LABORATORY ANIMAL BEDDING: A REVIEW OF SPECIFICATIONS AND REQUIREMENTS

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ABSTRACT

The literature is reviewed regarding existing specifications and requirements for laboratory animal bedding. The lack of comprehensive specifications in the guidelines of laboratory animal governing bodies, and the introduction of external variables by unsuitable bedding into experimental design, are discussed on the basis of examples from the literature.

Key words: Laboratory animals, contact bedding, specifications, requirements, variables.

Potgieter F.J.; Wilke P.I. **Laboratory animal bedding: A review of specifications and requirements.** *Journal of the South African Veterinary Association* (1991) 62 No. 3, 143-146 (En.) Animal Unit G 20, Faculty of Medicine, University of the Orange Free State, P.O. Box 339, 9300 Bloemfontein, Republic of South Africa.

INTRODUCTION

Laboratory animals are important research tools and every effort should therefore be made to ensure that they are accommodated in an optimal environment. Bedding is an important environmental component which is often ignored, but really needs thorough consideration before experiments are carried out.

Proper care of experimental animals, to uphold normal growth, reproduction and health status, is of the utmost importance during day-to-day husbandry, as well as during experimental procedures. Good laboratory practice will enable both the researcher and animal technician to provide these requirements and at the same time reduce the influence of external factors on experimental results. This produces results which are more consistent and which may mean a reduction in the number of animals required, resulting in more cost-effective research.

The environment of laboratory animals can be divided into the animal room (macro-environment) and the micro-environment in the animal cage. Between these 2 environments, there are major differences which are determined by factors such as the animal cage which acts as a partial barrier between the environments, room heating and ventilation, relative humidity, light intensity, population density, odours and dust. The use of cage filtertops, different types of cage lids and bedding materials can have a marked influence on the micro-environment.

The use of suitable cage bedding and nesting material is an essential husbandry practice that may result in a reduction of stress in animals[13]. It may also result in an improvement in the micro-environment by enhancing hygiene by means of reducing ammonia levels and absorption of moisture[7 20]. Changes in the environment can lead to abnormal biologic responses and so render unreliable results[15 30].

An example of such a change in environment, with major repercussions on experimental results, was reported by Sabine et al.[21]. They recorded a decline, from a virtual 100% to almost 0%, in the incidence of mammary and liver tumours in C3H-A^{vy} and C3H-A^{vy}fB mice, imported to Australia from the National Institute of Health in the United States. The low tumour incidence occurred when the mice were kept on sawdust bedding, derived predominantly from Douglas fir (*Pseudotsuga* spp.) and fed a commercial Australian diet. The high tumour incidence was seen when the animals were reared on the American diet and red cedar (*Juniperus virginiana* Linnaeus) bedding used at the National Institute of Health. Schoental[23] ascribes this phenomenon to a natural estrogen, possibly zearalenone, in the American diet and the lignan podophyllotoxin found in wood shavings from the red cedar.

The adverse effects of variables on experimental results were highlighted when Heston[11], the supplier of the C3H-A^{vy} and C3H-A^{vy}fB mice, replied to the findings of Sabine et al.[21]. He maintained that the decline observed in the occurrence of hepatomas and mammary tumours was related to the condition of the animals. On receiving animals back from Australia, he found them heavily infested with small mites. According to him, this as well as the difference in weight between the American (heavier) and Australian-reared counterparts, could be the only factor responsible for this phenomenon since any factor that decreases growth, would also decrease the occurrence of tumours.

The above-mentioned incident illustrates the importance of also defining and controlling the environmental factors, which are as important as the health and genetic status of the experimental animals. By applying the effect of variables on experimental data, variations in results can be minimised, not only within a specific laboratory, but also between different laboratories.

REQUIREMENTS FOR SUITABLE BEDDING MATERIALS

The type of bedding used is determined by the purpose for which the animals are utilised. For laboratory animals conditions must be optimised in order to eliminate variables, other than those imposed by the experiment[14].

