

Region Report
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RESEARCH LABORATORY

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December 10, 1959.

PROGRESS REPORT

ON ANALYSIS OF

3,4-BENZOPYRENE IN CIGARETTE SMOKE

Recovery of Ben. pyrene

The modified procedure for determination of 3,4-benzopyrene has been applied to the tars from cigarettes made with extracted and unextracted tobacco and the % recovery evaluated by the isotopic dilution method.

To each of the whole tars of the two samples labelled 7X and 7-1 respectively for tars from extracted and unextracted tobacco cigarettes, 5.28 µgm of 7,10-¹⁴C labelled 3,4-benzopyrene were added after three aliquots from each spiking sample had been removed for determination of counts by Dr. Huneckles proportional flow counter. After separation and quantitative U.V. evaluation of the benzopyrene from the tar samples three aliquots were again removed to determine the final count, and % recovery. The remainder of the samples were dried under nitrogen and shipped to Penrose Institute for their U.V. and radioactive evaluation. Table I summarizes the results.

TABLE I

Cigarette	7X		7-1	
	I.T.Co.	T.E.Co.	I.T.Co.	T.E.Co.
Total BP in sample (U.V.) in µgm	11.3	9.30	11.5	8.89
% recovery (by counting)	91.1	54.8	85.7	53.7

Continued

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Cigarette	7X		7-1	
	I.T.Co	T.E.Co.	I.T.Co	T.E.Co.
BP in whole tar	6.61	10.99	6.18	10.56
No. of cigarettes smoked	290		230	
Weight of whole tar in gm.	7.63		6.53	
µgm/100 cigs	2.30	3.8	2.62	2.5
ppm	0.95	1.57	0.95	1.62

It is difficult to reconcile the disparity between the T.E.Co. and I.T.Co. figures. During a recent visit to T.E.Co. (Nov. 30 - Dec. 1) the discrepancies were discussed but no satisfactory answer was arrived at. The I.T.Co. figures agree more closely to the values obtained for plain and cigarettes smoked to a 23 mm. butt length as well as with the conclusion that T.E.Co. has also arrived at that an extraction of tobacco reduces proportionally the whole tar and benzpyrene contents of the smoke. The above results by T.E.Co. appear to be a contradiction and imply a lowering of concentration of benzpyrene smoke content due to extraction of tobacco.

This laboratory repeated the determination on whole tar from unextracted tobacco cigarettes (Sample 7-2) without spiking and on the assumption that the recovery was again of the order of 85-87% achieved an identical result to that of the spiked run, that is, 2.62 µgm/cigt and 0.95 ppm of whole tar.

The isotopic dilution method will be used henceforth for all benzpyrene evaluations as it corrects for any inconsistencies in % recovery from run to run.

Until a radioactive sample is received from sources in the U.S.A. runs will be monitored using a sample kindly presented to us by Dr. Sievers of Tennessee Eastman. This sample is presently being purified.

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Benzpyrene content of cigarette smoke

The smoke from the tobacco-less Vanguard cigarettes was evaluated at the same time as the 7-2 sample. (See report to Research and Development dated Nov. 15, 1959) and the results compared to that of the 7-2 sample (N.B. The same % recovery was assumed).

The Vanguard cigarettes yield a significantly higher amount of benzpyrene per cigarette inspite of being a shorter and lighter weight cigarette and producing a lower whole tar/cigarette weight than the plain end tobacco cigarette (i.e. 3.73 $\mu\text{g}/\text{cigt.}$ as opposed to a 2.02 $\mu\text{g}/\text{cigt.}$ yield respectively).

Other determinations from the smoke of different cigarette were also completed (mainly 80 mm. Player's cigarettes to different butt lengths) but until the isotopic dilution method is systematically undertaken the results at best can only be considered approximations.

TABLE II

Cigarettes	Length	Length smoked	Butt Length	No. of cigarettes	Whole Tar		Benzpyrene		
	mm.	mm.	mm.		Wt. of sample	mg./cigt.	Total correc.	$\mu\text{g}/100$ cigt.	ppm
Player's	80	70	10	300	12.3	11.2	9.2	3.07	0.7
"	80	57	23	585	18.12	30.1	15.4	2.02	0.5
"	80	30	50	585	7.14	12.2	6.46	1.11	0.3

Same cigarettes smoked to a 50 mm. butt length then transferred to a second smoking machine and smoked to a 23 mm. butt length.

The values obtained were corrected assuming an 87.4% recovery.

Polycyclics in smoke

A qualitative identification study of other polycyclics in cigarette smoke has been initiated with a view of extending the study to a quantitative basis for some of the more readily separated and identifiable compounds. By comparing these results with those of benzpyrene for different smoking and cigarette variations, it will be possible to ascertain whether 3,L-benzpyrene can be accepted as a monitor for all smoke polycyclics.

