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16th May, 1974

Dr. E. Karbe,  
 Battelle Institut, eV.,  
 6 Frankfurt am Main 90,  
 Postfach 900160,  
 West Germany.

Dear Eberhard,

Following my visit at the end of last week, I would like to confirm the main points of our discussions as follows :

The B11 experiment is currently in progress and mortalities have increased as expected at this stage. The study is proceeding very satisfactorily.

The data from Experiment B7 has been coded and the forms dispatched to Southampton for preparation of the masterfile. I gave you the remaining computer print outs required for the B4 report, and also the tumour tabulations plus supplementary pathology data from Experiment B5 (Intercomparison of earlier samples). Some progress has been made with coding the data from Experiment B8; punched cards and a print out of the 80 column cards will be produced in Battelle.

Herr Preuss has produced nearly all the graphs and some of the preliminary tables for the report on Experiment B4. Work is proceeding with the sections on condensate production and supplementary pathology.

There are no promotion tests in progress. The current status is as follows :

- 1) Extended comparison of samples B9/1 and B9/6: Battelle are awaiting the statistical report from Southampton before writing their report. (The report on Experiment B4 will now be undertaken first.)
- 2) B11 samples, and
- 3) "D" series samples

The histological assessment of the slides from these experiments, which were run concurrently, is now complete. The results will be tabulated in the near future and sent to Southampton for statistical analysis. Separate reports will be written on these two experiments.

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You stated that, at present, facilities and staff are available to undertake a new promotion study for B-AT if required.

The assessment of the hyperplasia test conducted in January had been completed and the results sent to Southampton.

In answer to a question concerning future hyperplasia tests, I indicated that a number of samples were becoming available, but these would have to pass an internal screening test before they could be considered for biological short-term testing.

In the current inhalation experiment, the animals have now been exposed to smoke for 65 weeks. Since the last review, only a few additional results are available, since relatively few hamsters have died; almost all of these were in the lower vitamin A group (5) which were exposed to one round of cigarette smoke per day. The highest grade of lesion noted was Grade 3, compared to Grade 2 for animals receiving the same diet, but unexposed to smoke. It was pointed out that, compared with the lesions in the NMU experiment, the Grade 3 lesions were severe and more diffuse; i.e. extended throughout a larger area of the larynx.

Kari indicated that, in the controls, there was a dose-response relation with the level of dietary vitamin A. In Groups 0-3, the lesions were in classes 0 or 1, in Group 4, the lesions were in classes 1 and 2, while most of the lesions in Group 5 were in class 2. Additionally, it has been noted that in both controls and smoke exposed animals, vitamin A deficiency leads to localised bronchial metaplasia and severe keratin formation. In response to a question, you considered that the level of smoke exposure was probably too low to lead to the production of bronchial tumours.

The proposal and cost estimate for the inhalation promotion experiment had been received in Southampton shortly before the visit. Two particular points were discussed in detail. Firstly, I questioned the requirement for 450 hamsters, while only 324 were actually planned to enter the main part of the experiment. While it was appreciated that there would be losses due to the pre-treatment and in the acclimatisation period, I indicated that it was neither desirable nor essential (from a statistical point of view) to sacrifice animals simply to balance the numbers in each group. You replied that the additional hamsters would be allocated with more animals in the higher NMU groups. If the survival rate was higher than anticipated, the extra animals would be included in their appropriate groups in the main experiment.

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The second major point discussed was the validity of the original suggestion that some of the animals should be sacrificed at 4 and at 6 months, the remainder being exposed to smoke for 8 months. It was agreed that this was undesirable since it would lead to a very small number of hamsters (6) in each group. You considered that some adjustment should be made to all the groups if appreciable numbers of hamsters in the high NMU dose groups died in the first 3 months due to a late effect of the pretreatment. In further discussion, it was agreed that the adjustment would not be necessary if the early deaths were due to carcinomas.

You raised the possibility of increasing the smoke concentration in the experiment. I thought that you intended to increase exposure to 3 rounds during the experiment and outlined the problems that this might cause, i.e. premature deaths which could more than offset the hoped for increase in response. In further discussion, however, it was clear that you were considering increasing the concentration by a decrease in smoke dilution from the present level (1 smoke + 7 air) to 1 + 6 or 1 + 5. I considered that this was a reasonable suggestion, providing the change was made easily in the experiment. It was suggested further that, before deciding on the concentration to be used, a toxicity trial should be undertaken with about 50 hamsters and using smoke from cigarette B12/1. The cigarette is manufactured from the control brand which will be used for the inhalation experiment. However, the cigarette actually used may be manufactured to a revised specification in order to match the puff number to that of the PRT sample.

The following provisional planning for the experiment was suggested: hamsters should be ordered by 23rd May for delivery 6 weeks later. The NMU pre-treatment would take place over the three weeks beginning 8th July and exposure would commence on about 31st July. The programme could be delayed by up to two weeks, but a longer delay would mean that NMU pre-treatment could not be undertaken until September because of holiday arrangements and the special restrictions applying to the use of NMU.

The background reasons for the delays for proceeding with the development of the method for the analysis of inhalation experiments were discussed with Dr. Langbein. The main cause of this delay is that it is considered essential that the computer system and program should be fully documented, so that it can be transferred to Southampton at a later date. Dr. Langbein understood the requirement, but, as a theoretical physicist accustomed to writing his own programmes, he found considerable difficulty in writing the detailed documentation in the format required by B-AT. I suggested, and it was agreed, that the best solution appeared to be that he would collaborate with a member of staff from the Battelle computer section and that together they would document each stage of the computer system and programme. The computer in use in Battelle is a Burroughs B6700.

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Dr. Schwaier is now able to breed topias with considerable success. A number of basic biochemical and physiological parameters are being studied to form the basis of future project proposals and it was suggested that this small primate could be suitable for cardiovascular studies of cigarette smoke.

Topias are very active and it is easy to produce severe stress. Battelle have found that the animals have a very high blood pressure and the levels of adrenaline and noradrenaline are also much higher than those found in humans. In a preliminary study, topias are being exposed to chronic sub-acute stress; assessments will be made of any atherosclerotic changes, vascular lesions and the diabetic condition to which the animals are also prone.

In answer to a question, you stated that there was no information on the possible effects of carbon monoxide or of nicotine. One attempt had been made to expose topias to cigarette smoke and this indicated that topias did not tolerate smoke from 80 cigarettes. I indicated that B-AT was to some extent relying on the cardiovascular work being done at Harrogate; the major aim was to assess the relative importance of carbon monoxide and nicotine.

You will no doubt let me know if I have missed or mis-interpreted any important points.

With best wishes to all the Janus team,

Yours sincerely,



S. R. EVELYN

cc: Dr. F. A. Sacherer  
A. Percy, Esq.

noo: Dr. S. J. Green ✓

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