Environmental factors and effects on breeding performances and housing in small laboratory rodents: A technical report

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Introduction

A number of articles report on the incidence of environmental factors, as well on animal health, breeding performances, stress, etc as on physiological characteristics of animals. The importance of environmental factors is not unknown and this matter is classically treated in animal production and experimentation training cources.

Our breeding centre is located in the open country, about 30 km west of Lyon. It was created in 1966 and since then it has specialized in mouse, rat and guinea-pig production for biomedical research. These three animal species are bred in barrier units.

As laboratory rodent breeders for more than 20 years, our permanent goal has been to standardize animals for experimentation. Our efforts were first devoted to obtain sanitarilly and genetically defined animals but also to obtain stable environmental parameters. Unfortunately the fitting provided for occasional break downs. We could easily connect these events with variations in the permanently recorded productivity indexes of our animal strains. Furthermore, having on the same site several production units holding the same inbred strains of rodents, led us to conclude that some productivity variations are intrically linked to the environment.

Events observed in this industrial environment are reported in order to illustrate the impact of some environmental factors upon reproduction.

Methodological considerations

IFFA-CREDO rodents are maintained in barrier units. These units are disinfected with gaseous formaldehyde before being put into service. The animal caretakers are specially trained inside and outside the breeding centre.

Twice daily the caretakers enter the barrier unit through shower-rooms, where they change into a complete set of sterile garments including: hood, disposable face-mask, overall, boots and gloves.

At the time when the here reported observations were done, the environmental conditions were as follow:

* *Physical parameters:*
- Temperature: 21°C (but during summer time this could be as high as 25/26°C)
- Relative Humidity: registered but non graded
- CO_2 amount: < 5000 ppm
- Ammoniac gas amount: < 50 ppm
- 7 to 12 air changes per hour (with new air, not-recycled, filtered at 95 % DOP)
- Light: 600 lux at 1 m above the floor
- Nyctohemeral period: 12 h/12 h
- Noise: ≤ 60 dbs

* *Cages:* our breeders were housed in three different sizes of polypropylene cages:
- Monogamous pair: in 236 cm^2 cages (20.5 cm long × 11.5 cm wide × 12.9 cm high)
- Trios: in 424 cm^2 cages (26.5 cm long × 16.0 cm wide × 14.0 cm high)
- Harems: in 869 cm^2 cages (37 cm long × 23 cm wide × 17 cm high)

* *Food, water and bedding:*
- The pelleted food was Souriffarat (IFFA-CREDO diet for rat and mouse breeders, produced by UAR), sterilized at 107°C for 30 minutes and given ad libitum
- Drinking: water sterilized by 0.22 μm

microfiltration and given by hand in polypropylene bottles
- Bedding: made of saw-dust, autoclaved at 134°C for 25 minutes and renewed:
  . every week for harems
  . every two weeks for monogamous pairs and trios

Each barrier unit was quarterly health monitored as follows:
- . complete clinical examination of 30 animals four times
- . necropsy of 10 animals 3 times
- . screening for opportunistic bacteria from 10 animals
- . screening for specific pathogen bacteria from 10 animals
- . serological test of 10 animals, with 11 antigens per animal

Influence of a few environmental factors on the productivity of some rat or mouse strains

Influence of temperature

- On preweaning-mortality of inbred strains of rats and mouse:

During the years 1970 to 1976 we maintained a production unit which had no cooling system. In this unit the following inbred strains were maintained:

Rats: FISCHER/344Ico

Mice: C57BL/6RhoIco, AKR/RhoIco, DBA/2LacIco

During summer 1976, which was especially warm, we observed and recorded an increase in mortality rate in the colony of FISCHER/344Ico rats, this was closely linked to the maximal temperature curve in this unit (Fig. 1).

On the other hand no appreciable influence was recorded on the mortality rate in the mouse colonies maintained in the same unit.

