ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2015, Vol 22, No 1, 17–22
www.aaem.pl

Child car seats – a habitat for house dust mites and reservoir for harmful allergens

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Clarke D, Gormally M, Sheahan J, Byrne M. Child car seats – a habitat for house dust mites and reservoir for harmful allergens. Ann Agric Environ Med. 2015; 22(1): 17–22. doi: 10.5604/12321966.1141362

Abstract

Introduction and Objective. House dust mites produce allergens which can cause or aggravate diseases such as asthma, eczema and rhinitis. The objectives of this study are to quantify typical house dust mite and Der p 1 allergen levels in child car seats, and to determine external variables that may influence mite populations in cars.

Materials and Methods. Dust samples were collected from the child car seats and driver seats of 106 cars using a portable vacuum sampling pump over a two minute sampling period. Mites were counted and identified and results were expressed as mites per gram (mites/g) of dust, while Der p 1 content of samples were measured by enzyme-linked immunosorbent assay (ELISA). Questionnaires were completed by participants to identify environmental and behavioural effects on mite populations. Results were analysed using General Linear Model (GLM) procedures.

Results. Twelve species of mites, of which nine are known to produce harmful allergens, were recorded from 212 dust samples. Over 80% of drivers' seats and over 77% of child car seats harboured dust mites with a significant correlation ($p = 0.001$) between the mites/g of dust and Der p 1 content recovered from each seat. A mean of 53 mites/g of dust per seat was recovered, with a mean Der p 1 level of 1.1µg/g. Over 12% of driver seats and 15% of child car seats contained house dust mite levels sufficient to be risk factors for sensitisation and allergic reactions.

Conclusions. Child car seats and driver seats are habitats to a range of mite species which can be present in sufficient concentrations to cause or aggravate allergen related illnesses in individuals who are genetically predisposed.

Key words

house dust mites, house dust mite allergens, child car seats

INTRODUCTION

House dust mites, which are ubiquitous in the home (particularly in mattresses, carpets and upholstery) produce allergens, and are widely recognised as one of the leading factors in the development of house dust atopy, which can cause or aggravate symptoms of asthma [1, 2, 3], eczema [4] and rhinitis [5]. To date, the majority of studies investigating house dust mites and their associated allergens have been carried out in the home [4, 5, 6] or in public buildings, such as libraries and hospitals [6], schools [7] and hotels [8]. Fewer studies have examined different modes of transport as foci for dust mite populations, with many of these focussing primarily on allergen levels in public transport vehicles. Of these, a Japanese study of passenger trains recorded high mite antigen levels corresponding to >100 mites/m^2 [9] in contrast to low mite allergen concentrations reported for public transport systems in Helsinki, Finland [10], for aircraft in New Zealand [8], and for buses and trains in Manchester, UK [11]. In Brazil, a significantly greater percentage of taxis (42%) harboured sensitising levels (≥ 2 µg/g) of Der 1 allergen compared to private cars (5%) [12], while another study in the same country reported low levels of Der p 1 and Der f 1 from 60 private vehicles [13]. Of those few studies which examined mites in vehicles, 10 mite species were reported from 16 out of 22 samples collected from the seats of passenger trains in Glasgow, Scotland, with *Dermatophagoides pteronyssinus* being the most abundant species found [14]. In addition, a US study of automobile driver seats recorded 42.5 – 81.3 mean mites/g of dust from 139 automobile driver seats, with 23% of samples bearing sensitising Der 1 allergen levels exceeding 2 µg/g [15].

A recent survey conducted by the UK Department of Transport found that people spend an average of one hour per day travelling, with 64% of trips being made by car [16], while in the US, time spent travelling by car has been estimated at more than 18 hours per week [17]. The Irish Central Statistics Office (National Travel Survey 2012) reported that 69% of commuters and 62% of children travel to work and school by car, respectively, with the average person in Ireland spending 7 hours per week travelling by car [18]. With recent changes in European law with respect to child safety in vehicles (EU Directive 2003/20/EC), it is now compulsory for a child to use the correct child seat or booster cushion when travelling in a motorised vehicle. Upholstered seats in vehicles typically consist of polyester and/or cotton. These materials easily accumulate shed human skin scales and other organic detritus which form the main food component of house dust mites.

