active group ($n = 142$, vinyl flooring in child's room, allergen-impermeable cot mattress, hot-washable toy, mite allergen-impermeable encasings to parental bed and to child's bed when older, high filtration vacuum cleaner, hot-washing of bedding) or the Control group ($n = 136$, no intervention), in early pregnancy. Dust samples from the parental mattress, living room floor, child's mattress and floor at baseline (pregnancy), birth and at 3 years were analysed for Der p 1, Fel d 1 and Can f 1.

Results A total of 278 families completed the baseline visit, 259 the birth visit and 239 the 3-year visit. In the Active group at 3 years, 58% remained compliant with all measures likely to reduce the child's exposure to allergen and 77% of parents still used encasings on their bed. Levels of Der p 1, Fel d 1 and Can f 1 were significantly lower in the Active group in the child's floor and the child's mattress at 3 years compared to the Control group ($P < 0.001$). For the parental mattress, the levels of Der p 1 and Fel d 1 were lower in the Active group ($P < 0.001$) and there was a strong trend towards a lower level for Can f 1. There was no difference in the levels of any of the allergens between the groups in the living room floor. Children's bedrooms with no detectable mite, cat or dog allergen were significantly more common in the Active than the Control group (25 vs. 2, $P < 0.001$).

Conclusions Environmental control measures are effective in substantially reducing levels of Der p 1, Fel d 1 and Can f 1 in homes without pets in the long term and are acceptable to families. The effect of this environmental manipulation on the development of sensitization and allergic disease remains to be seen.

Keywords Can f 1, Der p 1, environmental control, Fel d 1, primary prevention of asthma

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Introduction

Many patients with asthma are allergic to house dust mites. Studies of mite allergen avoidance in adults with asthma have often used measures which were ineffective in reducing allergen exposure [1] or have been of too short duration to see a change in lung function [2, and reviewed in 3]. The Cochrane Review concluded that allergen avoidance should not be recommended in the management of asthma in adults [4]. However, studies in children have produced more promising results [5], and attention has now turned to the investigation of these environmental control methods in the primary prevention of asthma in high-risk infants.

As part of the National Asthma Campaign Manchester Asthma and Allergy Study (^{NAC}MAAS), we have designed and installed bedrooms for high-risk infants/young children, using stringent environmental control measures developed

originally to reduce mite allergen exposure. We have shown previously that such rooms are acceptable to parents and, when used in conjunction with mattress and bedding encasings in the parental bedroom, can significantly reduce exposure to mite allergen in the first year of life [6].

Sensitization to cats and dogs is a risk factor for asthma. The relationship between pet ownership, pet allergen and the development of sensitization to pets is confusing, and a wealth of recent publications has failed to clarify the issues. A prospective birth cohort study has, however, identified increasing exposure to Fel d 1 as a risk factor for sensitization to cat by age 7 years [7]. Many children who are sensitized to cats and dogs have never lived with these pets [8]. Levels of Fel d 1 and Can f 1 tend to be higher in homes with pets, but pet allergen is not only found in most homes without pets [9, 10] but can be detected on trains, planes and automobiles [11, 12], in the air of hospital outpatient clinics [13] and in nursery schools [14], i.e., places where pets have never been. A primary prevention of asthma study from Canada has reported that Fel d 1 levels can be reduced in the parental bed after intervention with encasings for up to 2 years, but found no effect on infant beds [15]. A similar effect on Fel d 1 was seen in an allergen avoidance

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study of children from the UK, with measurements taken to 6 months [16]. To our knowledge there are no published data on the effects of environmental control on Can f 1 levels.

The aims of this study were to investigate whether the environmental control measures had any long-term effect in reducing levels of dust mite and pet allergen levels in homes without pets.

Methods

The ^{NAC}MAAS is a pre-natally randomized parallel group study that follows the development of asthma and allergy in a group of infants considered to be high risk on the basis of both parents being atopic (skin prick test positive to one or more inhalant allergen). In the first trimester of pregnancy, half were randomly assigned to environmental control (Active group) and half to normal regimen (Control group). Pet ownership (cat or dog) was an exclusion criterion at this stage. Recruitment is described in detail elsewhere [6, 8].

