Journal of the American Association for Laboratory Animal Science
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Vol 47, No 2
March 2008
Pages 71–75

Comparison of Technicians' Ability to Detect Clinical Signs in Rats Housed in Wire-bottom versus Solid-bottom Cages with Bedding

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Rodent toxicology studies have historically been performed in wire-bottom cages, but the 1996 *Guide for the Care and Use of Laboratory Animals* recommends solid-bottom caging with bedding. Some investigators have expressed concern that changing to solid-bottom cages would interfere with technicians' ability to detect clinical signs. To test this hypothesis, rats were housed in both types of caging and given compounds to induce a variety of subtle clinical signs common to toxicology studies including chromodacryorrhea, soft stool, stereotypic behaviors, mild hypoactivity, abnormal postures, and discolored urine. For one comparison, fecal pellets were removed to simulate decreased production of feces. Technicians, blinded from knowing which animals had been treated, observed the rats and recorded the clinical signs they detected. The technicians who administered the treatments verified that clinical signs were present before and after the blinded technicians made their observations. The number of animals observed with clinical signs divided by the number of animals verified with signs was calculated for each compound and compared between the cage types by using the Fisher Exact Test. The only statistically significant difference observed was a diminished ability to detect discolored, dark urine from rats in wire-bottom cages. These results suggest that concerns about technical staff's inability to detect clinical signs in toxicity tests should not prevent investigators from using solid-bottom cages with bedding.

According to the *Guide for the Care and Use of Laboratory Animals*, "Rodents are often housed on wire flooring, which enhances sanitation of the cage by enabling urine and feces to pass through to a collection tray. However, some evidence suggests that solid-bottom caging, with bedding, is preferred by rodents. Solid-bottom caging, with bedding, is therefore recommended for rodents."[13] Despite this recommendation, most rodent toxicology studies in the United States still are performed in wire-bottom cages.[21] Reasons for preferring the use of wire-bottom cages in toxicology facilities include a desire to minimize secondary exposure to parent compounds and their metabolites, a need to use husbandry methods consistent with earlier work to maintain the validity of data comparisons, and the increased labor and equipment costs associated with changing to solid-bottom caging. Concern exists regarding whether technicians can detect clinical signs in rodents housed in solid-bottom cages with the same consistency as when the rodents are housed in wire-bottom caging. This study was designed to test whether technicians can detect subtle clinical signs better in wire-bottom caging versus solid-bottom caging with bedding.

Materials and Methods

This study was conducted according to the *Guide for the Care and Use of Laboratory Animals* and was approved by our Animal Care and Use Committee. Our animal facility is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

Sixty male, Sprague–Dawley rats (Hsd:SD), 7 to 8 wk old, were purchased from a commercial vendor (Harlan, Indianapolis, IN). The rats were pathogen-free for rat coronavirus (sialodacryoadenitis virus), Kilham rat virus, Toolan H1 virus, rat parvovirus, Sendai virus, pneumonia virus of mice, reovirus 3, Theiler murine encephalomyelitis virus, mouse adenovirus, lymphocytic choriomeningitis virus, Hantaan virus, rat cytomegalovirus, *Mycoplasma pulmonis*, *Clostridium piliforme*, cilia-associated respiratory bacillus, *Encephalitozoon cuniculi*, *Helicobacter* spp., pinworms, and fur mites.

Half of the rats were housed individually in wire-bottom cages (Lab Products, Seaford, DE) over white, noncontact bedding (Poly Pads, Shepherd Specialty Papers, Watertown, TN). The other half of the rats were housed individually in solid-bottom, clear, polycarbonate cages (Lab Products) with a white, cellulose-based contact bedding (Omega-Dri, Harlan Teklad, Indianapolis, IN). Both groups of rats were housed in the same room. All animals were fed a commercially available rodent diet (certified global rodent diet 2018C, Harlan Teklad) ad libitum. An automatic watering system that purifies tap water by reverse osmosis and then conditions the water by chlorination and acidification was used for all animals. The rats were maintained on a 12:12-h light:dark cycle in an environmentally monitored room with alarm set points of 18 °C and 26 °C for temperature and 30% and 70% for relative humidity.

Received: 10 Sep 2007. Revision requested: 23 Oct 2007. Accepted: 2 Jan 2008.
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Prior to each trial, the 30 rats in each cage type were randomized into 3 groups of 10: the control group, the vehicle-treated group, and the dosed group. A vehicle-treated group was included to prevent technicians from identifying which animals had been treated based on the presence of an injection site or other evidence of having been handled. The rats were left in their original housing locations, not moved together according to group, in order to have animals showing clinical signs randomly mixed throughout the rack of cages.

