Erratum

Environmental Health Perspectives
Vol. 97, pp. 277-280, 1992

Cage Allocation Designs for Rodent Carcinogenicity Experiments

by Agnes M. Herzberg† and Stephen W. Lagakos‡

Cage allocation designs for rodent carcinogenicity experiments are discussed and presented with the goal of avoiding dosage group biases related to cage location. Considerations in selecting a cage design are first discussed in general terms. Specific designs are presented for use in experiments involving three, four, and five dose groups and with one, four, and five rodents per cage. Priorities for balancing treatment groups include horizontal position on shelf and shelf of rack, nearest neighbor balance, and male-female balance. It is proposed that these balance criteria be considered together with practical issues, such as the ability to accurately conform to a design and to determine a sensible and efficient design for each experiment.

Introduction

The validity and sensitivity of rodent carcinogenicity experiments for assessing the safety of food additives, drugs, cosmetics, and other substances depend a great deal on the experimental design. Some of the many issues that need to be considered and implemented before initiating an experiment are the number of dose groups, the choice of dose levels, the strain of mouse or rat, the number of animals per dose, the number of animals per cage, and the allocation of animals to dose groups. There are also numerous design issues that are implemented during and upon completion of the experiment, such as feeding schedules, monitoring of animals, time of interim or terminal sacrifices, and pathology review. For detailed discussions of many of these issues, see Fox et al. (1), Gart et al. (2), Grice et al. (3), Krump (4), Portier and Hoel (5-7), Greenman et al. (8), Haseman (9), and Bickis and Krewski (10).

This paper considers a design issue other than those mentioned, namely, the allocation of dose groups to cages. In most laboratories, animal cages are arranged on racks having four to six shelves and holding from five to eight cages per shelf. Usually each rack is aligned with another rack; therefore, one can consider the pair as a single rack having a front and a back section. When racks are paired in this way, it also is common to place male animals in one section, for example, the front, and females in the other. The decision of where on the rack to locate the different dose groups is important because there can be environmental differences that influence a rodent’s longevity or risk of developing a tumor. For example, cages in different locations on racks experience different temperature, humidity, and lighting conditions based on height, proximity to ventilation devices, lights, or fans, and perhaps even noise. If dose groups are assigned to cages in a systematic way, these environmental effects can bias the statistical analyses used to assess whether dose has an effect on tumor production. For example, if each shelf on the rack corresponds to a single dose group and there are altitude effects, then there will be a systematic bias that could cause a spurious association between dose and tumor rates or mask a real association. Such effects have been noted in several experiments (8,11,12).

One approach to overcoming such a bias is by controlling for shelf location in the analysis of the data. In an examination of data on red dye 40 (11), this would not be possible because shelf and dose are completely confounded. Even if there were only partial confounding, this is not a very desirable solution because controlling cage position requires that the appropriate model be selected. Lagakos and Mosteller (11) state that good designs for these experiments should have included balance in the layout of the cages, i.e., “to arrange cages in a way that ‘balances’ treatment groups with respect to rows, columns, positions and racks.” They “prefer a balanced design to completely random allocation because it ensures that factors of interest will not be confounded, it leads to slightly more sensitive tests and it is easier to implement” (11).

A simpler, more foolproof, and more efficient solution than the above approach is to avoid the problem through design; that is, to prevent systematic bias from occurring. One way of achieving this is to use a completely random allocation of dose groups to cages. That is, once cages have been loaded onto racks, dose groups are assigned to cages in a completely random manner. Such an approach has a number of merits, the main one being that completely random allocation tends to prevent systematic biases. However, there are three potential difficulties: a) even though a fully randomized design will be balanced on average, imbalances can still occur; b) the process of randomly assigning doses to cages can be somewhat time consuming; and c) it might be more complicated for the laboratory technician who feeds the animals to keep track of the dose groups than in a

*This paper was first published in Environmental Health Perspectives, 96:199-202 (1991). As the result of a printing error regarding the omission of Figures 1, 2, and 3, we are reprinting the original article in its entirety.
†Queen University, Kingston, Ontario K7L 3N6 Canada.
‡Harvard University School of Public Health, Boston, MA 02115.
Address reprint requests to A. M. Herzberg, Queen’s University, Kingston, Ontario K7L 3N6 Canada.
systematic design, say where each rack row corresponded to a
different dose, thus increasing the risk that animals are given the
incorrect dose of the test compound.

The goal of this paper is to discuss the use of designs that force
certain types of balance and, in so doing, avoid the first two of
these problems. In the next section, specific designs for ex-
periments involving three, four, and five dose groups are presented,
along with the discussion of some related points. These designs are not an exhaustive selection but serve to il-
illustrate what can be done in practice. Once the design has been
determined, the assignment of dose levels, etc., should be done
at random. For methods of performing such randomizations, see,
for example, Cox (13).

