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Technical note Communication: Frequency of changing solid-walled cages does not affect pentobarbitone-induced sleeping time in rats and mice

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INTRODUCTION

Pentobarbitone-induced sleeping time in rats should be increased when animals are kept in a dirty environment (*Gillette* 1976) because under such conditions hepatic drug metabolism is impaired (*Vesell et al.* 1973). In these experiments, the rats were housed in cages with wire mesh bases, and urine and feces accumulated on trays under the cages. We have addressed the question whether pentobarbitone-induced sleeping time in rats and mice housed in cages with solid floors and a layer of wood shavings as bedding, would be affected by the frequency of cage changing.

MATERIALS AND METHODS

Prior to and during the experiments, the animals were housed in wire-topped Makrolon cages (UNO BV, Zevenaar, The Netherlands), that were placed on a rack. Bedding consisted of a layer of wood shavings (Woody Clean 8/15®; IFFA Credo/Broekman BV, Someren, The Netherlands). The cages were located in rooms with controlled temperature (20–22°C), humidity (50–70 %) and lighting (light: 07.00–19.00 h), and a ventilation rate of about 20 room air changes per h. A pelleted commercial diet (RMH-B®, Hope Farms, Woerden, The Netherlands) and tap water were provided *ad libitum*.

Four experiments, three with mice and one with rats, were carried out to study the effect of cage changing on pentobarbitone-induced sleeping time.

Experiment 1. Female NMRI mice, aged 19 weeks, were derived from a conventional breeding colony (Laboratory Animals Centre, Agricultural University, Wageningen). The mice were divided into two groups of 15 animals each with similar mean body weight distribution. They were housed individually in Makrolon type II cages (22.5×16×14 cm). The cages of the two groups were placed on racks in alternating order. After one week, all animals were weighed daily in random order between 09.00 and 10.00 h. The cage was taken from the rack and put next to an electronic balance. The animal was removed from its cage and weighed. Upon weighing, the animal was either placed into its home cage (sham cage changing) or into a clean cage with fresh bedding (true cage changing). In this way, handling was identical for both treatment groups.

After 15 days, all animals were placed in a clean cage and pentobarbitone-induced sleeping time was measured in random order by *ACPW* who at that time was blinded to treatment modality. The animals were premedicated with atropine-sulphate (i.p. 0.5 mg/kg; Kombivet®, Etten-Leur, The Netherlands). Subsequently, pentobarbitone was administered (i.p. 55.0 mg/kg; Nembutal®, Algin BV, Maassluis, The Netherlands). Possible loss of body heat after pentobarbitone injection was prevented by placing each animal on its back on a veterinary heat pad (Animed/Virbac, Barneveld, The Netherlands). Sleeping time was measured

as the time that elapsed from pentobarbitone injection until restoration of the righting reflex, which was defined as the capacity of the animal to roll over from back to belly twice within 15 sec.

Experiment 2. Female and male rats, aged 4 weeks, were derived from the conventional, outbred Wistar Cpb:WU strain of the Laboratory Animals Centre, Waageningen. The animals were housed individually in Makrolon type II cages. After one week, the animals of each sex were divided into two groups of 10 animals each, so that group mean body weights were similar. The cages were placed in racks at random. The animals were weighed daily between 09.00 and 10.00 h as described above. The rats were subjected to either true or sham cage changing. True cage changing took place once every three days. After 15 days, pentobarbitone-induced sleeping time was determined as described above, except that atropine-sulphate was administered subcutaneously at a dose of 0.1 mg/kg, pentobarbitone was given at a lower dose (i.p. 35.2 mg/kg), and the two righting reflexes had to occur within 30 sec.

Experiments 3 and 4. Female mice, aged 3 (expt 3) and 10 (expt 4) weeks, respectively, were divided into two groups of 10 animals each. They were housed individually in Makrolon type I cages (16×11×12 cm). After one week, the animals were placed into a set of two Makrolon type I cages that were connected by a traverse. The mice were weighed daily as described above. After weighing, either both cages were changed or one cage was changed. After 15 days, pentobarbitone-induced sleeping time was determined as described for experiment 1.

RESULTS AND DISCUSSION

Table 1 shows that the frequency of cage changing did not influence pentobarbitone-induced sleeping time in rats and mice. In other words, the accumulation of feces and urine for a period of 15 days did not affect the activity of microsomal enzymes that degrade pentobarbitone. The well-known fact that female rodents have shorter pentobarbitone-induced sleeping times than their male counterparts (*Lovell* 1986) was borne out in the present study. Thus, our measurements of sleeping times can be considered valid.

Vesell et al. (1973) showed that the accumulation of urine and feces on metal trays without bedding, under cages with wire mesh bases, caused decreased microsomal

Table 1. Pentobarbitone-induced sleeping times in mice and rats subjected to either frequent change of cage or kept in the same cage for 15 days.

Expt	Species	Sex	Cage changing	Body weight (g) Initial	Body weight (g) Final	Sleeping time (min)
1	Mouse	F	Daily	29.3 ± 2.1	30.6 ± 2.2	50.5 ± 22.9
			None	29.4 ± 2.3	30.2 ± 2.5	42.1 ± 20.5
2	Rat	F	Once/3 days	84.9 ± 8.6	138.2 ± 11.8	43.2 ± 11.8
			None	85.5 ± 8.6	140.0 ± 7.8	44.3 ± 11.8
		M	Once/3 days	97.2 ± 13.9	186.0 ± 19.6	115.2 ± 32.6
			None	98.0 ± 12.8	181.7 ± 18.1	123.1 ± 20.9
3	Mouse	F	Both cages[1]	17.3 ± 1.4	23.5 ± 1.6	29.6 ± 12.1
			One cage[1]	17.5 ± 1.5	22.3 ± 0.8	32.0 ± 12.2
4	Mouse	F	Both cages[1]	21.1 ± 1.8	24.4 ± 1.2	35.5 ± 26.5
			One cage[1]	21.1 ± 1.8	24.6 ± 1.6	31.4 ± 11.5

Results expressed as means ± SD for 10 or 15 animals. [1] The animals were housed in two cages connected by a traverse; either both cages were changed daily or one cage was changed.

enzyme activities in rats. This leads to longer pentobarbitone-induced sleeping times (*Gillette* 1976). Such effect was not seen in this study using cages with solid floors and wood shapings as bedding. This could imply that the bedding material absorbs those components of urine and feces that affect microsomal enzymes.

The frequency of changing did not clearly influence body-weight gain (Table 1). In previous work (*Beynen & Van Tintelen* 1989), frequent cage changing was found to lower weight gain in female rats. This discrepancy may be caused by differences in experimental design.

Summary

The effects on pentobarbitone-induced sleeping time of daily cage changing or once every three days versus no changing for 15 days, was investigated in mice and rats, respectively. The animals were housed individually in cages with solid floors and a layer of wood shavings as bedding. Sleeping times were not affected by the frequency of cage changing.

References

Beynen, A. C. & Van Tintelen, G.: Frequency of cage changing and weight gain in rats. Scand. J. Lab. Anim. Sci. 1989, *16,* 119–121.

Gillette, J. R.: Environmental factors in drug metabolism. Fed. Proc. 1976, *35,* 1142–1147.

Lovell, D. P.: Variation in pentobarbitone sleeping time in mice 1. Strain and sex differences. Lab. Anim. 1986, *20,* 85–90.

Vesell, E. S., Lang, C. M., White, W. J., Passananti, G. T. & Tripp, S. L.: Hepatic drug metabolism in rats: impairment in a dirty environment. Science 1973, *179,* 896–897.