To-date, there have been no attempts made to quantify typical house dust mite numbers, determine species composition or measure house dust mite allergen levels in child car seats. This indicates an important knowledge gap that currently exists, given that young children are particularly susceptible to becoming sensitised to house dust mite allergens [19, 20]. In the presented study, both house

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Received: 01 September 2014; accepted: 21 January 2015

dust mite populations and Der p 1 allergen levels in child car seats and driver seats were quantified. In addition, the densities and ranges of house dust mites and allergen levels in both seat types were compared, with a view to assessing, through questionnaires, the environmental and behavioural factors which influence house dust mite densities in cars.

MATERIALS AND METHODS

Collection and analysis of dust samples. Dust samples were collected from both child car seats and driver seats from 106 cars at 6 different locations in the west of Ireland from May – July 2011. Information leaflets outlining the research were distributed to 5 participating educational establishments, and subsequently sent to parents prior to the scheduled sampling days. In addition to collecting dust samples from cars, a questionnaire was given to each participant to elucidate those factors which may influence house dust mite populations within the car environment. Sampling was carried out using a battery powered pump, model Flite 2 made by SKC Inc. Air flow was set to the maximum 26 L/min. Two samples, one from the driver seat and one from the child car seat were taken from each car. Samples were taken by vacuuming the entire surface area of each seat over a 2 minute period. Dust was collected in a plastic cassette containing a track-etched polycarbonate membrane filter (Whatman), diameter 37 mm and pore size 0.4 μm, which was connected to the pump via plastic tubing. Dust samples were subsequently stored at –20 °C to: (a) kill any live mites present, thereby preventing artificially elevated numbers of mites as a result of breeding, and (b) preserve collected mites until counting and identification commenced. House dust mite extraction was carried out using a modified approach described previously [21]. Mites were counted and carefully identified with the aid of identification keys [22, 23] under a phase contrast compound microscope (Fisherbrand Max Bino II) at 100×magnification.

Der p 1 determination. In addition to house dust mite analysis, 71 samples (37 driver seats, 34 child car seats) with sufficient dust remaining (50 mg) were analysed for allergen content (Airmid Health Group, Ltd.). Dust samples were extracted in phosphate buffer saline pH 7.4 containing 0.05% Tween 20 (PBS-T). Samples were centrifuged and supernatants obtained were stored at -20 °C before being subjected to Group 1 *D. pteronyssinus* (Der p 1) allergen measurement using a two-site monoclonal antibody ELISA [24]. Four dilutions of each sample were added to the plate (1: 2, 1: 14, 1: 98, 1: 686). For determining the concentration of Der p1 in the samples, at least 2 points of the dilution series should fall within the linear part of the standard curve. The mean of these values was used as the final result for Der p 1 concentration. Results of the absorbance were expressed in micrograms per gram of dust (μg/g), with a detection limit of 0.025 μg/g for Der p 1.

Statistical procedures. Mite data were analysed using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, 2011) and Minitab 16 Statistical Software (2010). Statistical procedures used included Pearson correlation tests, paired-samples t-tests and GLM procedures. Although individual mites were counted from each sample, results, for the purpose of reporting means, medians and exposure thresholds, are expressed as mites/g of dust. At each combination of environmental and behavioural input variables used in the analysis, mite counts per gram in a random gram of dust tended to have a Poisson distribution; therefore, for the purpose of the inferential analysis, it was decided to transform to a variable defined as the square root of mites/g of dust. Using this as a response variable throughout the entire analysis, it was possible to conduct General Linear Model (GLM) procedures while adhering reasonably well to the assumptions on which these rely. This is an alternative procedure to using Poisson regression or negative binomial regression. Correlation coefficients and their significance were computed for several pairs of variables, partly to flag potential multicollinearity issues when GLM analyses were conducted. In addition, Tukey's *post hoc* test was used to determine significant differences between estimated mean effects of pairs of values of particular input variables in the GLM.