Environmental control measures

Measures introduced during pregnancy Allergen-impermeable covers were fitted to the mattress and duvet and all pillows of the parental bed (Intervet, WL Gore & Associates, Woking, Surrey, UK). For any non-standard sizes of beds, custom-made covers were provided. Parents were asked to hot-wash the bedding weekly at $>55^{\circ}\text{C}$, and to damp dust the allergen-impermeable covers once weekly. A high filtration vacuum cleaner was supplied (Nilfisk GM 210, Nilfisk, Brøndby, Denmark). All dust collection bags and filters were supplied and, in the event of breakdown, a replacement vacuum cleaner was supplied. Parents were asked not to use their old vacuum cleaner. The Nilfisk vacuum cleaner was tested for allergen leakage (using dust containing a high concentration of dog allergen Can f 1) prior to commencement of the study, and again after 6 months of use and was found not to leak allergen [17]. In areas of the home where Der p 1 levels were $>2\text{ }\mu\text{g/g}$ fine dust, parents were supplied with Acarosan to apply to the sofa or carpets.

Infant/child's bedroom At 36 weeks of pregnancy, vinyl cushion flooring was fitted in the infant's bedroom (Forbo-Nairn, Fife, UK), and if the family moved house, this was repeated in the new home. New custom-made crib and cot mattresses, encased in allergen-impermeable material were supplied (Slumberland, Wilmslow, UK) together with a hot-washable soft toy. Washing instructions were as above. The families were initially asked to comply with this environmental control regimen until the child was 1 year old, and encouraged and supported in continuing to use this regimen thereafter. When the child moved into a bed, allergen-impermeable covers were supplied for the mattress, duvet and pillows.

Visit schedule

Soon after recruitment (during early pregnancy), homes were visited by two technicians/nurses and dust samples were collected as described below (baseline visit). For the Active group, the avoidance measures were introduced after sampling, at this visit. The homes were then revisited 6 months later (just after the birth of the infant) and the dust sampling was repeated

(birth visit). Homes were visited again within 2 weeks of the child's third birthday (3-year visit).

Dust sample collection

Dust samples were collected at all visits from the living room carpet (LF) and parental mattress (M) and, for the birth and the 3 years visit, from the child's floor (CF) and child's mattress (CM), by vacuuming a 1-m^2 area using a Medivac dust sampler (airflow 45 L/s; Medivac Plc, Wilmslow, Cheshire, UK) for 2 min in a standardized fashion. The sampling head was loaded with a stainless steel mesh filter, to remove particles $>300\text{ }\mu\text{m}$ diameter, allowing a sample of fine dust to be collected onto a $5\text{-}\mu\text{m}$ pore size vinyl filter (Plastok Associates Ltd, Wirral, UK). Immediately after collection, the dust sample was transferred into a pre-weighed Petri dish and coded. The filled Petri dish was weighed to calculate the mass of fine dust collected and stored at 4°C until extraction. After each sample collection the sampling head was cleaned using 70% isopropyl alcohol.

Dust sample extraction and assay

A 100-mg aliquot of the dust was extracted by rotation with 2 mL borate-buffered saline with 0.1% Tween-20 pH 8.0, at room temperature (18°C) for 2 h before being centrifuged for 20 min at 1200 g at 4°C . The supernatant was stored at -20°C until analysed for allergen content.

Der p 1 was measured using a two-site monoclonal antibody-based ELISA as previously described [18]. The standard used to establish the control curve (UVA 93/02) was considered to contain 2500 ng Der p 1/mL relative to WHO/IUIS standard (NIBSC 82/518) which has been estimated to contain $12.5\text{ }\mu\text{g}$ Der p 1/ampoule.

Fel d 1 was measured using a two-site monoclonal antibody-based ELISA [19]. The UVA 91/01 standard used for Fel d 1 contained 2 Units Fel d 1/mL (relative to CBER Cat E5 standard containing 9.7 Units/mL; 1 Unit = $4\text{ }\mu\text{g}$ protein).

The Can f 1 assay was quantified using UVA 94/02 (10 000 IU Can f 1/mL) that has been substandardized against WHO/IUIS International Reference Preparation of dog hair and dander (NIBSC 84/685), which contains 100 000 IU/mL Can f 1. One International Unit is approximately 1 ng Can f 1 protein, and this value was used to calculate the results.

