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ORIGINAL RESEARCH

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Nesting materials may cause pneumonia-like findings in Sprague Dawley rats

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ABSTRACT

Aim/Background: In the studies conducted with laboratory animals, using wood shavings as cage nesting material may have an impact on study results. However, there is not sufficient information on the effects on the lungs due to using wood shavings. We have aimed to radiologically and histopathologically examine possible lung pathologies, which can be seen on laboratory animals in which wooden shavings are used as filling materials.

Method: In this study, 20 male Sprague Dawley rats have been divided into two groups and put into the cages one by one with and without filling materials. At the beginning and end of the 4-week monitoring process, their lung radiographies have been taken. Additionally, their lung tissues have been histopathologically examined at the end of the study, and ion chromatography and gas chromatography-mass spectrometry analyses were performed.

Results: Pneumonia-like findings were detected in the lungs of animals in cages where wooden shavings are used, and diagnosis has been confirmed histopathologically. Chlorate has been detected both in lungs of animals for which pathology has formerly been detected and in wooden shavings used; 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl] has also been detected in wood shavings and pathological lungs.

Conclusions: Detected chemicals may contribute to the lung pathology, which is seen on animals in which wooden shavings are used as filling material. It was concluded that avoiding wooden shavings as filling material in the experimental studies conducted with laboratory animals is going to be more reasonable in terms of reliability of results and health of animals.

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Introduction

A considerable number of medical improvements are introduced to the world by means of experimental studies conducted on laboratory animals [1]. However, it is very important to house these laboratory animals in reasonable and standard conditions in terms of obtaining healthy results and repeatability of these results. Besides conditions such as temperature, humidity, air conditioning, and light, it is known that filling materials used in the cages in which the animals are kept may have an impact on the results of studies and health of animals [2,3].

Laboratory animals need convenient surfaces during their daily routines, such as resting and sleeping. In addition, rodent laboratory animals especially want to live on a convenient surface filling for activities such as scratching, rasorial activities, digging the surface, hiding attempts, and arranging the place for themselves [4,5]. Surface fillings in cages enable the cages to be cleaned easily, to keep the place clean and hygienic, and to minimize the contact of animals with urine and fecal material by absorbing them [1]. Sufficient and type-specific cage filling contributes to the thermoregulation of animals [6]. Therefore, providing

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cage filling materials that are specific to animal species is extremely significant during the growing up, breeding, and housing throughout the study processes of laboratory animals [1].

There are some studies showing that various surface fillings may cause several health problems in animals; it has been shown that some filling materials produced from the paper that has tiny dust have caused periorbital abscesses in nude and hairless mice [7]; filling material produced from cotton may form conjunctivitis [8]. It has also been shown that filling material may affect mucosal immunity and cause endocytosis [9,10].

Wooden shavings are widely used as filling material but these materials may lead to some significant changes both at the stage of breeding of laboratory animals and evaluation of research results. Because the filling material emits aromatic hydrocarbons obtained from cedar trees, it has been pointed out that it inducts hepatic microsomal enzymes, may cause cytotoxicity, and also increase the frequency of cancer in animals [11–14].

Pine tree shavings may also change the results of experiments by affecting the metabolism of animals and damaging their cells [11,15–17]. Before using, exposing pine and cedar tree shavings to a high temperature may remove volatile compounds from the environment but negative effects may still continue on the animals. Additionally, all kinds of contaminants (pesticides, heavy metals, and various toxins) that are going to contaminate the filling material may affect animal health and may mislead the experiment results.

We have aimed to radiologically and histopathologically examine possible lung pathologies, which can be seen on experiment laboratory animals on which wooden shavings are used as filling materials.

Materials and Methods

All studies on rats conformed to the principles of the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Washington, USA) [18]. This study was also approved by the local ethical committee for laboratory animals (Local Animal Research Ethical Committee at Diskapi Yildirim Beyazit Training & Research Hospital, Ankara).

Twenty male Sprague Dawley rats weighing between 160 and 190 g and aged 30 days old were used in the study. Sprague Dawley rats are supplied by Gulhane Medical School.