Selection of input variables. One of the primary aims of this study was to determine the variables that influenced house dust mite populations in driver seats and in child car seats. Over 30 questions were included in a questionnaire in an attempt to obtain as much information as possible about the physical and behavioural characteristics of the participants which may have influenced mite populations. After preliminary analyses, these were reduced to 4 input variables for the purpose of statistical analyses. Where possible, these 4 variables were selected on the basis of existing knowledge of house dust mites. The input variables used (separately) in the statistical analyses for the driver seat and child car seat data were as follows: 1) time spent by the driver or child in the car per week; 2) age of the driver or child car seat; 3) time since the car was last vacuumed; 4) whether or not the driver or child was atopic (i.e. predisposed to developing hypersensitive allergic reactions).

The input variables concerning time spent in the car per week were partitioned into 5 categories: <1 hr, 1 – <3 hrs, 3 – <6 hrs, 6 – <10 hrs, and ≥10 hrs per week. The exact age, in years, of the driver seat was determined from the registration number plate on each car. However, only an approximate age (in years) of child car seats could be determined from the questionnaire to respondents. This was more difficult to determine since a number of child car seats were only a few months old, whereas others were used by several children of the same or different families over many years. For this reason, child car seat age was divided into the following 3 categories for the purpose of data analysis: <1 yr, 1 – <3 yrs, and ≥3 yrs. For the input variable 'time since the car was last vacuumed', 2 categories <2 months and ≥2 months were chosen, which coincide closely with the natural lifecycle of a typical house dust mite in optimum conditions [23].

RESULTS

Descriptive statistics. Mites were identified to species level where possible, but in some cases (due to missing appendages or imperfect specimens) were listed as members of a specific family or order. A total of 1,060 specimens were found in 212 samples of dust, with over 12 species of mites from 7 different families identified (Tab. 1). Nine out of the 12 species (75%) are known allergen producers [23, 25]. The pyroglyphid house dust mite species *D. pteronyssinus* was

Table 1. Species of mites found in the 106 cars sampled in this study

Family/Order	Species	Specimens found		
		Driver seats	Child car seats	Total (% of total)
Pyroglyphidae	*Dermatophagoides pteronyssinus**	464	366	830 (78.3%)
	*Dermatophagoides farinae?**	4	3	7 (0.7%)
	*Euroglyphus maynei**	38	33	71 (6.7%)
Acaridae	*Acarus farris**	7	4	11 (1.0%)
	Acarus sp.	2	4	6 (0.6%)
	Acaridae (unidentified)	2	7	9 (0.8%)
	Tyrophagus sp.*	6	15	21 (2.0%)
	*Lepidoglyphus destructor**	0	2	2 (0.2%)
Glycyphagidae	*Glycyphagus domesticus**	1	7	8 (0.8%)
	*Gohiera fusca**	2	2	4 (0.4%)
Cheyletidae	*Cheyletus* sp.*	8	4	12 (1.1%)
Tarsonemidae	tarsonemids (unidentified)	9	11	20 (1.9%)
Mesostigmata	mesostigmatids (unidentified)	8	5	13 (1.2%)
Oribatida sensu lato**	oribatids (unidentified)	0	1	1 (0.1%)
	unidentifiable desiccated mites	20	25	45 (4.2%)
	Total	571	489	1060

* Indicates species known to produce allergens. Species marked with "?" indicate that although most taxonomic characteristics of that species were present, a defining feature was either missing or obscured, preventing complete confirmation of the species.** The suborder Oribatida (formerly order Oribatida, now within the order Sarcoptiformes) has recently been re-classified to include the cohort Atigmatina, together with the families Pyroglyphidae, Acaridae and Glycyphagidae [31]. Hence, 'Oribatida sensu lato' refers to oribatids without astigmatid mites

the predominant species with 830 specimens found in total. Records of the pyroglyphid species *Euroglyphus maynei*, the second most abundant species (71 specimens found) and *Dermatophagoides farinae* (7 specimens found – see footnote for Table 1) have, to the best of the authors' knowledge, not been published previously for Ireland. Of the pyroglyphid species identified, various life stages were recorded, including larvae (3.0%), protonymphs (9.1%) and tritonymphs (12.3%). Of the 347 female pyroglyphid specimens identified, 45 (13.0%) were gravid specimens (Tab. 2).

