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The Manufacture, Shipping and Receiving and Quality Control of Rodent Bedding Materials¹

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Summary | The criteria for rodent bedding and nesting materials are discussed. The literature is reviewed regarding sources of bedding materials, manufacturing methods, quality control procedures (microbiological, physical and chemical), storage methods, shipment, methods of use and disposal, current knowledge concerning bedding effects on animals as related to research and testing and legal aspects. Future needs, especially with respect to the promulgation of standards, also are addressed.

Key Words | Animal bedding — Microbiological safety — Rodentia

In 1974, a journal published for the laboratory animal field cited 11 commercial sources of contact bedding for laboratory animals in the form of wood shavings or chips and six for pelleted products (1). In 1979, the same publication again listed 11 sources of shavings or wood chips, however, seven of those mentioned in 1974 were no longer listed (2). Thus seven new vendors had entered the market in the interim, and, whereas six suppliers offered pelleted bedding in 1974, there were only four in 1979. Of these, three were new vendors.

What are we to conclude from this? Do bedding vendors tend to be an evanescent lot? Or is the fault to be found in the nature of the products? (We assume, of course, that the journal personnel were equally industrious in soliciting vendors for listing in both issues).

While it might be more interesting to discourse on the vendors themselves, the purpose of this presentation is to examine their products, and even if we cannot reach a conclusion as to the precise reasons for the changes between 1974 and 1979, we should be able to infer from our discussion if the products themselves are to blame or if, perchance, the laboratory animal field might be found wanting.

For the most part, contact bedding will be discussed, although materials placed under suspended wire cages, as well as no bedding at all, also will be mentioned. Most of the data will center about mice and rats.

Specifications and Requirements: Types of Contact Bedding

The specifications and requirements for rodent contact bedding vary depending on whether one wishes only adequate or optimal, that is, ideal, conditions for the animals. In our view, adequate conditions are those that will permit an animal to reproduce and survive in reasonable health. Food and pet animals fall into this category. Optimal conditions, then, are those which follow when all variables, other than those imposed by experimentation, are known in detail and then are controlled. A *propos* of this comment is the fact that the ICLA (International Committee on Laboratory Animals) Governing Board (3) strongly urged in 1978 that bedding (type, changing schedule, sterilization status) always be described when the results of experiments using animals are reported.

Table 1 indicates a number of desirable characteristics for contact bedding that

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Table 1

Desirable criteria for rodent contact bedding

Moisture absorbent	Nondesiccating to the animal
Dust free	Uncontaminated
Unable to support microbial growth	Non-nutritious
Inedible	Nonpalatable
Nonstaining	Unlikely to be chewed or mouthed
Nontraumatic	Nontoxic
Ammonia binding	Nonmalodorous
Sterilizable	Nestable
Deleterious products not formed as a result of sterilization	Disposable by incineration
Easily stored	Readily available
	Relatively inexpensive
	Fire resistant

are thought to provide optimal conditions (4-13), while Table 2 shows some personal additions. While composing these lists, the author was reminded of the "little list" of the Lord High Executioner in Gilbert and Sullivan's "Mikado" as well as of their patter songs. Inspiration then brought into being the following doggerel:

I bet you any money that you cannot find a bedding

That will give a mouseie comfort from unmitigated wetting,

That will be both hygroscopic but yet dry the critter not,

That will burn just when you want it to, but not burn very hot,

That contains no toxins, viruses, no fast fermenting bug,

That is stored with ease in cubbyholes or underneath your rug,

That smells good both to man and beast before it's messed upon

As well as after usage when its wasted youth is gone,

That will do the universal task of cutting variation

To give test results with only zero standard deviation.

With aims so high

And motives so deep,

I wonder why

It should also be cheap.