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extractions of tobacco

Carbon tetrachloride extraction

Results from smoking cigarettes made with CCl₄ extracted tobacco failed to confirm the claims made by Bonnet and Neukom. The reduction of tars and nicotine in the smoke was less than 10%.

TABLE I/1

Cigarettes	% nicotine in tobacco	Whole tar mg/cigt.	nicotine mg/cigt.
Unextracted	1.59	27.1	2.09
Extracted	1.50	25.7	1.91
% reduction	3.0	5.2	6.6

The CCl₄ extracted tobacco shows a significantly better filling capacity than the control tobacco. However, the compression test on the respective cigarettes failed to indicate any significant difference although the trend was in the right direction (i.e. extracted cigarettes more resistant to compression).

acetone extraction of tobacco

The recycling extraction apparatus has been set up to anaemia and a hot extraction (50°C) with acetone carried out. After five 4 hour periods of extraction on 17 lbs. of tobacco a total of .3% (wet basis) had been removed. The rate of extraction had decreased to 0.5% in the last extraction period but there was no indication that no extraction was near completion.

The tobacco after removal of solvent and reconditioning is very brittle with a high percentage of skulls and quite light coloured. Due to small quantity of tobacco and high quantity of skulls cigarettes were not manufactured from this tobacco.

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Extraction of cigarettes with n-hexane, with toluene and with acetone

To obtain further information in respect to relationships between reduction in smoke tar and weights of extract removed from tobacco, four extractions of cigarettes were carried out. While the extraction of tobacco prior to the manufacture of cigarettes is preferable for these studies (thus avoiding change in weight of cigarettes, filtration efficiency, etc.) this method should give approximate values.

The data has been compiled in Table IV.

One very interesting observation was that hot toluene extractions (80°C-85°C) change the physical characteristics of the tobacco. This change was shown in that the cigarettes extracted in this way resulted in a very hard cigarette with a pressure drop approximately double that of the original cigarette (average pressure drop for 25 cigarettes = 5.5 ins. of water as compared to 2.5 ins.)

Cigarettes extracted with acetone showed a slight decrease in pressure drop (Average pressure drop of 15 cigarettes = 2.0 ins. of water). This decrease could be due merely to the decrease in weight of the cigarettes since the acetone extract represented 6.0% of the wet weight (moisture content = 13.5%).

Reduction in smoke tars have not as yet been determined but the acetone extracted cigarettes gave a very changed and unpleasant smoke. Comparing this adverse change in smoking qualities with the change in the smoking qualities of n-hexane extracted tobacco, it will be remembered that while our smoking panel could detect a difference between unextracted tobacco and tobacco extracted with n-hexane, no preference was shown for either. It could be surmised that the majority of compounds effecting "taste of smoke" are not extracted by n-hexane.

This ties in with an experiment carried out a number of years ago in comparing aromatic properties of Canadian and Havana cigar tobacco. Upon charring these tobaccos in crucibles, the aroma of the smoke from the Havana tobacco could be readily detected even after an extraction with petroleum ether. In fact, little difference was found in the tobacco before and after the petroleum ether extraction. On additional extractions of the two tobaccos with ethyl alcohol, the aromatic properties of the Havana tobacco was lost and no difference could be detected between the Canadian and Havana tobaccos.

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INVESTIGATION OF TOBACCO EXTRACTS

Since evidence has been obtained (see Progress Report April 1 - May 31, 1959) that would indicate that part of the extract was changed during molecular distillation, a breakdown of the extract by chromatography without prior fraction in the molecular still is now underway. The possibility that a preliminary fractionation into acidic, basic and neutral fractions might give cleaner cuts on chromatography has also been considered and is presently being investigated. A separation of the extract into two fractions by extracting n-hexane solution with 80% alcohol and subsequent chromatography has also been carried out.

1. Chromatography of total extract (Extract No. 45)

Preliminary chromatography on a small column was carried out to determine the most suitable activity of adsorbent (silica gel) and percentage recovery from the column. An activity was arrived at that gave good separation of the paraffin fraction and a good recovery (70% of starting material or a loss of 10 mg.) A larger scale chromatography of 3.25 gms. has been carried out using n-hexane, benzene in n-hexane in increasing proportions, benzene, trichloroethylene and a 100% recovery has been achieved.

2. n-Hexane and 80% alcohol fractions

n-Hexane solution of extract was extracted with 80% ethyl alcohol and 2% alcoholic KOH as shown in Flow Sheet 1.