Of the 106 driver seats and 106 child car seats sampled, 80.2% and 77.4%, respectively, contained dust mites (78.8% of car seats overall). The median/mean ± S.D. mites/g of dust for driver (28.34/53.03 ± 74.49) and child car seats (23.34/53.10 ± 80.34) were similar, although the variation between samples overall was large (Fig. 1). Of the driver and child car seats sampled, 12.3% and 15.1%, respectively (13.7% overall), exceeded the accepted lower threshold for sensitisation of 100 mites/g of dust [20]. The maximum number of mites found in a driver seat was 61 (equivalent to 406.67 mites/g of dust), while the maximum found in a child car seat was 23 (equivalent to 426.72 mites/g of dust); the samples containing these mite numbers were taken from separate cars.

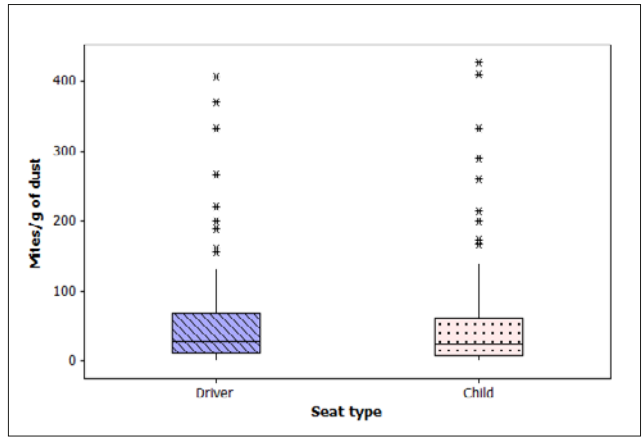

Figure 1. Mites/g of dust found in driver seats (median = 28.5, mean = 53.05) and child car seats (median = 23.5; mean = 53.11)

Der p 1 analysis. Of the 37 driver seats tested for Der p 1 concentrations, 34 (91.9%) had detectable levels of Der p 1, with a median/mean ± S.D. concentration of 0.53/1.08 ± 1.57 µg/g and a maximum concentration of 8.3 µg/g. Similar concentrations were found in 34 child car seats tested, with 29 (85.3%) bearing detectable levels of Der p 1 with a median/mean ± S.D. concentration of 0.43/1.12 ± 1.87 µg/g and a maximum of 10.03 µg/g. Of the driver seats tested, 6 (16.2%) were above the lower sensitisation threshold of 2 µg/g [20]. Five (14.7%) of the child car seats tested contained Der p 1 levels in excess of the lower sensitisation threshold, with one child seat exceeding the upper sensitisation threshold of 10 µg/g. There was a significant correlation (Pearson correlation test, $p = 0.001$ between the mites/g of

Table 2. Gender and life stages of identified pyroglyphid house dust mite specimens

Species	Adult Male (% total)	Adult Female (% total)	Gravid Female (% females)	Protonymph (% total)	Tritonymph (% total)	Larva (% total)	Gender/life stage unknown (% total)	Total (% total)
Driver seats								
D. pteronyssinus	143 (30.8)	184 (39.7)	22 (11.0)	41 (8.8)	69 (14.9)	10 (2.2)	17 (3.7)	464 (91.7)
E. maynei	12 (31.6)	16 (42.1)	1 (0.5)	0 (0)	3 (7.9)	3 (7.9)	4 (10.5)	38 (7.5)
D. farinae	4 (100.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (0.8)
Driver total	159 (31.4)	200 (39.5)	23 (11.5)	41 (8.1)	72 (14.2)	13 (2.6)	21 (4.2)	506
Child car seats								
D. pteronyssinus	125 (34.2)	134 (36.6)	22 (15.0)	41 (11.2)	38 (10.4)	10 (2.7)	18 (5)	366 (91.0)
E. maynei	13 (39.4)	13 (39.4)	0 (0)	1 (3)	2 (6.1)	4 (12.1)	0 (0)	33 (8.2)
D. farinae	3 (100.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.7)
Child total	141 (35.1)	147 (36.6)	22 (15.0)	42(10.4)	40 (10)	14 (3.5)	18 (4.5)	402
Overall	**300 (33.0)**	**347 (38.2)**	**45 (13.0)**	**83 (9.1)**	**112 (12.3)**	**27 (3.0)**	**39 (4.3)**	**908**

