Deciduous wood chips as bedding material: Estimation of dust yield, water absorption and microbiological comparison

by *Heli Haataja* [1], *Hanna-Marja Voipio* [1], *Aino Nevalainen* [2], *Matti J. Jantunen* [2] and *Timo Nevalainen* [1].
[1]National Laboratory Animal Center, Kuopio, Finland. [2]Department of Environmental Hygiene and Toxicology, National Public Health Institute, Kuopio, Finland. Address for correspondence: Heli Haataja, National Laboratory Animal Center, University of Kuopio, P. O. B. 6, 70211 Kuopio.

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INTRODUCTION

Bedding is probably the most neglected contact material to which laboratory animals are continuously exposed. It is not only inherent or chemical composition, nor residues e.g. pesticides which matter. Physical and microbiological properties of the bedding materials are of equal importance.

Optimally bedding should be:
- moisture absorbing,
- dust free,
- nontraumatic,
- nonstaining,
- nonpalatable and non-nutritious,
- ammonia binding,
- nestable,
- easily disposed and
- sterilizable (*Kraft* 1980).

Wood is the most commonly used bedding material for laboratory animals (*Kraft* 1980, *Teute* 1980, *Weichbrod et al.* 1986). The terminology of wood materials in this context should always be defined. Hardwood is commonly used as a synonym to deciduous wood while softwood refers to conifers. Scandinavian aspen and alder are thus considered as hardwood, although they are quite soft materials.

Bedding made from coniferous wood (softwood; red cedar, white and ponderosa pine, spruce etc.) is known to have effects on hepatic microsomal enzyme function (*Cunliffe-Beamer et al.* 1981, *Ferguson* 1966, *Nielsen et al.* 1984, *Vesell* 1967, *Vesell et al.* 1976). Aromatic compounds found especially in coniferous wood may also be carcinogenic or procarcinogenic (*Sabine* 1975, *Schoental* 1973 and 1974, *Vlahakis* 1977). Thus, deciduous wood (hardwood) which does not contain e.g. compounds with hepatic enzyme induction properties seems to be the material of choice for bedding.

Physical and microbiological characteristics of beddings commonly used in Scandinavia are poorly known. Inhalable wood dust can be harmfull for health by causing respiratory diseases, allergies and cancer (*Acheson et al.* 1968, *Whitehead* 1982). For experimental animals wood dust exposure is a part of background exposure, which affects the test and control animals alike.

Aspen (*Populus tremula*) and alder (*Alnus incana*) chips were chosen to this study, since they are commonly used bedding materials for rodents, and birch (*Betula sp.*) was included as a bedding material candidate. The choice of the materials was based on availability and price.

The purpose of this study was to compare dust forming properties, airborne microbes and water absorbing capacity of three new aspen beddings and a new alder and a birch bedding and compare them to two commercial qualities, one aspen and one alder.

MATERIALS AND METHODS

The chip manufacturing process included chip cutting, immediate drying with hot air and dust removal. Two sets of experiments were made with these chips (manufactured by Finn Tapvei Ltd., Finland).

I First set of experiments with commercial beddings

In the first set the bedding was unautoclaved chips of alder and aspen plus the bedding candidate birch. The chip size was 4 mm×4 mm×1 mm. This is the set value of the chipping machine and in practice the maximum chip size.

Airborne microbes were sampled in a laboratory (20 ± 2°C and relative air humidity, RH 50 ± 20%) with a six-stage impactor (Andersen Inc., USA). The samples were analyzed using nonselective media for bacteria and Hagem media for fungi and yeasts. The bacteria plates were incubated for 48 h at 37°C and fungi plates for 96 h at 20°C.

Water absorbing capacity was analyzed by immersing bedding samples (five samples of each bedding) in plastic mesh bags filled with 1350 ml of wood chips in water for 16 hours. Subsequently the bags were left hanging in room air (20 ± 2°C, RH 50 ± 20%) for 4 hours and weighed.

II Second set of experiments with experimental vs. commercial beddings

Six unautoclaved beddings of aspen and alder were studied (Table 1).

Dust yield was studied by pouring six litres of bedding back and forth from one Makrolon III^R cage into another for five minutes to simulate routine filling of cages. The nozzle of an optical particle counter (PKZV-905, V/O Mashibor Introg, USSR) was placed 50 cm above the dust source. Background dust levels were determined before each test.

The water absorption test was modified based on the experience of the first test. Plastic mesh bags filled with 100 g of one type of bedding were immersed in water for 21 hours, left hanging in room air for 4 hours and weighed. The increase of weight was used as amount of water absorbed.

Differences between the results, except dust yield results, in both sets of experiments were tested with the analysis of variance.