- On the number of weaned offspring in an outbred stock of SWISS mice:

Also in 1976, a unit equipped with a cooling system was entirely devoted to produce OF1/Ico mice. The number of weekly weaned mice was recorded. On the last week of August the cooling system failed. Fig. 2 shows that exactly six weeks after, this failure is noticeable on the weekly number of

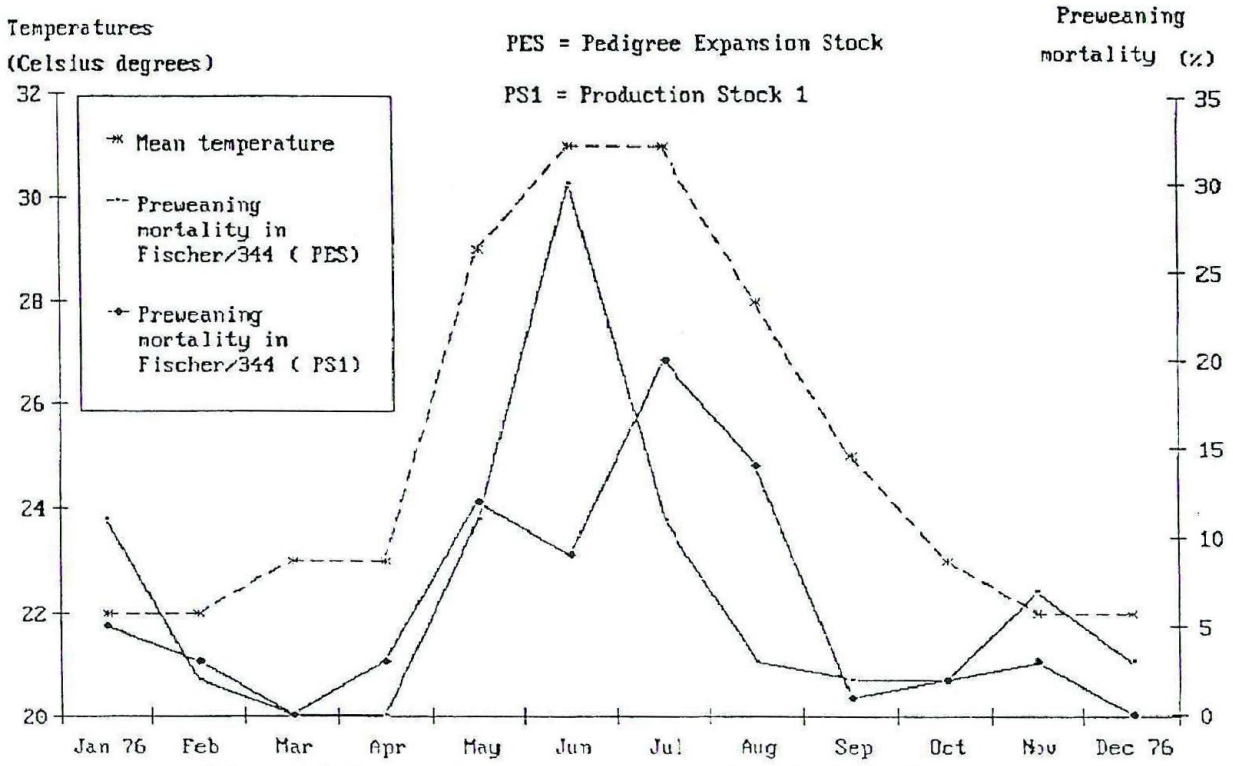

Figure 1. Influence of temperature on mortality i FISCHER/344 rats.