Back and front lung radiographies for all of the rats have been taken under general anesthesia [intraperitoneal ketamine (50 mg/kg) and dehydrobenzoperidol (2 mg/kg)] and have been evaluated radiologically. Radiological examinations have been conducted using a digital radiologic display device (Siemens-FD-X-VB21C). During the measurement, the voltage of the device is set to 40 V, the flow of the device is set to 1.8 mA, and the distance between the rat and device is set to 90 cm.

Following the detection that lung radiographies of rats were normal, they were randomly divided into two groups (each containing 10 animals). While the rats in the first group were put into the cages that contained wooden shavings, which had been sterilized in a vapored autoclave (at 121°C for 30 minutes) as filling material, the animals in the second group were put into a metabolic cage individually. Animals in both groups were marked according to their radiologic examination rank. The rats were provided free access to filtered tap water and a standard commercial rat diet throughout the study; also, environmental conditions were standardized (temperature 22°C–24°C, humidity 30%–60%, and 12 hours light/12 hours darkness). General situations of animals were checked daily and their weights were measured weekly. Cages with wooden shaving materials were cleaned and refilled every 3 days. At the end of 4 weeks of experiments, back and front lung radiographies of animals have been taken under general anesthesia [intraperitoneal ketamine (50 mg/kg) and dehydrobenzoperidol (2 mg/kg)], and each rat was evaluated by comparing its previous radiography.

At the end of the study, under general anesthesia [intraperitoneal ketamine (50 mg/kg) and dehydrobenzoperidol (2 mg/kg)], the thorax of each animal was opened and lung tissue samples were taken for histopathologic examinations, ion chromatography, and gas chromatography-mass spectrometry (GC-MS) analyses. Animals were sacrificed by decapitation. Moreover, unused wooden shavings, used shavings from cages, and urine samples (from animals kept in metabolic cages) were taken.

Histopathologic examination

Lung tissue samples from all animals of the groups were fixed in formalin solution for histopathological evaluation. Lung tissues were embedded in paraffin, cut into 4 μm sections, and mounted on the glass slides. Slides were stained with hematoxylin and eosin (H&E) and were examined under a light microscope to evaluate lung histopathology.

Ion chromatography analysis

One hundred microgram samples were taken from all rats' right lungs and were homogenized in 1 mg of distilled water. Obtained homogenate was filtered by using a 22 nm micro-filter (Sartorius Minisart, Goettingen, Germany). Chlorate, nitrate, and nitrite levels of filtered homogenate were analyzed by ion chromatography (Dionex ICS—1100, Sunnyvale, CA) [19,20]. Analyses for lung tissues were repeated for 100 µg unused shavings and 100 µg used shavings taken from normal cages. Ion chromatography measurement was repeated three times for each sample and mean values were recorded as the final result. Results were standardized as milligram per gram samples. Reference solutions used in measurements were provided by Dionex Company (Sunnyvale, CA).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analyses of lung tissue samples, used shavings, shavings from cages with shavings, and urine samples from metabolic cages were conducted. One hundred micrograms from each sample (100 µl from urine samples) were taken and (1:4 v/v) 1 ml hexane ethyl acetate was added to it. The mixes were extracted in ultrasound baths for 4 hours in room temperature. Then, these extracts were centrifuged in 6,000 rpm for 10 minutes (Hermle Z200A, Gosheim, Germany). 0.5 ml supernatant was taken from the mixes and transferred to the GC-MS sample tubes. After GC-MS analysis of samples (Trace GC Ultra & DSQ II, Thermo Scientific, Austin, USA), emerging chromatograms were compared with one another and different peaks were searched for in Wiley and NIST libraries [21].

All the obtained data were transferred to the computer and analyzed statistically by using the SPSS version 11.5 software for Windows (Chicago, IL). The data were presented as mean ± SD. By using frequency distribution graphs and skewness and kurtosis statistics that describe the shape and symmetry of the distribution, it was decided that our data were non-parametric. Therefore, non-parametric tests (Kruskal–Wallis and Mann–Whitney *U*-tests) were used to compare the groups [22].

Results

Animals were monitored daily in case of a possible pathologic situation throughout the research. No difference between weight rises and general situations of animals was encountered. At the end of the 4 weeks, weight change was calculated as 61.80 ± 8.17 g for animals in cages with wooden shavings, and 63.01 ± 7.69 g for animals in metabolic cages ($p > 0.05$).