Statistics

The analysis was performed using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) version 10.1. Results are expressed as total allergen recovered in ng [20]. Results were not normally distributed, so medians, ranges and interquartile ranges are presented. The Mann-Whitney *U*-test was used to compare the Active group to the Control group, and the Wilcoxon signed rank test was used to measure changes within the groups across different time-points. Samples which contained too small an amount of dust to assay or which were below the limit of detection of the assay were assigned an arbitrary value just below the limit of detection of the assay. In acknowledgement of the multiple testing, the *P*-value was set at <0.01 rather than the conventional $P=0.05$. Many samples contained no detectable allergen and chi square tests were used to compare the proportion of samples in each group which contained no measurable allergen.

Results

A total of 278 families (142 Active, 136 Control) completed the baseline visit. The birth visit was completed by 259 (134 Active, 125 Control). The 3-year visit was completed by 239 families (130 Active, 109 Control). All available data are presented for each time-point.

Compliance with environmental control at age 3 years

A total of 69 children still had vinyl flooring without a rug in their room at 3 years, and a further nine had a wooden floor, and 18 had a smooth floor (wooden or cushion floor) with a rug, leaving only 33 with a carpet (one was still sleeping in the parents' room).

Ninety-one children were using the allergen-impermeable covers on the bed; 12 were still in a cot, using the allergen-impermeable covers. A further 10 children were using the allergen-impermeable covers except for the duvet cover. Seventeen children were sleeping in a bed or cot, not using the allergen-impermeable covers, or were in the parents' bed. Seventy-six families remained fully compliant with environmental control in the child's bedroom (either vinyl or wooden flooring, no rug, allergen-proof bedding).

For the parents, 81 were still using the allergen-impermeable covers at 3 years, with a further 19 using the mattress and pillow covers but not the duvet cover, leaving 30 sets of parents not using any of the allergen-impermeable covers.

By age 3 years, 47 families were still fully compliant with every aspect of the environmental control measures and had not acquired a pet. Forty-five families were asked to apply Acarosan to the living room floor after visit 1 because of high Der p 1 levels. Thirty-six complied with this request.

By 3 years, four families in the Active group had acquired a cat and three had acquired a dog; in the Control group, seven had acquired a cat and six had acquired a dog. Between birth and 3 years, 37 families from the Active group and 33 families from the Control group moved house. For the Active group, environmental control measures were introduced into the new home.

Allergen levels in dust reservoirs

Child's floor Data are only available for two time-points, birth and 3 years and this is presented in Table 1. At birth levels of Der p 1, Fel d 1 and Can f 1 were all lower in the Active compared to the Control group ($P < 0.001$) and this effect was maintained at 3 years ($P \leq 0.001$), despite the fact that there was a significant rise in levels of all three allergens in the Active group ($P < 0.001$). This effect was maintained when those no longer using the vinyl floor at age 3 years were excluded from the Active group and those who had acquired pets were excluded from both groups ($P < 0.001$ for all three allergens).

Child's mattress The child's mattress was available for sampling at birth (cot) and at 3 years (mostly single bed) and the data are presented in Table 2. The levels of allergen were very low in both groups at birth. There was no difference in the levels of Der p 1 and Can f 1 between the two groups, but levels of Fel d 1 were lower in the active than the control group at birth. Although the levels of all three allergens tended to increase with time in both groups ($P < 0.001$), at 3 years the levels of all three allergens were significantly lower in the Active compared to the Control group ($P \leq 0.003$). This difference was maintained when those no longer using the allergen-impermeable covers at age 3 years were excluded from the Active group and those who had acquired pets were excluded from both groups ($P < 0.01$).

Parental mattress The medians and ranges for all three allergens at three time-points are shown in Table 3.