In any event, fulfilling the criteria (some of which seem antithetical as they stand) should provide the basis for standards for the specific use of a bedding material, with the ultimate aim of removing bedding from the list of environmental variables that beset the investigator who attempts to obtain reproducible

Table 2

Additional desirable criteria for rodent contact bedding

Remains chemically stable during use
Manifests batch to batch uniformity
Optimizes normal animal behavior
Nondeleterious to cage washers
Noninjurious and nonhazardous to personnel

results in a cost responsible fashion. Whether or not we now have all the data necessary to formulate such standards is, as we shall see, somewhat in question.

A number of authors (4-11) have mentioned the types of contact bedding and bedding supplements (as for nesting) that have been utilized in the past or are still in use: white, ponderosa, sugar and lodgepole pine shavings; redwood, aspen or other types of poplar, basswood, cedar and maple shavings; white pine sawdust and flakewood; flax stems; shredded newspaper, both used and unused; chopped and ground corncobs; wood wool, excelsior, shreds or filaments of aspen; peat moss; paper sheets; pine or hardwood chips; cotton batting; attapulgite (hydrated magnesium aluminum silicate); clays, such as bentonite; filter paper, paper towels; peanut hulls; hay (for guinea pigs); bagasse (crushed sugar beet or sugar cane pulp); cellulose fibers; alfalfa stems; dry sand; shredded computer printouts; nonabsorbent cotton; and polyethylene granules (prepared from ethylene polymerized with organic peroxides). It may be safe to say that, of these, white pine shavings are the most widely used. Hardwood or pine chips and aspen shreds probably follow some distance behind. Without an extensive survey, however, the precise usage cannot be stated with certainty.

A few bedding types mentioned have fallen out of favor for a variety of reasons. Cedar, and in some instances pine, shavings may not be used in pharmacological studies. Hay is edible. Peat moss, used newsprint and alfalfa stain the animals' coat, although peat moss does have the apparent advantage of relatively high acidity and therefore may control ammonia. Attapulgite is too hygroscopic for some animals, and, together with sand, it is not combustible and thus difficult to dispose of. Pelleted peanut hulls are relatively new on the market. Comparative studies with these have not, to our knowledge, been reported. Regardless of the advantages or disadvantages of a

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particular product, polyethylene granules and their variability and at which one is chosen "continues to be a" (11).

Even re very specific on guidelines. The Go (GLP), for example, bedding, indicating rated from areas h protected against inf (14); it also states cages or pens shall pose or conduct c changed as often a mals dry and clear

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particular product, it is true that, except for the polyethylene granules, all are natural substances and therefore subject to considerable variability and at least some contamination. Which one is chosen in a particular instance "continues to be a matter of personal preference" (11).

Even regulatory agencies are not very specific concerning requirements or guidelines. The Good Laboratory Practices Act (GLP), for example, addresses storage areas for bedding, indicating that they are to be separated from areas housing test systems and protected against infestation or contamination (14); it also states "bedding used in animal cages or pens shall not interfere with the purpose or conduct of the study and shall be changed as often as necessary to keep the animals dry and clean."

The Animal Welfare Act makes no reference to bedding specifically. The National Cancer Institute, on the other hand, specifies that its bioassay contractors use only heat treated hardwood chips.

The *Guide for the Care and Use of Laboratory Animals* (12) gives the following: "In general, bedding or litter should be absorbent and free of substances that could injure animals or personnel . . . or a type not readily eaten by the animals. Enough . . . placed in cage to keep animals dry between cage changes . . . should not come into contact with watering tubes. Some bedding materials may contain substances that affect the biologic responses of animals to some experimental procedures . . ." It states further that "Bedding materials may contain substances that have significant effect on an animal's biological responses. Pine and cedar, for example, are known to cause changes in hepatic microsomal enzymes of mice and rats: therefore, they should not be used . . ."

In the report of the Committee on Long-term Holding of Laboratory Rodents (13), bedding criteria are restricted to the statement: "The type of bedding, source and availability for the duration of the experiment should be known."

