

Research

SRE/SEV/3.5

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1st November, 1972

Dear Eberhard,

Below I would like to confirm the main points of our discussions in Frankfurt last week.

GENERAL

(a) Proposal on Inhalation of Lead Aerosol

Battelle are putting forward a proposal to the German Ministry of the Interior on the effect of lead pollution. Three different areas of work are involved and although two of these do not involve tobacco smoke, I asked whether there was any risk of infecting animals used in the JANUS project with the virus introduced in one of the work areas. Dr. Sacherer explained that the project would be undertaken in another division of the Biology Department housed in a separate building. I could not see any serious objections which might be raised in relation to the project involving tobacco smoke since the expertise and background knowledge gained in this type of experiment could be useful for the JANUS project in the future.

I was concerned, however, that the facilities available for inhalation work could be strained and assurance was sought that the JANUS inhalation projects would not be affected. You and Dr. Sacherer stated that this was a problem and that additional smoking facilities would be required; in any instance the project would not be undertaken to the detriment of the JANUS work.

(b) Animal Models for Studying Bronchitis/Emphysema

Battelle are considering the recruitment of a specialist to work in the area of bronchitis and emphysema. Based on the consideration that these diseases are often due to at least two factors, you suggested that by "priming" animals, models could be developed which were sensitive to bronchitis. You asked whether B-AT would be interested in projects in this area.

I indicated that the recruitment of staff could not be based entirely on B-AT's interest but you assured me that the

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recruitment was being considered to fill a general need to develop expertise in this area. Although considerable effort had been put into various goblet cell tests, I thought that there was a need for relevant tests related to these diseases, and considered that suitable proposals would be given detailed consideration.

### SKIN-PAINTING

#### (a) Long-Term Experiment

The current experiments are proceeding satisfactorily. From the B9 series it is clear that Carverth animals have a higher death rate than the ASL(Edwards) animals used in the experiments up to B8.

The B11 animals appear to be satisfactory; none were lost during the quarantine period but some routine health checks are still in progress.

I indicated that a number of suggestions had been made for experiment B12. As detailed plans have to be formulated, it was unlikely that the next long-term test could start before mid-1973.

#### (b) Promotion Experiments

The draft report on the promotion experiment (B9-2/B9-5) was considered and discussed in detail. One of the main conclusions from this study is that the promotion results parallel the long-term experiment and also those found in the short-term hyperplasia test.

The extended comparison of B9-1 and B9-6 was still in progress and it was agreed that this experiment should be terminated at week 36.

Termination of this experiment will leave sufficient B9-1 cigarettes for the continuation of the long-term experiment (on this sample) until week 126. If necessary, surplus condensate will be collected during the last four weeks of smoking so that the painting could be continued after 126 if this procedure is deemed desirable.

#### New Promotion Tests

A new test with seven samples and using 126 animals per sample over a period of about six months was planned. The samples and condensate yields would be similar to those in experiment B10-j. Consideration was given to the inclusion of an eighth sample and it was agreed that facilities would be available if smoking started after 19th January. It was decided to order the mice necessary for this experiment so that they would arrive early in January. Following pre-treatment with a single dose of DMBA (probably 180 ug), condensate treatment would start in the last week of January. The levels of condensate to be used were discussed and I will advise you of the final choice when this has been

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discussed in Southampton.

I informed you that a further promotion study could be undertaken to examine the condensates currently being used in the long-term experiment. It was agreed that this could best be undertaken during the course of the long-term experiment (B11), and that fewer control animals would be required if the two promotion experiments were started at the same time. Following discussion of the inhalation and other projects, it was agreed that this experiment should be started in January 1973.

(c) Hyperplasia Tests

The reasons for the delays in the assessment of recent hyperplasia tests were explained by Herrn Preuss. The results for the series undertaken in June were given to me during the visit.

INHALATION RESEARCH

(a) Vitamin A Experiment

Difficulty was still being experienced in obtaining a satisfactory supply of pregnant hamsters for this experiment. You had already contacted TMO in Holland, who are reputed to provide good quality animals, but unfortunately their maximum number for sale is about 200 hamsters (male and female) per week.

Correspondence with Roberts of Basingstoke was continuing but he appeared reluctant to give some guarantee that the 200 pregnant animals will provide an adequate number of litters within a relatively short time interval. You had specified that the pups should be born within a ten-day period. In a telephone call, however, Roberts indicated that he would accept a time criteria if it was extended to twelve days. You agreed to the slightly longer period and asked Roberts to put the matter in writing.

The information from TMO and Roberts was considered in relation to the vitamin A experiment and also to the further promotion (inhalation) experiment (see point (b) below). TMO could not meet the requirements for the vitamin A project but I suggested that their supplies would be sufficient for the promotion experiment if treatment could be started sequentially. Although this approach could be used for a long-term test, you considered that it would introduce greater variability in a prized experiment.

