

Mattresses as a winter refuge for house-dust mite populations

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House-dust mites are a major source of allergens. Beds are often heavily infested. It is conceivable that, during the winter period in temperate climates, the ambient indoor air is too dry for their survival, but that mites can still survive in beds where the humidity is raised when the bed is occupied. To investigate whether house-dust mites can take advantage of daily episodes with elevated humidity to restore water balance, we exposed mites to spells of high humidity (90% relative humidity [RH]), lasting 0, 1.5, 3, 6, or 12 h, once every day, while the humidity was low (10% RH) during the rest of the time. Survival was markedly promoted when only 1.5 h of moist air per day was given. Three hours of moist air per day allowed the mites to reproduce. The same average humidity but at a constant level would have been lethal to the mites. Thus, average humidity can be misleading as an indicator of mite survival conditions. Measurements of relative humidity were made inside the foam core of a mattress and underneath the ticking. The rise of humidity occurring when the bed was occupied was often insufficient for the mites, because the temperature rose at the same time. At higher temperatures, mites required a higher relative humidity, abolishing the effect of the simultaneous rise in absolute humidity. Thus, it seems quite possible to regulate house-dust mite numbers also in beds by reducing the water content of the ambient air. The rules governing changes in temperature and humidity inside a bed may be simple enough to allow assessment of house-dust mite living conditions by measurements in the ambient air.

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House-dust mites constitute a major source of house-dust allergens (1). In contrast to the allergens from cats and dogs, removal of the source of mite allergens is not hindered by any emotional obstacles. But, technically, it is very difficult. Live dust-mite populations can be found in carpets, rugs, stuffed furniture, mattresses, bedding, cushions, etc. Only one control method tackles all these populations at the same time; namely, reduction of the moisture content of the indoor air. House-dust mites satisfy their demands for water by extraction of water vapour from the air (2–4). This is not possible unless the relative humidity (RH) is above a certain minimum value. This value, called the critical equilibrium humidity (CEH), depends on the temperature. During the winter in the temperate climatic regions of the world, it is relatively easy to keep the indoor RH at a low level, because then the absolute humidity (AH) of the atmosphere is

low. However, even during the winter, it may be difficult to maintain the indoor RH below the CEH without interruption. In beds, where house-dust mites live deep inside the foam core (5–7), the moisture content of the air is intermittently supplemented by water vapour emanating from the occupant's body (8–10). It has been suggested that populations can thereby survive the winter in beds, while the conditions in other parts of the home are too dry (11). Indeed, in a survey of some Dutch cottages by van Bronswijk (12), live mites were found in beds only during the winter. In contrast, Korsgaard (13) reported on a number of homes in Denmark where the humidity was generally low and house-dust mites accordingly scarce in all the items sampled; the beds were no exception. Similar findings were reported by Turos (14) and Wickman et al. (15). Hospital beds and beds in hotels have also been found to contain very low numbers of

mites or levels of allergen (16–20). Harving et al. (21) reported that dust mites disappeared from mattresses after the indoor humidity was reduced through the installation of a mechanical ventilation system. Prolonged use of a dehumidifier in 10 bedrooms also resulted in a decreased concentration of mite allergens in mattress dust, in contrast to 10 matched controls (22). These findings are difficult to explain if the occupant of a bed indeed provides sufficient moisture for house-dust mite populations to survive the dry winter period. The availability of winter refuges determines the number of mites that survive to the following spring. It seems quite likely that the number of survivors largely determines the speed by which the mite numbers increase and allergens accumulate during the subsequent spring and summer. Therefore, it is important to understand how beds can remain clear of house-dust mites, in spite of the extra moisture that is provided during the night. In the present study, this problem is approached in two steps. Firstly, it is determined how many hours per day the RH must be above the CEH for the house-dust mite population to survive. Secondly, the changes in temperature, RH, and AH in the foam core of an occupied bed are studied. On the basis of information of this kind and the published data on CEH values at various temperatures, it should be possible to define the temperature and RH conditions that must be maintained in the bedroom to prevent the colonization of mattresses by house-dust mites.

Material and methods

Mite cultures and cohorts

A long-established laboratory culture of *Dermatophagoides pteronyssinus*, reared on a medium of dried, ground *Daphnia* and yeast (1:1, v/v), was used. Mites of known age were obtained by sieving eggs from a culture and collecting them on filter paper. Hatchlings of a single day were allowed to walk off the filter paper into food medium, where they continued their ontogenic development. For exposure of mites to various regimens of RH, these hatchlings were confined, with or without food medium, in stainless steel cylindric cages (diameter: 7 mm, height: 10 mm), of which the flat sides were made of stainless steel gauze (mesh: 50 μ m).

