



HAL
open science

Endotoxin concentration in poultry houses for laying hens kept in cages or in alternative housing systems

Adeline Huneau-Salaün, Sophie Le Bouquin, Valérie Bex-Capelle, Didier Huonnic, Loïc Balaine, Marie-Thérèse Guillam, Fabrice Squizani, Claire Ségala, Virginie Michel

► To cite this version:

Adeline Huneau-Salaün, Sophie Le Bouquin, Valérie Bex-Capelle, Didier Huonnic, Loïc Balaine, et al.. Endotoxin concentration in poultry houses for laying hens kept in cages or in alternative housing systems. *British Poultry Science*, 2011, 52 (05), pp.523-530. 10.1080/00071668.2011.617728 . hal-00745615

HAL Id: hal-00745615

<https://hal.science/hal-00745615>

Submitted on 26 Oct 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Endotoxin concentration in poultry houses for laying hens kept in cages or in alternative housing systems

Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2010-306.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	16-Dec-2010
Complete List of Authors:	Huneau-Salaün, Adeline; Anses, EBEAC LE BOUQUIN, Sophie; Anses, UEBEAC Bex-Capelle, Valérie; LHVP Huonnic, Didier; Anses, UEBEAC Balaine, Loïc; Anses, UEBEAC Guillam, Marie-Thérèse; SEPIA-Santé Squizani, Fabrice; LHVP Ségala, Claire; SEPIA-Santé Michel, Virginie; Anses, UEBEAC
Keywords:	Laying hens, dust, endotoxin

SCHOLARONE™
Manuscripts

NOTE; Please
remove box and
grid lines from
Figure 1 and 2;
axis title needs to
be changed. This
may require
authors input.

CBPS-2010-306
Edited Randall July 2011

CBPS-2010-306 Edited
Hocking 13 August 2011

**Endotoxin concentrations in poultry houses for laying hens kept in cages or in
alternative housing systems**

A. HUNEAU-SALAÜN, S. LE BOUQUIN, V. BEX-CAPELLE¹, D. HUONNIC, L.
BALAINE, M.- T. GUILLAM², F. SQUIZANI¹, C. SEGALA², AND V. MICHEL

*Anses-French Agency for food, environmental and occupational health safety, BP 53
Route de Beaucemaine, 22440 Ploufragan France; ¹LHVP- Laboratory of Hygiene of
Paris, 11 rue Georges Eastman, F-75013 Paris, France; and ²SEPIA-Santé, 31 rue de
Pontivy, F-56150 Baud, France*

Running title:
Endotoxin concentrations in henhouses

Accepted for publication:
15th Jan 2011

Correspondence to: Adeline Huneau-Salaün, Anses, BP 53, F-22440 Ploufragan, France. Tel: + 33 296 01
62 22, Fax: + 33 296 01 62 23, E-mail: Adeline.HUNEAU@anses.fr

1
2
3
4
5 22 **Abstract** 1. Endotoxins as components of organic dust may have adverse effects on the
6
7 23 respiratory health of workers in poultry buildings. The move towards more welfare-
8
9 24 friendly housing systems for layers may increase worker exposure to air contaminants
10
11 25 due to the use of litter.
12
13
14 26 2. The endotoxin concentrations in the inhalable fraction of airborne dust (below 100
15
16 27 μm) from cage and alternative system houses (on-floor, free range and aviaries) were
17
18 28 compared under both experimental and commercial conditions.
19
20
21 29 3. The endotoxin concentration was higher in experimental aviaries (median: 565
22
23 30 EU/m^3 , range: 362-1491 EU/m^3) than in cage housing (98 EU/m^3 (51-470)).
24
25
26 31 4. In field conditions, the endotoxin concentration in the air of 13 alternative houses was
27
28 32 higher (35 to 3156 EU/m^3) than in cage system buildings (n=8, 78-576 EU/m^3). It was
29
30 33 correlated to the respirable dust concentration (fraction below 5 μm) and to the
31
32 34 temperature inside the henhouse but no seasonal variation was observed.
33
34
35 35 5. The present study emphasises that considerable worker exposure to endotoxins may
36
37 36 occur in laying houses, especially in alternative systems.
38
39
40
41
42
43
44
45

42 INTRODUCTION

46
47 40 The high frequency of respiratory health problems among workers in poultry
48
49 41 confinement buildings has often been reported (Radon *et al.*, 2002b; Kirychuck *et al.*,
50
51 42 2003; Rylander and Carvalheiro, 2006). The air in poultry houses is known to be
52
53 43 contaminated by various potentially hazardous materials including gases (e.g. NH_3),
54
55 44 chemicals such as disinfectants, and organic and inorganic dust. Organic dust in poultry
56
57 45 houses consists of a complex combination of feed, litter, animal material such as
58
59
60