There was no difference in allergen levels in the parental mattress between the groups at baseline. At birth, the levels of all three allergens were significantly lower in the Active than in the Control group ($P < 0.001$) and this effect was maintained at 3 years for Der p 1 and Fel d 1 ($P \leq 0.001$), despite the fact that Der p 1 levels in the Active group increased significantly during this time ($P < 0.001$). This is shown for Der p 1 in Fig. 1. For Can f 1, there was a strong trend towards lower levels in the active group at 3 years ($P = 0.018$). When those who were

Table 1. Total allergen recovered from the child's floor (ng)

	Active group Median (interquartile range)	Control group Median (interquartile range)	Mann-Whitney U-test
Der p 1	1.25	16.4	$P < 0.001$
Birth	1.25–1.25	1.25–176.1	
Der p 1	1.25	420	$P < 0.001$
3 years	1.25–205.1	94.6–2050.8	
Fel d 1	1.99	65.78	$P < 0.001$
Birth	1.99–1.99	12.6–232.9	
Fel d 1	1.99	118	$P < 0.001$
3 years	1.99–57	40.4–343.4	
Can f 1	5	93.43	$P < 0.001$
Birth	5–5	5–199.8	
Can f 1	5	96	$P = 0.001$
3 years	5–131.4	5–443	

Table 2. Total allergen recovered from the child's mattress (ng)

	Active group Median (interquartile range)	Control group Median (interquartile range)	Mann-Whitney <i>U</i> -test
Der p 1	1.25	1.25	$P = 0.131$
Birth	1.25–9.2	1.25–32.1	
Der p 1	49.5	456	$P < 0.001$
3 years	1.25–258.2	57.7–2882.8	
Fel d 1	1.99	1.99	$P = 0.007$
Birth	1.99–9.9	1.99–35.7	
Fel d 1	1.99	75	$P < 0.001$
3 years	1.99–55.2	1.99–493.4	
Can f 1	5	5	$P = 0.239$
Birth	5–22.8	5–42.3	
Can f 1	5	24	$P = 0.003$
3 years	5–138.2	5–497.5	

Table 3. Total allergen recovered from the parental mattress (ng)

	Active group Median (interquartile range)	Control group Median (interquartile range)	Mann-Whitney <i>U</i> -test
Der p 1	325	240	$P = 0.07$
Baseline	41.6–2979.4	2.1–1526	
Der p 1	1.25	245	$P < 0.001$
Birth	1.25–46.4	8.6–2017.7	
Der p 1	19	569	$P < 0.001$
3 years	1.25–216.4	48.1–2182.9	
Fel d 1	87	109	$P = 0.78$
Baseline	33.7–231.3	32.5–447.1	
Fel d 1	1.99	97.4	$P < 0.001$
Birth	1.99–22.8	1.99–381.4	
Fel d 1	1.99	1.99	$P = 0.001$
3 years	1.99–5.08	1.99–201.2	
Can f 1	132	92	$P = 0.79$
Baseline	65.98–373.8	43.2–215.5	
Can f 1	5	47.4	$P < 0.001$
Birth	5–54.4	5–339.4	
Can f 1	5	5	$P = 0.019$
3 years	5–5	5–261.7	

non-compliant with the allergen-impermeable covers and those who had acquired a pet were excluded from the analysis, levels of all three allergens were significantly lower in the Active compared to the Control group at 3 years ($P < 0.008$).

Living room floor There was no significant difference between the levels of Der p 1 in the living room floor between the two groups at any of the three time-points. However, levels of Der p 1 did increase with time, so that at age 3 years, levels were significantly higher than during early pregnancy or at birth ($P < 0.001$) for both groups. When all those who had moved house during the study period were excluded from the analysis, Der p 1 levels were still significantly higher at 3 years than in early pregnancy or at birth for both groups (e.g. Active group birth median 185 ng, range 1.25–31 571 vs. 3 years median 1264 ng, range 1.25–33 523, $P < 0.001$). For those who applied

Acarosan after the early pregnancy visit, no reduction was seen in Der p 1 levels at the birth ($P = 0.649$).

For Fel d 1 and Can f 1, there was no significant difference between the groups at any time-point, but the levels at 3 years were lower than during early pregnancy ($P \leq 0.004$, for both groups) and this effect was maintained when those who had acquired a pet were excluded.