At the start of each trial, dosing technicians administered a low dose of a compound to 10 animals, administered the vehicle without compound to another 10 animals, and then verified which of the treated animals exhibited clinical signs. The dosing technicians then left the room, and 4 experienced toxicology technicians, 2 at a time, examined the animals for clinical signs and recorded their individual findings in a computerized data collection system (Artemis II In-Life Data Collection Software, Instem Life Sciences Data Systems, Stone, Staffordshire, England). The examinations mirrored those performed on routine toxicology studies including cage side observation and, at the technicians' discretion, removal of the rats from their cages for a hands-on physical examination. All of the observing technicians were fully trained in rodent clinical observations, and their training was documented through records compliant with Good Laboratory Practices. Each of the observing technicians had several years of experience performing rodent clinical observations under Good Laboratory Practices conditions, and their ability to make and document such observations accurately had been assessed and validated by supervisors, study directors, clinical veterinary staff, and members of our facility's quality assurance unit. After the technical observers completed their examinations, the dosing technicians again entered the room and confirmed that clinical signs were still present in the treated animals. Although the technical observers knew at each trial that rats had been treated, they did not know what compound had been given or what clinical signs to expect. Animals were given at least 3 d to recover between dosing trials.

The compounds and doses administered were selected from the literature in an attempt to induce only mild clinical signs common to toxicology studies. An attempt was made to avoid producing severe clinical signs as it was felt that severe signs would be easily detected regardless of the caging type. The compounds administered, the doses used, and the vehicle controls for trials 1 and 3 through 8 are listed in Table 1. For trial 2, approximately 75% of the fecal pellets were removed from the cages of 'treated animals' to simulate decreased fecal output.

Because low doses of compounds were used, not all of the 10 animals in each caging type administered a compound actually displayed clinical signs. Due to this biologic variation, the number of animals with signs available for detection was often inconsistent between caging types. Therefore, to make valid cage type comparisons, the mean number of animals detected with a clinical sign by observing technicians was divided by the actual number of animals verified to be exhibiting the sign by dosing technicians. Those ratios were compared between caging groups using the Fisher Exact Test.[1] P-values < 0.05 were considered significant.

Results

Tables 2 through 8 list the clinical signs recorded by the dosing (confirming) technicians and the technical observers. In trial 1 (Table 2), dosing with *p*-chloroamphetamine induced increased salivation, an abnormally low posture, chromodacryorrhea, and chromorhinorrhea. For data analysis purposes, clinical signs of chromodacryorrhea, chromorhinorrhea, or red material around the eyes, face, or muzzle were combined into a single sign of 'red material on face.' In trial 2 (Table 3), removal of approximately 75% of the fecal pellets from each cage produced a simulated clinical sign of scant fecal production in all animals. In trial 3 (Table 4), dosing with polyethylene glycol 400, a vehicle commonly used in toxicology studies, induced loose stool in all animals. Dosing with serotonin in trial 4 at a dose selected from the literature induced unexpectedly severe clinical signs including recumbency, dyspnea, and cyanosis. As the severe signs were obvious to all technicians, the results were excluded from this study, and comparisons between caging types were not made (data not shown). Dosing with xylazine in trial 5 (Table 5) induced ataxia and hypoactivity in most animals. Dosing with apomorphine in trial 6 (Table 6) induced stereotypic behaviors in all rats and hyperactivity in most animals. Unfortunately, the effects were short-lived and resolved before the last 2 observing technicians could perform their observations. The dosing technicians verified that no clinical signs were present when the last 2 technicians performed their observations. For this trial, statistical analysis was performed by using only the clinical signs reported by the first 2 technical observers. Dosing in trials 7 and 8 (Tables 7 and 8) produced discolored urine, red or yellow from phenolsulfonphthalein or blue or dark due to new methylene blue.

To compare the effects of caging type on technicians' observational ability, the mean numbers of animals detected with a clinical sign versus the numbers of animals verified to exhibit that clinical sign were compared between caging types using the Fisher Exact Test. Only treatment of animals with new methylene blue resulted in a significant difference in the number of observations between caging types. Significantly ($P < 0.05$) more animals with dark or blue urine were detected when housed in solid-bottom versus wire-bottom cages. In the other trials, the intertechnician variability appeared to be greater than the variability caused by caging type.

