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Volatile organic compounds in commonly used beddings before and after autoclaving

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Introduction

Already three decades ago *Ferguson* (1966) showed that red cedar bedding shortens sleeping time in mice. Simultaneous studies of *Vesell* (1967) revealed the mechanism responsible for the effect: Induction of liver microsomal enzymes caused by softwood (red cedar, white pine and ponderosa pine) bedding. This basic finding has been subsequenty confirmed in several studies *(Wade et al.* 1968, *Bang & Ourisson* 1975, *Sabine* 1975, *Cunliffe-Beamer et al.* 1981, *Nielsen et al.* 1984, *Weichbrod et al.* 1988).

Far less attention has been paid to description of the exposure from the bedding materials or the route of exposure. *Bang & Ourisson* (1975) attributed the effect found to inhalation of cedrol or α-cedrol emitted from cedar bedding. *Nielsen et al.* (1984) combined the same effect with α-pinene, a natural compound found in both pine and spruce. Yet, in all studies using beddings made of wood or other natural raw materials, the case is that of mixed exposure, in contrast to single compound exposures traditionally used in biomedical research.

The definition of volatile compounds imply that these compounds evaporate in rather low temperatures. Most of bedding materials are heated either for drying before package or for sterilization or both. Due to variations in treatment process it is reasonable to expect that even the same bedding at its final destination, the cage, can have quite variable concentrations of volatile organic compounds. However, there is only one study in which the effect of autoclaving of bedding on barbiturate sleeping time has been assessed *(Cunliffe-Beamer et al.* 1981), but found to be insignificant. The aim of this study was to characterize exposure of animals due to bedding volatile organic compounds before and after autoclaving. The bedding samples assessed represented commonly used laboratory animal beddings in Europe.

Materials and Methods

Beddings. Samples of seven commercial and one noncommercial bedding were collected. The beddings are identified through letters A to H, the explanations of which are as follows:

Bedding	Type / Brand name / Manufacturer
A	Noncommercial wooden shaving / Local saw mill, Tartu, Estonia
B	Wooden chip / 4HV / Tapvei, Kaavi, Finland
C	Wooden chip / GLP-bedding / B & K Universal AS, Nittedal, Norway
D	Wooden shaving / Gold Shaving / Witham, SDS, UK
E	Wooden chips / Spanish Woodchip / B&K Universal Ltd., N. Humberside, UK
F	Pelleted hay / Litterrite / B&K Universal Ltd., N. Humberside, UK
G	Wooden chip / Gold chip / SDS, Witham, UK
H	Cellulose chip / Alpha Dri / SDS, Witham, UK

Bedding treatment. One liter of each bedding was placed into stainless steel solid bottom cage (42.0 cm x 24.5 cm x 15.0 cm). Cages were placed into a rack, and the rack was forwarded to autoclave chamber. Two autoclaving cycles were used. Treatment of cycle I was 134°C and 12 min, which is the standard cycle for stainless steel

equipment and that of cycle II was 121°C and 20 min, again the standard cycle for polycarbonate equipment and diet.

Chemical determinations. The bedding sample (1.5-5.5 g), taken either directly from manufacturer's package or treated with one of two autoclaving cycles, was weighed in glass bottle connected to purge and trap injector (Chrompack M 16234-89-1, 4330 EA Middelburg, The Netherlands). The bottle was heated to 55 °C for ten minutes and evaporated compounds were collected to cold trap (-120 °C). After collection of the volatile compounds the cold trap was heated to 200 °C and the compounds were analyzed with GC-MSD (GC Hewlett-Packard 5890II, MSD Hewlett-Packard 5971) equipped with a fused silica capillary column (DB-VRX, 30 m, 0.25 mm, ID 1.4 µm). Identification of compounds was performed by using NBS-spectral library containing 75,000 compounds. Quantification was performed by a total ion recording method using 1,3,5-trichlorobenzene as an external standard. The compounds processed were propanal, pentanal, hexanal, heptanal, toluene, α-pinene, β-pinene, camphene, 3-carene and limonene.

