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Clinical Pathology Laboratory Values of Rats Housed in Wire-Bottom Cages Compared with Those of Rats Housed in Solid-Bottom Cages

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Rats are used routinely for the discovery of new pharmaceuticals and for toxicology testing to fulfill regulatory requirements. In 1999, a survey showed that 80% of all rodents housed in toxicology studies were housed in wire-bottom cages. However, both the National Research Council and Association for the Assessment and Accreditation of Laboratory Animal Care, International, recommend housing rats in solid-bottom cages with bedding. In this study 2 groups of male Sprague Dawley rats were housed in the same room for 4 wk and provided the same food and water by the same husbandry staff person. The only variable in the study was the type of housing. One group was housed in solid-bottom polycarbonate cages with bedding and the other group in standard wire-bottom caging. Clinical pathology laboratory evaluations of complete blood count, serum chemistries, urinalysis, urine creatinine, urine corticosterone, blood coagulation, and hepatic cytochrome P450 isoenzyme mRNA levels were performed. No clinically relevant differences were found between the 2 groups for any of the laboratory data.

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Care, International; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBC, complete blood count; Ct, cycle threshold; CYP-450, cytochrome P-450; DNA, deoxyribonucleic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPF, high power field; LPF, low power field; m RNA, microsomal ribonucleic acid; RBC, red blood count; RNA, ribonucleic acid; RT-PCR, reverse transcription polymerase reaction; SD, standard deviation; WBC, white blood count

The *Guide for the Care and Use of Laboratory Animals*[11] states, "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." In 1999, 3 y after the National Research Council published this recommendation, a survey showed that 80% of all rodents housed in toxicology studies in the United States were kept in wire-bottom cages.[17]

Toxicologists may be reluctant to change the type of housing used for their study rats in part for fear that a change may induce significant differences in laboratory test results. Such differences would interfere with their ability to compare data between differently housed groups and to use the vast amount of historical data collected from animals housed in wire-bottom cages. Toxicology data collected in safety studies are subject to compliance with Good Laboratory Practice and scrutiny by the Food and Drug Administration.

The present study was designed to compare the clinical pathology laboratory values commonly evaluated in rats on a 4-wk toxicology study between rats housed in wire-bottom cages and those in solid-bottom cages. Specifically, this study evaluated body weight, serum clinical chemistries, complete blood count parameters, blood coagulation values, urinalysis data, urine corticosterone concentration, and urine creatinine concentration. Although not a routine lab test for toxicology, analysis of cytochrome P450 (CYP450) isoenzymes also was performed because changes in the levels of these enzymes might alter the metabolism of drugs under study.[8]

Materials and Methods

The following study was conducted in accordance with guidelines set forth in the *Guide for the Care and Use of Laboratory Animals*[11] and was approved by our institutional animal care and use committee. The animal facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC).

Animals. Male Sprague-Dawley (Hsd:Sprague Dawley SD) rats (*Rattus norvegicus*) approximately 8 to 9 wk of age were obtained from a commercial vendor (Harlan, Indianapolis, IN). The rats were specific pathogen-free for *Mycoplasma pulmonis*, Sendai virus, rat coronavirus/sialodacryoadenitis virus, Kilham rat virus, Toolan H-1 virus, rat parvovirus, pneumonia virus of mice, reovirus 3, Hantaan virus, Theiler mouse encephalomyelitis virus, mouse adenovirus, lymphocytic choriomeningitis virus, *Clostridium piliforme*, *Encephalitozoon cuniculi*, and cilia-associated respiratory bacillus. Animals were fed a standard laboratory diet (Harlan Teklad Global Diet 2018) and were allowed food and water ad libitum. Water was purified by reverse osmosis. Lighting was controlled on a 12:12-h light:dark cycle (lights on, 06:00; lights off, 18:00), temperature was maintained at 23.3 ± 1.1 °C, and relative humidity was maintained between 40% and 60%. Techboard (Shepherd Specialty Papers, Watertown, TN) placed under the wire-bottom cages was changed 3 times per week.

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Wire-bottom cages were removed and sanitized every 14 d. Solid-bottom shoebox cages were changed twice per week. Racks and feeders were removed and sanitized every 14 d.