Radiologic findings

In radiologic examinations conducted at the beginning of the study, no pathologic and suspicious finding was encountered in the lung radiographies of rats. In an examination conducted following the 4 weeks, while the lung radiographies of rats in metabolic cages were defined as normal, pathology was detected in the lung radiographies of all animals in cages with wooden shavings. Abnormal detected radiography findings are defined as retrocardiac peribronchiovascular thickening, blunting in costophrenic sinus, paracardiac and perihilar density rise, retrocardiac density rise, diaphragmatic density rise, focal parenchymal density rise, peribronchial density rise in the retrocardiac zone, and medium and low zone density rise (Fig. 1; Table 1).

Lung tissue histopathology

Bronchopneumonia with acute inflammation lesions was seen in the walls of the bronchial tubes, in peribronchiolar alveoli, and in the alveolar areas of the lungs in all lung tissue sections of the wood shavings group (Fig. 2). No inflammation finding was encountered in the lung tissues of animals kept in metabolic cages (Table 1).

Ion chromatography analysis

Chlorate, nitrate, and nitrite levels in the tissue samples of rats from both groups were investigated. While there is no meaningful difference between nitrate and nitrite levels of groups, it is observed that the chlorate level of rats in wooden shaving cages was higher than rats in metabolic cages (2.07 ± 0.07 mg/g *versus* 0.18 ± 0.09 mg/g, respectively) ($p < 0.05$).

Following the analysis of used and unused shavings samples, chlorate was detected in the wooden shavings used in cages for 2 days, and the amount was 6.12 ± 0.16 mg/g. Chlorate was not found in unused wooden shavings.

GC-MS analysis

Gas chromatographic analysis of lung homogenates, bedding materials, and urine showed

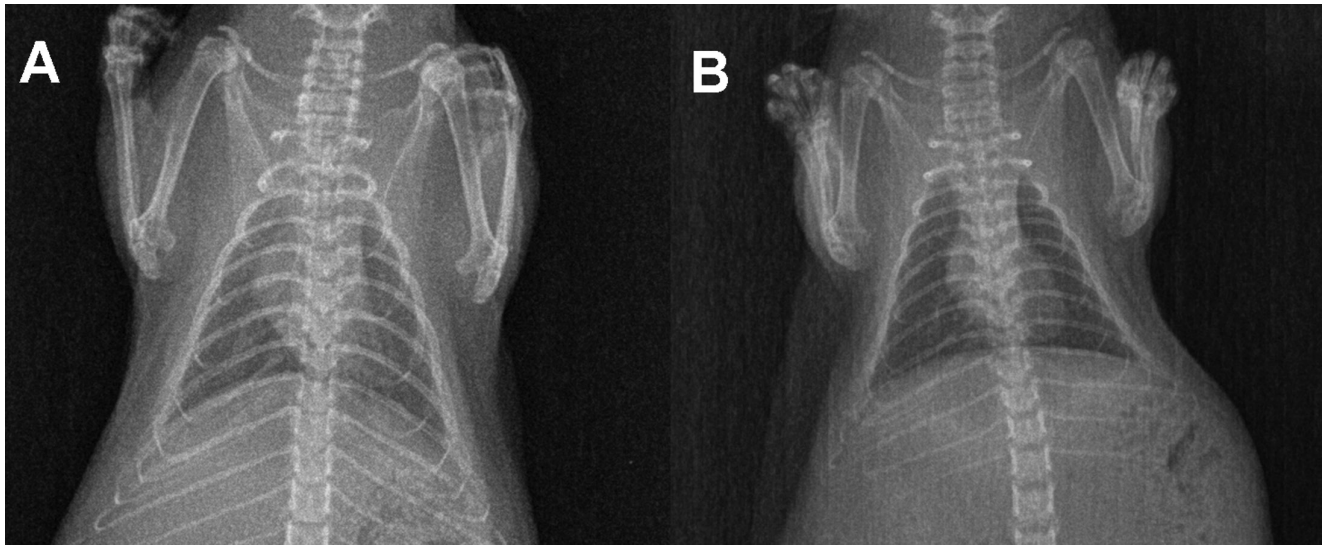

Figure 1. Pathologic and normal lung radiography. (A) Pneumonia-like lung radiography of a rat in wood shavings group (severe inflammatory infiltration seen in the walls of the bronchial tubes and alveolar areas). (B) Normal lung radiography of a rat in a metabolic cage.