1
2
3
4
5 46 feathers and skin, and faecal particles (Ellen *et al.*, 2000). It also contains high
6
7 47 concentrations of airborne microorganisms such as fungi, viruses, bacteria, and their
8
9 48 constituents (Jones *et al.*, 1984; Seedorf *et al.*, 1998; Radon *et al.*, 2002a; Lee *et al.*,
10
11 49 2006). Endotoxins, derived from the outer membrane of Gram negative bacteria,
12
13 50 constitute a major component of organic dust (Rylander, 2002). Endotoxins exhibit
14
15 51 proinflammatory properties and are therefore implicated in the aetiology of occupational
16
17 52 lung diseases including asthma-like syndrome, organic dust toxic syndrome (ODTS)
18
19 53 and chronic airway obstruction (Schenker *et al.*, 1998; Rylander, 2002; Douwes *et al.*,
20
21 54 2003). High levels of endotoxins in poultry confinement buildings have been reported
22
23 55 (Clark *et al.*, 1983; Jones *et al.*, 1984; Seedorf *et al.*, 1998; Schriel *et al.*, 2007). A
24
25 56 relationship between poultry worker exposure to endotoxins and the occurrence of
26
27 57 respiratory symptoms has been established in some studies (Thelin *et al.*, 1984;
28
29 58 Donham *et al.*, 2000; Kirychuk *et al.*, 2006). In laying hen and turkey buildings an
30
31 59 exposure concentration higher than 614 EU/m³ endotoxins was associated with
32
33 60 pulmonary function decrements in workers (Donham *et al.*, 2000). Interestingly, aerial
34
35 61 concentrations of endotoxins and their consequences on human health appear to vary
36
37 62 according to the bird housing system. Kirychuk *et al.* (2006) reported that endotoxin
38
39 63 concentrations in cage-housed poultry farms tended to be greater than in on-floor broiler
40
41 64 farms, and that workers in cage systems more frequently reported current and chronic
42
43 65 phlegm. In experimental layer houses, Larsson *et al.* (1999) observed that exposure to
44
45 66 airborne dust from on-floor houses induced a more acute inflammatory reaction in the
46
47 67 upper airways of naïve subjects than exposure to dust from cage system houses. This
48
49 68 was related to higher concentrations of inhalable dust and ammonia, but not of
50
51 69 endotoxins, in the air of the non-cage buildings. However, these studies have not
52
53
54
55
56
57
58
59
60

1
2
3
4
5 70 completely taken into account recent developments in hen housing systems, especially
6
7 71 in Europe. Indeed, to improve animal welfare, the European Directive 1999/74/EC
8
9 72 requires the abolition of conventional cages for housing laying hens from 2012
10
11 73 onwards. Alternatives such as furnished cages with litter or loose systems have been
12
13 74 proposed. In France, 80% of laying hens are still kept in cages but conventional cages
14
15 75 are gradually being replaced by furnished cages which include a nest box, a pecking and
16
17 76 scratching area with litter, and 15 cm of perch per bird. The most common alternative
18
19 77 system in France is the on-floor henhouse in which the building is divided into a slatted
20
21 78 area with perches and nest boxes, and a litter area. The development of housing systems
22
23 79 where hens can move freely and are provided with litter seems to lead to an increase in
24
25 80 airborne dust concentration, but few comparisons of cage and alternative hen housing
26
27 81 systems have been carried out since the adoption of the European Directive for the
28
29 82 protection of laying hens (Protais *et al.*, 2003; de Reu *et al.*, 2009; Nimmermark *et al.*,
30
31 83 2009). There is therefore a lack of data to assess the impact of housing system
32
33 84 modifications on the working environment. Thus a French epidemiological study, called
34
35 85 AIRPOUL project, was carried out to characterise more precisely the air quality and
36
37 86 worker exposure to aerial dust in cage and alternative systems for laying hens. This
38
39 87 project was based on an experimental assay, followed by an observational field survey.
40
41 88 The experimental assay was focused on measuring the personal exposure of workers to
42
43 89 air pollutants, while the field study assessed air quality in poultry buildings under
44
45 90 commercial conditions. The first objective of the present study, within the framework of
46
47 91 the AIRPOUL project, was to determine the personal exposure to endotoxins of
48
49 92 stockmen working in a cage system and an aviary system under experimental
50
51 93 conditions. Secondly, the ambient endotoxin concentrations were determined and
52
53
54
55
56
57
58
59
60

1
2
3
4
5 94 compared with cage and on-floor buildings for laying hens under commercial
6
7 95 conditions.
8
9
10 96

11 97 MATERIALS AND METHODS

12 98 **Experimental trial**

13 99 *Experimental facilities*

14
15
16
17
18
19 100 The study was performed in 2006 in two laying houses on an experimental farm located
20
21 101 in Brittany (France). In the first building, hens were kept in two identical aviaries
22
23 102 separated by a wire netting fence. Each aviary consisted of platforms on three levels and
24
25
26 103 housed 2,680 hens at a density of 9 hens per m² (409 cm² of litter per hen). The second
27
28 104 poultry-house was equipped with three batteries of cages on three levels. The cages
29
30 105 were conventional with 5 birds per cage (580 cm² per hen). There were 5760 hens. A
31
32
33 106 high-extraction forced ventilation system operated in both buildings. Hens and
34
35 107 husbandry management (feeding, watering and lighting programs) were the same in
36
37 108 both the cage and aviary houses.
38
39
40 109

41 110 *Endotoxin and dust sampling.*

42
43
44 111 The endotoxin concentrations in the inhalable dust fraction (diameter < 100 µm) were
45
46
47 112 measured 7 times in the ambient air when the hens were between 59 and 66 weeks of
48
49 113 age. The air sampler for endotoxin sampling (CAP 10, ARELCO, Auxerre, France) was
50
51
52 114 placed 1.5 m above the ground in the middle corridor of the cage building, and near the
53
54 115 separating fence at the height of the second platform in the aviary house. The air flow
55
56 116 was 1 l/min as specified by the manufacturer and was checked before and after sampling
57
58
59 117 with a soap bubble flowmeter (BUCK Calibrator M5, ARELCOA 5% change of flow
60

1
2
3
4 118 rate between the two measurements was considered acceptable. Sampling took place
5
6
7 119 from 0800 h to 1600 h during the same working day in both the cage building and
8
9 120 aviary house. Personal exposure to endotoxin of the workers taking care of the birds
10
11 121 was assessed three times during the same period. The workers wore the air sampler in
12
13 122 the breathing zone during a 6-hour work shift. During this period, their main activities
14
15 123 were collecting and sorting eggs, and making bird and mortality checks. Endotoxin
16
17 124 samples were collected on 37 mm diameter glass fibre filters with a pore size of 0.5 μm
18
19 125 (Millipore AP4003705, St Quentin, France), aseptically placed in three-part polystyrene
20
21 126 filter holders (Millipore M000037AO) in a constant airflow pump (SKC 224-PCTX8,
22
23 127 ARELCO) operating at a rate of 1 l/min (air flow checked before and after measuring).
24
25 128 The two workers involved in the study were non-smokers; one worked in the cage
26
27 129 building and the other in the aviary system. Neither suffered from chronic respiratory
28
29 130 diseases.