Is it possible that the reason for the general nature of the requirements and criteria given above is that everything is known about contact bedding that needs to be known, and therefore, it is not necessary to be more specific? On the other hand, could it be that we are

still afloat in a sea of relative ignorance, and therefore, it is prudent not to be too specific?

Requirements of the animal: To this point, we have been almost entirely anthropocentric in dealing with requirements for bedding. But what does the animal require? Few studies in which mice or rats have been given free choice of bedding for parturition and lactation or for extended holding periods have been conducted (15-17). Even in these studies, it must be recognized that the test bedding materials were first selected by the investigator. Of those provided, wood products were preferred in all cases by the animals, with aspen in the form of shreds (excelsior, wood wool) chosen by all the animals. Of more than passing interest is the fact that combinations were chosen. Thus all pregnant mice in one study chose aspen bedding, 75% added cedar shavings to this, 45% added pine shavings, 5% added hardwood chips and 5% chose a corncob product as supplement. Some chose as many as four different materials. Rats similarly tested manifested essentially the same behavior.

In a study using mice over a 10-week maintenance period, four materials were offered: shredded aspen, cedar shavings, ground corncobs and dehydrated alfalfa. On the first day, all animals had chosen cedar shavings. Thereafter, the animals chose various combinations for the duration of the test, but aspen and cedar combinations predominated (17).

In another study, pregnant mice were on one of four different bedding materials up to the eighteenth to twentieth day of gestation (18). Thereupon they were each offered the four choices, three of which now differed from that with which they had become familiar. The choices were: ground corncob, pine shavings, dehydrated and pelleted alfalfa, and flax fiber. The only clearcut preference in this case concerned the alfalfa. Whether the mice had been on it previously or not, they almost totally ignored the product. A study of habitat preference in inbred mice has shown that selection of birth site for the next generation is affected by prior experience of the parents, males being affected more than females by that experience (19).

Other comparative studies, without offering free choice to the animals, also have been performed (20-22). Further, comparisons

have been made between results in solid bottom and suspended wire cages (23-25). The results of these are certainly of pragmatic value, for they took into account several important variables including reproduction, lactation, litter weight and size. Experimental designs, however, do not always permit us to draw firm conclusions. For example, did the mice and rats in the free-choice trials choose the aspen because it was aspen or because it was in shreds or for both reasons? Would they have chosen pine over aspen if the pine had been shredded? Did they like the cedar shavings because of its undesirable pharmacologic characteristics, or would they have accepted it equally if the cedrene and cedrol had been removed?

Other questions are also brought to mind. Is it a mistake to provide only one kind of bedding material as routine? Are we depriving the animals of an important factor for their well-being by doing so, and what effect might this have on their usefulness for certain types of testing and research? Can free-choice experiments give new insight into rodent behavior and therefore increase their usefulness in research? Suitably designed experiments are surely needed in order to answer these as well as other problems that may justifiably be posed.

Requirements for special rodents: Some requirements for special rodents should be mentioned. In spontaneously hypertensive rats (SHR), one investigator found that they seemed healthier on pine shavings as compared with dehydrated alfalfa, clay or corncobs. He ascribed this to the rat's ability to nest on the shavings (26). The Institute of Laboratory Animal Resources publication concerning these animals makes no mention of special bedding needs for them (27). Bedding should be carefully chosen for the nude mouse to avoid irritation of the mouse's vulnerable skin. The bedding should be dust- and splinter-free. Hardwood chips and corncobs are regarded as beddings of choice, although pine shavings may also be used. Nesting material is regarded as optional (28). For gnotobiotics, recommendations include that the material be easy to sterilize, not be readily eaten by the animals and not yield toxic compounds as a result of sterilization (29).

Requirements for special experiments and testing: No bedding currently available is suitable for most toxicologic studies or for any

trace element research. Suspended wire cages generally are used for the former, and a number of systems have been described for the latter (30-32).