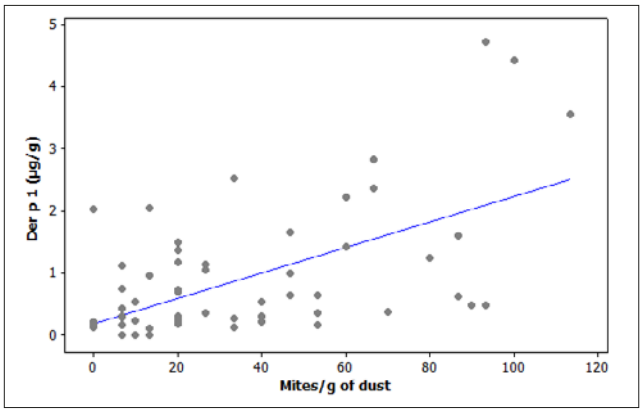

Figure 2. Correlation of house dust mites (mites/g dust) with concentrations of Der p 1 allergen (µg/g) measured from 71 car seats (Pearson correlation test, $p = 0.001$)

dust recovered and the corresponding concentration of Der p 1 detected in the car seats tested (Fig. 2).

Inferential analysis. A GLM was fitted to the driver and child car seat data using 'square root of mites/g of dust' as the response variable in each case. For the driver seat data, input variables 1 – 4 were used in the analysis i.e. (1) 'time spent by driver in the car per week', (2) 'age of the driver seat', (3) 'time since the car was last vacuumed', and (4) 'whether the participant was atopic or not'. The resulting p-values obtained from these analyses are shown in Table 3. Input variable 1 'time spent by driver in the car per week' showed a significant ($p = 0.010$) effect on the response variable, with more mites/g of dust recovered from seats where drivers spent 6-<10 hours per week in their car, compared to 1-<3 hours (Tukey's test, $p = 0.017$). Also for driver seats, input variable 3 'time since the car was last vacuumed' showed a significant effect ($p = 0.049$), while input variable 4 'whether the participant was atopic or not' showed a significant effect ($p = 0.042$) on the response variable for the child car seat data (Tab. 3; Fig. 3). Although the GLM did not show any evidence of an effect of the age of child car seats on the response variable ($p = 0.181$), there was a slight upward trend, indicating that more mites were present with increasing age of the child car seats (Fig. 4). A significant positive correlation (Pearson correlation test, $p = 0.000+$) existed between the mites/g of dust found in driver seats and child seats of the same car (Fig. 5), illustrating that, in general, if densities of mites were high in one seat in the car they were also high in the other seat (ignoring the possible effect of other variables). Based on this information, it was unsurprising that there was no significant difference (paired-samples t-test) between the mean mites/g of dust in driver seats compared to that of child car seats of the same car ($p = 0.749$).

Table 3. General Linear Model effects of input variables (with p-values) on the square root of mites/g of dust recovered from driver seats and child car seats

	Driver seats	Child car seats
Atopic person present	$p = 0.460$	$p = 0.042$*
Seat age	$p = 0.242$	$p = 0.181$
Seat vacuumed	$p = 0.049$*	$p = 0.513$
Time spent in car	$p = 0.010$*	$p = 0.713$

* indicates a statistically significant p-value

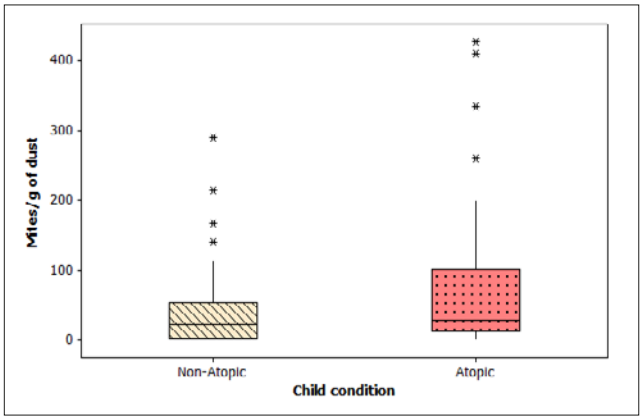

Figure 3. Mites/g of dust found in child car seats where the child was non-atopic (median = 21.10, mean 40.25) and atopic (median = 28.34, mean = 80.30). Child condition showed a statistically significant effect on the square root of mites/g of dust in the General Linear Model analysis ($p = 0.042$)