30
31
32
33
34
35 131 Ambient dust was measured fortnightly when hens were 19 to 63 weeks old.
36
37 132 Samples for the respirable dust fraction ($< 5\mu\text{m}$) were collected using a stationary
38
39 133 sampler (CAP 10, ARELCO), equipped with a pre-weighed filter with a pore size of 4
40
41 134 μm . The samplers were located in the same places as the stationary air samplers for
42
43 135 endotoxin sampling. They ran for about 8 h during the day of measurement. The suction
44
45 136 pump was operated at a rate of 10 l/min and was checked before and after sampling. All
46
47 137 exposed filters were subsequently reweighed (AG 104, Mettler Toledo, Viroflay,
48
49 138 France) after desiccation for 12 h at 37°C. The results were calculated according to air
50
51 139 volume and expressed as mg/m^3 .

52
53
54
55
56
57
58
59 141 **Field trial**
60

1
2
3
4
5 142 *Farm sample*

6
7 143 The field study was carried out in 2008 on a sample of 21 laying houses stratified
8
9 144 according to housing system: 8 poultry houses where hens were kept in cages, and 13
10
11 145 buildings where they were housed in an alternative system. Ten of the 13 alternative
12
13 146 farms were specialised in free-range production, and in the remaining three houses the
14
15 147 hens were housed in aviaries. Farms were selected according to the willingness of the
16
17 148 owners to participate in the study. The main characteristics of these farms are shown in
18
19 149 Table 1. Cage buildings were characterised by their large size and forced ventilation
20
21 150 system, whereas the smaller poultry houses in free-range systems were equipped with a
22
23 151 natural ventilation system. The cages on two farms were furnished with a nest box and
24
25 152 perches. The aviary systems differed from the other alternative systems in that they had
26
27 153 a higher rearing density: median density of 13.6 hens/available m² (min: 13.1 - max:
28
29 154 18.5) versus 7.9 (5.7-10.7) in the free-range systems; a forced ventilation system; a
30
31 155 manure disposal system with belts; and no access to an open-air range.
32
33
34
35
36

37
38 156 Table 1 near here

39
40 157 *Dust and endotoxin sampling.*

41
42 158 One stationary sample of the respirable dust fraction and one stationary sample of the
43
44 159 inhalable endotoxins were collected twice from each poultry house: once during the
45
46 160 autumn/winter period (from October to March) and once during the spring/summer
47
48 161 period (from April to September). Thus, two dust samples and two endotoxin samples
49
50 162 were taken on each farm with the exception of two free-range farms and the three
51
52 163 aviaries, which were only visited once during the autumn/winter period. This was due to
53
54 164 veterinary problems in the two free-range farms in the spring/summer period; and to the
55
56 165 fact that the three aviaries were recruited for the study later than the other farms. The
57
58
59
60

1
2
3
4 166 endotoxin and dust samples were collected using the same methods as those used for
5
6
7 167 ambient air in the experimental assay. No personal exposure measures for endotoxins
8
9
10 168 were performed in the field study because the equipment required for this was too
11
12 169 cumbersome to be worn by the farmers during a working day under field conditions.
13

14 170

171 **Endotoxin analysis**

172 At the end of sampling, the filters were sent in their holders to the Laboratoire
173 d'Hygiène de la Ville de Paris and stored at 4°C. Within 48 h of sampling, the
174 endotoxins were extracted with 5 ml of pyrogen-free water in borosilicate vials by
175 shaking them horizontally (1500 rpm) at room temperature for 60 min. The extracts
176 were centrifuged at 1000g for 15 min in borosilicate tubes, and the supernatants were
177 then analysed. Endotoxin analysis was performed using a microtitre plate (Falcon, 96
178 flat bottomed, sterile wells) with a quantitative kinetic chromogenic Limulus
179 Amoebocyte Lysate (LAL) test (Endosafe, Charles River, L'Arbresle, France). 100 µl
180 of LAL reagent was added to each 100 µl sample. The plate was then incubated at 37
181 +/-1°C in a spectrophotometer (Sunrise, Tecan, Männedorf, Switzerland) and the optical
182 density was read kinetically at 405 nm every minute. Each sample value was compared
183 to a standard curve. *Escherichia coli strain O55:B5* endotoxin (CSE Lot: EX51722;
184 LAL Reagent Lot: V2702E; potency 17 endotoxin units (EU) per ng) was used to
185 construct the standard curve. Data were analysed using Endoscan software. Inhibition
186 and enhancement phenomena were checked by adding a defined amount of standard
187 endotoxin to each sample. These interferences were reduced by serial dilution of the
188 sample (e.g. full-strength, 1:10 and 1:100). The endotoxin analysis followed the
189 European Standard EN 14031 (2003). Results were expressed in EU per cubic metre of
190

1
2
3
4
5 190 air (EU/m³). The detection limit was 0.005 EU/ml and the upper limit of the standard
6
7 191 curve was 50 EU/ml. A value of half that of the detection limit (0.0025 EU/ml) was
8
9 192 assigned to samples with concentrations below the detection limit. Given that sampling
10
11 193 and analysis required the use of pyrogen-free material, the polystyrene filter holders
12
13 194 were cleaned by sonication in 0.05 % triethylamine for 10 min, rinsed three times in
14
15 195 pyrogen-free water and dried at 50°C in an oven. The glass fibre filter and glassware
16
17 196 were heated at 250°C for 90 min. Each set of 20 filter holders and filters was tested in
18
19 197 order to exclude sets with endotoxin levels higher than the detection limit. Blank field
20
21 198 filters were used as controls for endotoxin contamination during transport and sampling
22
23 199 at each sampling campaign.
24
25
26
27
28
29