Table 1. Radiological and histopathological findings in animals.

Parameters	Animals in cages containing wood shavings	Animals in metabolic cages
Radiological findings	• retrocardiac peribronchiovascular thickening • blunting in costophrenic sinus • paracardiac and perihilar density rise • retrocardiac density rise • diaphragmatic density rise • focal parenchymal density rise • peribronchial density rise in retrocardiac zone • medium and low zone density.	Normal lung radiography
Histopathological findings	Acute inflammation lesions in the walls of the bronchial tubes, in peribronchiolar alveoli, and the alveolar areas of the lungs.	Normal histologic appearance of lung tissue

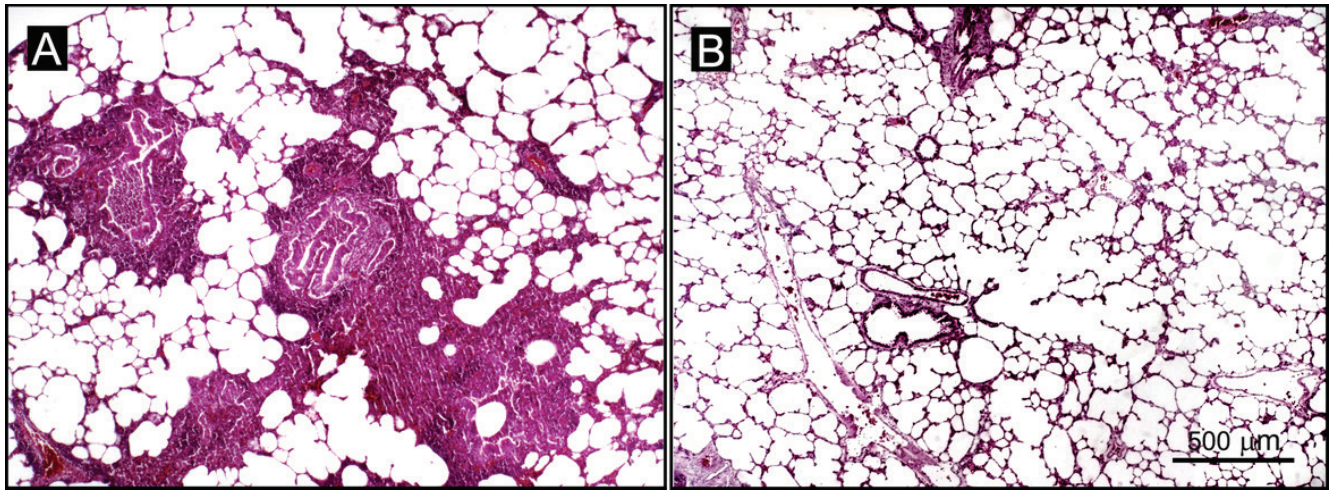

Figure 2. Representative sections of the lung tissues for the groups. (A) In the wood shavings group, severe inflammatory infiltration seen in the walls of the bronchial tubes and alveolar areas. (B) In the metabolic cages group, normal lung tissue is brightly seen in walls of the bronchial tubes and alveolar areas (H&E, Scale bar for two images is 500 µm).