Study design. The study was designed to mimic a 4-wk toxicology study as it would be performed at our institution. The only variable between the 2 groups of rats was their type of housing. We placed 23 rats in solid-bottom polycarbonate cages (30.5 × 43.2 × 21 cm) with bedding (Alpha-Dri, Shepherd Specialty Papers) to a depth of approximately 1.25 cm. Another 22 rats were housed in standard hanging wire-bottom caging made of stainless steel (30.5 × 39.4 × 35.6 cm). In both types of cages, rats were housed 3 per cage, which allowed more than adequate floor space per rat based on recommendations from the *Guide for the Care and Use of Laboratory Animals*.[11] All rats received a physical examination and were weighed and orally gavaged with saline daily to mimic a daily oral dose of test compound. Blood was collected for serum chemistries and hematology at the beginning of the study and at 2 and 4 wk thereafter. Urine was collected for urinalysis and urine corticosterone and creatinine concentrations over an 18-h period on day 22 of the study. At the end of the study (28 d), the rats were euthanatized by an overdose of sodium pentobarbital, blood was collected for hemostasis evaluation, and liver tissue was collected for reverse transcription–polymerase chain reaction (RT-PCR) analysis of CYP450 isoenzymes.

Collection of blood, urine, and liver tissue samples. To obtain blood for serum chemistries and hematology, each rat was removed from its cage and placed for 3 to 6 min in an incubator set at 52 °C . The rat then was removed from the incubator and placed in a cloth restraining device. A blood sample (1.5 ml) was taken from the tail vein by using a 21-gauge "butterfly" needle and collected into ethylendiaminetetraacetic acid and serum separator tubes. On day 22 of the study, rats were removed from their cages and placed individually in metabolism cages (model 650-0350, Nalgene, Rochester, NY) for 18 h to collect urine. At the end of the study (day 28), rats were euthanatized by an overdose of sodium pentobarbital (100 mg/kg intraperitoneally). Blood for coagulation studies was collected by cardiocentesis in buffered sodium citrate (9:1) tubes. Liver tissue for evaluation of CYP450 isoenzymes was collected from 7 to 9 animals from each group, snap-frozen in liquid nitrogen, and stored at –70 °C pending analysis.

Blood sample analysis. Serum chemistries were evaluated on an automated clinical chemistry analyzer (Hitachi model 917, Roche Diagnostics, Indianapolis, IN). Samples were analyzed for glucose, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, globulin, cholesterol, triglycerides, total bilirubin, calcium, phosphorous, sodium, potassium, and chloride. CBC samples were evaluated on an automated hematology system (Advia 120 model 254, Bayer, Norwood, MA). Samples were analyzed for red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, red cell distribution width, platelets, white blood cell count, absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, absolute eosinophil count, absolute basophil count, and absolute large unstained cell count. Blood smears were evaluated visually for platelet clumping and WBC and RBC morphology. The coagulation parameters were run on an automated coagulation analyzer (STA Compact, Diagnostica Stago, Parsippany, NJ). Coagulation parameters tested were prothrombin time, partial thromboplastin time, and fibrinogen.

Urine sample analysis. Urinalyses were performed using Multistix 10SG reagent strips (Bayer, Elkhart, IN). Analytes measured were specific gravity, pH, glucose, ketones, bilirubin, blood, urobilinogen, RBCs, WBCs, epithelial cells, calcium oxalate crystals, calcium carbonate crystals, triple-phosphate crystals, amorphous material, casts, bacteria, and sperm. Urine specific gravity was determined using a refractometer (Shuco model 5711-7020, American Caduceus Industries, Carle Place, NY). Urine microscopic analysis was performed using the Kova Standardized Microscopic Urinalysis System (Hycor Biomedical, Garden Grove, CA). A standard volume of 2 ml urine was placed in the Kova tube and placed in a centrifuge (Heraeus Sepatech Contifuge 17RS, Osterode, Germany) and spun for 5 min at 323 × *g*. A well-mixed sediment sample was evaluated microscopically (Nikon Eclipse E 400, Tokyo, Japan). The urine creatinine and urine corticosterone samples were evaluated on a multiwell gamma counter (Genesys 5000, Laboratory Technologies, Maple Park, IL).