Many different types of contact bedding and nesting materials, have been utilised in the past. This includes processed wood products such as shavings, wool, chips, shreds, filaments and sawdust, paper products, peat moss, cotton, ground corncobs, peanut hulls, hay, and inorganic substances such as attapulgite

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(hydrated magnesium aluminium silicate) and certain clays, and even organic compounds such as polyethylene granules[14]. Some of these have become unpopular for a variety of reasons. Raw wood products, derived from cedar and pine trees for example, contain substances that may interfere with certain enzyme systems which may render this kind of bedding unsuitable for pharmacological studies[5 6 19 29 31 33]. Other examples are hay which is edible, peat moss and used newsprint which tend to stain the animals' coat and attapulgite which is too hygroscopic for some animals[14]. Kraft[14] proposed a number of desirable and self-explanatory criteria for laboratory animal contact bedding (Table 1).

experimentation, no definite standpoint on specifications for bedding is taken. The Institute of Laboratory Animal Resources, National Institutes of Health[17 18], do however address the problem to some extent by determining standards for bedding. They describe how beech, birch and maple or any mixture thereof should be processed as well as how the quality assurance is to be done. In contrast, codes governing laboratory animals in South Africa, do not mention bedding materials specifically, except that paraphrases like, "hygienic surroundings" and "animals must be kept in optimal conditions at all times"[26], could be regarded as an indication that bedding material should be used to achieve this goal.

Table 1: **Desirable criteria for laboratory animal contact bedding according to Kraft[14]**

Ammonia binding	Disposable by incineration
Dust free	Fire resistant
Easily stored	Manifests batch to batch uniformity
Inedible	Non-deleterious to cage washers
Moisture absorbent	Non-desiccating to the animal
Nestable	Optimises normal behaviour
Non-malodorous	Readily available
Non-palatable	Relatively inexpensive
Non-staining	Remains chemically stable during use
Non-toxic	Unable to support microbial growth
Non-traumatic	Uncontaminated
Sterilisable	Unlikely to be chewed or mouthed
Deleterious products not formed as a result of sterilisation	
Non-injurious and non-hazardous to personnel	

SPECIFICATIONS FOR LABORATORY ANIMAL BEDDING

It seems that personal preference and what is available in the market place often determines the choice of contact bedding, while the objective should rather be to select a product that will create an optimal environment for the animals and not interfere with the experiment in any way. This trend can also be observed in the rather nonchalant way that specifications for laboratory animal bedding is treated by different regulatory bodies, worldwide.

Although the International Committee on Laboratory Animals[12], the Canadian Council on Animal Care[2], the European convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes[4] and the Good Laboratory Practices Act[28] of the United States Food and Drug Administration do, directly or indirectly, acknowledge the fact that bedding material is a source of variation in animal

Comparing Kraft's criteria[14] for animal bedding with those set by some other governing bodies, we find that only 5 feature regularly. They are:

a) "Dust free" - dust as found in fine sawdust is contra-indicated in breeding boxes[2], bedding shall be inspected for sandings and dust[17 18] and should be non-dusty[4].

b) "Uncontaminated/non-toxic" - wood products may (i) carry pollutants (insecticides, fungicides, etc.) (ii) introduce disease (particularly mites and tape worms) into the colony, (iii) significantly affect experimentation by influencing response to pharmacologic agents[2], (iv) Bedding should be non-toxic and free from infectious agents or vermin or any other form of contamination, (v) care should be taken to avoid bedding material derived from wood which has been chemically treated[4] and (vi) storage areas should be protected against infestation or contamination[8 28].

c) "Ammonia binding" - must help to control ammonia generation[2].

d) "Non-traumatic" - (i) the type of litter material chosen may exert a considerable influence on the physiological responses of the test animals, (ii) wood shavings have been reported to cause injury to foot pads with eventual granuloma formation in hamsters. Synthetic bedding materials including shredded paper are suggested for these species[2], and (iii) bedding should not interfere with the purpose or conduct of the study[28].

e) "Moisture absorbent" - bedding should be absorbent[4] and it should be changed as often as necessary to keep the animals dry[8].

Regulatory bodies not only omit many of the important criteria for bedding as stipulated by Kraft[14], but those that they do address, are often vague and/or ill-defined.