Proportion of dust reservoirs with no detectable allergen

Comparisons were made between the proportions of the homes in each group that contained no detectable allergen, in each reservoir, at each time-point. These results are shown for all three allergens in Tables 4–6 for the child's floor, child's mattress and parental mattress. There was no difference between the groups for the living room floor at any time-point. When those who had acquired a pet and those who were non-compliant with the relevant part of the environmental control

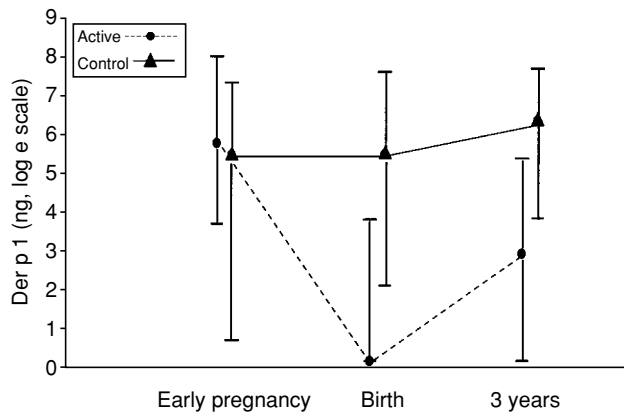


Fig. 1. Changes in Der p 1 in the parental mattress across the three visits (medians and ranges) for the Active (●) and Control groups (▲).

Table 4. Chi square test comparing the percentage of homes in the active and control group containing no detectable Der p 1

	Active group	Control group	χ^2	P-value
Child's floor				
Birth	83%	40%	32.68	$P < 0.001$
3 years	53%	11%	40.04	$P < 0.001$
Child's mattress				
Birth	73%	64%	2.13	$P > 0.05$
3 years	33%	16%	9.12	$P < 0.005$
Parental mattress				
Baseline	20%	25%	0.83	$P > 0.05$
Birth	59%	23%	33.36	$P < 0.001$
3 years	39%	17%	13.49	$P < 0.001$

were excluded, the results were very similar. The most striking results were for the child's floor, where far fewer homes contained detectable allergen in the Active compared to the Control group (e.g. 47% vs. 89% for Der p 1 at age 3 years). Children's bedrooms containing no detectable mite, cat or dog allergen were identified and were far more common amongst the Active ($n = 25$) than the Control ($n = 2$) groups ($\chi^2 = 18.26$, $P < 0.001$).

Discussion

This is the first study to show that environmental control measures are tolerable to families with small children and are still effective more than 3 years after their introduction. In addition to their previously reported effect on Der p 1 levels [6], they also reduce exposure to Fel d 1 and Can f 1 levels in homes without pets.

At recruitment to the study, families were advised of the environmental control protocol and were asked to follow the regimen until the child was 1 year old. Of the 145 initially recruited into the Active group, 133 families complied with the suggested measures until the child was aged 1 year [6]. After this time, families were encouraged to continue with the environmental control measures, but failure to do so was not a reason for withdrawal from the study, as the main outcome measures were development of allergy and asthma in the children. Despite

Table 5. Chi square test comparing the percentage of homes in the active and control group containing no detectable Fel d 1

	Active group	Control group	χ^2	P-value
Child's floor				
Birth	82%	20%	59.34	$P < 0.001$
3 years	72%	21%	52.2	$P < 0.001$
Child's mattress				
Birth	73%	59%	5.62	$P < 0.02$
3 years	59%	40%	8.13	$P < 0.005$
Parental mattress				
Baseline	1%	1%	0.001	$P > 0.05$
Birth	63%	32%	22.8	$P < 0.001$
3 years	74%	57%	6.96	$P < 0.01$

Table 6. Chi square test comparing the percentage of homes in the active and control group containing no detectable Can f 1