200

201 **Statistical analysis**

202 The data collected in the field study were not normally distributed and therefore results
203 are presented as median and range. Seasonal effect and correlation between respirable
204 dust concentrations and endotoxin concentrations were assessed using rank-based tests
205 (Spearman coefficient for correlation analysis, Kruskal-Wallis test for rank
206 comparison). The 5 farms where measures were obtained only during the autumn/winter
207 period were excluded from the seasonal-effect analysis (Wilcoxon test for rank
208 comparison on paired data). One sample, taken from a cage house during the
209 spring/summer period, was invalidated due to the high blank concentration and
210 consequently, this farm was also excluded from the seasonal effect study. Statistical
211 calculations were performed with SAS® 9.1 software.

212

213

RESULTS

1
2
3
4
5 214 **Experimental trial**

6
7 215 The endotoxin concentrations in the ambient dust samples obtained from the aviary
8
9 216 house were higher than in samples from the cage building for all but one of the
10
11 217 sampling days (Table 2). The median endotoxin concentration was thus higher ($P<0.05$)
12
13 218 in the air of the aviary house than in the cage house. Consequently the median exposure
14
15 219 of workers to airborne endotoxins was at least three times lower in the cage system than
16
17 220 in the aviary system for a 6 h work shift. Similarly, the ambient dust concentration was
18
19 221 lower in the cage system than in the aviary system for all 23 days of measurements
20
21 222 (Figure 1).

22
23
24
25
26 223 Table 2 & Fig 1 near here

27
28 224 **Field trial**

29
30 225 The median respirable dust concentrations and endotoxin concentrations in each
31
32 226 housing system are given in Table 3. As expected, the respirable dust concentrations
33
34 227 were higher ($P<0.01$) in the alternative farms than in the cage farms, especially in the
35
36 228 three aviaries. The measurements showed great variability in the alternative systems
37
38 229 compared with the cage system. As shown in Figure 2, the endotoxin content of
39
40 230 inhalable dusts was also higher and more variable in the alternative than in the cage
41
42 231 houses: the median concentration was 684 EU/m³ (35-3156) in the alternative systems
43
44 232 compared with 204 EU/m³ (78-576) in the cage systems ($P<0.01$). No seasonal effect
45
46 233 was observed on respirable dust concentrations or on endotoxin concentrations (Table
47
48 234 4), although the temperatures measured inside the buildings were actually lower during
49
50 235 the autumn/winter period than during the spring/summer period: the median of the
51
52 236 average temperature inside the buildings was 19.0°C (10.2-23.2) during the
53
54 237 autumn/winter period compared with 21.7°C (16.8-23.7) during the spring/summer
55
56
57
58
59
60

1
2
3
4 238 period ($P=0.03$). The inhalable endotoxin concentration was positively correlated to the
5
6 239 respirable dust concentration (Spearman coefficient $r=0.53$, $P<0.01$) and was negatively
7
8
9 240 correlated to the average temperature inside the poultry house during the sampling
10
11 241 period ($r=-0.40$, $P<0.01$).

12
13
14 242 Tables 3, 4 & Fig 2 near here

15
16 243
17
18
19 244
20
21 245
22
23
24
25
26 247
27
28 248
29
30
31 249
32
33 250
34
35 251
36
37
38 252
39
40 253
41
42 254
43
44
45 255
46
47 256
48
49
50 257
51
52 258
53
54 259
55
56
57 260
58
59 261
60

DISCUSSION

21 245 This study shows that high dust and endotoxin concentrations can occur in the air of
22
23 246 experimental and commercial laying houses. The endotoxin concentrations in the
24
25
26 247 ambient air, and to which workers were exposed, appeared to be high in comparison
27
28 248 with the threshold of 50 EU/m³ over 8 h proposed by the Dutch Expert Committee on
29
30 249 Occupational Standards. The American International Commission on Occupational
31
32 250 Health (Schenker *et al.*, 1998) identified that short-term exposure to concentrations less
33
34 251 than 10 ng/m³ (100 EU/m³ assuming a conversion factor of 10 EU/m³ for 1 ng/m³) had
35
36
37 252 no impact on workers' health, while a concentration above 10 ng/m³ was associated
38
39 253 with inflammatory symptoms of the airways; a concentration above 100 ng/m³ (1000
40
41 254 EU/m³) with systemic effects on health; and a concentration higher than 200 ng/m³
42
43
44 255 (2000 EU/m³) with ODTs. The effect of exposure to endotoxins on the health of
45
46 256 stockmen, particularly in alternative housing systems, may thus give cause for concern
47
48
49 257 because the common concentration limits (not specific to poultry working
50
51 258 environments) are regularly exceeded in these buildings. However, the exposure-
52
53 259 response threshold of 614 EU/m³ for workers in a poultry environment, proposed by
54
55
56 260 Donham *et al.* (2000), only appeared to have been exceeded for one working day in the
57
58
59 261 aviary system. Indeed, the exposures of workers to endotoxins in the experimental
60

