

148/5

FILE NOTE NO.0844

BBW/CAE/46D-2

17th March 1969

THE MONITORING OF MOUSE-SKIN PAINTING EXPERIMENTS

Both the TPI (1) and the IMI (2) have been proposed as monitors of long-term skin painting experiments. Using these methods, single line graphs are produced which may be extrapolated by eye to forecast the likely final result of the experiment. More recently I have been considering the possibility of using more than one line on a graph as an indicator of the progress of the experiments. If these lines could be so chosen as to be reasonably smooth, of characteristic shape, i.e. of similar shape in all experiments, and to meet or intersect at the end of the experiment, then the extrapolation of both would be a rather more reliable forecast than either one alone. Also, it is obviously an advantage to be able to predict the age standardised result; that is to say, age standardised recording to a previously selected method, using a previously selected standard population. Of all the methods of age standardisation currently being considered, the arithmetically simplest is actuarial simulation coupled to fixed base direct standardisation. This method however can occasionally produce standardised responses of over 100%, and these are sometimes difficult to visualise. For this reason it is preferable to use actuarial simulation with double decrement age standardisation. This method is arithmetically somewhat more complex, but still within the scope of most desk calculators, and cannot produce results of over 100%. The details of this method are shown in the Appendix.

Having standardised the data, graphs of Σd_i (the cumulative number of tumour-bearing animals) and ΣD_i (the cumulative number of tumour-less deaths)

100995273

satisfy the desiderata described. They are both of characteristically sigmoid shape, and at the end of the experiment $\Sigma D_1 + \Sigma D_2 = 100\%$ of animals entering the experiment. Thus by plotting the curves in the manner shown, with the zero for ΣD_1 at the opposite end of the scale to the zero for ΣD_2 , the two lines meet when the standardised experiment terminates.

Also shown plotted is the PSR (Predicted Standardised Response). The PSR is also defined in the Appendix, and from its definition it may be seen to be a quite simple weighting up of ΣD_1 according to the proportion of surviving animals. Here again, at the end of the standardised experiment $PSR = \Sigma D_1$, so that extrapolation by eye of these three lines gives a three-way guess at the final result.

As mentioned earlier, the standard population must be previously selected. The population chosen to produce the attached tables and graphs was selected to fit experiment B0, and is very similar to the standard population now in use at Harrogate. Examination of the tables however shows that the standard population, although fitting the B0 and B1 experiments reasonably well, is a bad fit to the later experiments. For example, considering experiment B2 at the 25 mg dose level, the standardised experiment is virtually complete, whereas in the actual experimental group 71 animals are still alive; 25% of the starting number. Any tumours now produced by these 71 animals cannot influence the standardised result. This is an unsatisfactory situation, and arises because the life-span of the standard population is too short with respect to the life-span of the experimental population. The same situation can be seen to exist in the results from B3 and B4. Since it is our intention in general to be studying tobacco products tending towards the B3/B4 type in future experiments (i.e. lower toxicity and tumorigenicity than B0) it is

100995274

obviously preferable to select a standard population curve more suited to these longer-lived experiments than the standard shown in the tables.

It is of course inevitable that the standard population cannot fit all experiments. If we choose a longer-lived standard than that now being used, the effect will be to increase the calculated standardised response from the experiments, since in general more standardised animals will be alive at all ages, so that there will be more animals at risk overall. The ideal situation is that in which the standard population is representative of the mean life-span of all the experiments. In this case, the depression of the tumour rate caused by the remaining animals in the longest lived groups, and the accentuation of the tumour rate in the shortest lived groups, is minimised. As already remarked, it is the general intention to move towards tobacco products of the B3/B4 type. Therefore I propose to create a standard life curve based upon the mean mortality rate of experiment B4. The reason for choosing B4 is that not only is B4 representative of the level of toxicity/tumorigenicity towards which we are moving, but also it is proposed to adopt B4 for control purposes in B5 onwards. Experiment B4 is now only 63 weeks old, so the actual mean mortality curve is not available; however, enough data exists to create a life curve which will fit this experiment quite well. The proposed method of monitoring of experiments will use this B4 based standard mortality rate rather than the standard mortality rate shown in the tables.