Evaluation of liver CYP450 isoenzyme levels. CYP450 isoenzyme mRNA levels were evaluated from the livers of 7 to 9 rats from each group. Hepatic mRNA was extracted using a standard institutional protocol. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA), followed by further purification using RNeasy Minikit (Qiagen, Valencia, CA). RNA (1 µg) was reverse-transcribed to cDNA (Superscript II, Invitrogen). The resulting cDNA (5 µl, equivalent to 5 ng RNA) was used as template for each PCR reaction; the primers used were validated for rat tissue expression using mRNA extracted from normal rat spleen and liver (Table 1). The PCR reaction was amplified using an automated DNA sequencer (model 7900 Sequence Detection System, Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations for primer concentrations and number, temperature, and duration of cycles. All samples were run in triplicate for each rat. Cycle threshold (Ct) values (the number of cycles for the signal intensity to exceed the background signal threshold) in the linear, exponential phase of amplification were determined for each gene. Ct values are inversely proportional to the amount of mRNA present. The Ct values for each gene target were normalized to the housekeeping gene cyclophilin (PPIA) to allow intersample comparison of data. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a second internal housekeeping gene for comparison. The levels of expression of both housekeeping genes were similar among all samples evaluated and across both experimental groups (data not shown). The comparative quantification of hepatic mRNA expression from solid-bottom cage-housed versus wire-bottom cage-housed rats was determined using the comparative Ct method arithmetic formula recommended by Applied Biosystems.[1] Results are reported as fold increase over control.

Statistical analysis. Because a number of the body weights, hematology, and serum chemistry variables exhibited statistically significant differences at baseline, the initial differences were compensated for by analyzing the data collected at the middle and end of the study by using analysis of covariance, with the baseline value used as the covariate.[12] Because only 3 values (0, 0.1, and 0.2) were observed in all of the total bilirubin data, this variable was analyzed using the Wilcoxan rank-sum test at all time points.[9] All other variables except CYP450 expression were compared using *t* tests. PC SAS, version 9.1, TS Level 1M3[16] was used for these analyses. CYP450 expression levels from each group were compared using the Dunnett Comparison of Means test, with rats housed in wire-bottom cages as the control group (JMP 5.1.2 Software, SAS, Cary, NC). For all analyses, a P value of ≤0.05 was considered statistically significant.

Table 1. Primer sequence information for rat cytochrome P450 isoenzyme amplification

CYP450 isoenzyme	Forward primer sequence (3′ to 5′)	Reverse primer sequence (3′ to 5′)	Amplicon length (bp)	GenBank mRNA source
P450 1A1	CAC AGA CAG CCT CAT TGA GCA T	ATT GTG TCA AAC CCA GCT CCA	123	GI:6978732
P450 1A2	CTT TCC GGT CCT GCG CTA C	CGC CTG TGA TGT CCT GGA TA	143	GI:203762
P450 3A1	GGT CAG TGG TCA TGA TTC CAT CT	GGC GAA ATT CCT CAG GCT CT	75	GI:56038
P450 3A2	GAC TTG GAA CCC ATA GAC	CAT GTC AAA TCT CCC TAA G	116	GI:498863
P450 4A1	GCT GAA TGG ACA ACC GTG GT	TGC CAG CTG TTC CCA TTT G	133	GI:56046
P450 2B1/2	TTC CTG CTG ATG CTC AAG TAC C	TAC TGC GGT CAT CAA GGG TTG	103	GI:203784
P450 2C11	TGA ATG TCA CAG CTA AAG TC	CTT GGG AAT GAA GTA GTT TC	200	GI:944945
P450 2E1	CCA TTG CCT TGC TGG TGT G	AGC GCT TTG CCA ACT TGG T	172	GI:3126850
GAPDH	ACT TTG GCA TCG TGG AAG GG	CAT ACT TGG CAG GTT TCT CCA GG	267	NM_017008
PIPA	TGA GGA TGA GAA CTT CAT CCT GAA	CTC AGT CTT GGC AGT GCA GAT AA	112	NM_017101

bp, base pairs; start location on mRNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IPIA, peptidylprolyl isomerase A (cyclophilin A).