Determining the relative numbers of live and mobile mites

At the end of an experiment, the subjected subculture was spread over a block of open-porous polyurethane foam (50 mm $\times$ 50 mm $\times$ 8 mm). Through gentle

tapping and shaking, the material sank into the foam, which was then covered by a thin layer of clean foam (50 mm $\times$ 50 mm $\times$ 4 mm). Adhesive tape was applied to the upper surface of this layer, and then the block was warmed from below (1 h, 40°C). Mites captured on the sticky tape were counted under a dissecting microscope ($\times$ 50 magnification) (23).

Counting eggs

Other subcultures were washed through sieves (90- and 63- μ m mesh) and spread over black filter paper in a Büchner funnel under suction, which was subsequently examined through a dissecting microscope ($\times$ 50) to count eggs and larvae.

Exposure of mites to a constant RH

Mite cages were suspended in airtight 100-ml vials, with, on the bottom, either a layer of dry silica gel (RH<5%) or a saturated salt solution (MgCl₂: 33%, MnCl₂: 58%, MgNO₃: 56%, NaBr: 60%, K₂CO₃: 44%, NaCl: 75%, Mg(SO₄)₂: 90%; at 20°C [24]).

Exposure of mites to 24-h cycles of high (>CEH) and low (<CEH) humidity

Six identical, specially designed appliances were used, as described previously (25). Briefly, the mite cages were placed in a perspex chamber. This chamber was fitted into the appliance so that its interior could exchange air only with the interior of one of two 1-l air-containers. The air in the containers was continuously stirred by a small fan, while the RH was controlled by a saturated salt solution (container 1) or dry silica gel (container 2) on the bottom. At set intervals, controlled by a time-switch and driven by a pneumatic system, the connection with the chamber was switched from container 1 to container 2, in which a different RH was maintained or vice versa. For the present experiments, the apparatuses were placed in a climatized room at 19 $\pm$ 0.5°C.

Temperature and humidity measurements and CEH at different temperatures

One chamber, with dimensions similar to the mite chamber, was adapted to fit the RH sensor of a Testo 451 humidity measurer (Testoterm GmbH, Lenzkirch, Germany). The sensor was fitted inside a gauze basket, to simulate more closely the situation inside a mite cage. For measuring temperature and RH in the core of a mattress, a sword probe (Hygrotest 80, Testoterm GmbH, Lenzkirch,

Germany) was introduced into a deep slit made in the side of the mattress with a sharp knife. RH and temperature were displayed directly by the instrument. AH was calculated according to Buck (26). The following simplified formulae were used: $\text{ews} = 6.1365 \cdot e^{17.502T/(240.97+T)}$ mbar, where T = temperature in °C, $\text{ew} = \text{ews} \cdot \% \text{RH} / 100$ mbar, and $\text{AH} = 0.614 \cdot \text{ew}$ g/kg. From weight changes of partially dehydrated *D. farinae*, Arlian & Veselica (4) observed that mites can maintain water balance and survive at humidities between 45 and 55% RH at 15°C, 55 and 65% RH at 25° or 30°C, and 65 and 75% RH at 35°C. The 0 intercept for weight change of a line connecting the high and low RH probably gives a close approximation of the CEH for each temperature; i.e., 53% at 15°C, 58% at 25°C, 62% at 30°C, and 68% at 35°C. An estimate of the CEH at intermediate temperatures was obtained by linear interpolation and used for comparisons and ecologic interpretations. The calculations were done expediently with a spreadsheet program (Excel). Graphs were prepared with the Cricket Graph program.

Weighing mites

For measurement of changes in body mass, single mites were anaesthetized in CO₂ gas and weighed seven times consecutively with a Sartorius Supermicro S4 balance accurate to 0.1 µg (Sartorius GmbH, Göttingen, Germany), zero-point correction being applied after each weighing (25).

Statistical evaluation

The distribution of replicate measurements (mite numbers and mite body mass) was determined as described by Sokal & Rohlf (27) (pp 412–14). Mite numbers were ln-transformed for statistical tests. Outliers were detected by the Grubbs test (27). Nonparametric statistical tests were done when a (pseudo-) normal distribution was not present or doubtful.