E.S. Wilkes

E.S. Wilkes

REFERENCES

1. Sir Charles Ellis. IARC Document No. F431.
2. File Note No. 0838 (46D-2). "The Modified Tumorigenic Index".

CCP: 151 DR. S. J. GREEN
DR. D. G. FELTON

100995275

APPENDIX

ACTUARIAL DOUBLE DECREMENT STANDARDISATION

There are three populations of living tumourless animals to be considered.

1. The tumourless animals in the experiment, called the Actual Population.
2. The tumourless animals in the Standard Population. This is a hypothetical set of tumourless animals with a known mortality curve. In any interval of time t_i to $t_i + 1$ the proportion surviving will be MF_s .
3. The Simulated Population. This is the hypothetical population of animals we construct by combining the tumour survival rate of the Actual Population with the mortality survival rate of the Standard Population.

In the Actual Population, at any point in time t_i let there be N_i tumourless living animals, and in the succeeding time interval $t_i - t_i + 1$ let there be δ_i new tumour bearing animals and Δ_i tumourless deaths. Then during the time interval $t_i - t_i + 1$, if there were no tumours, the average number of animals alive would be $N_i - \frac{1}{2} \Delta_i$. Let this be the number of animals at risk of developing tumours. Then the probability of tumourless survival in the absence of mortality for the time interval $t_i - t_i + 1$ is $TF = 1 - \frac{\delta_i}{N_i - \frac{1}{2} \Delta_i}$.

Similarly, the probability of survival in the absence of tumours is

$$MF = 1 - \frac{\Delta_i}{N_i - \frac{1}{2} \delta_i}$$

Using the same approach, we may define the same quantities for the Simulated Population as

$$TF \text{ (sim)} = 1 - \frac{d_i}{N_{is} - \frac{1}{2} D_i}$$

$$MF \text{ (sim)} = 1 - \frac{D_i}{N_{is} - \frac{1}{2} d_i}$$

100995276

where N_{iS} is the number of tumourless animals alive in the Simulated Population at time t_i , and d_i and D_i are the number of new tumour-bearing animals and the number of tumourless deaths produced by the Simulated Population during the time period $t_i - t_{i+1}$. But we have defined the Simulated Population as one which has the same tumour survival rate as the Actual Population, and the same mortality survival rate as the Standard Population. Hence

$TF (sim) = TF$ and $MF (sim) = MF_S$, so that

$$TF = 1 - \frac{d_i}{N_{iS} (1 - \frac{1}{2} D_i)}, \quad MF_S = 1 - \frac{D_i}{N_{iS} - \frac{1}{2} d_i}$$

Eliminating D_i between these two equations, we have

$$d_i = \frac{2 N_{iS} (1 + MF_S) (1 - TF)}{4 - (1 - MF_S) (1 - TF)}$$

which is the number of actuarially simulated tumour bearing animals produced during the time interval $t_i - t_{i+1}$. Thus $\sum d_i$ is the standardised tumour rate produced by this method.

$(N_{iS} - N_{(i+1)S})$ is the total loss of healthy living animals in the Simulated Population during $t_i - t_{i+1}$. Having calculated d_i , we have

$$D_i = N_{iS} - N_{(i+1)S} - d_i$$

This is the number of tumourless deaths during $t_i - t_{i+1}$ in the Simulated Population. Hence we may calculate $\sum D_i$.

The Predicted Standardised Response (PSR) is defined as

$$PSR = \left(\frac{N_0}{N_0 - N_{iS}} \right) \sum d_i$$

where N_0 is the number of animals at t_0 in the Simulated Population. Obviously PSR is just a straightforward weighting up of $\sum d_i$ by a factor proportional to the number of survivors to estimate the likely final value of $\sum d_i$. It will of course only be accurate if $\sum d_i$ is linear with time. Also, at the beginning of the experiment $\sum d_i$ is subject to fairly wide fluctuations as the first few

100995277

tumours occur; these fluctuations are reflected in the PSR. However, usually by about the 76th week onwards the PSR has settled to a reasonably smooth curve (as the attached graphs show) and hence may be combined with the curves for Σd_1 and $\Sigma \Delta_1$ to forecast the end-result of the experiments.

100995278