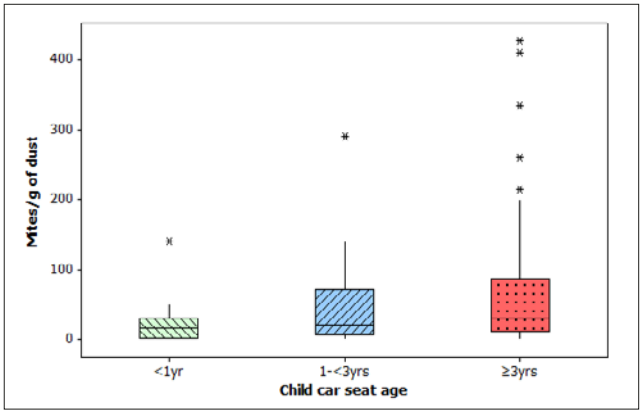

Figure 4. Square root of mites/g of dust for 3 child car seat age categories: <1 year (median = 15.12, mean = 26.05); 1-<3 years (median = 20, mean = 41.42); and ≥3 years (median = 30, mean = 67.28)

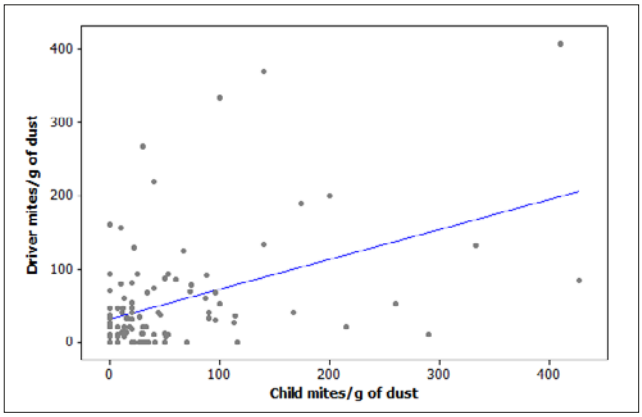

Figure 5. Correlation of square root of mites/g of dust with driver seats and child car seats of the same car (Pearson correlation test, $p = 0.000+$)

DISCUSSION

The presented study examined the presence of house dust mites in cars, and to the authors' knowledge, is the first to make particular reference to child car seats as a reservoir for mites and their associated allergens. The presence of pyroglyphid larva, protonymph and tritonymph life stages in addition to gravid female specimens indicate that the

mites recovered were breeding, resident populations living in the car seats, as opposed to dead or desiccated specimens transferred from an indoor location via clothing or some other mechanism. Twelve species of mites were identified, of which 9 are known to produce allergens associated with inducing sensitisation [23, 25]. This highlights that while pyroglyphid mites such as *D. pteronyssinus*, *D. farinae* and *E. maynei* are probably the most important and widely studied mites from an epidemiological point of view, there are other allergen producing mites which could also play a role in inducing or aggravating atopic conditions. Recent discoveries of cross-reactivity allergens in tarsonemid mites, which were found in this study, suggest that they may be important in triggering IgE antibodies in the human body [26], while other species found, such as *Acarus farris*, *Glycyphagus domesticus*, *Lepidoglyphus destructor* and *Cheyletus eruditus,* have all been linked to inducing sensitisation [25]. While these results show that there is a varied acarofauna present in car seats, the species composition is reflective of that found in typical homes [6, 23].

A mean of 53.03 mites/g of dust from 106 driver seats in this study fits within the range of 42.5 – 81.3 mites/g of dust found in 139 automobile driver seats in the USA [15]. Although mean Der p 1 levels were slightly lower (1.1 µg/g) than mean Der 1 levels in Ohio, USA (1.3 µg/g), they exceed mean levels from 60 private cars in Brazil (0.24 µg/g) [13]. A significant correlation ($p = 0.001$) between mites/g recovered and Der p 1 allergen content was observed, a result similar to those previously recorded in samples from sofas and bedding [27]. In addition, more mites were found in older child car seats than in newer ones, an observation analogous to similar studies conducted on mattresses [28], which have found that, in general, older mattresses contain more dust and bear higher concentrations of house dust mite allergen.