Results and Discussion

The CEH at 20°C

The CEH of our strain of *D. pteronyssinus* was established by weighing individual, partially dehydrated (1 week at RH < 5% at 25°C) females, exposing them to a test humidity at 20°C without food, and weighing them again (4). An increase in weight indicated uptake of water vapour, which is possible only at RH > CEH. Preliminary tests had indicated that the CEH lay between 56% and 60% RH. Five of nine mites kept at 60% RH gained

weight significantly (t -test, one-sided, $P < 0.001$ for two mites, $P < 0.01$ for two mites, and $P < 0.05$ for one mite), showing that the CEH was below 60%. None of seven mites kept at 56% and none of 10 mites kept at 58% showed a significant increase of weight, confirming that the CEH at 20°C was probably between 58 and 60% RH (i.e., 8.4–8.6 g/kg AH). The CEH of this strain at 16°C was previously determined to be in the region of 56–58% RH. The CEH of the *D. farinae* strain, referred to in this study (4), seemed to be somewhat lower still.

Cyclic (24-h) changes of RH and population growth at 20°C

For each of seven equivalent experiments, a mite culture, containing mites, eggs, and food medium, was thoroughly homogenized by gentle shaking. Portions of approximately 35 mg were weighed and introduced into separate mite cages. Of four such portions, the number of live mites was determined immediately. Other portions were subjected to one of four humidity regimens: 1.5, 3, 6, or 12 h per day in moist air (approximately 90% RH, Mg[SO₄]₂) and dry air (approximately 10% RH, dry silica gel) during the rest of the time. Invariably, four portions were subjected to the same regimen. Four portions were also placed in continuously moist air (75% RH, NaCl), and another four in continuously dry air; namely, at 33% RH (MgCl₂). At the end of the exposure period, the cages were placed at 75% RH for 48 h to allow dehydrated mites to rehydrate. Subsequently, the heat-escape method was applied, and the numbers of live and active mites were compared with the numbers recorded at the start (Table 1). Seven separate experiments were completed. Differences between equivalent experiments may have been due to differences in population structure and in the condition of the mites at the start. Therefore, different regimens were compared within experiments (t -test, two-sided; Table 1).

It is remarkable that some, though very few, mites survived 7 weeks of continuous exposure to 33% RH (4.7 g/kg AH). However, when 1.5 h of moist air was given every day, the survival rate was much higher (Mann-Whitney U-test, experiments 1, 2, and 3 combined, $n_1 = n_2 = 12$, $P < 0.001$, two-sided). During the dry period, these mites were subjected to an RH far below 33% and consequently experienced, on average, a much lower humidity; namely, 15% RH or 2.2 g/kg AH. Thus, the average air humidity may be a misleading indicator of mite survival. Although it promotes the survival of a population, a daily moist period of 1.5 h does not allow an increase of mite numbers. In all three cases, the mite density decreased significantly (Table 1) (final mite density)/(initial

Table 1. Ratios (average [$n=4$] final mite numbers)/(average [$n=4$] initial mite numbers). For each experiment, one culture of *D. pteronyssinus* was subdivided into subcultures which were exposed to humidity regimen for 7 weeks

Regimen	a	b	c	d	e	f	Mites/100 mg at start range ($n=4$)
RH	33%	90%/10%	90%/10%	90%/10%	90%/10%	75%	
h/day	24	1.5/22.5	3/21	6/18	12/12	24	
Exp. 1	0.015	0.304***	—	—	—	2.086*	548–821
Exp. 2	0.003	0.183***	—	—	—	1.870 NS	373–563
Exp. 3	0.008	0.419*	—	2.493***	2.526**	3.848***	575–808
Exp. 4	—	—	—	1.554 NS	1.723 NS	3.136**	342–842
Exp. 5	—	—	0.684 NS	—	—	1.302 NS	436–1009
Exp. 6	—	—	2.133 NS	—	—	3.190*	299–850
Exp. 7	—	—	0.328*	—	—	1.000 NS	509–1295

Significance of change in number of mites/100 mg compared to values at start was tested by *t*-test, two-sided, after log-transformation. NS: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

mite density) < 1). With 3 h of moist air per day, the mite numbers remained roughly unchanged; with 6 h per day, a significant increase was recorded. Experiments 3 and 4 underwent analysis of variance for the regimens 6, 12, and 24 h of moist air per day. The results are given in Table 2.