Results

The concentrations of the ten volatile organic compounds assessed from bedding samples both before and after autoclaving are shown in Figures 1 and 2. For illustration reasons Figure 1 shows results of the top four group, ie the beddings with highest sum of concentrations of the compounds

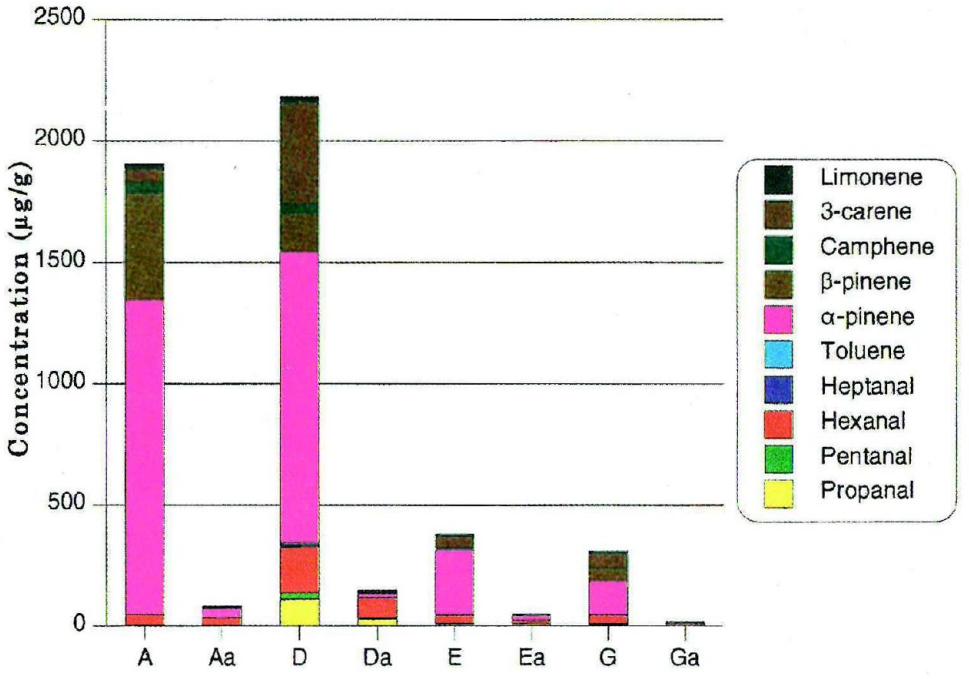

Figure 1. Summative concentrations of ten volatile organic compounds of four beddings before and after autoclaving. This figure shows the top four, ie. with the highest sum. Letters by x-axis depict each bedding by code. For explanations see materials and methods. The capital letter alone represents results before autoclaving and when followed by letter a, it indicates results after autoclaving.

determined. Similarly Figure 2 depicts results from ranking order five to eight. All compounds chosen were detected at least in some of the untreated beddings. Variation of the values was tremendous, hence it was decided to use quite different y-axis scaling in the figures.

Both autoclaving cycles decreased sum of, as well as indvidual, concentrations by an order of magnitude except with the beddings with initial sum below 10 µg/g. Because there were only minor differences between the autoclaving cycles, postautoclave values in both figures are those of autoclave cycle I.

Discussion

Emphasis on quality has yielded screening and control of the bedding for toxic residues. The same is not true with other likely negative effects associated with bedding. Despite convincing evidence on untoward effects of sofwood bedding on animals, beddings high on α- and β-pinenes, indicative of softwood origin, are still commercially available and widely used.

Small differences in some volatile organic compounds, for instance α- and β-pinenes, found in beddings made of similar raw materials obviously reflect differences in treatment process prior to package by the manufacturers. Even in those cases it was still possible to decrease those concentrations with autoclave. It appears that if you have to use bedding high on volatile organic compounds, you can get rid of most of them through autoclaving. Whether this is enough to avoid unwanted effects on animals is to be evaluated.

