

DGF/BK/460

22nd December, 1960

NEUKOM/BONNET PROCESS

Visit to SEITA, Paris 22nd December, 1960

Present:

Dr. J. Guzin	}	SEITA
M. Vaissereau		
Mr. G.F. Todd	}	T.M.S.C.
Dr. D.G. Felton		

Mr. T. Russell-Cobb, Campbell-Johnson Ltd.

Dr. Guzin opened the discussion by introducing M. Vaissereau, a statistician, who directs research for the Regie at Orleans and Bergerac. He then stated that SEITA had taken an interest in the Neukom/Bonnet process in Switzerland and, by arrangement with Neukom, had been afforded an opportunity of testing it. Samples of "Gaullois" tobacco were subdivided into two parts, one of which was treated in the N/B machine at Serrieres by Dr. Bonnet in the presence of Guzin. The two samples were then manufactured into cigarettes by Rinsez et Ormand et Cie (Yvey), the Swiss manufacturer of SEITA brands. Twenty thousand cigarettes of each type were produced and the samples carefully matched statistically.

It was intended to use these samples to evaluate the N/B process most thoroughly as follows:-

1. Complete Chemical Analyses

(a) of tobacco, special attention being paid to paraffins and other possible pre-cursors of polycyclic hydrocarbons.

(b) of smoke, especially for paraffinic hydrocarbons and polycyclic hydrocarbons, e.g. 3,4-benzopyrene, pyrene, anthracene, benzo [g,h,i] perylene, dibenzanthracene and 3,4,9,10-dibenzopyrene.

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These analyses would aim to check the detailed findings of the Matthey report.

2. Biological Testing

This would be undertaken in two parts:-

(a) Quick Test - SEITA have developed a rapid test which is a combination of the sebaceous gland suppression test and a test for hyperplasia developed by Guerin. The Guerin test is carried out by painting mice at two-day intervals for four times and sacrificing after ten days total time. Tissue sections are made both transversely and in the plane of the skin and the Malpighian zone of the epidermis is studied for sebaceous gland involution and invasive hyperplasia (indicative of irritation response). The test is said to correlate excellently with carcinogenic potency for polycyclic hydrocarbons only (5 - 8 rings).

(b) Long term skin painting - This would only be undertaken if the quick test proved to be positive.

Cuzin then stated that the Regie must accept any good method for making cigarettes safe. The N/3 process raised an important question since the choice of any method implied the possible exclusion of another process which might be better in the long run. For that reason, Cuzin needed to be convinced that the N/3 process was sufficiently effective.

He then proceeded to describe the experimental methods used by SEITA for quantitative measurement of polycyclic hydrocarbons in smoke condensate and handed copies to G.F.T. and D.G.F. Briefly, the analysis, which is carried out in condensate from five hundred cigarettes (10.g. condensate), depends upon solvent partition between hexane and aqueous

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methanol, followed by chromatography on silica gel/celite and then on alumina. Assay is made by spectrophotometry or by spectrophotofluorimetry. Cuzin and D.G.F. briefly discussed the relative merits of the Aminco-Bowman and Unicam spectrophotofluorimeters.

Cuzin criticised the Bonnet experimental techniques on three grounds:-

(i) The use of acid and alkali washings to produce a neutral fraction.

Cuzin believes that Neukom's "brown polymeric substances" are an artefact, due to this technique.

(ii) The use of zinc chloride as a clarification step.

(iii) The method of assay by spectrophotometry using an artificial background. Cuzin thinks Bonnet is a highly competent chemist and that in his hands the method is probably reliable. However, other people, including SEITA chemists, find the method less precise. Cuzin discounts Matthey's results, saying that the agreement between these and those of Bonnet are probably coincidences. Moreover, the Bonnet technique does not allow for interactions between two or more hydrocarbons.

Cuzin was then asked whether he believed that the active components in smoke were only poly-cyclic hydrocarbons. He replied affirmatively, adding that SEITA had shown by the quick Guerin test that crude paraffins from smoke gave a positive response, as Neukom found by new test. However, the presence of benzopyrenes in this material could be shown fluorimetrically and after boiling the crude paraffins with concentrated sulphuric acid, the fraction showed no activity biologically.

The quantitative aspect was then mentioned and Cuzin was asked whether he, therefore, believed in the theory of a "super-carcinogen" of the poly-cyclic type, which as yet was unknown. He replied that he had no faith

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In the quantitative argument as applied to condensate, since there were no grounds for believing that it was in this form that smoke-tissue interaction took place. He could envisage solid droplets of pure polycyclic hydrocarbon or a monomolecular layer of polycyclic hydrocarbon on the exterior of aqueous aerosol droplets; in this way the concentration on impact on lung tissue and hence the effective concentration could be much greater than the concentration as measured in condensate.

Cuzin referred to Wynder's belief in the hydrocarbon causation and, on being checked on this point, admitted that with the advent of Hoffman, Wynder had changed his argument in favour of phenols as co-carcinogens. He described Wynder to Vaissereau as being like Denoix - "a passionate fanatic". The possible effect of atmospheric pollution as a co-adjutant factor was dismissed as "purely psychological". Cuzin then restated his guiding beliefs, namely that polycyclics were the main factors responsible for smoke carcinogenesis, with relatively minor roles being played by co-factors.

(a) Co-carcinogenesis - synergistic influences as postulated by Berenblum were probably no longer valid.

(b) Phenols - acting as irritants and inhibiting ciliary activity of bronchial epithelium.

(c) Positive ions - also inhibiting ciliary activity.