1
2
3
4
5 262 assay, even in the aviaries (181-667 EU/m³), were lower than those reported in a
6
7 263 previous experimental study (83-175 ng/m³ or 830-1750 EU/m³) (Larsson *et al.*, 1999).
8
9 264 For the field study, stationary rather than personal sampling was chosen to determine
10
11 265 the exposure of both workers and animals. Indeed, deterioration of air quality may also
12
13 266 have an impact on poultry health (Michel and Huonnic, 2003), although no critical
14
15 267 concentrations of organic dust or endotoxins in the air have been established for
16
17 268 livestock. However, the stationary sampling method might lead to an underestimation of
18
19 269 human exposure to airborne endotoxins, because workers are close to endotoxin sources
20
21 270 and can even handle them during cleaning operations (Renström, 2002). Therefore, the
22
23 271 endotoxin measurements obtained by stationary sampling in our study cannot be
24
25 272 compared with the personal exposure measurements performed in studies designed
26
27 273 solely to assess human exposure. Furthermore, the endotoxin concentrations in the
28
29 274 ambient air, observed in both parts of our study, were lower than the average
30
31 275 concentrations measured with stationary samplers in 43 laying houses by Seedorf *et al.*
32
33 276 (1998) (860 ng/m³ or 8604 EU/m³); or the concentrations reported by Schriel *et al.*
34
35 277 (2007) in three on-floor houses (3,389 UE/m³ (100-21933)). These comparisons have to
36
37 278 be considered with caution because endotoxin analysis methods may differ from one
38
39 279 study to another, although Schriel *et al.* (2007) also used the European Guideline EN
40
41 280 14031.

42
43
44
45
46
47
48
49 281 A multicentric European survey under commercial conditions reported a
50
51 282 deterioration of air quality in alternative systems compared to cage systems for dust
52
53 283 concentrations (Takai *et al.*, 1998) and endotoxin concentrations (Seedorf *et al.*, 1998).
54
55 284 However, these studies date from the early 1990s, before the implementation of
56
57 285 Directive 1999/74/EC, which is likely to have led to substantial modifications to
58
59
60

1
2
3
4
5 286 housing systems in Europe and to have altered air quality in poultry houses. Our study
6
7 287 thus confirms that air quality may deteriorate after the adoption of modern alternative
8
9 288 housing systems which comply with the recent European regulation. Differences in dust
10
11 289 and endotoxin concentrations between the cage and alternative systems may be due to
12
13
14 290 the presence of litter and to the greater activity of the hens in the on-floor buildings. In
15
16 291 addition, the natural ventilation systems in the alternative houses, in contrast to the
17
18 292 forced ventilation systems in the cage buildings, could lead to a lower ventilation rate
19
20
21 293 and thus a lower clearance rate of air-borne dust and endotoxins. For example, higher
22
23 294 concentrations of bacteria and gases were found in the air of poultry houses with
24
25
26 295 ventilation through porous inlets than in buildings equipped with automatic ventilation
27
28 296 systems (Radon *et al.*, 2001).

29
30 297 As described in previous studies of personal exposure of poultry workers to
31
32 298 airborne dust and endotoxins (Thelin *et al.*, 1984; Simpson *et al.*, 1998a; Simpson *et al.*,
33
34 299 1998b; Donham *et al.*, 2000), a strong correlation between dust and endotoxin
35
36
37 300 concentrations was observed in the field studies, although the endotoxins were
38
39 301 measured in the inhalable fraction, and not in the respirable fraction as for dust.
40
41
42 302 According to Simpson *et al.* (1998b), this correlation may be difficult to demonstrate in
43
44 303 personal exposure studies as the endotoxin concentrations in dust could vary in the
45
46 304 different rooms and sites where people were working during the sampling period. This
47
48
49 305 problem does not occur in ambient exposure studies with stationary samplers. The
50
51 306 correlation between dust and endotoxin concentrations in layer houses could be useful
52
53 307 for implementing monitoring programs and corrective measures; assessment of
54
55 308 exposure could be limited to measurements of dust concentration, and control measures
56
57
58 309 taken to reduce dust concentration should also reduce the endotoxin concentration. High
59
60

1
2
3
4
5 310 ventilation rates dilute dust concentration inside the house, but reduce ambient
6
7 311 temperature leading to thermal discomfort for birds and to discharge of airborne
8
9 312 pollutants in the environment. Various methods of air treatment to reduce dust
10
11 313 concentrations (physical and electrical filtration) have been tested with success under
12
13 314 experimental conditions (Lyngtveit and Eduard, 1997; Mitchell *et al.*, 2000; Ellen *et al.*,
14
15 315 2010). However, their application in commercial conditions is difficult on account of
16
17 316 the large volumes of air to be treated. In contrast, spraying oil or fogging with water
18
19 317 droplets are inexpensive and effective methods which are relatively easy to use in
20
21 318 commercial poultry houses. A 50% reduction in airborne dust concentration in an aviary
22
23 319 was obtained using water fogging without deterioration in the conditions of the hens'
24
25 320 feathers (Gustafsson and Von Wachenfelt, 2006). Local manual application of oil on
26
27 321 litter of an experimental aviary gave a reduction of 20 to 30% of fine dust emissions,
28
29 322 but no effect was observed when oil was applied with an automatic system (Ellen *et al.*,
30
31 323 2010). This technique has thus to be improved before being used under commercial
32
33 324 conditions.

34
35
36
37
38
39
40 325 No differences in dust or endotoxin concentrations were observed in our field
41
42 326 study between the measures obtained during the autumn/winter period and during the
43
44 327 spring/summer period. This result is in line with the observations of Seedorf *et al.*
45
46 328 (1998). By contrast, Schriel *et al.* (2007) noted a higher concentration of endotoxins in
47
48 329 the dust samples taken in winter than in those taken in summer and spring. Although the
49
50 330 seasonal effect was not significant in our study, a negative correlation was observed
51
52 331 between the average temperature inside the house and the endotoxin concentration. The
53
54 332 temperature inside the building, and the ventilation control measures taken in response
55
56
57
58
59
60

1
2
3
4
5 333 to this temperature, are likely to influence the endotoxin concentration more than the
6
7 334 season.