Increasing the length of the moist period above 6 h per day seemed to result in a larger increase of mite numbers, certainly for the step from 12 h to 24 h per day. This is an important observation because it shows that an interruption of the hydrating (RH $>$ CEH) conditions by 12 h, though it will not prevent the survival of a population, will retard reproduction and no doubt also production of allergens. It could be objected that an important part of the population – namely, eggs and quiescent stages – was missed. However, in *D. pteronyssinus*, the quiescent stages are relatively brief and never (as far as we know) prolonged, as in *D. farinae*. When a stable age distribution is attained, the eggs and quiescent stages constitute a constant proportion of the population. Laboratory cultures do not always have a stable age distribution (28). Yet it seems unlikely that a general trend in the total number of mites after 7 weeks will be seriously obscured by changes in the stage distribution.

Cyclic (24-h) changes of RH and egg production

For these investigations, four independent, replicate experiments were completed (Table 3). For each one, four groups of young adult females (< 1 week after ecdysis) were confined in mite cages without food and exposed to a regimen of 1.5, 3, 6, or 12 h of moist (90% RH) air, and dry (10% RH) air during the rest of the time. A fifth group was exposed continuously to 75% RH. After 2 days, each group was transferred to a clean mite cage, and plenty of food medium was added. Three weeks later, eggs and larvae were counted (Table 3; one cage was lost by accident). None of the 118 females

Table 2. Two-way analysis of variance of log (mite number/100 mg) of experiments 3 and 4, concerning regimens with 6, 12, and 24 h of moist air per day (see Table 1)

	<i>P</i> value
Differences between experiments	0.0035
Differences between regimens	0.0033
Interaction experiments and regimen	0.5812
6 vs 12 h moist air per day	0.7156
12 h moist air per day vs continuous moist air	0.037

that received 1.5 h of moist air daily produced offspring. The 98 females exposed to a regimen with 3 h of moist air daily produced altogether 96 eggs, i.e., one egg/female over 3 weeks. It should be noted that the average RH was 20% (2.9 g/kg AH), which is far below the CEH. Yet the mites were reproducing. Again, average RH (or AH) turned out to be misleading. With 6 h of moist air per day, the egg production was significantly higher (*t*-test after ln-transformation, $n_1 = 3$, $n_2 = 4$, $P < 0.05$, one-sided). With 12 h of moist air per day, the egg production was again higher ($n_1 = n_2 = 4$, $P < 0.05$). The highest egg production was recorded for the mites that were exposed continuously to 75% RH, but the difference from the former group was not significant ($n_1 = n_2 = 4$, $P = 0.19$).

Changes of temperature and RH in occupied beds

Recordings of temperature and RH were made underneath the upper ticking and inside the foam core, in a horizontal slit made 4.5 cm underneath the upper surface of a mattress. Measurements were recorded every 5 min before, during, and after a man (70 kg), wearing pyjamas, lay down on his back on top of it, while covered by a duvet. The sword probes were inserted so that the sensors were underneath the centre of the back or the hips. The course of temperature and humidity changes directly underneath the ticking is shown in the Figs. 1 and 2.

Table 3. Egg production (eggs/female per week) by young *D. pteronyssinus* females, while subjected to various humidity regimens. In parentheses: number of females

Regimen	b	c	d	e	f
RH	90%/10%	90%/10%	90%/10%	90%/10%	75%
h/day	1.5/22.5	3/21	6/18	12/12	24
Exp. 1	0 (14)	—	1.67 (10)	3.17 (18)	5.26 (9)
Exp. 2	0 (31)	0.67 (24)	2.55 (41)	4.45 (38)	4.62 (37)
Exp. 3	0 (38)	0.28 (39)	2.22 (40)	3.48 (35)	4.04 (31)
Exp. 4	0 (35)	0.14 (35)	1.32 (35)	5.27 (35)	4.26 (35)
Average	0	0.36	1.94	4.09	4.54
SD	0	0.27	0.55	0.96	0.53

Initially, the AH approached equilibrium at a faster rate than the temperature. This resulted in a sharp increase of RH followed by a decrease due to an ongoing increase of temperature (Figs. 1B and 2B). When the bed was vacated, the opposite effect was seen. The RH fell to a level below the ambient RH because the water vapour dissipated very fast, reaching ambient concentrations after roughly 30 min, when the temperature was still elevated (the duvet was removed as soon as the bed was vacated). Most of the water vapour emanated from the occupant's body, but, initially, when the temperature was rising, some vapour was also released by the mattress material (polyurethane foam), which absorbs varying amounts of water depending on the temperature (5). The opposite effect occurred when the bed was vacated and the temperature fell again. In the example given in Fig. 2, the RH rose above the CEH (based on *D. farinae* data) very briefly (<5 min) only in the beginning. Its duration would certainly be insufficient for house-dust mites to restore water balance. It should also be remembered that the CEH curve for *D. pteronyssinus* probably lies somewhat higher on the RH scale. The possibility that the RH remains effectively below the CEH in spite of water vapour emanating from the occupant's body is hereby illustrated.