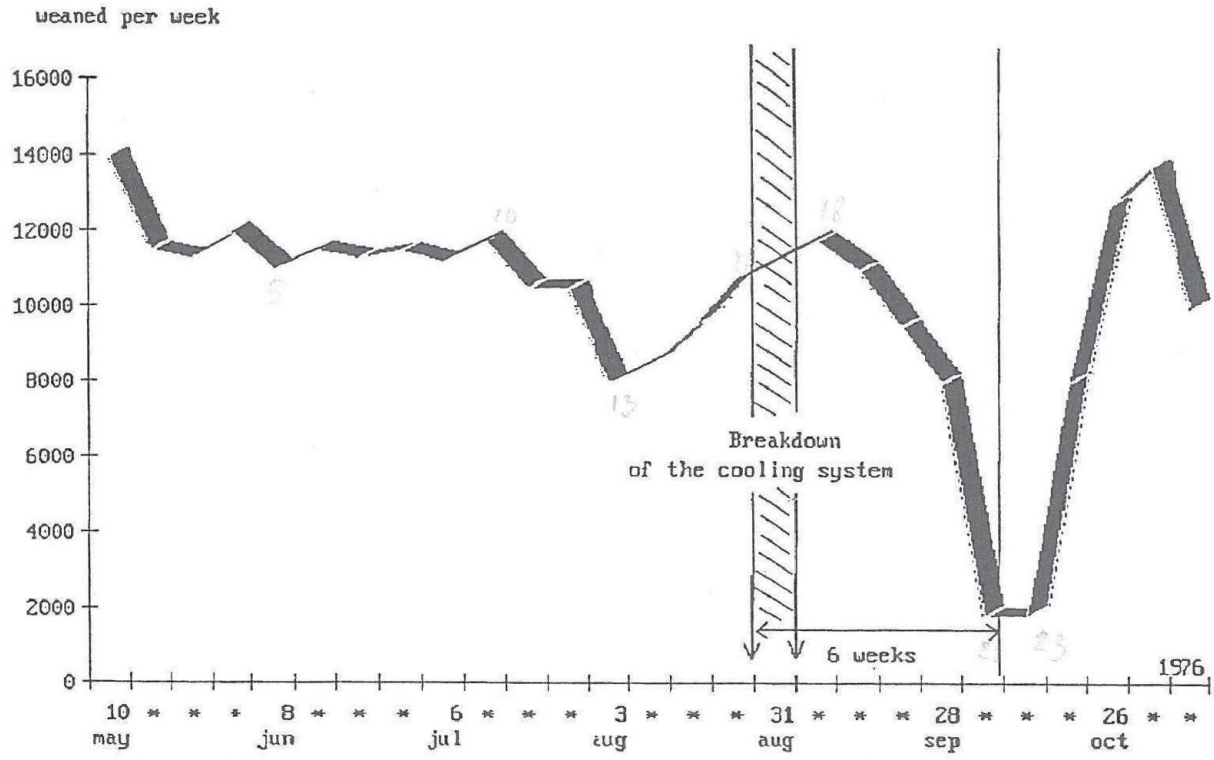

Figure 2. Influence of temperature on productivity in OF1/Ico SWISS mice (unit A41).

weaned mice. It can be concluded that the high temperature in this unit (more than 30°C) for a week, stopped gametogenesis and/or the sexual activity of the mice.
On the other hand, three weeks later this event did not produce any noticeable effect, that is to say on birth and mortality.

Light influence

a) Permanent lighting

In mammals the influence of light on reproduction is well known; it is stimulative and regulating sexual activity through the classical axis: retina-hyphothalamus-pituitary gland-ACTH.
In the breeding unit, the nyctohemeral cycle is totally artificial, regulated by an electric clock on a 12/12 hours light/dark basis (light from 6 a.m. to 6 p.m.).
During the winter 1984–1985 we noticed an important decrease in fecundity (number of born litters) in a production colony of FISCHER/344 rats. From the clinical examinations performed every six week in this breeding unit, the rats were healthy and the few born litters were quite normal. We tried to find an explanation to this variation until one day when some of the staff, working occasionally out of diurnal schedule, noticed that the lighting was permanently on the unit. Fig. 3 illustrates our observations.
Parellelly, in a number of various inbred strains of mice and hybrid F_1 maintained in the same unit, we noticed no change in the breeding performances.
The same event occurred twice in our rodent breeding unit maintained in isolators. The misfunctioning lasted less long but resulted each time in a decrease of the number of born litters in outbred SPRAGUE-DAWLEY rats, whereas no change in productivity was recorded in the outbred SWISS mouse colony maintained in the same room.

b) Light intensity

The CBA/JIco strain of mouse is known to have a very high preweaning mortality rate (30 to 35 %). In 1985 during a 3-month pe-