8
9 335 The present study highlights that considerable exposure to endotoxins may occur
10
11 336 in laying houses under both experimental and field conditions. The personal exposures
12
13 337 to endotoxins recorded in the experimental houses exceeded the limits proposed by the
14
15 338 Dutch Expert Committee on occupational standards on all measurement days, and thus
16
17 339 give grounds for concern about the health of poultry workers. Effective methods to
18
19 340 reduce worker exposure to air contaminants in laying houses still need to be developed.
20
21 341 Higher dust and endotoxin concentrations were measured in alternative housing systems
22
23 342 than in cage houses under commercial conditions. Therefore further research is required
24
25 343 to focus on working conditions in these alternative systems because of the ban on
26
27 344 conventional cages in European Union from 2012.
28
29
30
31
32

33 345

34 346

ACKNOWLEDGEMENTS

35 347

36
37
38 348 The authors are grateful to the Experimental Services for Poultry and Rabbit Husbandry
39
40 349 of Anses, and to the egg production companies and farmers who collaborated in this
41
42 350 study. Funding for the AIRPOUL project was provided by the Conseil Régional de
43
44 351 Bretagne.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

353 REFERENCES

354

355 CLARK S., RYLANDER R. & LARSSON L. (1983) Airborne bacteria, endotoxin and fungi in
356 dust in poultry and swine confinement buildings. *American Industrial Hygiene Association*
357 *Journal*, **44**: 537-541.

358 DE REU K., RODENBURG T.B., GRIJSPEERDT K., MESSENS W., HEYNDRICKX M.,
359 TUYTTENS F., SONCK B., ZOONS J. & HERMAN L. (2009) Bacteriological contamination,
360 dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: An
361 international on-farm comparison. *Poultry Science*, **88**: 2442-2448.

362 DONHAM K.J., CUMRO D., REYNOLDS S.J. & MERCHANT J.A. (2000) Dose-response
363 relationships between occupational aerosol exposures and cross-shift declines of lung function
364 in poultry workers : recommendations for exposure limits. *Journal of Occupational and*
365 *Environmental Medicine*, **42**: 260-269.

366 DOUWES J., THORNE P., PEARCE N. & HEEDERIK D. (2003) Bioaerosol health effects and
367 exposure assessment: progress and prospects. *Annals of occupational Hygiene*, **47**: 187-200.

368 ELLEN H.H., AARNINK A., MOSQUERA J., WINKEL A., CAMBRA-LOPEZ M., VAN
369 HARN J. & OGINK N. (2010) Dust Reduction Options for Poultry Houses. *World's Poultry*
370 *Science Journal*, **66**: S26.

371 ELLEN H.H., BOTTCHE R.W., VON WACHENFELT E. & TAKAI H. (2000) Dust levels
372 and control methods in poultry houses. *Journal of Agricultural Safety and Health*, **6**: 275-282.

373 GUSTAFSSON G. & VON WACHENFELT E. (2006) Airborne Dust Control Measures for
374 Floor Housing System for Laying Hens. *VIII Agricultural Engineering International*
375 *Conference*, pp. 1-11.

376 JONES W., MORRING K., OLENCHOCK S.A., WILLIAMS T. & HICKEY J. (1984)
377 Environmental study of poultry confinement buildings. *American Industrial Hygiene*
378 *Association Journal*, **45**: 760-766.

- 1
2
3
4 379 KIRYCHUCK S., DOSMAN J.A., REYNOLDS S.J., WILLSON P., SENTHILSELVAN A.,
5
6 380 FEDDES J.J.R., CLASSEN H. & GUENTER W. (2006) Total dust and endotoxin in poultry
7
8 381 operations: comparison between cage and floor housing and respiratory effects in workers.
9
10 382 *Journal of Occupational and Environmental Medicine*, **48**: 741-748.
11
12 383 KIRYCHUCK S., SENTHILSELVAN A., DOSMAN J.A., JUORIO J.A., FEDDES J.J.R.,
13
14 384 WILSON P., CLASSEN H., REYNOLDS S.J., GUENTER W. & HURST T.S. (2003)
15
16 385 Respiratory symptoms and lung function in poultry confinement workers in Western Canada.
17
18 386 *Canadian Respiratory Journal*, **10**: 375-380.
19
20 387 LARSSON B.-M., LARSSON K., MALMBERG P., MARTENSSON L. & PALMBERG L.
21
22 388 (1999) Airway responses in naive subjects to exposure in poultry houses : comparison between
23
24 389 cage rearing system and alternative rearing system for laying hens. *American Journal of*
25
26 390 *Industrial Medicine*, **35**: 142-149.
27
28 391 LEE S.-A., ADHIKARI A., GRINSHUPUN S.A., MCKAY R., SHUKLA R. & REPONEN T.
29
30 392 (2006) Personal exposure to airborne dust and microorganisms in agricultural environments.
31
32 393 *Journal of Occupational and Environmental Medicine*, **3**: 118-130.
33
34 394 LYNGTVEIT T. & EDUARD W. (1997) Reduction of dust exposure by negative air ionisation.
35
36 395 *Annals of Agricultural and Environmental Medicine*, **4**: 229-232.
37
38 396 MICHEL V. & HUONNIC D. (2003) A comparison of welfare, health and production
39
40 397 performance of laying hens reared in cages or aviaries. *British poultry science*, **43**: 775-776.
41
42 398 MITCHELL B.W., HOLT P.S. & SEO K.H. (2000) Reducing dust in a cage layer room: an
43
44 399 electrostatic space charge system. *Journal of Applied Poultry Research*, **9**: 292-296.
45
46 400 NIMMERMARK S., LUND V., GUSTAFSSON G. & EDUARD W. (2009) Ammonia, dust
47
48 401 and bacteria in welfare-oriented systems for laying hens. *Annals of Agricultural and*
49
50 402 *Environmental Medicine*, **16**: 103-113.
51
52 403 PROTAIS J., QUEGUINER S., BOSCHER E., PIQUET J.C., NADARD B. & SALVAT G.
53
54 404 (2003) Effect of housing systems on the bacterial flora of the air. *British Poultry Science*, **43**:
55
56 405 788-789.
57
58
59
60