Results

Physical examination. Rats were examined daily for general condition and health when they were weighed and dosed. No abnormalities were noted in any of the rats during the 28-d period. No foot lesions were observed.

Body weight. Rats were ordered from the vendor by age, and initial body weights ranged within the normal parameters for that age. At the outset, the rats housed in solid-bottom cages weighed 299 ± 14 g (mean ± 1 standard deviation), and the rats housed in wire-bottom cages weighed an average of 288 ± 10 g (Table 2). At the end of the 28-d study, both groups of rats had gained weight. The solid-bottom cage group weighed 349 ± 22 g, and the wire-bottom cage group weighed 338 ± 16 g. There was no statistically significant difference in body weight between the two groups at the end of the study.

Serum chemistries. The rats in the solid-bottom cages showed a positive bias for the hepatic enzymes ALT and AST that was evident in the prestudy baseline data (Table 2). This bias persisted with no change throughout the study indicating that the cage type was not the cause but rather the result of randomization. There were no biologically relevant differences in serum chemistries between rats housed on wire- or solid-bottom cages.

Hematology. At the midstudy time point (2 wk), there were statistically significant differences ($P \leq 0.05$) in values for RBC count, hemoglobin concentration, mean corpuscular volume, and absolute neutrophil and eosinophil counts (Table 2). At the end of the study there was a statistically significant difference ($P < 0.05$) in hematocrit between the rats housed on solid-bottom cages (49.5) and those in wire-bottom cages (48.1); however, all values were well within the laboratory reference range for that value. All other values for hematology showed no statistical difference at any of the 3 time points. All statistically significant differences ($P < 0.05$) between cage types at the same time point are indicated by asterisks in Table 2.

Coagulation studies, urinalysis, urine corticosterone, and urine creatinine. There were no statistically significant differences between the 2 groups for any of the parameters analyzed (Tables 3 to 5). Although partial thromboplastin time in the rats from this study was greater than the reference interval, the samples were handled identically, and this increase was consistent for both groups.

CYP450 isoenzymes. The mean expression level for each CYP450 isoform from the rats housed on solid-bottom cages was compared with the mean expression level from the rats housed on wire-bottom cages, which served as the control group (Table 6). The comparison data are presented as the fold increase in expression of each CYP450 in the solid-bottom cage group as compared with controls. There was no significant difference in the expression levels of the CYP450 isoenzymes examined from rats housed in solid-bottom cages with bedding compared with rats housed in wire-bottom cages.

Discussion

Historically, toxicology and safety studies in the United States have been performed with rats housed in wire-bottom cages. The National Research Council and AAALAC have recommended housing rats in solid-bottom cages since the 1996 publication of the *Guide for the Care and Use of Laboratory Animals*,[11] in light of studies indicating that rats appear to prefer solid-bottom caging over wire-caging when presented with a choice.[11,13] In addition, rats fed ad libitum and housed on wire-bottom cages over a long period can develop painful foot lesions.[14] Still, a 1999 survey found that 80% of United States pharmaceutical companies continued to house rats for toxicology and safety studies on wire-bottom cages.[17] Although more recent studies have indicated some trends toward the use of solid-bottom caging,[5,18] the issue remains a challenge for both regulatory agencies and institutional animal care and use committees in contract research organizations and pharmaceutical companies.

Toxicologists may be reluctant to change the type of housing used for their study rats in part for fear that such a change may result in significant clinical differences in clinical pathology data. It is important to be able to compare results from concurrent studies and to compare current results with historical data. Differences in clinical data due solely to caging type could potentially complicate such comparisons. There has been a trend among toxicologists to attempt to harmonize the methods used in toxicologic studies so data can be compared more easily. A joint international committee, formed to provide expert recommendations for clinical pathology testing of laboratory animal species used in safety and toxicology studies,[20] addressed some methods, such as bleeding intervals, but did not discuss caging methods specifically.