RESULTS AND DISCUSSION

Airborne microbes

Microbe results are shown in Figure 1. There were no significant differences in the airborne bacteria or yeast counts between bedding materials during the filling. The counts of airborne fungi were significantly ($p<0.01$) lower during handling of aspen beddings compared to alder and birch. However, the total fungi counts were so low that the observed differences have no practical consequences. Since birch was not superior in any way to aspen and alder, and it is considered a valuable raw-material for industry (more expensive and not always readily available), it was omitted from further evaluations of dust.

Inhalable dust yield

The total mass of dust yield was dominated by large particles, optical $d>10$ µm. However, our interest is focused on the smaller, $d<10$ µm, particles which can be inhaled by animal technicians and animals (Task Group on Lung Dynamics 1966).

The inhalable dust yield from simulated cage

Table 1. Characteristics of the deciduous wood chips used in the second series.

Wood material	Chip size* mm	Abbrev.	Weight/Volume g/l (mean)
Aspen, unsoftened	4×4×1	AS1	170
Aspen, softened	4×4×1	ASS1	190
Aspen, unsoftened	1×2×1	AS2**	170
Aspen, softened	1×2×1	ASS2**	170
Alder, unsoftened	4×4×1	AL1	170
Alder, softened	4×4×1	ALS1**	190

*) The set value of the chipping machine.
**) Specially made for this study.

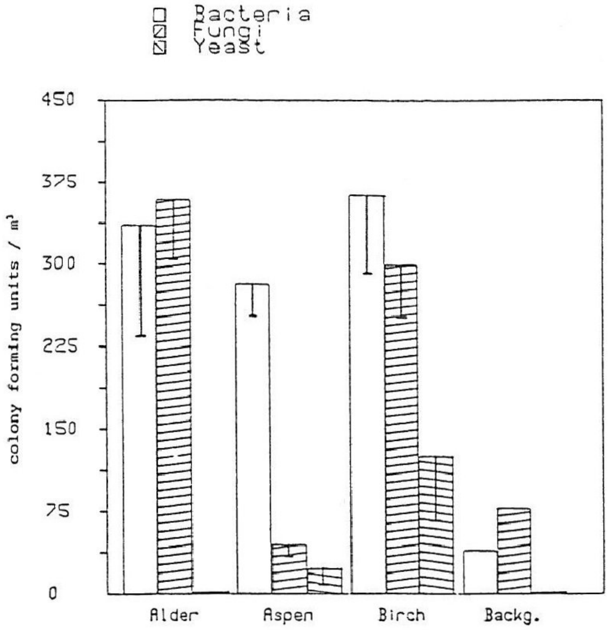

Figure 1. Airborne, microbes during filling of cages with alder, aspen or birch bedding (mean ± SEM, n = 5 in each column, except background n = 1).

filling is shown in Figure 2. The ASS2 bedding is left out of the figure, because its dust yield repeatedly exceeded the capacity of the counter.

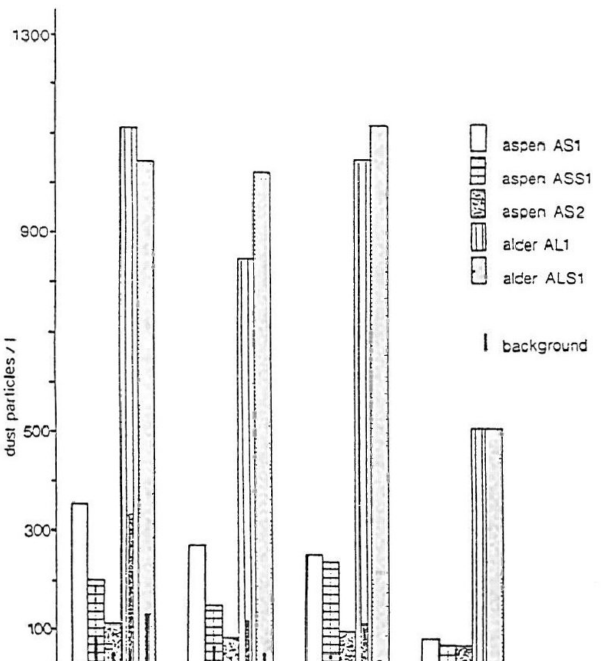

Figure 2. Dust yield of the beddings during simulated cage filling.

Aspen chips were generally less dusty than alder chips, ASS2 being an exception. The results also indicate that softening of large size aspen chips reduces dust release, and that the reduction is even more pronounced with unsoftened small chip size. This effect can be seen in the three smallest dust particle size groups.

Dust production of alder surmounted aspen manyfold. With alder no advantage was gained with the softening treatment and hence it should not be softened with this method. It is obvious that the processing method should be compatible to the specific wood material, since even hardwood species differ from each other.