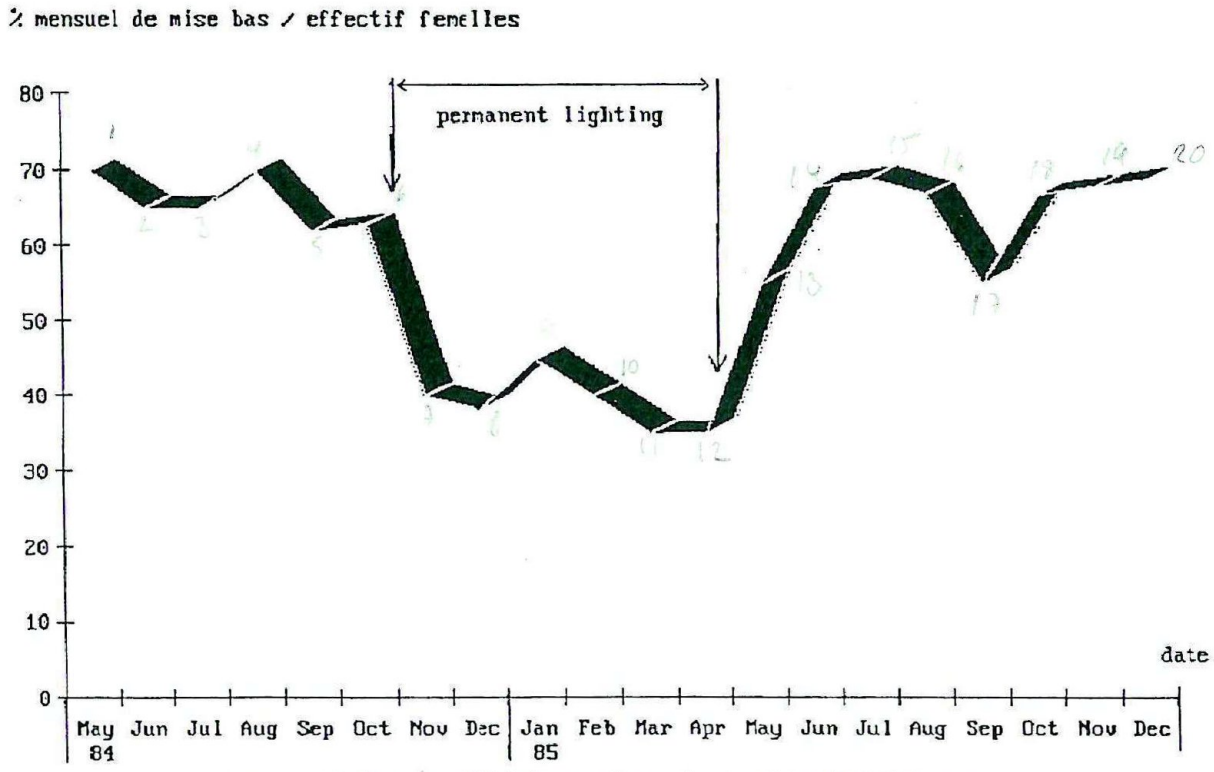

Figure 3. Influence of lighting on fecundity in FISCHER/344 rats.

riod, in a production colony of monogamous pairs the number of born offspring and mortality were recorded in relation to the position of the cages on the stabulation racks in a protected unit. As a matter of fact the way the cages were piled upon the rack-shelves modified the intensity of the light coming from the ceiling of the room.

Table 1 shows the results obtained under usual production conditions. All other environmental conditions possible to control were strictly identical between the three breeding groups.

These results indicate differences concerning the parameters studied: fecundity and pre-weaning-mortality rate.

Thus a normal lightning (300 to 400 lux) is favourable to fecundity (number of born offsprings per litter) and unfavourable to the survival rate of these mice between birth and weaning. This lact point could probably be explained by considering the natural living conditions of wild rodents.

Table 1. Influence of light intensity on fecundity and mortality of inbred CBA/JIco mice in monegameous pairs.

Measurements Ranking of the rack shelf	Light intensity (Lux)	Number of females in each group	Born/female in 13 weeks 13 weeks of mating	Preweaning mortality rate (%)	Weaned/female in 13 weeks of mating	Weaned/female/week
Upper shelf	388	81	12.85	39	7.8	0.61
Middle shelf	108	81	11.12	30	7.7	0.60
Lower shelf	55	81	10.76	32	7.3	0.56