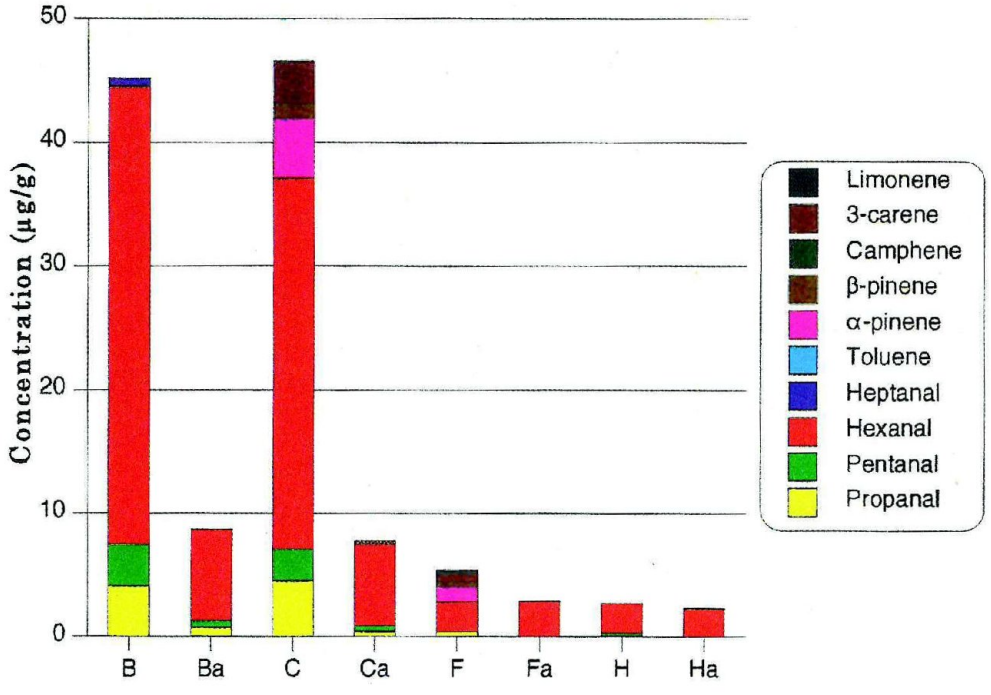

Figure 2. Summative concentrations of ten volatile organic compounds of four beddings before and after autoclaving. This figure shows the ranking order from 5-8, ie. with the lowest sum. Letters by x-axis depict each bedding by code. For explanations see materials and methods. The capital letter alone shows results before autoclaving, and when followed by letter a, it indicates results after autoclaving.

Studies so far suggest that this may not be the case *(Cunliffe-Beamer et al.* 1981).

It is a major drawback of the studies, where animals have been exposed to bedding, that none of them had made an attempt to characterize the exposure, and only two of them evaluated a single compound each *(Bang & Ourisson* 1975, *Nielsen et al.* 1984). Hence the rest of the studies ended up using mixed and unknown exposure, which cannot be reproduced afterwards due to sensitiveness of volatile organic compound concentrations to heat treatment.

In conclusion the deleterious effects of softwood bedding and the mechanism behind have been validated in a convincing way. Yet, numerous studies on the topic are hampered by lack of characterization of the exposure. Hence, we do not quite know which one(s) of the volatile compounds are responsible for the effects reported. It leaves us with the rational that we should, whenever feasible, to use beddings lacking or low in volatile organic compounds.

Summary

How to describe bedding, that is the question. So far it has been acceptable to write down the type and manufacturer of the bedding. And maybe for quality purposes screen the bedding for pesticide and heavy metal residues. This study focused on assessing of ten volatile organic compounds, which as a group has been combined with negative effects on animals. The commonly used European beddings were found to contain tremendously variable concentrations of these arbitrarily chosen volatile compounds. Furthermore, in most cases the concentrations went down by an order of magnitude after autoclaving. In conclusion, description of bedding in sensitive studies remains vague unless there is data on volatile organic compounds of the bedding at its final destination, the animal cage.

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