- 1
2
3
4 406 RADON K., DANUSER B., IVERSEN M., MONSO E., WEBER C., HARTUNG J.,
5
6 407 DONHAM K.J., PALMGREN U. & NOWAK D. (2002a) Air contaminants in different
7
8 408 European farming environments. *Annals of Agricultural and Environmental Medicine*, **9**: 41-48.
9
10 409 RADON K., MONSO E., WEBER C., DANUSER B., IVERSEN M., OPRAVIL U.,
11
12 410 DONHAM K.J., HARTUNG J., PEDERSEN S., GARZ S., BLAINEY D., RABE U. &
13
14 411 NOWAK D. (2002b) Prevalence and risk factors for airway diseases in farmers - summary of
15
16 412 results of the European Farmers' Project. *Annals of Agricultural and Environmental Medicine*,
17
18 413 **9**: 207-213.
19
20 414 RADON K., WEBER C., IVERSEN M., PEDERSEN S. & NOWAK D. (2001) Exposure
21
22 415 assessment and lung function in pig and poultry farmers. *Occupational Environmental*
23
24 416 *Medicine*, **58**: 405-410.
25
26 417 RENSTRÖM A. (2002) Exposure to airborne allergens: a review of sampling methods. *Journal*
27
28 418 *of Environmental Monitoring*, **4**: 619-622.
29
30 419 RYLANDER R. (2002) Endotoxin in the environment - exposure and effects. *Journal of*
31
32 420 *Endotoxin Research*, **8**: 241-252.
33
34 421 RYLANDER R. & CARVALHEIRO M.F. (2006) Airways inflammation among workers in
35
36 422 poultry houses. *International Archives of Occupational and Environmental Health*, **79**: 487-
37
38 423 490.
39
40 424 SCHENKER M.B., CHRISTIANI D., CORMIER Y., DIMICH-WARD H., DOEKES G.,
41
42 425 DOSMAN J., DOUWES J., DOWLING K., ENARSON D., GREEN F., HEEDERIK D.,
43
44 426 HUSMAN K., KULLMAN G., LACASSE Y., LAWSON B., MALMBERG P., MAY J.,
45
46 427 MCCURDY S., MERCHANT J., MYERS J., NIEUWENHUIJSEN M., OLENCHOCK S.,
47
48 428 SAIKI C., SCHWARTZ D., SEIBER J., THORNE P., WAGNER G., WHITE N., XU X. &
49
50 429 CHAN-YEUNG M. (1998) Respiratory health hazards in agriculture. *American Journal of*
51
52 430 *Respiratory and Critical Care Medicine*, **158**: S1-S76.
53
54
55
56
57
58
59
60

- 1
2
3
4 431 SCHRIEL R., HEISE A., SCHNEIDER F., EICHELSER R., NESER S. & NOWAK D. (2007)
5
6 432 Endotoxin concentration in modern animal houses in Southern Bavaria. *Annals of Agricultural*
7
8 433 *and Environmental Medicine*, **14**: 129-136.
9
10 434 SEEDORF J., HARTUNG J., SCHRÖDER M., LINKERT K.H., PHILLIPS V.R., HOLDEN
11
12 435 M.R., SNEATH R.W., SHORT J.L., WHITE R.P., PEDERSEN L.J., TAKAI H., JOHNSEN
13
14 436 J.O., METZ J.H.M., GROOT KOERKAMP P.W.G., UENK G.H. & WATHES C.M. (1998)
15
16 437 Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings
17
18 438 in Northern Europe. *Journal of Agricultural Engineering Research*, **70**: 97-109.
19
20 439 SIMPSON J.C.G., NIVEN R.M., FLETCHER A.M., OLDHAM L.A. & FRANCIS H.M.
21
22 440 (1998a) Prevalence and predictors of work related respiratory symptoms in workers exposed to
23
24 441 organic dusts. *Occupational Environmental Medicine*, **55**: 668-672.
25
26 442 SIMPSON J.C.G., NIVEN R.M., PICKERING C.A.C., OLDHAM L.A., FLETCHER A.M. &
27
28 443 FRANCIS H.M. (1998b) Comparative personal exposures to organic dusts and endotoxin.
29
30 444 *Annals of occupational Hygiene*, **43**: 107-115.
31
32 445 TAKAI H., PEDERSEN S., JOHNSEN J.O., METZ J.H.M., GROOT KOERKAMP P.W.G.,
33
34 446 UENK G.H., PHILLIPS V.R., HOLDEN M.R., SNEATH R.W., SHORT J.L., WHITE R.P.,
35
36 447 HARTUNG J., SEEDORF J., SCHRÖDER M., LINKERT K.H. & WATHES C.M. (1998)
37
38 448 Concentrations and emissions of airborne dust in livestock buildings in Northern Europe.
39
40 449 *Journal of Agricultural Engineering Research*, **70**: 59-77.
41
42 450 THELIN A., TEGLER O. & RYLANDER R. (1984) Lung reactions during poultry handling
43
44 451 related to dust and bacterial endotoxin levels. *European Journal of Respiratory Diseases*, **65**:
45
46 452 266-271.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 453 FIGURE LEGENDS
5
6

7 454

8
9 **Figure 1.** *Respirable dust concentrations in the ambient air of cage and aviary systems*
10
11 *in the experimental trial.*
12

13 457

14 458

15
16
17
18 **Figure 2.** *Endotoxin concentrations in the inhalable fraction of dust in different*
19
20 *housing systems in the field study. Means are represented by a “plus” and the extreme*
21
22 *values depicted by a square are outside the interval defined as the 1st or 3rd quartile ±*
23
24 *1.5 interquartile range.*
25
26
2728 463
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