Requirements during shipping: Except for recommending that animals be kept warm or cushioned from shock during shipment, there are no particular requirements or guidelines. What may be of importance to the investigator, however, is to know if there are different bedding types in the breeding unit, the shipping unit and the research laboratory. Shifting animals from one type to another may have profound effects.

Effects of Bedding Materials on the Animals

Work on the induction of hepatic microsomal enzyme activity in rodents as a result of bedding them on cedar shavings has received considerable attention (33). One is cautioned against keeping such shavings in the vicinity of rodents even though, as we have seen, the animals themselves seem to enjoy them. Further, even when cedar shavings were placed beneath suspended wire cages, the threshold for clonic seizures due to pentylentetrazol was significantly decreased (34). Pine shavings also have been shown to be effective pharmacologically in that they increase the activity of sulfabromophthalein sodium (BSP)-s-aryl transferase in rats as compared with those bedded on polyethylene granules (35).

One report describes increased rat pup mortality caused by cedar shavings as compared with crushed corncob and shredded aspen beddings (36). In studying growth and several hematologic parameters of rats housed in suspended wire cages with half of the floor covered with either cotton wool, polypropylene fibers or shavings (type not stated), it was determined that those bedded on shavings showed significantly higher leukocyte counts and decreased β -globulin levels than those kept on the other materials (37). Growth patterns, concentrations of albumen and of other globulins, hemoglobin, hematocrit, erythrocyte counts, total serum protein were not significantly different.

The question of carcinogenicity of cedar shavings, as well as other types of bedding, has been alluded to on occasion. It was

thought at one time that cedar shavings increased the occurrence of adrenal gland tumors in C3H mice. However, this was not the case. The tumor incidence subsequent to the condition of the general health) rather than the bedding. One still finds references to the carcinogenic effects of wood shavings on their podophyllotoxin content (40,41), although these have not yet been adequately studied. A firm statement about the carcinogenicity of the literature also cited. The increased incidence of tumors among workers in the furniture industry, particularly where the predominant woods are mahogany, walnut, and other hardwoods. The tumors are primarily adenocarcinomas on the epithelium of the respiratory tract with latent periods of several years.

Of additional interest is the fact that corncob bedding, which is rich in lignin, resulted in cholesterolemia and increased tumor incidence. Ethylene glycol was used as a control. Although the incidence of adenocarcinoma was increased, it was not statistically significant. The safety of ethylene glycol as a bedding, nevertheless,

Although ammonia evolution is a major concern. For pigs exposed to 170 ppm ammonia for 18 hours/day, 5 days/week for 18 weeks, there were significant pathological changes in the spleen, kidneys, and adrenal glands. The spleen showing the most marked congestion with increased cellularity and some necrosis of the kidneys, and adrenal glands showing degenerative lesions. In rats, the changes observed in the control group were of known pathogenesis. The results in morphology and histology after 18 passages, while in control rats, the severity of the changes was directly with ammonia concentration. On this basis, it was concluded that ammonia plays an important role in murine respiratory

thought at one time that cedar shavings increased the occurrence of mouse mammary gland tumors in C3H sublines (38). However, this was not the case: the factor in altering tumor incidence subsequently was shown to be the condition of the mice (weight gain and general health) rather than their bedding (39). One still finds reference to the possible carcinogenic effects of wooden bedding products due to their podophyllotoxin (in softwood) or lignan content (40,41), although these compounds have not yet been adequately tested to make a firm statement about them. On the other hand, the literature also contains reports concerning increased incidence of nasal cancer in woodworkers in the furniture industry in Great Britain where the predominant woods are oak, mahogany, walnut, ash, beech and elm - all hardwoods. The tumors were mostly adenocarcinomas on the ethmoid or turbinate bones with latent periods of 20-40 years (42).