Water absorbing capacity

In the first set the water absorbing capacity when calculated per volume of bedding was best in alder chips followed by birch and aspen. The difference between alder and aspen (9%), and alder and birch (9%) were statistically significant ($p < 0.001$). Birch was omitted from further evaluations for the reasons mentioned in connection with dust.

In the second set ASS2 was the most absorbent bedding (Fig. 3). ASS2 absorbed about 35%, AS2 22%, ALS1 19%, ASS1 18% and AL1 15% more water than AS1 bedding. The small chip size and the softening treatment improved the water absorbing capacity of bedding

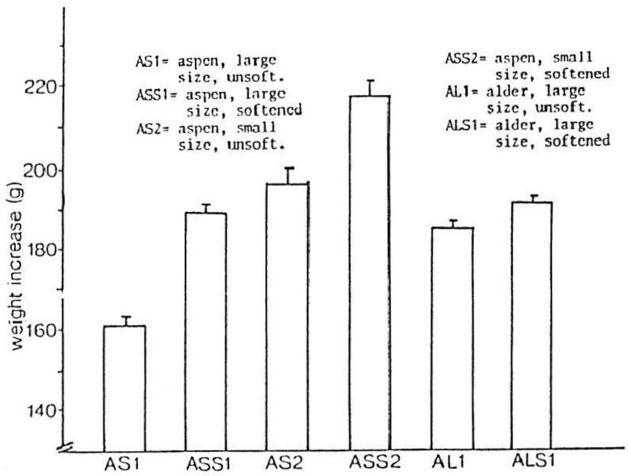

Figure 3. Water absorbing capacity of bedding as weight increase per 100 g of dry bedding (mean ± SEM, n = 4 in each column).

($p < 0.001$). Alder (AL1) was followed by aspen (AS1, $p = 0.05$). This is in accordance with the results of the first set.

Conclusions

The studied aspen and alder beddings can all be used as animal bedding, except the small, softened aspen chips. The combination of the small chip size and the softening treatment with aspen produces an absorbing, but a very dusty bedding unsuitable for experimental animal use. We conclude that aspen bedding, with the above mentioned exception, should be preferred to alder, because it is less dusty (inhalable particles).

Water absorbing capacity of unsoftened, large size aspen chips seems to be adequate to soak up all urine with the normal change interval. When necessary, the absorbing capacity can be improved by using softened or small chips or changing the bedding more often. The softening treatment increases the water absorbing capacity of all these bedding materials, and it also increases its density in the large chip size.

Summary

Dust yield, water absorbing capacity and airborne microbes of laboratory animal beddings made of deciduous wood were compared. Three different bedding materials, aspen, alder and birch, two chip sizes and effects of a softening treatment were studied. Overall dust yield from chips was relatively low. Aspen should, however, be preferred to alder, because it was less dusty. Small (1 × 2 × 1 mm) aspen chips yielded less inhalable dust than the large chips (4 × 4 × 1 mm). Water absorbing capacity was better in small chips than in large chips and could also be increased with a softening procedure. In this study softening of small aspen chips resulted in unacceptable high dust yield. Though alder had a better water absorbing capacity, aspen seems to soak up urine well enough during a normal change cycle.

Sammendrag

Denne artikel handler om strøelse, fabrikeret af løvtræssorter. Der sammenlignes strøelse og vandabsorption, ligesom der foretages en mikrobiel sammenligning.

Yhteenveto

Tässä tutkimuksessa verrattiin kuuden lehtipuuhakkeen ominaisuuksia koe-eläinten kuivikkeena. Tutkimuksessa on käsitelty kolmen eri puumateriaalin, haavan, lepän ja koivun, kahden palakoon ja pehmennyskäsittelyn vaikutuksia kuivikkeen imukykyyn, pölyävyyteen ja pölyn mikrobipitoisuuteen. Tutkimuksen perusteella haapa on suositeltavin tutkituista materiaaleista lähinnä vähäisen pölynsä vuoksi. Pieni palakoko (1 × 2 × 1 mm) haapakuivikkeessa vähensi hengitysteihin kulkeutuvan pölyn määrää. Suurikokoisen (4 × 4 × 1 mm) haapahakkeen vedenimukyky riittänee normaalin vaihtovälin aikana, mutta tarvittaessa kuivikkeen imukykyä voidaan lisätä käyttämällä pienikokoista tai pehmennettyä kuiviketta tai lyhentämällä vaihtoväliä. Tässä tutkimuksessa pienikokoisen haapahakkeen pehmentäminen tuotti imukykyisen, mutta erittäin pölyisen kuivikkeen.

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