

APPENDIX D

RESEARCH & DEVELOPMENT MEMORANDUM

THE RANDOMIZATION OF ANIMALS INTO EXPERIMENTAL GROUPS

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I. INTRODUCTION

Randomization of experimental animals is a necessary step in study conduct to assure that differences among and between groups are distributed evenly. Randomization based on ranked body weight reduces variance among and between experimental groups and eliminates the probability of introducing experimental bias into the study. This note describes a randomization procedure that uses a computerized algorithm to produce homogeneous groups.

The procedure was written so as to produce "censored" randomization: the aim is to produce body weights of test animals in each group that are not significantly different from those in the other groups. This is done after animal assignment to groups, by analyzing group weights both for homogeneity of variance and analysis of variance.

This note has been written in the form of a Standard Operating Procedure (SOP).

II. EQUIPMENT

The equipment required to accomplish randomization of experimental animals is listed below.

1. Balance to weigh animals. For rodents, Mettler PM 2000 or equivalent. The balance should integrate signals over a fixed period of time to minimize error associated with movement of the animal in the weighing receptacle.
2. IBM-PC, XT, AT or compatible computer with at least 64K of RAM memory, DOS operating system version 2.0 or higher with BASIC, a single 360 K disk drive, and a printer configured as LPT1
3. Program diskette containing the randomization program "RANDOM" and the homogeneity program "CCBART" (listings attached).

III: PROCEDURE

1. Weigh all of the experimental animals. Record the body weight alongside the original number the animal was assigned at receipt. It is important during the weighing process to tare the balance between animals, because of the possible accumulation of urine and feces in the animal receptacle. The balance should be calibrated with weights close to those of the animals to be weighed.

2. Rank the animals in ascending order according to their body weight. Male and female animals are ranked separately during the randomization process. Assign the animals a rank number based on their position in the rank order. Some computer programs are helpful in the ranking process. A particularly useful program is MINITAB, where the commands "SORT" and "CARRY OVER" are used (see page 167 in the reference manual, available from MINITAB Inc., 3081 Enterprise Drive, State College PA 16801).

You should now have a ranked list of male animals and a separate ranked list for female animals. Make sure that the original animal number is correctly recorded when the animal receives a rank number. Each animal should now have recorded a rank number, body weight for randomization, and the original number. Carefully check for transcription errors before continuing. For example, a portion of a list of animals is shown below. The animals shown are from the first five animals in the ranked list.

Males Rank Number *****	Randomization Weight *****	Original Number *****
1	312.6	67
2	314.3	16
3	317.5	43
4	317.8	121
5	318.1	96

3. Discard extra animals from the randomization process. If, for example, 126 animals were received and only 100 animals will be assigned to the study, 26 animals will be discarded. The animals are now ranked from 1 to 126 in ascending order of body weight. Thirteen animals are discarded from the top (rank numbers 1-13) and bottom (rank numbers 114-126) of the ranked list to reduce the total number for randomization to the 100 required. The remaining animals in the ranked list (e.g., 14-113) should be re-numbered from 1-100.

Randomization Procedure

4. Start the computer and boot it up with DOS version 2.0 or higher in the 360 K drive, preferably the "A" drive. When the DOS is correctly booted you should see the A> prompt.

5. Load the BASIC computer language into the computer by typing BASIC and then strike the "Enter" key. When the BASIC language is loaded into the computer, the screen will display a few lines of information about the BASIC language version number and amount of memory available. You will no longer see the A> prompt on the screen but you will see OK instead.

6. Turn on the printer and make sure that the paper is situated in the printer correctly.

7. Insert the program diskette containing the RANDOM program into the "A" drive and close the disc drive door.

Type LOAD"RANDOM" and strike the Enter key. The quote marks are important and should be typed. The computer will load the RANDOM program from the diskette into the computer memory. You will again see the OK prompt on the screen.

8. Type RUN and strike the Enter key. The program will prompt you several times for information. Type in the requested information and press the Enter key to continue. You can interrupt the program at any time by holding down the Ctrl key and pressing the Break key at the same time.

9. The program will print some information on the screen and will ask a question concerning page breaks. The program is designed to start a new page on the printer after every 25 animals. For example, it may be more convenient to have only 15 animals printed on the page before skipping to a new page. If you wish to print fewer than 25 animal numbers on the page then answer the question with the character Y and press the Enter key.

You will then answer the question concerning position of page breaks with a number of 25 or less.

10. The program will now ask for information concerning the study number, date, and initials. You can answer these questions in any format you wish except for the inclusion of commas. For example, enter the date as July 19 1987 or 19 July 1987 instead of July 19, 1987.

11. The next question will be concerning the sex of the experimental animals. It is usually considered essential to keep the sexes separate during the randomization process. You should answer this question with M or MALES for the male animals, F or FEMALES for the female animals, or the answer can be BOTH if the sexes are not kept separate during the randomization process.

Randomization Procedure

12. The next question will be concerning printing of the ID Numbers on the randomization form on the printer. There may be an occasion that you want to repress printing of the ID numbers. If you wish to repress printing of the ID numbers answer Y and press enter. Otherwise, answer N and press the enter key.