The low bedroom temperature on this cold, dry winter day was compensated for by a relatively strong temperature increase. This had two effects. Firstly, the raised temperature was associated with a higher CEH. Secondly, the temperature increase inhibited the rise in RH. In Fig. 1, the ambient RH, i.e., the RH in the unoccupied bed, is above the CEH. This favourable situation for house-dust mites was reversed when the bed was occupied, because mites need a higher humidity when the temperature rises. The extra water vapour emanating from the occupant's body is insufficient. Changes

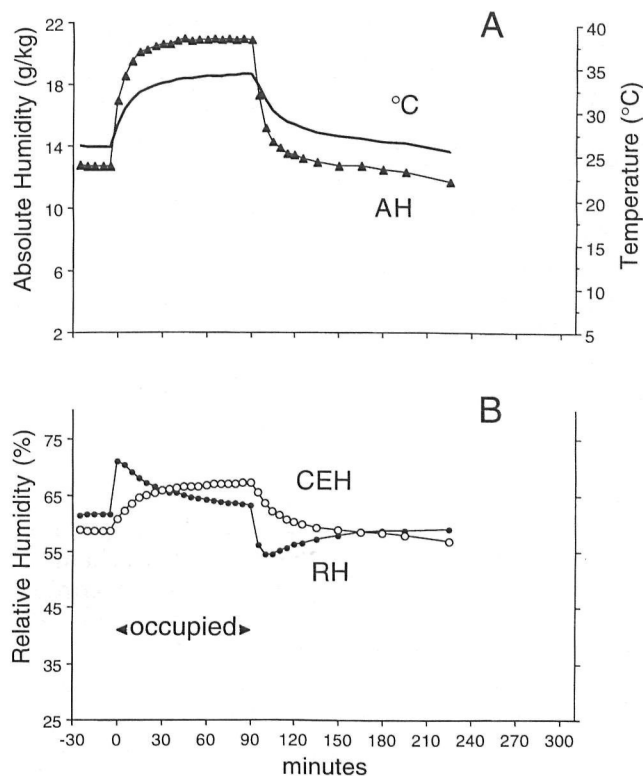


Fig. 1. Changes of temperature (°C), absolute humidity (AH), and relative humidity (RH) in occupied bed. Occupant was lying on his back. Recordings were taken directly underneath ticking at centre of occupant's back on 14 July 1992.

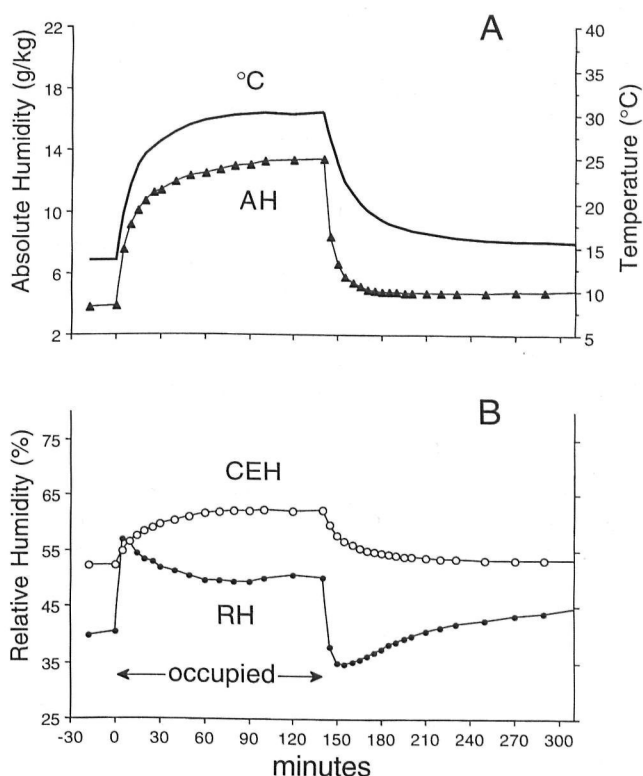


Fig. 2. As Fig. 1. Recordings were taken directly underneath ticking at centre of occupant's back on 22 February 1992.