Of additional interest is the finding that corncob bedding sterilized with ethylene oxide resulted in chronic toxicity in male mice and increased tumor incidence in females (43). Ethylene glycol was recovered from the sterilized bedding. Although the findings with regard to carcinogenicity were more suggestive than statistically significant, doubts as to the safety of ethylene oxide sterilization of bedding, nevertheless, have been raised.

Although not caused directly by bedding, ammonia evolution by animal waste is a major concern. For example, in male guinea pigs exposed to 170 ppm of ammonia up to 6 hours/day, 5 days/week for 12 weeks, no significant pathological changes could be found. After 18 weeks, there were mild yet definite changes in the spleen, kidney, adrenals and liver. The spleen showing the greatest change: marked congestion with increased hemosiderin. Cloudy swelling and some casts were observed in the kidneys, and adrenals showed some early degenerative lesions. None of these changes were observed in the control animals (44). Exposure of known pathogen-free rats to ammonia alone results in morphologic changes in the nasal passages, while in *Mycoplasma pulmonis* infected rats, the severity of the disease increased directly with ammonia concentration (45,46). On this basis, it was concluded that ammonia plays an important role in the pathogenesis of murine respiratory mycoplasmosis.

Investigators also have shown that mice in a dirty environment (one in which ammonia levels are high) manifest significant decreases in some microsomal enzymes, aniline hydroxylase and ethylmorphine N-demethylase (33). Additional unknowns may abound. Of some concern is the possibility that steam sterilization may leave residues on bedding. Cyclohexylamine, frequently added to water to prevent corrosion of pipes, is such an unknown (47). We are "assured" that the dose of such a chemical will be too low to produce an effect. But cyclohexylamine is a skin irritant. Could it, under the proper conditions, cause problems in rodents during long-term contact, for example? Lest we feel too confident that, since low doses produce no effect, lower doses will also produce none, attention is called to a paper which points to a number of dose-response relationships in nature where that is not necessarily true (48). Sometimes less is indeed more!

Manufacture of Contact Bedding: Quality Control at the Source and by the Vendor

Wood shavings: These are byproducts of the lumber or woodworking industry and result from the planing of wood. Except for the fact that the lumber may have been kiln dried at some time before the shavings are produced, they are not likely to be heat treated before shipment to the user. Quality control is in general virtually lacking. A manufacturer may be able to claim that his shavings are softer, contain less debris, are free from pesticides or other pollutants depending upon the source of the wood, but few if any can assure the user of microbiological or toxicological purity by means of assay data. Packaging of the product is variable. It is not necessarily protected from the environment or from vermin. Some vendors use simple paper bags while others may use only wooden slats and bailing wire to contain the shavings. In some cases, deliveries are made in bulk. Still others may package them so that they may be conveniently sterilized at the user laboratory.

Wood chips: The process by which wood chips (byproducts of the manufacture of particle board, the heels of women's shoes, etc) are produced is a marked improvement over that which provides shavings. It is stated that

only green logs are used. These are debarked prior to production of the chips, thus avoiding the presence of wood preservatives or other surface contaminants in the final product. Particle size is controlled by means of sieves, heat (up to 350°C) is applied for drying, dust is aspirated, steam is introduced to bring the humidity up to about 6-9%, and then it is packaged by means of an auger in three-ply paper bags. These are constructed of "Kraft" paper with autoclavable glue, requiring no stitching that could result in a portal of entry for vermin during transit and storage.

In addition to control of particle size, dust and humidity, vendors or manufacturers also on occasion (frequency not known) submit their product to independent laboratories to test for the presence of microbiological, toxicological and other chemical agents. In addition, particle uniformity, absorptivity, ammonia evolution and skin irritant properties may be determined by independent laboratories.

Corn cob products (contact): These are the byproducts of both the seed and feed corn industry, animal bedding beintoonly one of about 70 uses to which they are put. The wood of the cob is separated from the remainder and ground. The granules then pass through screens for sizing, residual dust is aspirated, the granules are heated to 100-300°C, cooled and immediately placed in bags similar to those described above. Quality control may consist of some or all of the items mentioned above.