Discussion

The use of wire-bottom versus solid-bottom cages with bedding in toxicology studies remains a controversial issue. Although the *Guide for the Care and Use of Laboratory Animals*[13] recommends solid-bottom cages with bedding because they are "preferred by rodents," the references cited to support this claim do not describe rodent preference studies. The cited studies describe nervous system lesions associated with wire-bottom caging.[3,6,14] Other studies have demonstrated rodents' preference for bedded cages,[11,12] including one that showed a strong preference in rats for solid-bottom cages when resting, a weak preference for solid-bottom cages when awake and exploring, but no difference in body weight gain, food consumption, water consumption, or docility to being handled.[12]

However, animals do not always demonstrate a preference for husbandry conditions that truly enhance their wellbeing. For example, gerbils prefer sunflower seeds to nutritionally complete rodent diets, although the former are too low in calcium and too high in fat to meet their metabolic needs.[9] Preference testing alone, in the absence of other measurements of animal wellbeing, should not be the sole determinant for selecting one husbandry condition over another.

Both pros and cons apply to the use of either type of caging in toxicology studies. Wire-bottom caging may be more economical to use, because it often requires less frequent cage changes (up to 2-wk intervals) compared with solid-bottom cages (generally weekly or more often). Stainless steel cages have a much

Table 1. Compounds administered and doses used

Trial	Treatment	Vehicle	Dose (mg/kg)	Volume (ml/kg)	Route	Source
1	*p*-chloroamphetamine	saline	5	1	SC	Sigma–Aldrich, St Louis, MO
2	fecal pellet removal	NA	NA	NA	NA	NA
3	polyethylene glycol 400	water	16500	15	PO	Mallinckrodt Baker, Phillipsburg, NJ
4	serotonin	saline	250	1	SC	Sigma–Aldrich, St Louis, MO
5	xylazine	saline	8	0.4	SC	Phoenix Science, St Joseph, MO
6	apomorphine	saline	0.9	0.12	IP	Wedgewood Pharmacy, Swedesboro, NJ
7	phenolsulfonphthalein	saline	1	1	SC	Sigma–Aldrich, St Louis, MO
8	new methylene blue	water	10	10	PO	Sigma–Aldrich, St Louis, MO

NA, not applicable

Table 2. Trial 1: Signs detected in *p*-chloroamphetamine-treated rats in solid- or wire-bottom cages

	Dosing technicians (confirmed)		Technical observer A (detected)		Technical observer B (detected)		Technical observer C (detected)		Technical observer D (detected)		Mean detected		Ratio of mean detected to confirmed	
	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire
Salivation	7	10	5	9	6	10	3	6	6	10	5	8.8	0.71	0.88
Red material on face[a]	5	8	1	3	0	1	3	6	0	0	1	2.5	0.20	0.31
Low posture	9	10	0	0	0	0	0	0	4	3	1	0.75	0.11	0.08
Hypoactivity	0	0	0	0	0	7	0	0	0	0	0	1.8	c	c
Splayed posture	0	0	0	0	0	0	0	0	1	0	0.25	0	c	c
Stereotypic behavior[b]	1	0	0	0	0	0	1	1	0	0	0.25	0.25	0.03	c

[a]Red material on face includes red material around eyes (chromodacryorrhea), nose (chromorhinorrhea), or muzzle.
[b]Stereotypic behavior includes stereotypic head movement, stereotypic sniffing, and stereotypic biting.
[c]Ratio invalid because no animals were confirmed with the clinical sign (denominator is 0).

Table 3. Trial 2: Signs detected when fecal pellets were removed from solid- or wire-bottom cages

	Dosing technicians (confirmed)		Technical observer A (detected)		Technical observer B (detected)		Technical observer C (detected)		Technical observer D (detected)		Mean detected		Ratio of mean detected to confirmed	
	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire
Scant feces	10	10	0	0	9	10	0	0	0	10	2.3	5	0.23	0.50
Chromodacryorrhea	0	0	0	1	0	1	0	0	0	0	0	0.25	a	a

[a]Ratio invalid because no rats were confirmed with the clinical sign (denominator is 0).

longer lifespan than do either polycarbonate or polysulfone cages, greatly reducing capital expenses for replacement cages. Although animal wellbeing should take precedence over cost savings, in the absence of obvious improvements in wellbeing, cost remains an important factor.