of temperature and RH further away from the occupant's body were much more gradual, as shown in Fig. 3. In this example, the circumstances in the occupied bed approach very closely the optimal circumstances for both *D. pteronyssinus* and *D. farinae* (25°C and 75% RH [29–34]). We never observed the RH in the occupied bed to rise above the CEH, whereas the ambient air was below it. However, it is quite likely that this situation would arise if the conditions shown in Fig. 3 were just a little different, with only a slightly lower ambient AH. A summary of equilibrium values, observed on days with divergent ambient air con-

ditions, is given in Table 4. The AH was increased by 8–10 g/kg, which seems to be independent of ambient conditions. In contrast, the rise in temperature seemed to be inversely related to the ambient temperature. This meant that equilibrium temperatures were little influenced by ambient conditions, being within the range of 29–34°C directly underneath the ticking and 26.5–29.5°C at 4.5 cm below the surface. It also meant that the temperature, and, in its wake, the CEH were more strongly influenced by the occupant's body heat when the ambient air was cool. The changes of temperature and humidity occurring in occupied, upholstered chairs are probably similar. van Bronswijk has suggested that the RH may be high especially near the outside of the blankets in an occupied bed (10). More observations from near the underside of the mattress are certainly desirable. From such information, a preliminary assessment can be made of the effect that may be expected from the installation of a mechanical ventilation system (21) or of a dehumidifier (22, 35), by simply measuring humidity and temperature changes before expensive and time-consuming experiments are done.

In conclusion, house-dust mites can take good advantage of limited periods of elevated humidity, certainly when these occur every day. Findings that beds can sometimes be free of house-dust mites, in spite of regular occupation by a sweating person, can be explained by the concomitant rise of temperature when the bed is occupied. At the higher temperature, the mites require still more moisture than is supplied by the occupant. Nevertheless, it is conceivable that situations occur where house-dust mites can survive the winter period only in beds or in stuffed chairs, but these may be more exceptional than previously thought.

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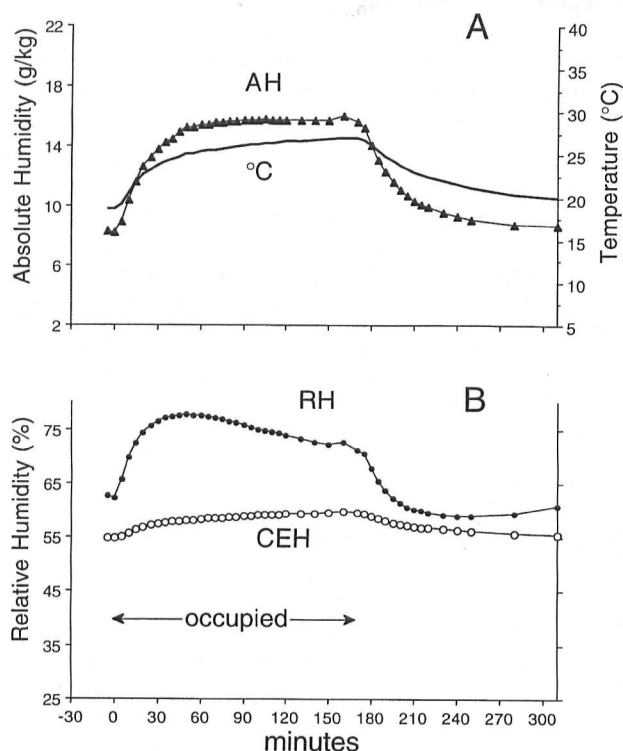


Fig. 3. As Fig. 1. Recordings were taken 4.5 cm below surface of mattress, at centre of occupant's back on 20 September 1992.

Table 4. Differences in absolute humidity (Δ AH, g/kg) and temperature (Δ temperature, °C) between ambient air and either of four positions inside occupied mattress (below centre of back or hips, directly underneath ticking, or 4.5 cm below surface), when humidity and temperature have nearly reached equilibrium values

Date (1992)	Position of probe							
	Ambient		Back, underneath ticking		Back, 4.5 cm below surface		Hip, underneath ticking	
	AH	Temperature	Δ AH	Δ Temperature	Δ AH	Δ Temperature	Δ AH	Δ Temperature
22/02	4.5	15	+9.5	+15	—	—	> +10	+15
20/09	8	18	—	—	+7.5	+8.5	+8.5	+11
25/08	11	24.5	—	—	+8	+5	+10	+9
14/07	13	26	+8	+8	—	—	+10	+7

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