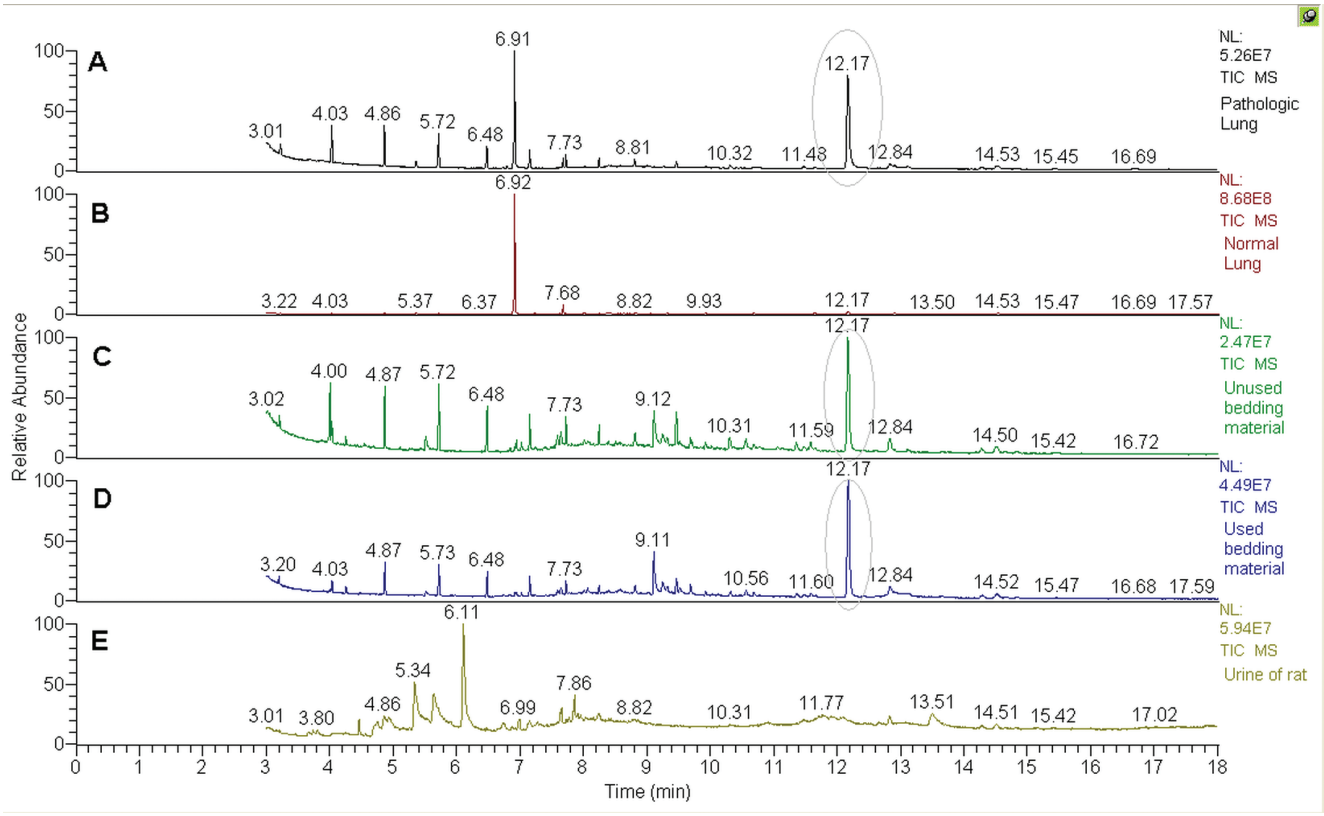

Figure 3. GC-MS analysis of lung homogenates, wood shavings, and urine: (1) chromatogram of lung homogenate of rat in cage containing wood shavings, (2) chromatogram of lung homogenate of rat in metabolic cage, (3) chromatogram of unused wood shavings, (4) chromatogram of used wood shavings in normal cages, and (5) chromatogram of urine collected from the rats that lived in metabolic cages. The peak at 12.17 retention time represents 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl].

different spectrums. An unusual peak was found in pathologic lung homogenate, unused wood shavings, and used wood shavings, while it was not seen in urine and healthy rat lung homogenates. This common peak was defined as 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl] (MBMBP, CAS Registry Number 119-47-1) in Wiley Library of GC-MS. MBMBP is a synthetic chemical that is produced by the reaction of 2-*tert*-butyl-*p*-cresol with formaldehyde through a carbonyl condensation. MBMBP is widely used in industry as a stabilizer in styrenic and olefin polymers, and as an antioxidant in the acrylonitrile-butadiene-styrene copolymer, polypropylene, polyacetal, rubber, latex, and adhesives [23] (Fig. 3).