The use of solid-bottom cages with bedding has the potential to increase rodents' exposure to parent compounds and excreted metabolites, especially those in the urine. Rats will be exposed to some fecal metabolites and will be re-exposed to some unmetabolized parent compound in either caging type because they are coprophagic, ingesting some of their own feces directly from the anus. Although not been shown experimentally, in theory, increased exposure to urinary and fecal metabolites and re-exposure to parent compound may come from oral, dermal, or respiratory exposure to soiled bedding. Compounds and metabolites, especially those that are lipophilic, could be absorbed directly through the skin.[17] Normal grooming behavior could lead to ingestion of compound and metabolites on the fur, and volatile or particulate compounds and metabolites could be inhaled. Re-exposure to parent compounds theoretically could lead to a falsely elevated estimate of expected exposure levels in humans. Early in the drug development process, the identification, activity, relative concentrations, and excretion of metabolites from a new compound are often unknown. Increasing exposure to a toxic metabolite may falsely lead researchers to conclude that a compound produces noteworthy toxicity and result in a decision to discontinue development of an important new drug.

The use of polycarbonate solid-bottom cages, especially older cages that become cracked or 'crazed,' can expose rodents to bisphenol A. Bisphenol A has estrogenic activity and has been shown to alter reproductive parameters in some rodents.[8,10] In addition, some bedding materials, especially pine and cedar, can alter hepatic enzyme activity in rats.[22]

Solid-bottom cages have some advantages over wire-bottom cages for toxicology studies. The use of bedding and nesting material can provide environmental enrichment for rodents.[5] When used with filter tops, solid-bottom cages help decrease the exposure of control animals and also the exposure of animal care technicians to test article.[18] With highly potent test articles

Table 4. Trial 3: Signs detected in rats treated with polyethylene glycol 400 and housed in solid- or wire-bottom cages

	Dosing technicians (confirmed)		Technical observer A (detected)		Technical observer B (detected)		Technical observer C (detected)		Technical observer D (detected)		Mean detected		Ratio of mean detected to confirmed	
	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire
Loose stool	10	10	1	8	7	10	7	10	10	10	6.3	9.5	0.63	0.95

Table 5. Trial 5: Signs detected in xylazine-treated rats in solid- or wire-bottom cages

	Dosing technicians (confirmed)		Technical observer A (detected)		Technical Observer B (detected)		Technical observer C (detected)		Technical observer D (detected)		Mean detected		Ratio of mean detected to confirmed	
	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire
Low posture	4	7	0	0	0	6	0	0	6	6	1.5	3	0.38	0.43
Hypoactivity	7	6	7	7	7	7	7	6	8	7	7.3	6.8	1.04	1.13
Recumbency	5	6	0	2	1	0	0	0	0	0	0.25	0.50	0.05	0.08
Respiration labored or decreased	0	0	0	0	4	6	0	0	2	5	1.5	2.8	a	a
Ataxia	9	10	0	0	7	6	0	0	2	3	2.3	2.3	0.25	0.23
Excessive urine or wetness	0	5	0	0	0	0	0	5	0	0	0	1.3	a	0.25

[a]Ratio invalid as no animals were confirmed with the clinical sign (denominator is 0).

Table 6. Trial 6: Signs detected in apomorphine-treated rats in solid- or wire-bottom cages

	Dosing technicians (confirmed)		Technical observer B (detected)		Technical observer D (detected)		Mean detected		Ratio of mean detected to confirmed	
	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire
Ptosis	2	0	4	1	0	0	2	0.5	1.00	c
Hypoactivity	0	1	0	0	0	0	0	0	c	0.00
Hyperactivity	5	9	0	0	0	0	0	0	0.00	0.00
Stereotypic licking	6	8	0	2	1	2	0.50	2	0.08	0.25
Stereotypic biting	3	5	0	3	0	3	0	3	0.00	0.60
Stereotypic grooming	0	0	0	0	1	0	0.5	0	c	c
Stereotypic sniffing	9	9	9	10	10	5	9.5	7.5	1.05	0.83
Stereotypic behaviors[b]	10	10	9	10	10	8	9.5	9.0	0.95	0.90

[a]The signs listed above were short-lived and only present for assessment by 2 of the 4 technical observers.
[b]Stereotypic behaviors include stereotypic licking, biting, sniffing, and grooming because the animals were eliciting multiple behaviors that varied temporally.
[c]Ratio invalid because no animals were confirmed with the clinical sign (denominator is 0).

or with potentially carcinogenic compounds, minimizing environmental contamination and employee exposure (therefore health risk) are critically important.