It is important to note that direct comparisons between this and other studies needs to be treated with caution, given that: (a) all studies consist of different sample sizes, (b) the climates and geographical locations of each study site are different, and (c) no other studies have previously sampled child car seats for house dust mite content. House dust mites have been reported to be present in higher densities in damp humid climates in contrast to dry, arid ones [29] with populations known to fluctuate seasonally [5, 6, 11, 20]. While it must be noted that the majority of studies deal specifically with dust samples collected from homes and not from car seats, it is likely that the same basic requirements for mite survival, such as suitable temperature and relative humidity, apply. Sampling in this study took place in the summer months of May, June and July, which are some of the warmer months of the year in Ireland and are likely to coincide with optimum growth conditions for mite proliferation [19]. It is probable that resident mite populations in car seats fluctuate seasonally also, with associated implications for sensitisation risks.

The input variables 'time spent in the car per week' and 'time since the car was last vacuumed' showed a significant effect on mite densities in driver seats. There was a significant difference between the number of mites recovered from seats where the drivers spent 6-<10 hours per week in their car, compared to 1-<3 hours (Tukey's test, $p = 0.017$), with higher mite densities recovered from the former category. Additionally, there was a significant difference ($p = 0.049$) between the number of mites recovered from driver seats that were vacuumed within the previous 2 months than those that were not vacuumed during this time, with lower mite densities recovered from the former category. Although the input variables 'time spent in the car per week' and 'time since the car was last vacuumed' had an effect on the response variable in driver seats, the same was not true for child car seats. The latter could be explained by the fact that in reality, the frequency of vacuuming for child car seats could indeed be higher than reported in the questionnaires, as these seats are often subjected to vacuuming or washing after a wetting episode or spillages of food or drinks. The disruption caused by such events, in addition to the fact that child seats (especially infant carriers) are often removed temporarily from cars after journeys or during vacuuming/washing, may also have had a bearing on the house dust mite densities of these seats.

There was a significant difference ($p = 0.042$) between the mean mites/g of dust recovered from seats of atopic children compared to that found in seats of non-atopic children, with higher mean mites/g of dust recovered from the former (Fig. 3). Previous investigations have reported that mattresses of atopic dermatitis sufferers tend to have denser house dust mite populations compared to those of healthy non-atopics [23], which also seems to be reflected in the results from the presented study, for children, at least. A statistically significant correlation ($p = 0.000+$) was observed between the mites/g of dust found in child car seats and driver seats of the same car, suggesting that the conditions that influenced mite propagation within the car seats were common to both seat types. It may be possible that other variables not measured in this study, such as relative humidity and temperature, may play an important role in determining house dust mite proliferation in the micro-habitat of the car seat fabrics.

CONCLUSIONS

This study confirms that child car seats are home to a range of species of house dust mites, which can be present in concentrations high enough to induce or aggravate allergen associated conditions diseases or symptoms, especially in children who are genetically predisposed. Under EU law, children under the age of 12 are required to use the appropriate child restraint in cars, an environment which until now had previously been unexplored with regard to house dust mite populations. In order to minimise exposure to house dust mites and their allergens, it is recommended that car seats are subjected to regular vacuuming, a process which has been shown to be effective in removing mites and allergens from carpets [5]. Older child car seats had higher mean house dust mite densities than newer seats, which suggests that frequent replacement or washing of child car seat covers may be required in cases where a child is genetically predisposed towards hypersensitivity due to the inhalation of house dust mite allergens. While washing clothes in hot water at temperatures >55 °C has been found to be effective at removing mites and allergens [20], it is not yet clear whether this is effective for car seat covers. Recent research involving the irradiation of house dust mite cultures with UV-C light has shown effective mortality of adults and reduction in hatchability of eggs [30], although this has not been tested on resident mite populations in their natural environments. The findings of this research should instigate future studies in an attempt to understand further

the environmental conditions which play a role in house dust mite propagation in child car seats, with a view to developing mitigation measures to abate or eradicate populations to help reduce instances of allergen associated conditions.

Acknowledgements
The authors would like to express gratitude to Owen Doherty, Erica Dix and Kevin McCaffrey for their help in the collection of dust samples and to all of the willing parents who made this study possible. Thanks to Dr. Anne Baker, Natural History Museum, London, for advice on mite identification. Also a special thanks to Airmid Health Group Ltd. who performed the allergen analysis on dust samples and to the NUI Galway College of Science Fellowship for providing the funding to carry out this research.

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