Since a number of aspects relating to the promotion experiment still required evaluation and detailed planning, I agreed that the vitamin A study should be started at a convenient date providing Roberts confirmed that he could meet the slightly revised specification.

(b) Promotion (Inhalation) Experiment

Dr. Köster had finished grading the laryngeal lesions.

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Numerous checks have still to be undertaken together with the assessment of the slides from the trachea and lungs. At this preliminary stage, the most severe lesions in each animal have been placed into the following grades: 0, 1, 1-2, 2, 2-3, 3 and benign and malignant tumours. Grades 4 and 5 described by Dentonwill at Gatlinburg have not been observed. The lesions in the control (unsmoked) animals are mainly in grade 0 or 1, although some (3) benign tumours were recorded. All the malignant tumours occur in animals exposed to smoke following pre-treatment with methylnitrosourea.

I stated that B-AT would like to undertake a further promotion experiment:

- (i) To examine the effect of two smoke exposure regimes.
- (ii) To confirm the encouraging results found in the preliminary study.

You suggested that it would also be desirable to examine the smoke from two different cigarettes in the one experiment and I agreed that this would be ideal providing such an experiment was practicable and could be accommodated within the inhalation facilities.

As a guide to planning such an experiment, the provisional results were discussed in more detail. You considered that, although the removal of mucus from the larynx had resulted in one additional tumour being produced in this group, the possible advantage of this technique was not great enough to outweigh the disadvantages, additional stress, etc. Thus, in outline it was suggested that the experiment should include three levels of MNU pre-treatment (0, 1 and 2) and three levels of smoke exposure (0, 1 and 2). If this was replicated for a second cigarette, a total of fifteen groups would be needed (one set of control eliminated).

It was difficult to assess the number of hamsters required for each group, but a group size of eighteen was suggested.

(c) Analysis of Laryngeal Lesions (Dr. Langbein)

I put forward the comments and criticisms which had been made by E.H. Wilkes, and asked Dr. Langbein to consider these. Many of the detailed criticisms were not valid because the values given in Dr. Langbein's paper were derived from the curves drawn from the raw data and had not been calculated. A more serious criticism was the large number of parameters which Dr. Langbein's approach produced and which had to be combined to evaluate a particular cigarette or treatment.

Dr. Langbein argued that the number of parameters could be reduced by making assumptions similar to those put forward by EHV and he went on to suggest that these could be examined before they were incorporated. For example, using Dentonwill's data, it

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appeared feasible to combine the rate curves for the transitions to class 1 with class 2, and for class 3 and 4, since the curves had the same shape. Dr. Langbein also suggested that there was no reason why the average transition rate should not be calculated and used as an overall index.

The problem of the increasing width of the confidence limits which would be worst for the most severe lesions was raised. Dr. Langbein indicated that this aspect could be improved very considerably if the curves were defined.

After you had outlined the initial results of the first inhalation experiment, and some discussion had followed on the possible dose levels of initiator, the question of the duration of the experiment was raised. It appeared feasible to incorporate some degree of serial sacrifice so that the time period of four to eight months could be examined. This range would appear to be desirable to cover differences likely to result from the use of two smoke exposure regimes.

Assuming that Dr. Langbein's approach was relatively simple, I considered that it had merit and could provide a useful insight into the biological mechanisms even if it required further development for use as a basis for comparison of samples. Subsequently I asked Dr. Sacherer and you to obtain an estimate for developing the method and using it for the analysis of the promotion experiment just completed. Dr. Sacherer did not think that Dr. Langbein would have any interest in using the method routinely once development had been completed and the method proved.

#### MISCELLANEOUS

##### (a) Report on Experiment B1

The various sections of the B1 report had been prepared but the amalgamation and discussion section remains to be undertaken. It was apparent that priority had been given to the inhalation work and to the second promotion report. I indicated that the statistical analysis had been compiled and that a copy of the report by KBW would be sent to Battelle as soon as it was produced.

##### (b) Histology and Coding

The data from experiments B5 and B6 was complete except for the final "book-keeping" checks. Dr. Königsmann indicated that he would start coding B5 when these were completed; meanwhile about 50% of the histology for experiment B7 had been undertaken.

Copy No. 3 of the System Definition (2500J/04) for the analysis of supplementary pathology was given to Dr. Königsmann. The corrections requested by him at the draft stage had been

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incorporated and I asked Dr. Knaigsmann to confirm that the System Definition was now correct, or to advise any necessary amendments.

As usual, I assume that you will write to me if you do not agree with any of the points outlined above, or if I have missed out anything of importance.

With kind regards,

Yours sincerely,



S.R. EVELYN

cc : Dr. P.A. Sacherer  
Hattelle, London

noo : Dr. S.J. Green ✓  
Sir Charles Ellis, FRS

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