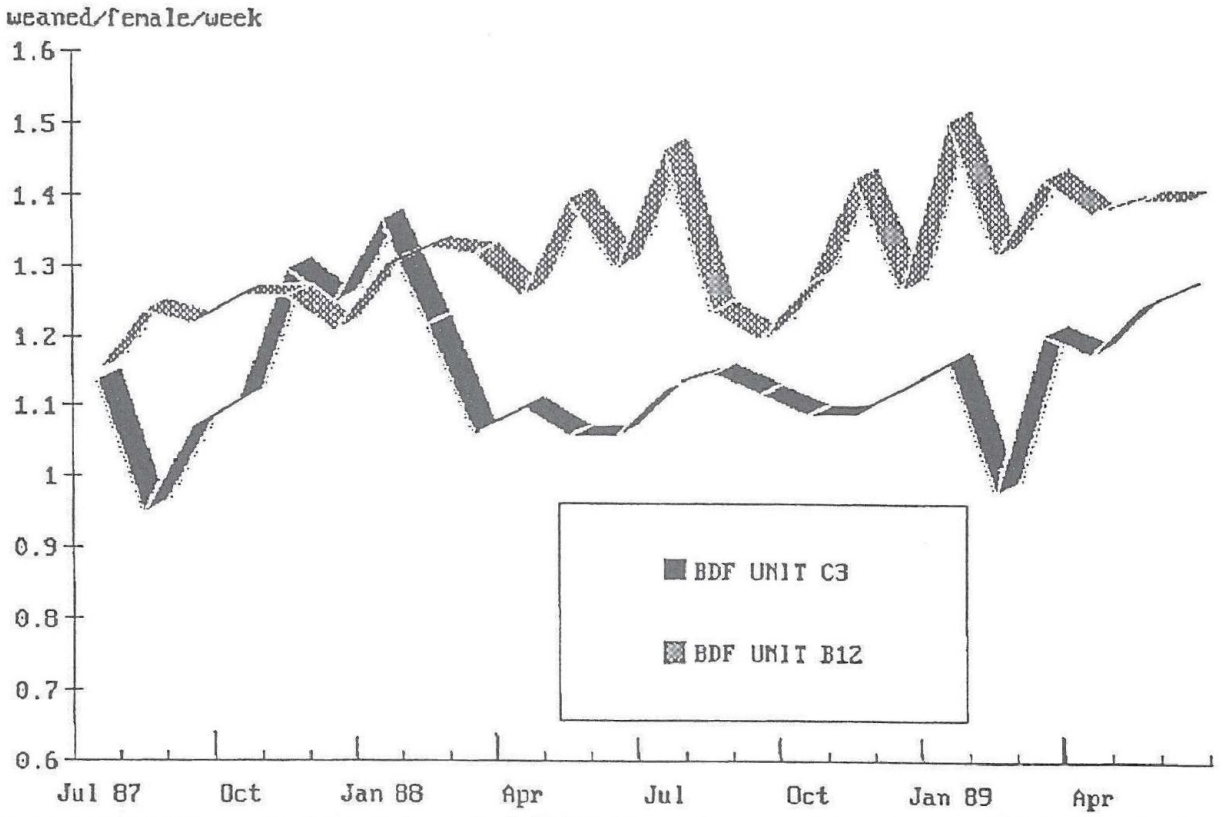

Figure 4. Monthly productivity indexes in B6D2F1/JIco mice, maintained in two different production units.

"Building" effect

– Under this title we shall refer to the effects of combined factors such as: noise, vibrations, air composition (ionisation for instance), magnetic fields, odors, etc. and factors that cannot be actually identified but that can be noticed through comparing breeding performances between two breeding units.

– These observations were done in a population of F_1 hybrid mice ($B6D2F_1$/JIco), which are rather rustic and well defined from a genetical point of view. The stock was important (about 50 000 born mice/-year/unit) and was kept in two different production units with remarkable differences in breeding performances. (Fig. 4).

– This "building" effect was noticed because there were several distinct production units (protected areas) and a sanitary security rule consisting of a partition of one strain production (Hybrid F_1) into at least two different units.

Identical observations have been done on mice belonging to various inbred strains, even though they were less demonstrative.

– From our own experience as breeders it has rather constantly been noted that good breeding performances are obtained in concrete buildings built under the ground level (totally or partly) and in which the air conditioning system is totally independent from the breeding area.

– On the other hand, rubble-built buildings with a suspended brick ceiling and an attic harbouring the air-conditioning system give generally less good performances.

Finally very bad breeding performances were recorded in a small protected unit situated in a module (mobil-home).

Influence on breeding performances of filtertop equipped cages

– To obtain more satisfactory results (from a sanitary point of view) procedures achie-

Table 2. Influence of adding a filter cover on the cages of breeders, on productivity of five "classical" inbred strains of mouse and one hybrid F_1.