Cuzin mentioned some studies by scientists of the French Navy (Toulon), published in Acts of the Congress of Otolaryngology. This was in connection with smoke removal from Atomic submarines, in the course of which they found that ciliary activity was inhibited by positive ions and by water-soluble fractions of cigarette smoke, probably carbonyl in nature.

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The work of Koflin and of Krueger was mentioned and Cuzin indicated he was well aware of it. The Regie were at present constructing an ionisation chamber. He thought Koflin's approach was unsound, since physiological studies were not included.

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Cuzin then changed the course of the discussion abruptly by producing a copy of the letter by Ruffy of the Swiss Federal Public Health Service, permitting the industrial application of the N/B process. He described this document as "dynamite". The meeting then considered the report by Prof. Nicod, on which the Ruffy letter was based.

G.F.T. and D.G.F. described their conversations with Neukom while Cuzin produced a report by Dr. Anguera, a medical statistician with SEITA, in which it was stated that the biological results of Nicod were statistically improved at either the 5 or 10% levels of significance, even when the results of both tests were combined. Anguera drew the following Conclusion:-

"Nicod furnishes interesting data on the effects of the N/B treatment which permit the presupposition of favourable action in decreasing the toxicity and carcinogenicity, but proof of this remains to be made."

Cuzin mentioned the supposed extension of the latent period between appearance of papillomata and carcinomata, for the treated group of Nicod, and agreed with D.G.F.'s argument that, in the absence of individual markings, this extension was almost meaningless. G.F.T. reinforced the argument by referring to the false negatives admitted by Neukom. G.F.T. referred to the apparent discrepancy between expected and observed numbers

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of tumours for the group (J + E). Cuzin had raised the same question with Neukom and had not received a satisfactory answer, either.

Cuzin then referred to the fact that dosage in the Nicod experiments had been on a per cigarette basis. D.G.F. then told him that, in fact, the treated cigarettes had been lighter in weight but, nevertheless, of the same firmness. Cuzin had obviously heard of this through Rinsor or Grand and enquired whether cigarette firmness had been measured by "Compacimeter". He became quite excited at this point, laughing and joking and saying this was the major justification of the process.

D.G.F. went on to make the points that, since the cigarettes were lighter, there was less tobacco to burn to yield condensate and, therefore, that, unless there was a reduction in the specific biological activity of the condensate, the same effect could be obtained by filtration. Cuzin agreed with this and stated that the Regie tests would be made in order to measure specific activities, but on a "crude tar" basis including the weight of water present ("wet tar" basis). He said that Neukom argued that man smoked on a "per cigarette" basis, and this justified Nicod's methods, but he (Cuzin) believed that man smoked for equivalent physiological and psychological satisfaction.

Cuzin mentioned that he had seen Nicod, who had told him that this was the first carcinogenicity test on tobacco smoke condensate which he had performed!

The Regie tests were scheduled to start at the beginning of January and Cuzin indicated he expected to receive the first consistent results by mid-February. The arrangements used by SEITA for biological testing were explained. The Regie worked with the "Groupe de l'etude de la fumee

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de tabac", which consisted of:-

Prof. Lacassagne - Radium Institute
Prof. R. Latarjet - Curie Labs, Radium Institute
Prof. Ng.-Zn Bud-Hoi - ditto.
Prof. Guerin - Villejuif
Prof. R. Truhak - Ecole de Pharmacie

Guerin carried out the biological tests at Villejuif, using a basic colony of 2,000 mice for quick testing, with one long term test every two years (but on four "items", which were not clarified).

The observation that Truhak had made that atmospheric pollution alone in Paris could cause skin cancers in mice was referred to, but Guzin said that this had not been done quantitatively.

G.F.T. then told Guzin on a confidential basis that I.M.S.C. were planning to establish a Research Unit, but that this had not yet been built. Guzin then enquired whether I.M.S.C. would like to approach the W/B process on an international scale in conjunction with SEITA, and it was indicated to him that this would indeed be valuable. The Ruffy letter suggested that pressure might be brought to bear both on the Swiss and international levels, through SASMOCC, S.A. Guzin remarked that one of the biggest shareholders in SASMOCC was Schürch of F.T.R. (Serrieres). F.R.-C. enquired whether Guzin thought that pressure should be brought to bear on Hsgl of the Swiss Federal Public Health Service to withdraw the Ruffy letter of authorisation. Guzin replied that legally the letter existed and he knew of no way of forcing its withdrawal. Guzin was asked whether he knew of other tobacco companies who were dealing with Neukom. He replied that he didn't know about the Germans, (Weber had been non-committal);

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the Italians would have no facilities for checking the claims; the Austrian Regie might be interested, but he had no information about them, or of the Swedish Monopoly.

It was indicated to Curzin that the results of the thorough testing being given to the N/B process by SEITA would be of the greatest interest to T.M.S.C.

Curzin then reverted to his point that the choice of the N/B process might well exclude subsequent processes employing additives. He referred to Bentley and Burgan's paper in "Analyst" and added that SEITA had found calcium carbonate was an effective inhibitor of benzopyrene formation. They were working on the temperature and oxido-reduction conditions necessary for polycyclic formation and had found, contrary to Law that 300°C was much more effective than the 600 - 800° cited by the Danish worker.

At the close of the meeting, D.G.F. extended an invitation to Curzin to visit R. & D.E., B.A.T. at Southampton during his proposed visit to England in mid-January. Curzin agreed to write suggesting a date when his arrangements are fixed.

D.G.F.

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