464 **Table 1.** *Characteristics of cage houses and alternative systems monitored in the field*
 465 *study. The median (range) is given for continuous variables.*

Variable	Cage	Alternative systems	
		Free-range	Aviary
Number of houses studied	8	10	3
Area (m ²)	1,426 (1062-1650)	625 (412-864)	350 (350-784)
Volume of the henhouse (m ³)	9,600 (4400-11700)	2,040 (1600-3500)	1,600 (1600-5800)
Housing capacity (hens/house)	46,400 (32000-65000)	4,600 (2900-4900)	4,700 (4600-16100)
Density (hens/m ²)	31.9 (26.1-54.2)	7.9 (5.7-10.7)	13.6 (13.1-18.5)
Access to an open-air run (number of houses)			
- Yes	-	10	0
- No	8	0	3
Ventilation (number of houses)			
- Natural	0	10	0
- Forced	8	0	3
Manure disposal system (number of houses)			
- Manure belts	5	0	3
- Dip pit	3	10	0

1
2
3
4
5 Number of hens per cage 9 - -
6
7 (5-60)
8

466

9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

1 **Table 2.** Median (range) of *endotoxin concentrations (EU/m³) of inhalable dust*
 2 *fraction per housing system in the experimental trial.*

Housing system	Ambient air (8 h)		Personal exposure (6 h)	
	n	Concentration	n	Concentration
Cage	7	98 (51-470)	3	90 (88-97)
Aviaries	7	565 (362-1491)	3	450 (181-667)

3

1 **Table 3.** *Respirable dust and endotoxin concentrations (median and range) in the*
 2 *inhalable dust fraction in laying houses for each housing system in the field study*

Housing system	n ¹	Respirable dust concentration (mg/m ³)	Endotoxin concentration (EU/m ³)
Cage	15	0.125 (0.000-0.264)	204 (78-576)
Alternative	21	0.438 (0.020-2.850)	684 (35-3156)
- Free-range	18	0.386 (0.020-1.010)	669 (35-3156)
- Aviary	3	1.200 (0.825-2.850)	771 (465-1543)
<i>P</i> ²		<0.01	<0.01

3 ¹Number of measures.

4 ²Probability for the Kruskal Wallis test for comparison of cage and alternative housing
 5 systems.

Table 4. *Respirable dust concentrations and endotoxin concentrations in the inhalable dust fraction in laying houses for each season in the field study (median and range)*

Season	n ¹	Respirable dust concentration (mg/m ³)	Endotoxin concentration (EU/m ³)
Autumn/winter	15	0.189 (0.000-0.599)	320 (135-1822)
Spring/summer	15	0.143 (0.000-0.733)	385 (35-2329)
<i>P</i> ²		0.55	0.65

¹ Number of measures

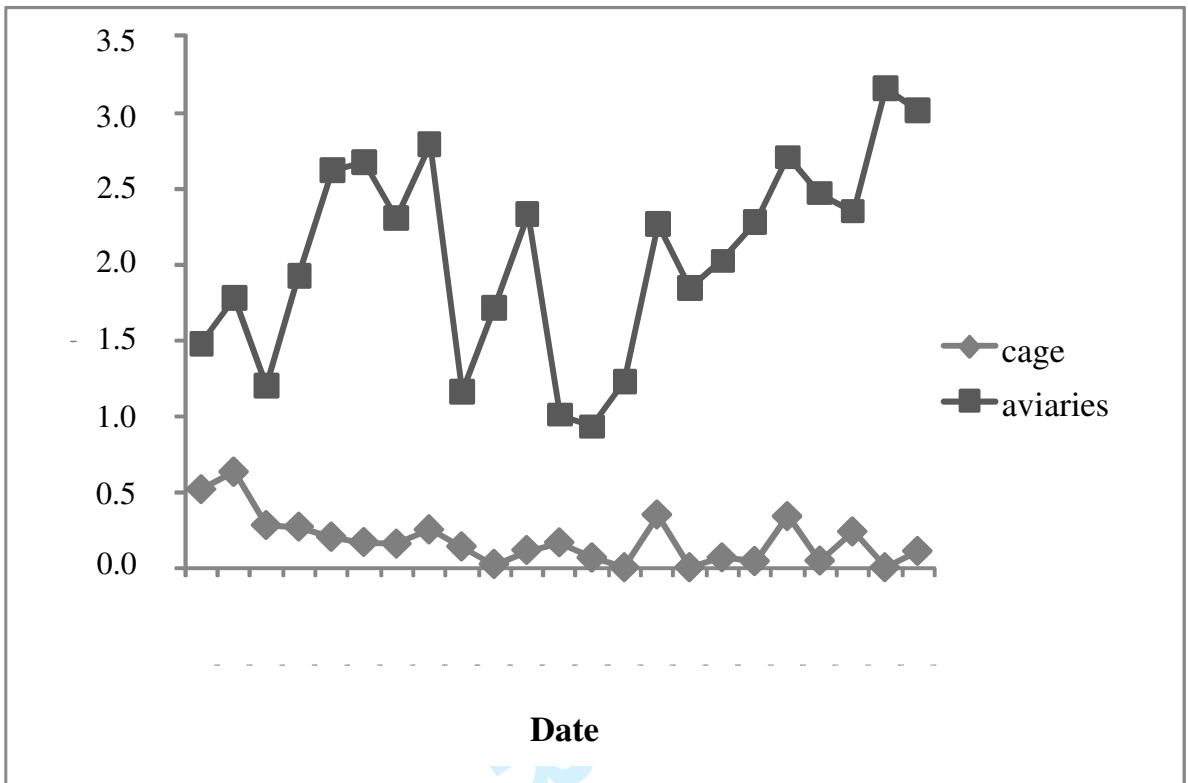
² Probability for the Wilcoxon test for comparison between seasons

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1

For Peer Review Only

Figure 1



Review Only

Figure 2