	Active group	Control group	χ^2	P-value
Child's floor				
Birth	85%	27%	56.7	$P < 0.001$
3 years	66%	45%	11.03	$P < 0.001$
Child's mattress				
Birth	72%	66%	1.12	$P > 0.05$
3 years	59%	49%	2.52	$P > 0.05$
Parental mattress				
Baseline	1%	1%	0.37	$P > 0.05$
Birth	64%	45%	8.8	$P < 0.005$
3 years	77%	66%	3.41	$P > 0.05$

this, by 3 years of age, 58% of families were compliant with measures likely to reduce the child's allergen exposure in the child's bedroom. The allergen-impermeable covers were introduced to the parental bed in an attempt to reduce the exposure of the mother and fetus during pregnancy and the infant in early life and yet 77% of parents were still using them when the child was 3 years old. We suggest three possible explanations for this. Many small children continue to spend some time in the parental bed and so the parents may leave the covers in place to protect the child. Alternatively, the parents may have continued to use the allergen-proof covers because of perceived benefit for their own allergies. Thirdly, the parents may have found it difficult to remove the mattress cover after fitting. In contrast, in the Control group only five parents had fitted covers to their mattress and bedding which were likely to be allergen-impermeable during the course of the study.

Therefore we have shown that not only are these environmental control measures acceptable to parents during the first year of life, but after this time, the majority of families continue to use at least some of the environmental control measures.

By the time the child was born, 6 months after the introduction of the parental mattress cover, the levels of all three allergens had fallen dramatically in the Active group and were undetectable in many mattresses. This effect was maintained at 3 years for all three allergens in those who were still compliant with the allergen-impermeable covers. This suggests that these covers are durable and remain effective with prolonged use.

For the child's mattress, levels in both groups tended to be very low at birth. In the Active group, the mattresses were supplied and so all were new. It is likely that many of the parents in the Control group bought new cot mattresses for the baby also, explaining why the allergen levels should be so low in both groups. We have previously shown that in the Control group over the first year of life, the ordinary cot mattresses accumulated allergen much more readily than the allergen-impermeable manufactured mattresses of the active group [6]. At some time between the ages of 1 and 3 years, most children in the study moved from sleeping in a cot to sleeping in a bed, and in the Active group, the families were supplied with allergen-impermeable covers for the mattress, duvet and pillow. As was seen for the parental bed, the allergen-impermeable bedding used by the Active group in the child's bed was much more effective at reducing the rate of accumulation of all three allergens when compared to the uncovered, ordinary beds of the Control group.

The vinyl flooring used in this study was extremely effective at reducing the amount of recoverable allergen in the active group. The smooth surface of such flooring allows for very effective cleaning as there is nowhere for dust particles to get trapped, and so dust samples collected from these floors tended to be small. Because appointments to collect dust samples were pre-booked by the visiting technicians, it is possible that families vacuumed immediately before the visit. However, this behaviour is likely to have occurred in both the Control and Active groups, and still the levels of all three allergens were lower in the Active group.

The Acarosol had no effect on the level of Der p 1 in those homes in which it was used. This problem has been encountered with Acarosol before; this product works well in a laboratory setting [21], but needs to be applied vigorously and repeatedly to carpets and so families fail to comply with this control measure. Our findings concur with those of previous studies that Acarosol is ineffective in reducing mite allergen levels in the clinical trial setting [5]. The levels of Der p 1 increased in the living room floor between birth and age 3 years. This finding is difficult to explain. During this time the carpets have got older and we have previously shown that increasing age of the carpet is a significant risk factor for higher Der p 1 levels in the carpet [22]. However, we know that at recruitment in early pregnancy, 32% of the carpets were already more than 5 years old, with 42.3% between 1 and 5 years old; that is the carpets sampled during pregnancy and at birth were not brand new carpets which have acquired a high allergen load by 3 years. Almost one in three subjects moved house between birth and age 3 years, but the difference in Der p 1 levels between birth and 3 years is even more significant when this group is excluded from the analysis. Clearly, the levels of Der p 1 have increased within the study homes, and the effect is not because some families have moved to high allergen homes (just the reverse in fact). We have also investigated the effect of siblings/number of children living in the home on levels of Der p 1 in the carpet, but there was none (data not presented).

We were surprised to find that cat and dog allergen levels fell within the living room floor over time, and this finding persisted when those who had acquired pets and those who had moved house during the study were excluded from the analysis and we have no explanation for this.

We conclude that environmental control measures are acceptable to families and once in place are effective over more than 3 years. In addition to the effects on mite allergen levels, they are also effective at reducing exposure to cat and dog allergen in homes without cats. It remains to be seen what effect this will have on the development of sensitization and allergic disease in the two groups of children.

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