An additional concern is whether the contact bedding used in solid-bottom cages could have any effect on study data. It has been established that varieties of both soft and hardwood can induce hepatic drug-metabolizing enzymes in rodents that are housed on bedding made from this wood.[2,7,15] Cedar-chip bedding has been shown to cause a significant increase in CYP450 enzymes in rats, and use of pine products in bedding has been associated with rat liver endosome acidification.[2] It also has been shown that when rats are removed from housing containing pine bedding products, the liver enzyme induction is reversible.[4] Another in vivo study compared cedar chips, corncob bedding, heat-treated pine bedding, and wire-bottom cages. The corncob bedding had no effect on the liver enzymes

Table 2. Body weight, serum chemistry, and hematology values for rats housed in solid-bottom or wire-bottom cages

Analyte	Units	Start (day 0)						Midpoint (day 14)						End (day 28)						Reference range
		Solid-bottom			Wire-bottom			Solid-bottom			Wire-bottom			Solid-bottom			Wire-bottom			
		n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	
Body weight	g	22	299	14	22	288	10*	21	332	21	22	325	13*	23	349	22	22	338	16	NA
Glucose	mg/dl	23	93	14	22	97	15	23	85	13	22	93	15	22	76	14	22	87	15*	91–145
Blood urea nitrogen	mg/dl	23	21.8	2.4	22	22.3	3.6	23	20.7	2.1	21	21.0	2.6	22	23.5	3.0	22	21.3	2.7*	13.0–29.0
Creatinine	mg/dl	23	0.4	0.1	22	0.4	0.1	23	0.4	0.1	21	0.4	0.1	22	0.5	0.1	22	0.5	0.1	0.2–0.5
Alanine aminotransferase	U/l	23	69	15	22	42	5*	23	57	10	22	41	3*	22	62	8	22	41	4*	34–69
Aspartate aminotransferase	U/l	23	113	28	22	101	17	23	112	26	22	98	14	22	125	26	22	103	16*	73–125
Alkaline phosphatase	U/l	23	141	16	22	131	11*	23	120	12	22	119	17	22	109	13	22	104	12	86–218
Total protein	g/dl	23	6.2	0.2	22	6.1	0.2	23	6.7	0.2	22	6.7	0.1	22	6.8	0.2	22	6.8	0.2	5.4–7.5
Albumin	g/dl	22	3.4	0.1	21	3.3	0.1	23	3.5	0.1	21	3.4	0.1*	22	3.6	0.1	22	3.5	0.1	2.8–3.7
Globulin	g/dl	22	2.8	0.1	21	2.8	0.1	23	3.3	0.1	21	3.2	0.1	22	3.2	0.1	22	3.3	0.1	2.6–4.0
Albumin:globulin ratio	none	22	1.2	0.05	21	1.2	0.04	23	1.1	0.04	21	1.1	0.03	22	1.1	0.04	22	1.1	0.05	NA
Cholesterol	mg/dl	23	119	12	21	101	12*	23	108	11	21	106	7*	22	108	12	22	108	8*	71–117
Triglycerides	mg/dl	23	37	15	21	24	13*	23	27	9	21	26	9	22	27	12	22	22	10	6–52