Discussion

In this study, it has been shown that some lung pathologies have developed in the laboratory animals that are housed in cages in which wooden shavings are used as filling material, but for those that are housed in metabolic cages, no pathology development has been observed. As a consequence of radiologic examinations, pneumonia-like findings have been detected in the animals housed in wooden shaving cages (retrocardiac peribronchiovascular thickening, blunting in costophrenic sinus, paracardiac and perihilar density rise, retrocardiac density rise, diaphragmatic density rise, focal parenchymal density rise, peribronchial density rise in retrocardiac zone, and medium and low zone density). Pneumonia-like acute inflammation findings have been detected histopathologically, and it is pointed out that the pathology in lungs may be evaluated as chemical pneumonia.

Nitrate and nitrite analyses were performed in order to obtain general information about oxidative stress in animals and to see whether nitrogen and nitrogen-related metabolites had increased in animals. But we did not find a significant difference between nitrate and nitrite levels of groups. These findings, when taken into account with general physical properties of animals, could help to eliminate the possibility that lung pathologies were a

result of general health deficiency. Although there is some information about ammonia-related respiratory problems in rats, it was shown that ammonia in cages may cause nasal lesions in rats in some well-designed studies [24]. If these animals were contaminated with mycoplasma, lung pathologies may then develop; furthermore, there are findings indicating that environmental ammonia may promote mycoplasma invasion in a rat's respiratory tract [24,25]. In this study, we analyzed biological materials, so environmental gaseous ammonia is beyond the scope of the study. But because there is no increase in nitrogen-related chemicals in analyzed samples, we could consider that the current pathological changes were not related to urinary ammonia in cages.

It is known that chlorate and compounds with chlorate may cause upper and lower respiratory tract and lung irritation [26–28]. However, chlorate has not been detected in unused wooden shavings, but it has been detected in wooden shavings taken from cages and urine samples from animals. Therefore, chlorate in wooden shavings may be assumed to have passed from animal urine. It is considered that chlorate has passed to wooden shavings by means of urine and that it has been taken by animals via respiration. Lungs being exposed to chlorate by air may create the pneumonia-like findings detected in animals.

In GC-MS analysis conducted within the study, a chemical called 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl] has been detected in unused wooden shavings, in wooden shavings from cages, and in lung tissues of animals in which pathology has been detected. It is predicted that this synthetic chemical matter can be found in the formation of adhesive matters used in the production of chipboard and that these chipboard wastes may be mixed with wooden shavings, which have been used in cages for nesting [23]. Although there is not a certain finding that 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl] causes lung pathology [23], it may be claimed that observing this matter in all animals which have lung pneumonia-like findings and not observing this matter in animals that are housed in metabolic cages can be responsible for lung pathologies or can contribute to it. However, acute and chronic toxicity studies are required to confirm it.

As a conclusion, it is considered that while it can easily be proven by lung radiography, this situation is generally ignored in studies and endangers the health of animals and study results because there is not a routine radiographic monitoring system available. Therefore, to avoid using wooden shavings as nesting material in the experimental studies conducted with laboratory animals is going to be more reasonable in terms of reliability of results and health of animals.

References

[1] NRC. Guide for the care and use of laboratory animals. 8th edition, National Academies Press, Washington, DC, 2011.

[2] Sharp J, Azar T, Lawson D. Effects of a cage enrichment program on heart rate, blood pressure, and activity of male sprague-dawley and spontaneously hypertensive rats monitored by radiotelemetry. Contemp Top Lab Anim Sci 2005; 44(2):32–40.

[3] Smith E, Stockwell JD, Schweitzer I, Langley SH, Smith AL. Evaluation of cage micro-environment of mice housed on various types of bedding materials. Contemp Top Lab Anim Sci 2004; 43(4):12–7.

[4] Armstrong KR, Clark TR, Peterson MR. Use of cornhusk nesting material to reduce aggression in caged mice. Contemp Top Lab Anim Sci 1998; 37(4):64–6.

[5] Ivy AS, Brunson KL, Sandman C, Baram TZ. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. Neuroscience 2008; 154(3):1132–42.

[6] Gordon CJ. Effect of cage bedding on temperature regulation and metabolism of group-housed female mice. Comp Med 2004; 54(1):63–8.

[7] White WJ, Hawk CT, Vasbinder MA. The use of laboratory animals in toxicology research. In: AWH (Ed.). Principles and methods in toxicology. 5th edition, CRC Press, Boca Raton, FL, pp 1055–101, 2008.