Pelleted peanut hulls: Once all foreign objects are removed from the harvested peanuts and the nuts themselves removed, the hulls are recleaned, ground to a small particle size, mixed with bentonite (5%) as a binder and pelleted under steam pressure. The pellets are cooled, the fines and dust removed by aspiration, and the pellets are directly bagged.

Quality control is based on the vendor specification of granule size uniformity, bulk density, absence of additives, moisture content of 8-12%, lack of effect on hepatic microsomal enzyme function and inability to support mold growth. Tests by independent laboratories are occasionally run (frequency not known).

Shredded aspen: In general, this is derived from trees in Colorado and Wisconsin. The shreds are produced by fracturing the wood. They are heat treated at 160-300°C.

Dust is removed by vacuum aspiration, and the product is bagged in four-ply paper bags. Quality control is physical: no sharp ends on the shreds. Other laboratories are utilized for microbiological and toxicological determinations.

Shipping, Receiving and Storage of Bedding

Depending on the distance involved, bedding is shipped by railroad box car (long haul) or truck (short haul), usually to a dealer. There can be considerable friction and jostling during transit, and even though the material may be dust-free at the source, it can become dusty before receipt.

Because bedding has a long shelf-life, a dealer may store the bedding for a long time. That being the case, his warehouse should be protected from vermin, should be dry, and the bedding should be palletized. Dating of production lots is, to our knowledge, not practiced, either as to time of manufacture or receipt at the warehouse.

The receiving of bedding at the user laboratory often is fraught with the same problems that apply to the receipt of animals, that is, there is seldom a special area set aside for those purposes. Sometimes such areas even lack adequate protection from the elements. Add to that the usually inadequately sized storage areas provided in an animal care facility, and it becomes important for the vendor (dealer) of bedding to have suitable storage space to maintain sufficient stock to service his customers frequently. Delivery from the warehouse is by truck, which should be a closed van.

Bedding Usage: Quality Control by the User: Cost

It is axiomatic that all contact bedding should be sterilized before use, although under certain circumstances (with conventional animals, for example, and with a low order of microbiological and other contamination) this may be judiciously dispensed with.

Recently, investigators reported on the development of a paper strip containing an indicator sensitive to both heat and steam that can be inserted into bags of bedding to monitor sterilization (49). It is claimed to be an improvement of the indicators that detect heat alone. The strip is so designed that the integrity of a barrier system, for example, is not compromised.

Quality control on his needs and on his health. Often, it consists of testing for the dustiness. Testing is expensive, the cost of the item. The currently being done by producer are summarized in a recent article. These be performed? of the various chemical animals that spend time in contact with them? These still open for discussion.

In terms of cost, a detailed account as to various types are used, another paper. Sufficiently expensive bedding is locally, direct from a quality control. The almost any bedding vendor have run out requiring a supply to the holidays just before strike.

In these days of some value to men, found that he could do by adding an equal volume of shredders to pine shavings. This was thus proved to be improved, and better controlled. Also, than with shavings, may not yet be the possible that new bedding be investigated for cost operations.

Here we see the addition of odor suppressants, counterproductive because the ability to monitor hormones cannot be effective.

Table 3

Tests of bedding

Chemical properties
Pesticides and polychlorinated biphenyls
Aflatoxins
Detergent residues
Ether extractable substances
Heavy metals

Quality control by the user depends on his needs and on his ability to afford testing. Often, it consists of nothing more than checking for the dustiness of a particular bagful. Testing is expensive and, therefore, adds to the cost of the item. The kinds of tests that are currently being done on bedding by user and producer are summarized in Table 3 and discussed in a recent article (50). How often should these be performed? What are allowable limits of the various chemicals and microbiota for animals that spend their lives in intimate contact with them? These are additional questions still open for discussion and determination.