Total bilirubin	mg/dl	23	0.1	0.02	22	0.1	0.00	23	0.1	0.03	21	0.1	0.03	22	0.1	0.1	22	0.1	0.00*	0.1–0.2
Calcium	mg/dl	23	10.2	0.3	22	9.6	0.5*	23	10.1	0.3	22	9.8	0.6*	22	10.7	0.3	20	10.4	0.2*	9.3–10.7
Phosphorous	mg/dl	23	8.8	0.7	21	8.4	0.6*	23	7.9	0.5	21	7.5	0.6	22	7.3	0.7	22	7.0	0.6	6.1–10.8
Sodium	mEq/l	23	142	1	22	143	1*	23	143	1	22	143	1	22	144	3	22	144	1	140–149
Potassium	mEq/l	23	5.4	0.8	22	5.0	0.5	23	5.2	0.2	22	5.1	0.3	22	5.0	0.2	22	5.0	0.3	3.9–6.0
Chloride	mEq/l	23	100	1	22	102	1*	23	98	1	22	100	1*	22	98	2	22	100	1	98–110
Red blood cells	$\times 10^6/\mu l$	21	8.1	0.4	23	8.2	0.5	22	8.9	0.3	22	8.6	0.3*	22	9.1	0.5	22	8.9	0.3	7.8–9.0
Hemoglobin	g/dl	21	15.4	0.6	23	15.4	0.8	21	16.8	0.5	22	15.9	1.1*	22	16.6	0.8	22	16.2	0.5*	14.3–16.9
Hematocrit	%	21	45.8	1.8	23	45.8	2.6	22	49.2	1.5	22	47.1	1.9*	22	49.5	2.6	22	48.1	1.9*	44.6–48.7
Mean corpuscular volume	fl	21	56.5	1.4	23	56.0	1.6	22	55.3	1.3	22	54.7	1.8	22	54.7	1.4	22	54.3	1.5	53.1–59.5
Mean corpuscular hemoglobin	pg	21	19.0	0.4	23	18.8	0.5	21	18.9	0.5	21	18.7	0.6	22	18.4	0.4	21	18.2	0.5	17.8–20.8
Mean corpuscular hemoglobin concentration	g/dl	21	33.6	0.5	23	33.5	0.5	21	34.1	0.4	22	33.8	2.2	22	33.6	0.6	22	33.7	0.6	31.9–35.9
Red cell distribution width	%	21	12.2	0.5	23	11.9	0.4*	22	12.0	0.4	22	12.0	0.3	22	11.6	0.4	22	11.9	0.2*	10.8–12.5
Platelets	$\times 10^3/\mu l$	15	1198	213	15	1075	131	18	1079	140	20	1053	143	18	1088	162	21	1033	177	810–1873
White blood cells	$\times 10^3/\mu l$	21	12.51	2.91	23	11.70	2.87	22	11.94	2.36	22	12.17	2.21	22	10.94	2.01	22	11.16	2.42	11.09–25.85
Absolute neutrophils	$\times 10^3/\mu l$	21	1.56	0.76	23	1.50	0.70	22	1.71	0.59	22	2.20	0.83*	22	1.29	0.44	22	1.25	0.37	0.54–2.78
Absolute lymphocytes	$\times 10^3/\mu l$	21	10.41	2.52	23	9.70	2.41	22	9.65	1.93	22	9.39	1.77	22	9.15	1.74	22	9.43	2.20	9.27–23.54
Absolute monocytes	$\times 10^3/\mu l$	21	0.31	0.13	23	0.27	0.15	22	0.34	0.12	22	0.31	0.10	22	0.24	0.08	22	0.22	0.10	0.14–0.74
Absolute eosinophils	$\times 10^3/\mu l$	21	0.08	0.04	23	0.10	0.04	22	0.10	0.04	22	0.14	0.05*	22	0.13	0.05	22	0.14	0.04	0.05–0.28
Absolute basophils	$\times 10^3/\mu l$	21	0.04	0.02	23	0.04	0.02	22	0.04	0.02	22	0.04	0.02	22	0.04	0.01	22	0.04	0.02	0.02–0.16
Absolute leukocytes	$\times 10^3/\mu l$	21	0.11	0.04	23	0.09	0.04	22	0.10	0.04	22	0.08	0.04	22	0.08	0.04	22	0.08	0.03	0.05–0.21