DISCUSSION

The Canadian Council on Animal Care[2] is rather vague on what kind of wood shavings would induce liver microsomal enzymes, whilst it is a well-documented fact that soft wood (wood derived from the Gymnospermae, especially pine, cypress and cedar) could be a source of organic compounds such as the tricyclic sesquiterpenes, cedrol and cedrene that exercise an adverse effect on the animals' response to certain pharmacologic substances[5 6 19 29 31 33], and the carcinogens coniferaldehyde and sinapaldehyde; constituents of wood lignins[22 24], and podophyllotoxin[23]. From the literature reviewed, hardwood could also present problems, due to the fact that it contains tannins, alkaloids, and lignin[34] (the same constituents encountered in softwoods). Silverman & Adams[25] further confirm the fact that bedding manufactured from hardwood is not the ultimate in laboratory animal bedding as N-nitrosamines, which are carcinogenic to laboratory animals, were detected in 50% of the heat-treated (815°C) hardwood chip bedding samples they examined. Acheson et al.[1], postulated that the aetiological agent, causing nasal cancer in wood workers, could be a constituent or constituents of wood dust that are inhaled and are present in such commonly-used hardwoods as oak and beech. The National Institutes of Health make no specific statement in their specification[17] on the reasons for the use of only hardwood bedding materials but it could with reasonable safety, be deduced that the effect of the organic compounds in softwood, as illustrated by the events with red cedar in Australia[21 23], moved them to totally ban the use of bedding materials derived from softwood trees. Wood is according to Wirth[34] still the most suitable raw material for animal bedding. Firs and spruce are, according to this author, the best source of raw material for the manufacturing of contact bedding. Species belonging to these two genera *(Abies* and *Pseudotsuga)* however also contain terpenoids such as pinene[3 16 32], limonene[3], carene[26], camphene[3], phellandrene[16] and santene[3]. An oleoresin, i.e., a mixture of mostly resins and essential oils, of the turpentine type is obtained from the common Douglas fir, *Pseudotsuga menziesii* (Mirbel) Franco[16].

Heston's[11] argument on the reasons for the decline in the occurrence of spontaneous tumours might be true, but the effect of the bedding or a constituent in the bedding on the animal is still ignored. Some of the previously mentioned authors[5 19 30 31] have identified the presence of hepatic microsomal enzyme-inducing substances such as cedrene and cedrol, in red cedar shavings. Couldn't this, or another substance such as podophyllotoxin[23], also exercise an effect on the natural occurrence of tumours in mice?

Heston[11] acknowledged the fact that the addition of at least some cedar shavings to the bedding of experimental animals, as a normal husbandry procedure, prevents infestation of the animals by ectoparasites. He, however, does not elaborate on the pesticidal properties of this bedding. The manufacture of "fragrant mothproof" chests from the wood of the white cedar *(Calocedrus decurrens* [Torrey] Florin) and the "insect repellant" properties of the wood from the Lawson cypress *(Chamaecyparis lawsoniana* [A Murray] Parlatore)[3] confirm the presence of such an inherent insecticidal substance in some wood species. A reasonable assumption would be that this substance, possibly a terpenoid, and in the case of Heston's findings, cedrene and cedrol, the 2 main constituents of oil of cedar, could be responsible for this insecticidal action. Irrespective of the beneficial insecticidal properties of red cedar bedding, the active substance would also have exercised an adverse effect on the enzyme system of these animals.

According to Hartwell et al[9], extraction of plant material from red cedar yields 0,10% podophyllotoxin, one of the isolation products of podophyllin. Topical application of podophyllin can, according to them, cure condyloma acuminatum, whilst its isolation products were found to damage experimental tumours. The question thus arises whether housing animals on this type of bedding is acceptable, whilst the occurrence of tumours, either spontaneous or induced, or the progressive changes occurring in vivo during the development of cancer, are studied.

To justify this rather non-specific attitude towards specifications for bedding the assumption could be made that comprehensive knowledge on physical and chemical properties of the different bedding materials in use, does exist. This is totally untrue. Although a fair number of scientific papers mention the type of bedding materials used, most of them, especially with regards to wood, sadly lack precise information about the species of tree(s) utilised as bedding source. The absence of this information plus:

(a) the continuation of the use of sawdust, an undefined byproduct originating from wood, used in the building and furniture industries and thus treated with poisonous wood preservatives such as the tributilic tin compounds, pentachloric phenol and chromium and copper salts,

(b) the utilisation of wood, rich in tannins, alkaloids, hydrocarbons, etc., for the manufacture of bedding materials, thus introducing variables into the experimental model that could exercise adverse effects on experimental results, and

(c) the continued use of vermiculite, especially in South Africa, notwithstanding the evidence that the long-term maintenance of mice on vermiculite causes a reduction in both the number of litters born and their growth rate, and that histological changes occur in the lungs of these animals, similar to industrial pneumoconiosis[10],

are rather indicative of the contrary, or perhaps even of an ignorance regarding the possible ill effects variables could impose on experimental results.

The time has probably arrived to urgently address this seemingly insignificant, but still essential aspect of animal experimentation, to at least impose specific minimum specifications for laboratory animal bedding materials, not only for the benefit of the animals but also to obtain reliable experimental results. Until the materialisation of such specifications, users of this commodity should remember that the majority of modern-day bedding materials are of natural origin and regardless of the advantages or disadvantages of any particular product, contamination of and variability between different batches and types are and remain important factors. These factors should not be ignored during experimental design and the interpretation of results.

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