[8] Bazille PG, Walden SD, Koniar BL, Gunther R. Commercial cotton nesting material as a predisposing factor for conjunctivitis in athymic nude mice. Lab Anim (NY) 2001; 30(5):40–2.

[9] Sanford AN, Clark SE, Talham G, Sidelsky MG, Coffin SE. Influence of bedding type on mucosal immune responses. Comp Med 2002; 52(5):429–32.

[10] Buddaraju AK, Van Dyke RW. Effect of animal bedding on rat liver endosome acidification. Comp Med 2003; 53(6):616–21.

[11] Potgieter FJ, Torronen R, Wilke PI. The in vitro enzyme-inducing and cytotoxic properties of South African laboratory animal contact bedding and nesting materials. Lab Anim 1995; 29(2):163–71.

[12] Torronen R, Pelkonen K, Karenlampi S. Enzyme-inducing and cytotoxic effects of wood-based materials used as bedding for laboratory animals. Comparison by a cell culture study. Life Sci 1989; 45(6):559–65.

[13] Weichbrod RH, Cisar CF, Miller JG, Simmonds RC, Alvares AP, Ueng TH. Effects of cage beddings on

microsomal oxidative enzymes in rat liver. Lab Anim Sci 1988; 38(3):296–8.
[14] Vlahakis G. Possible carcinogenic effects of cedar shavings in bedding of C3H-Avy fB mice. J Natl Cancer Inst 1977; 58(1):149–50.
[15] Vesell ES. Induction of drug-metabolizing enzymes in liver microsomes of mice and rats by softwood bedding. Science 1967; 157(3792):1057–8.
[16] Vesell ES, Lang CM, White WJ, Passananti GT, Tripp SL. Hepatic drug metabolism in rats: impairment in a dirty environment. Science 1973; 179(76):896–7.
[17] Vesell ES, Lang CM, White WJ, Passananti GT, Hill RN, Clemens TL, et al. Environmental and genetic factors affecting the response of laboratory animals to drugs. Fed Proc 1976; 35(5):1125–32.
[18] ILAR. Guide for the care and use of laboratory animals. National Academy Press, Washington, DC, 1996.
[19] Oztosun M, Akgul EO, Cakir E, Cayci T, Uysal B, Ogur R, et al. The effects of medical ozone therapy on renal ischemia/reperfusion injury. Ren Fail 2012; 34(7):921–5.
[20] Ogur R, Uysal B, Ogur T, Yaman H, Oztas E, Ozdemir A, et al. Evaluation of the effect of cola drinks on bone mineral density and associated factors. Basic Clin Pharmacol Toxicol 2007; 100(5):334–8.
[21] Gulec M, Ogur R, Gul H, Korkmaz A, Bakir B. Investigation of vasoactive ion content of herbs used in hemorrhoid treatment in Turkey. Pak J Pharm Sci 2009; 22(2):187–92.
[22] Acikel CH, Kilic S. Selecting statistical methods in medical researches. TAF Prevent Med Bull 2004; 3(7):162–3.
[23] HSDB. Hazardous substances data bank: bis (2-hydroxy-3-tert-butyl-5-methylphenyl) methane. 2011. Available via http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (Accessed 7 December 2011).
[24] Broderson JR, Lindsey JR, Crawford JE. The role of environmental ammonia in respiratory mycoplasmosis of rats. Am J Pathol 1976; 85(1):115–30.
[25] Saito M, Nakayama K, Muto T, Nakagawa M. Effects of gaseous ammonia on Mycoplasma pulmonis infection in mice and rats. Jikken Dobutsu 1982; 31(3):203–6.
[26] Abdel-Rahman MS, Couri D, Bull RJ. The kinetics of chlorite and chlorate in rats. J Environ Pathol Toxicol Oncol 1985; 6(1):97–103.
[27] Bacci M, Cavaliere A, Fratini D. Lung carcinogenesis by procarbazine chlorate in BALB/c mice. Carcinogenesis 1982; 3(1):71–3.
[28] Abdel-Rahman MS, Couri D, Bull RJ. Metabolism and pharmacokinetics of alternate drinking water disinfectants. Environ Health Perspect 1982; 46:19–23.