NA, not applicable; SD, 1 standard deviation.

*Value for rats on solid-bottom cages statistically significant different ($P < 0.05$) from that of rats on wire-bottom cages at the same time point.

Table 3. Coagulation times and factors for rats housed in solid-bottom or wire-bottom cages

		Solid-bottom (n = 23)		Wire-bottom (n = 19)		
Analyte	Unit	mean	SD	mean	SD	Reference range
Prothrombin time	s	19	2	19	1	15–19
Partial thromboplastin time	s	23	4	23	3	8–15
Fibrinogen	mg/dl	232	72	259	48	261–391

SD, 1 standard deviation.

Table 4. Urine chemistries for rats housed in solid-bottom or wire-bottom cages

		Solid-bottom (n = 23)		Wire-bottom (n = 22)	
Analyte	Units	mean	SD	mean	SD
Corticosterone	ng/18 h	7.46	4.48	6.46	3.30
Creatinine	mg/18 h	23.0	11.0	23.2	9.9
Corticosterone:creatinine ratio	none	0.317	8.230	0.277	7.010

SD, 1 standard deviation.

Table 5. Urinalysis results for rats housed in solid-bottom or wire-bottom cages

		Solid-bottom (n = 23)		Wire-bottom (n = 21 or 22)[a]		
Analyte	Units	mean	SD	mean	SD	Reference interval
Specific gravity	none	1.034	0.006	1.032	0.007	1.016–1.066
pH	none	6.7	0.4	6.5	0.4	6.5–8.5
Glucose	g/dl	0	0	0	0	0–9.9
Ketones	mg/dl	37	14	33	11	0
Bilirubin	0–3	0.2	0.4	0.2	0.4	0
Blood	0–3	0	0	0	0	0
Urobilinogen	mg/dL	0	0	0	0	0
Red blood cells	no./hpf	0	0	0	0	0
White blood cells	no./hpf	0	0	0	0	0
Epithelial cells	no./hpf	0	0	0	1	0–3
Ca_2O_4 crystals	no./hpf	0	0	0	0	0
Ca_2CO_3 crystals	no./hpf	0	0	0	0	0
Triple-phosphate crystals	no./hpf	4	0	3	1	0–5
Amorphous material	no./hpf	0	0	0	0	0
Casts	no./lpf	0	0	0	0	0–3
Bacteria	no./hpf	0	0	0	0	0
Sperm	no./lpf	4	0	3	1	0–many

hpf, high-power (×100) field; lpf, low-power (×40) field; SD, 1 standard deviation.
[a]Values represent 22 samples, except those for Ca_2O_4 and Ca_2CO_3 crystals (21 samples each).

Table 6. Expression of cytochrome P450 isozymes in rats housed in solid-bottom cages compared with rats housed in wire-bottom cages

			CYP450 isoenzymes (fold increase over control value)							
Group	GAPDH	PPIA	1A1	1A2	2B1/2	2C11	2E1	3A1	3A2	4A1
Wire-bottom (n = 7)	1	1	1	1	1	1	1	1	1	1
Solid-bottom (n = 9)	0.65	1	0.84	0.94	0.92	1.3	1.44	0.95	1.14	0.63

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IPIA, peptidylprolyl isomerase A (cyclophilin).

of the rats, the cedar significantly increased enzyme activity, and the pine bedding significantly decreased activity.[19] Such changes in enzyme function could affect metabolism of a drug under study.[3,4,8,21] To date, there have been no in vivo studies measuring the induction of hepatic enzymes in rodents housed on cellulose products.

There may be specific instances in which wire-bottom cage housing for rats is preferred, such as in breeding colonies in which a copulatory plug needs to be identified,[5] studies in which polyuria is anticipated, or studies in which compounds are known to create a hyperthermic effect.[5] However, these situations are uncommon in standard toxicology studies.

Overall, the data from the present study showed that clinical pathology analyses of the type commonly performed in toxicology studies was similar regardless of whether rats were housed in wire-bottom or solid-bottom cages. There were no biologically relevant differences between the 2 groups. In addition, this study showed that Alpha-Dri used as contact bedding had no effect on the activity of CYP450 isoenzymes in rats.

Although our data includes several instances of statistically significant differences in some analytes at certain points, specifically among clinical chemistry and hematology results, these differences are not deemed to be biologically relevant. The actual mean values were within or very close to established reference intervals for species, sex, and age of the animals. A joint committee formed to provide expert recommendations

for clinical pathology testing has stated that "appropriate statistical methods should be used to analyze clinical pathology data. Regardless of the outcome of statistical analysis, scientific interpretation is necessary for the ultimate determination of test material treatment effects. Statistical significance alone should not be used to infer toxicological or biological relevance of clinical pathology findings."[20] A large coefficient of variation, for example in alanine and aspartate aminotransferase values,[10] may lead to statistically significant differences that have no biological relevance. Our laboratory data were evaluated by a board-certified veterinary clinical pathologist, who determined that there were no clinically relevant differences between the two groups.

Because housing rats in solid-bottom cages does not appear to introduce any significant bias for routine clinical pathology analyses commonly required by toxicology studies, selection of cage type for animals used in toxicology studies should be based on scientific and animal welfare considerations. Concerns about study reproducibility or the ability to compare current data with historical data should not preclude housing of toxicology study animals in solid-bottom cages, as recommended by the *Guide for the Care and Use of Laboratory Animals*.[11]

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