Proposed Designs

Design Priorities

Experiments consisting of 50 animals per sex per dose group,
which is customary in experiments conducted by the National
Toxicology Program and in numerous privately conducted ex-
periments, are considered. It is also assumed that any balancing
by weight, litter, etc., has already taken place (8), and all that re-
mains is the arrangements of cages onto racks and the allocation
of cages to dose groups. A helpful guide on how to choose fac-
tors in an experiment is given in Cox (13).

In selecting designs for balancing dose groups by cage posi-
tion, our priorities are a) Latin square balance: dose groups are
balanced with respect to the "rows" (i.e., horizontal position) and
"columns" (i.e., vertical position) within a rack, with each
dose group appearing the same number of times in each row and
column; b) nearest neighbor balance: cages to the north, south,
east, west of each cage are balanced by dose group (14); and c) 
front–back balance: dose groups are balanced with respect to
cages in the front and back of racks, referred to as ortho-
gonality. It is usually not possible to achieve perfect balance
according to these criteria; therefore, one attempts to use designs
that come as close as possible. Similarly, given the number of
animals per cage, it is not always possible to achieve exactly 50
animals per dose and sex.

Designs that satisfy the first and third of these criteria are
referred to in the literature as orthogonal Latin squares or
Graeco–Latin squares. For the analysis of such designs, see, for
example, Box et al. (15) and Davies (16). Because one has
balanced as much as possible and randomized where it is not
possible to balance, the analysis of the designs satisfying the
criteria will be as precise as possible.

Designs for Three Dose Groups

Let A, B, and C denote the three dose groups. Then 150 male
and 150 female animals are needed for the experiment. Consider
first the situation with four animals per cage; then 38 cages for
each sex are needed, two cages having three animals. Figure 1
gives a design for this situation with eight cages per shelf. If
shelves cannot hold this many cages, two racks can be used. The
18 interior cages in each rack are balanced in the rows and col-
umns and for nearest neighbors, with each cage having two cages
each of the other two dose groups as nearest neighbors. Also,
there is front–back balance, i.e., orthogonality between the front
and back racks, and the Latin square property holds. The boun-
dary cages of each rack cannot be completely balanced under
the three criteria in the previous section, but are balanced as near-
ly as possible. The design is implemented by randomly assign-
ing the letters A, B, and C to the three dose groups and then plac-
ing animals in their appropriate cages.

If five animals are housed in each cage, 30 cages per sex are
necessary. Figure 2 gives a design for this situation. The middle
three rows of cages are balanced for the three criteria of the
previous section. With one animal per cage, 150 cages are needed
for each sex. The design given in Figure 2 can be repeated five
times for this.

Designs for Four Dose Groups

Let A, B, C, and D denote the four dose groups. Then 200
animals per sex are necessary for the experiment. Consider
first the situation with four animals per cage; then 50 cages are needed
per sex. An example of a design for this situation is given in
Lagakos and Mosteller (11). Their design is balanced for rows
and columns, orthogonality between the racks, has the Latin
square property, and is also such that each of the four 2 × 2
quadrants in each 4 × 4 Latin square contains all four dose groups.
Figure 3 gives an alternative design based on the criteria discus-
sioned earlier. In this design, 48 cages are used for each sex, which
results in the use of 192 animals. The design is balanced for the
criteria except for orthogonality and the Latin property in each
column. Each cage has as its nearest neighbors all four dose
groups.
CAGE DESIGNS FOR CARCINOGENICITY EXPERIMENTS

279

Figure 4. Cage layout for four dose groups, denoted, A, B, C, and D with five animals per cage. Two letters within each cage denote dose groups for front and back of rack, respectively.

With five animals per cage and four dose groups, 40 cages are needed for each sex. Figure 4 gives a design for this situation. The design is balanced inside the boundary, i.e., the inner 32 cages of each satisfy all criteria except for orthogonality and the Latin property in the columns.

Designs for Five Dose Groups

Let A, B, C, D, and E denote the five dose groups. Then 250 male and 250 female animals are needed for the experiment. With four animals per cage, approximately 62 cages are needed. Figure 5 gives a design for this situation with 60 cages for each sex. The design is balanced inside the boundary for the three criteria discussed earlier. The cages on the boundary are not balanced for nearest neighbor or orthogonality. Inside the boundary each cage has each of the four other dose groups as its nearest neighbor.

With five animals per cage, 50 cages are necessary. Such a design is given by the cages inside the boundary of the design in Figure 5. For one animal per cage, 250 cages are necessary, and a design is given by five replicates of the design with five animals per cage.

Discussion

Two alternatives to the proposed designs are completely randomized designs and partially randomized designs that control for fewer factors. In most applications, it is our view that the designs proposed in this paper are preferable to a completely randomized design. The main reason for this is simplicity: to use one of the designs given in this paper, one only needs to allocate the numbers 1, 2, ..., k to the k dose groups. In contrast, a completely randomized design essentially requires 100k random allocations. The proposed designs also have the advantage of ensuring balance of dose groups with respect to shelf, location on shelf, and nearest neighbor, whereas the completely randomized design leaves this to chance.