In terms of cost effectiveness, a detailed account as to the manner in which the various types are used could easily comprise another paper. Suffice it to say that the least expensive bedding is one that can be obtained locally, direct from a lumber mill without any quality control. The most expensive bedding is almost any bedding that both the user and the vendor have run out of at the same time, thus requiring a supply to be shipped by air around the holidays just before the airlines might go on strike.

In these days of recycling, it might be of some value to mention that one investigator found that he could cut his bedding cost in half by adding an equal volume of paper from paper shredders to pine shavings (51). Nesting material was thus provided, water absorption seemed improved, and odor appeared to be better controlled. Also, cages were easier to clean than with shavings alone. Even though this may not yet be the wave of the future, it is possible that new bedding combinations should be investigated for cost responsible animal care operations.

Here we should mention that the addition of odor suppressants to bedding is counterproductive both because of resulting inability to monitor husbandry and because pheromones cannot be effective in maintaining high

fertility under such circumstances. Five rodent contact beddings for guinea pigs were studied with respect to odor control (52). They were hardwood chips, pine chips, poplar filaments, cellulose pellets and cellulose pellets with chlorophyll. Reduced ammonia levels were found with both types of cellulose pellets and poplar shreds as compared with the others. The use of specially designed ventilated cage racks for this purpose has recently been described (53). The aim was to reduce cage changing from once a week to once every 2 weeks. Others attempt to solve the problem by more frequent cage changes or by removing only the soiled portion of the bedding, leaving the clean part for the animals to continue nesting upon. The problem is not easily solved. Each solution may add to cost, all other things being equal, but as we know, reduced ammonia levels do result in better animals.

Disposal of Soiled Bedding

Within an animal room, cage changing can produce hazardous aerosols. In order to control these, safety cabinets have been devised (54-56).

Incineration is by far the most economical means of destroying both the bedding and the microbiological and toxicological contaminants (radioactive wastes are a separate problem, of course) (57,58). With regard to disposal or re-use of soiled bedding (as a mulch or fertilizer), even after sterilization, it should be observed that the Resource Conservation and Recovery Act, PL 94-580, as amended in 1978, provides that an Office of Solid Waste be established with the Environmental Protection Agency. Subtitle C relates to hazardous waste, which is defined as ignitable, corrosive, reactive, toxic, radioactive, teratogenic or mutagenic. It might be difficult to prove that soiled bedding, even though it might have been sterilized, would not fall into one of these categories.

Table 3

Tests of bedding quality

Chemical properties	Physical properties	Microbiological properties
Pesticides and polychlorinated compounds	Particle uniformity	Standard plate count
Aflatoxins	Absorptivity	Yeasts and molds
Detergent residues	Ammonia evolution	Coliforms and <i>Salmonella</i>
Ether extractable substances	Visible trauma and irritant potential	<i>Pseudomonas</i>
Heavy metals		

Conclusion

We have posed a number of questions since we initially wondered what happened to bedding vendors between 1974 and 1979. Considering the questions that may yet arise from the use of the materials currently in fashion, perhaps we should be surprised that so many vendors have remained to serve the field of laboratory animal science. The real task, however, still lies in the future. Taking into account scientific, economic, humane and legal aspects of laboratory animal bedding, is there agreement that there should be standards for bedding? Should there be standards covering more than one species or type of animal (hairless, HSR, germfree, pregnant, etc)? If so, how can the promulgation of such standards be abetted? What are the questions that still require hard answers before they can be written? And who will do the work in order to obtain the results on which the standards are to be based? It is perhaps a "cop-out" to end this discussion with so many queries, but it is to be hoped that they will stimulate action in the form of interest and concern on the part of investigators followed by support, both moral and monetary, from appropriate agencies.

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Questions and Answers

Question from Robert Whitney Jr,
National Institutes of Health

Do you feel that all bedding should be sterilized?

Answer by L M Kraft
Yes.

Question from R Whitney

Do you feel that there may be products or by-products in some of the bedding from sterilization itself that may be a problem?