A considerable drawback to the use of wire-bottom caging in toxicology studies is the development of foot lesions. In rats, these lesions begin to appear around 1 y of age.[15] Early lesions may require moving the rats from wire-bottom cages to solid-bottom cages with bedding. Advanced lesions may become ulcerated and infected, requiring euthanasia for humane reasons. Lesions develop more frequently in larger rats than in smaller animals. The lesions are clearly detrimental to rats' wellbeing and make solid-bottom cages with bedding preferable for long term (>1 y) studies when there is no scientific justification to use wire-bottom cages.

The type of caging, solid-bottom with bedding or wire-bottom, has been shown to affect some research studies. For example, housing in wire-bottom cages increased the severity of experimentally induced dental caries in rats,[20] although a study comparing the effects of magnesium and phosphate supplementation on the formation of dental caries found the opposite.[7] Housing mice in wire-bottom cages increased the incidence of mouse urologic syndrome.[2] Wood and colleagues reported that changing from wire-bottom cages to solid-bottom cages with bedding altered the consumption of sugar water in successive negative-contrast studies.[23]Cage type affected multiple thermoregulatory variables, and thermogenesis in response to 3,4-methylenedioxymethamphetamine administration was markedly higher in rats housed in solid-bottom cages versus wire-bottom cages.[4] Housing in wire-bottom cages increased nighttime activity levels and food consumption in rats.[16] However, another study showed no difference in clinical pathology parameters in Sprague-Dawley rats housed in the 2 types of caging.[19]

In routine, preclinical toxicology studies, animals are administered test articles at quantities expected to produce clinical signs in higher dose groups. Therefore, technicians performing clinical observations expect to see some clinical signs, although which signs to expect is often unknown for novel compounds. This scenario is analogous to the conditions of our caging comparison study in which technicians knew that some animals would be showing clinical signs but did not know which signs to expect.

Table 7. Trial 7: Signs detected in phenolsulfonphthalein-treated rats in solid- or wire-bottom cages

	Dosing technicians (confirmed)		Technical observer A (detected)		Technical observer B (detected)		Technical observer C (detected)		Technical observer D (detected)		Mean detected		Ratio of mean detected to confirmed	
	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire
Hypoactivity	0	0	0	1	0	0	0	0	0	0	0	0.25	a	a
Pink, red, or yellow discoloration	10	10	8	10	7	8	2	8	7	6	6	8	0.60	0.80

[a]Ratio invalid as no animals were confirmed with the clinical sign (denominator is 0).

Table 8. Trial 8: Signs detected in rats treated with new methylene blue and housed in solid- or wire-bottom cages

	Dosing technicians (confirmed)		Technical observer A (detected)		Technical observer B (detected)		Technical observer C (detected)		Technical observer D (detected)		Mean detected		Ratio of mean detected to confirmed	
	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire	Solid	Wire
Urine, blue or dark	10	9	8	1	10	4	9	2	10	3	9.3	2.5	0.93[a]	0.25[a]

[a]Denotes a statistically significant ($P < 0.05$) difference between caging types, as determined by the Fisher Exact Test.

Intertechnician variability in the number of animals called with clinical signs was high in this study because the clinical signs produced were subtle, and the observations were qualitative, not quantitative. There was no measuring device available to assist technicians in determining how much moisture around the muzzle to call 'salivation' or how much additional movement around the cage to call 'hyperactivity.' The results relied on the individual technicians' judgment regarding when a difference between one animal and others was so marked that it merited documentation as a clinical sign.

In light of the results of this study, toxicologists need not be concerned that using solid-bottom caging with bedding will inhibit technical staff's ability to detect common clinical signs in study animals. Neither will the use of solid-bottom cages with bedding improve the detection of clinical signs in most studies. When selecting the type of caging to use in toxicology studies, researchers should evaluate the risks and benefits of both caging types and select the most appropriate type based on scientific need and animal welfare. Institutional animal care and use committees should assess the reasons for selecting a given type of caging when reviewing protocols.

Acknowledgments

The authors wish to thank Jeff Bottoms, Ron Cox, Danny Deig, Dennis Jourdan, Chris Carnahan, and Dean Wilkinson for their assistance with dosing and observing the animals and for their dedication to quality animal research and humane care.

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