The choice between the designs proposed in this paper and simpler balanced designs is less clear. One such design balances only with respect to shelf by having the same dose group in each column of a rack (8, 10). This type of design will prevent biases from altitude effects, but does not guard against horizontal environmental effects. However, if there are concerns about the ability of laboratory technicians to give the appropriate doses to cages in feeding experiments, or if vertical spilling of feed is a real possibility, this design may be preferable. One must weigh the trade-offs of unexpected errors in food distribution versus unexpected environmental effects. In general, if the use of a particular design is likely to lead to serious error in the delivery of the assigned doses, then it would be prudent to use a cage allocation design that would minimize or avoid this problem.

When a restricted randomization is used in the design of an experiment, failure to account for this in the analysis can lead to conservatism in statistical tests for a dose effect on tumor rates, but in most situations this will be slight and thus of no concern (17, 18). Alternatively, cage location can be controlled for in the analysis by regarding shelf height or location as explanatory variables and by using the regression model generalizations of the standard statistical methods of analysis (19, 20).

Finally, note that the proposed designs can be used in conjunction with any type of scheme for allocating animals to dose groups. For example, if animals are assigned to dose groups in a completely or restricted randomized way to control for possible effects of weight, litter, etc., this allocation can precede the allocation of dose groups to cages.

Nothing is sacred about the design sizes that have been used here. They have been chosen only because of their similarity to the sizes used in actual experiments. They have been used as illustrations to show what is available in practice.

REFERENCES

1. Fox, J. G., Thibert, P., Arnold, D. L., Krewski, D. R., and Grice, H. C. Toxicology studies II. The laboratory animal. Food Cosmet. Toxicol. 17: 661-675 (1979).
2. Gart, J., Chu, K., and Tarone, R. E. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62: 957-974 (1979).
3. Grice, H. C., Munro, I. C., Krewski, D. R., and Blumenthal, H. In utero exposure in chronic toxicity/carcinogenicity studies. Food Cosmet. Toxicol. 19: 373-379 (1981).
4. Krump, K. S. Designs for discriminating between binary dose response models with application to animal carcinogenicity experiments. Commun. Stat. Theory Methods II: 375-394 (1982).
5. Portier, C. P., and Hoel, D. G. Optimal design of the chronic animal bioassay. J. Toxicol. Environ. Health 12: 1-19 (1983).
6. Portier, C. P., and Hoel, D. G. Design of animal carcinogenicity studies of goodness-of-fit multistage models. Fundam. Appl. Toxicol. 4: 949-959 (1984).
7. Portier, C. P., and Hoel, D. G. Type I error of trend tests in proportions in the design of cancer screens. Commun. Stat. Theory Methods 13: 1-14 (1984).
8. Greenman, D. L., Kodel, R. L., and Sheldon, W. G. Association between cage shelf level and spontaneous and induced neoplasms in mice. J. Natl. Cancer Inst. 73: 107-113 (1984).
9. Haeman, J. Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58: 385-392 (1984).
10. Bickis, M., and Krewski, D. Statistical Design and Analysis of the Long-term Carcinogenicity Bioassay. Toxicological Risk Assessment. CRC Press, Boca Raton, FL, 1985.
11. Lagakos, S. W., and Mosteller, F. A case study of statistics in the regulatory process: the FD&C no. 40 experiments. J. Natl. Cancer Inst. 66: 197-212 (1981).

12. Teas, J., Harbison, M. L., and Gelman, R. S. Dietary seaweed and mammary carcinogenesis in rats. Cancer Res. 44: 2758-2761 (1984).

13. Cox, D. R. Planning of Experiments. John Wiley and Sons, New York, 1958.

14. Herzberg, A. M., and Wynn, H. P. Nearest neighbor balance and interblock and intra-block analysis in the design of experiments. Util. Math. 31: 243-254 (1987).

15. Box, G. E. P., Hunter, W. G., and Hunter, J. S. Statistics for Experimenters. An Introduction to Design, Data Analysis and Model Building. John Wiley and Sons, New York, 1978.

16. Davies, O. L., Ed. The Design and Analysis of Industrial Experiments, 2nd ed. Oliver and Boyd, Edinburgh, 1956.

17. Green, S. B., and Byar, D. P. The effect of stratified randomization on size and power of statistical tests in clinical trials. J. Chron. Dis. 31: 445-454 (1978).

18. Kalish, L. A., and Begg, C. B. The impact of dose groups allocation procedures on nominal significance levels and bias. Control. Clin. Trials 8: 121-135 (1987).

19. Cox, D. R. Regression models and life tables (with discussion). J. R. Stat. Soc. B 34: 187-220 (1972).

20. Dinse, G. E., and Lagakos, S. W. Regression analysis of tumour prevalence data. J. R. Stat. Soc. C 32: 236-248 (1983).