Answer by L M Kraft

Yes. There has really been so little work done on bedding that we don't know very much about it. It's perfectly true that there are some beddings on the market, the heat treated ones, that are so low in bacterial count that it is hard to find the bugs, but there they are. I just think it's an open question.

Comment by Edwin P Les, The Jackson Laboratory

I'd like to comment a little bit about the behavior aspect of bedding. One of the things that at least mice require to lead a normal life is nesting material, but more than that, they have to build nests. They are nest builders, and they need to have material that they can lug around in the cage and build a nest with. In a study that I was doing with bedding, I was testing a pelleted bedding and comparing it with white pine shavings. I found that there was a bizarre type of behavior going on in the cage that had the pelleted bedding. This behavior consisted of two mice standing head to toe so to speak, front to rear, each mouse picking up the tail of the mouse in front of it and walking around in a circle. Not only that, but sometimes even a single mouse in a cage would pick up its own tail and walk around in a circle. I thought that this was so unusual that I contacted our art and photo department to have movies made of this, but in the meantime I did what people should do and looked in the library and found that this was very well described by people who are studying animal behavior. They ascribed this abnormal behavior to the lack of nest building material and the animal's environment. They just have to have something to build a nest with. If you deprive them of it, they're not normal any longer.

Comment by L M Kraft

I have not done any work on it, but I would not be surprised to find that the fact that the aspen material was in shreds, of which a nest can easily be made, is the reason the animals like the aspen excelsior material so much. It's easier for them to build a nest out of it.

Comment by Jim Halkett, New England Nuclear

Many years ago, we were breeding some mice, and to each cage we added one sheet of kleenex, and the mice had a ball. They just shredded the thing up and got their young in amongst it, and it was just terrific. Probably most of you have tried this, but it is a 100 percent.

Comment by L M Kraft

One of the things that I did not mention because I didn't want to go overtime was trying to breed wild rodents in the laboratory.

There are some papers on this as you doubtless know. Here, too, they're ridiculous animals, and giving them a cotton wad or an old computer printout that's been shredded or anything like that, anything that's in the form of a shred or something that they're capable of shredding, seems to turn them on to nest building.

Comment from Gordon W Newell, National Academy of Sciences

Dr Kraft, you mentioned one paper that we also are interested in but in a different manner, and I bring this up only so that we don't always jump to too many conclusions. This is a rather interesting and extensive paper done in England on the incidence of tumors among furniture workers, and the kinds of nasal tumors they have developed are described as being very similar to tumors which have been shown to occur in an on-going experiment currently underway in exposing mice and rats to formaldehyde. Formaldehyde is also, as you know, a component of many urea formaldehyde resins, and there is much interest in this at the moment. Whether or not these particular furniture workers are involved with urea formaldehyde resins is something we are going to attempt to try to investigate with the people in England. However, there are these possible other types of interpretations of information that develops in the laboratory, and I bring it up because life is so complex today, and it's not easy to jump to a conclusion and be sure that one is correct.

Question from Bob Sedlacek, Massachusetts General Hospital

I have observed the same thing that Dr Les alluded to with a similar type bedding, but I have another question. I'd appreciate your comments on the use of antibiotics in bedding. This seems like a two-edged sword. We've already got more medication resistant bugs than we know what to do with now.

Answer by L M Kraft

The people with whom I speak don't want it. The only way they would have it is under a suspended wire cage in the cardboard substance. But in the contact bedding, most people with whom I have spoken don't want it at all because it usually interferes with their experiments.

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Comment by Ralph Anslow, University of Wisconsin

I'd like to comment on one of the things we see very commonly in some of our most prestigious areas and that is mites on mice. I suspect that bedding may very likely be

the source. As you heard what Dr Kraft said about storage of shavings from furniture factories, most are blown out of the facility where they may become nesting spots for wild rodents as well as birds. I think this is a logical explanation of the source of mites.