Strain Mating parameters	C57BL/6J trio	DBA/2J Trio	BALB/cByJ Trio	C3H/HeOuJ Mono-gamous	CBA/J (1) Mono-gamous	B6D2F1/J Trio
Number of females Control batch	98	98	98	42	42	42
Number of females Filter-top cages	98	98	98	42	42	42
Productivity: Weaned/female/week Control batch (A)	0.80	0.76	0.88	0.67	0.58	1.06
Productivity: Weaned-female/week Filter-cover cages (B)	0.77	0.73	0.78	0.73	0.76	1.18
$\frac{B - A}{A} \times 100$	– 3.75 %	– 3.94 %	– 11.36 %	+ 8.95 %	+ 31 % (1)	+ 11.32 %

(1) With the CBA/J strain of mouse (with rather poor breeding performances) a study realised earlier showed a large increase on the productivity index (about 50 %) in favour of the filter-cover cages system.

ved, while maintaning SSC^{up} (1) immunodepressed mice (SWISS-nude) in filtertop cages and handling them under the protection of a laminar air flow cabinet, were decided to be used also for the production of a small quantity of congenic BALB/cAJcIIco-nude mice.

– A better productivity index was observed with this filter-top caging system than previously obtained in isolators or in protected areas.

. A comparative test was performed on five inbred strains of mice and one hybrid ($B6D2F_1$) under the following conditions.

. Filter cover cages should only be opened when under the protection of a laminar air flow cabinet and be maintained in a protected area.

. Standard cages (without filter) should be maintained in the same protected area during the same period of the year.

These tests lasted over one generation (28 weeks) in previously randomised PS2 (Production stock no 2) colonies.

– The results of these comparative tests are presented in table 2.

– The results are very heterogenous. Accordingly they differ considerably in relation to the strain, from unfavourable (for BALB/-cByJIco strain) to very favourable (about + 30 %) for CBA/J strain. Moreover the BALB/caJIcoIco-nude congenic strain evidenced better breeding performances when using the filter-top caging system.

In such system, there are certainly many possible environmental parameters which effects, whether favourable or unfavourable, are unknown. Those listed here are certainly involved.

. Increased temperature in the cage, linked to an important decrease of the air exchange

(1) SSC^{up} = Status Sanitaire Controlé pour Usages Particuliers.
Controlled health status for special use.

between the inside and the outside of the cage.
. Increased CO_2 and NH_3 levels for the reason hereabove.
. Increase species odours.
. Decrease noises and/or vibration.
. Decrease draughts that could come from the air diffusers in large breeding units.
. Decrease light intensity.
. Less ocular stimuli: much more quiet life environmental conditions.

Discussion

The results reported are obtained either from events that have pappened in our breeding units or from tests intended to evaluate the best breeding conditions. Thus they are rather heterogeneous and constitute only contributions to a better knowledge about certain environmental factors and the variation of their effects on rodent breeding performances.

On the other hand we are quite aware that the zootechnical parameters illustrating these effects are not always those on which the mentioned factors play. No statistical analysis has been done on the comparative data. Important deviations only are taken into account.

Conclusion

From the few cases observed in our breeding centre basic principles have emerged:

1/ The effects of an environmental factor on reproduction must not be generalized.
. between two different species
thus rats seem much more sensitive to the "lightning factor" (permanent lightning) than mice.
. between two strains
the effect obtained when using filtertop cages is particularly conclusive.

2/ The effects can be different in relation to the measured parameter: either favourable or unfavourable from a zootechnical point of view. Consequently only the environmental parameter affecting the animal as a whole or organ/organ systems should be taken into account.

Example: we recorded a decrease in the number of born litters in rats maintained in a permanent lighting. Which parameter does the lighting affect:
spermatogenesis, sexual activity, ovogenesis . . .?

3/ The effects even of defined environmental factors are difficult to study as they often interfere with each other and sometime they also act in a concealed manner.

Consequently, it is quite important to define the optimum environmental parameters for a given species (or even a strain) and to have reliable equipment to meet the fixed requirements.

Furthermore, procedures to check the regulating and recording apparatuses of environmental factors (temperatures, air conditioning, lighting cycle, CO_2 and NH_3 levels in cages, etc.) will have to be implemented in order to prevent equipment from unfortunate dysfunction in breeding units.

Summary

Based on their professional experience in large scale production of laboratory rodents, the authors report on some documented cases showing the effects of environmental factors on reproductive performances of the rodent strains they produce. Beyond confirmation of the effect of a simple environmental factor (temperature, light) the reported cases show that environmental effects must not be systematically generalized to several strains or, even less, to several species.

Moreover, the incidence of associated (complex) factors, which cannot be evaluated without the opportunity of having a control sample must be emphasized. This last point is of major importance when comparing results coming from different laboratories although the maintain environmental factors are a priori identical.

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