An example is shown below to indicate what the randomization form will look like with the ID numbers printed and with the ID numbers repressed.

This example has the ID numbers printed:

RANDOMIZATION FOR ANIMALS TRD-ATS-000 SEX= MALES 19 JULY
 1987
 ***** OPERATOR = PHA

I D #	RANK NUMBER	ORIGINAL NUMBER	RANDOMIZATION WEIGHT (GRAMS)
*****	*****	*****	*****
G 1	25	_____	_____
G 2	34	_____	_____
G 3	47	_____	_____
G 4	15	_____	_____

The following example has printing of the ID Numbers repressed:

RANDOMIZATION FOR ANIMALS TRD-ATS-000 SEX= MALES 19 JULY
 1987
 ***** OPERATOR = PHA

I D #	RANK NUMBER	ORIGINAL NUMBER	RANDOMIZATION WEIGHT (GRAMS)
****	*****	*****	*****
_____	25	_____	_____
_____	34	_____	_____
_____	47	_____	_____
_____	15	_____	_____

13. The computer will now prompt you for the number of experimental units (animals). Input the number of animals that you wish to randomize.

14. The next question will be concerning the first ID number to assign to an animal. Commonly, ID numbers start at 1 but they can start with any number. Answer the question and continue to the next question. The computer will ask for an alphabetic character that will be printed with the ID number. Commonly, ear tags for rodents have an alphabetic character associated with a digit such as A 001 or G 001. If you do not wish to print an alphabetical character with the ID number, press the Enter key without entering an alphabetical character.

15. The last question you have to answer is a random seed number that starts the randomization function in the computer. The number must be between -32768 and 32767. Commonly a random seed number is supplied that equals the Julian date plus the year. For example, if the randomization process is occurring on the 30 th day of 1987 you might wish to use $1987 + 30$ or 2017.

The computer may require several seconds before it starts printing the randomized list of numbers to assign to the experimental animals. Figure 1 is an example of a page when only 12 animals were randomized.

16. In Figure 1 the ID number and the rank number are printed. The columns for original number and randomization weight are left blank. The rank numbers are randomized by the computer program. The ID number A 1 would be assigned to the animal that has rank number 4 in the ranked list (refer to step 2 above). Refer to the ranked list and record on the randomization form the original animal number and randomization weight that corresponds to the animal with rank number 4.

Make sure that all numbers are transcribed correctly. For example, Figure 2 is the same as Figure 1 except that the original number and randomization weight have been transcribed to the randomization form.

When all of the original numbers to match the rank numbers have been transcribed to the randomization form the randomization process has been completed. From Figure 2 it can be seen that ID number A 1 will be assigned to the animal with original number 3. ID number A 2 will be assigned to the animal with original number 11 etc...

17. Identify the experimental animals with the assigned ID numbers on the randomization form. The method of identification of laboratory animals is not the subject of this SOP.

18. Verify that the body weights in the groups are in fact homogeneous. This can be done using a simple analysis of variance; the command "AOVONEWAY" in MINITAB is typical of

the techniques used to ascertain the success of the randomization process.

It is usual to compare the variances among the sets of data before using analysis of variance; this can be done using the program "CCBART" which performs Bartlett's test for homogeneity of variance. A listing of this BASIC program is also attached.

IV. QUALITY ASSURANCE

Transcription of numbers from the original animal list to the randomization form is a likely source of error. As indicated in step 2 above, make sure that all numbers are entered or transcribed correctly in steps 1 and 2 before continuing with the procedure. Weighing of the animals is an important first step in the randomization procedure. To reduce variation among and between groups, animals to be randomized should be weighed on the same day.

After the randomization process has been completed and a randomized identification number has been assigned to the experimental animals, the actual identification process can occur over the course of a few days.

V. MAINTENANCE

To assure that the programs run successfully on the computer the diskette must be kept in a cool, dry place that is relatively free of dust. The diskette should also be kept so that it will not become warped. The program requires at least 1 page of printed output for each 25 animals randomized. Make sure that the printer has enough paper to finish printing all of the randomization forms so that the program will not be interrupted by printer failure.

VI. REFERENCES

S.C. Gad and C.S. Weil, 1986. "Methods for Data Preparation and Exploration". Chapter 5 in Statistics and Experimental Design for Toxicologists. Telford Press, Caldwell, New Jersey.

VII. ATTACHMENTS

Figure 1. Example of randomization form.

Figure 2. Example of randomization form with original number and randomization weight transcribed to form.

Listing of programs RANDOM.BAS and CCBART.BAS

OBJECTIVES

To devise a system for randomizing experimental animals into groups, using body weight; to verify that the system produces homogeneous groups.

SUMMARY

Two programs were written in BASIC to achieve the objectives. The programs were shown to produce homogeneous groups of animals using body weight as the criterion.

STATUS

The programs are in routine use and do not need any further work.

KEYWORDS

Randomization; homogeneity; analysis of variance; body weight; experimental animals; ranking.