To determine to what degree of reproducibility this type of separation could be obtained, this extraction was repeated on another portion of the same extract. 50.2% remained in the n-hexane phase after extracting the n-hexane solution with 80% alcohol as compared to 53.6% in the first sample. The alcoholic KOH extraction was not carried out in the second instance.

Chromatography of IV (See flow sheet) gave very

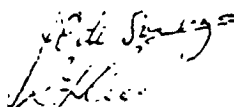
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promising results and a good recovery (93%) was achieved, using the same eluents as described in the chromatography of total extract. The n-hexane and benzene cuts appear to be cleaner than those obtained with total extract.

Chromatography of III was carried out on the same silica gel and with the same solvents. While a good recovery was obtained, it is believed that a different activity adsorbant would improve the separation.

Breakdown of extract into acidic, basic and neutral fractions prior to chromatography. -----

This separation has not been completed.



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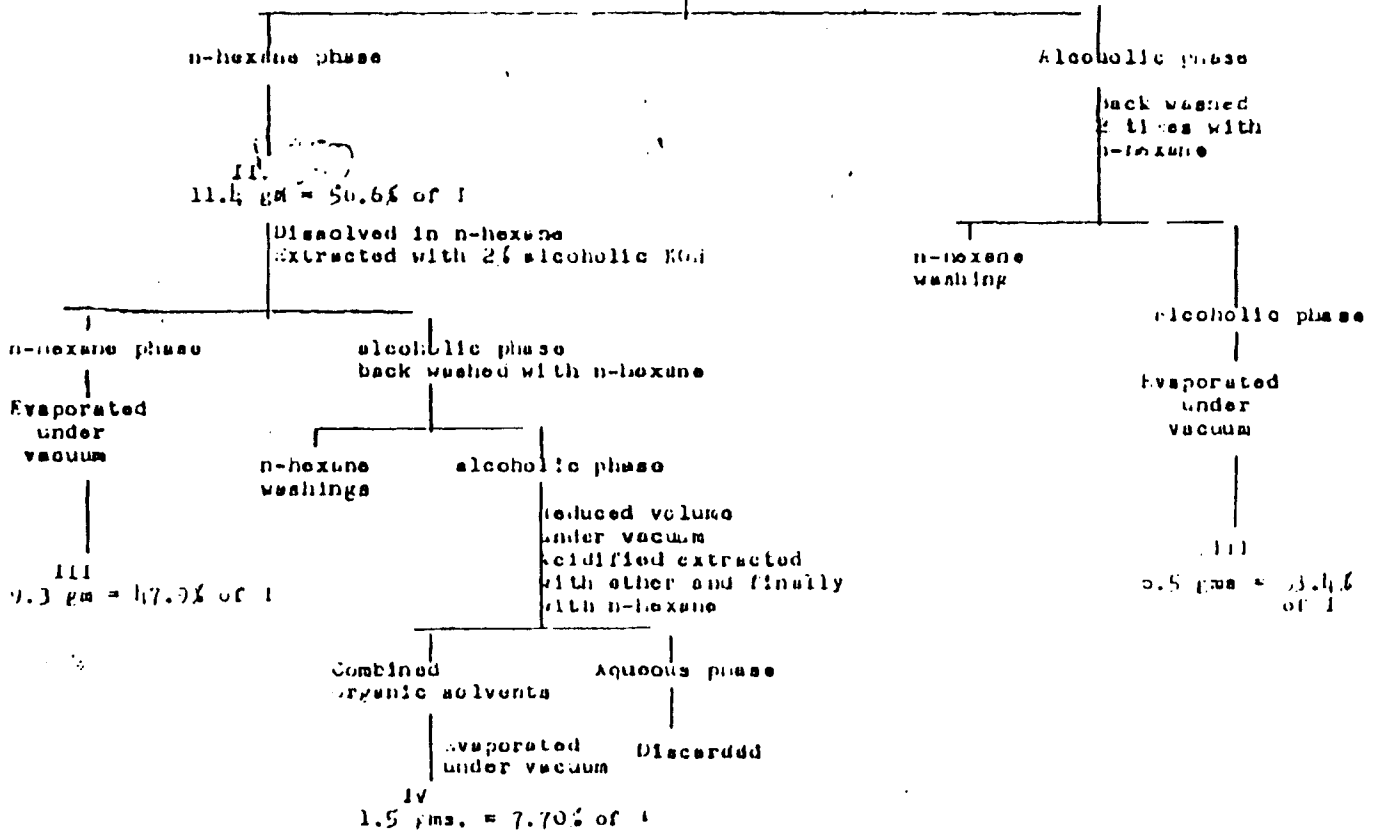
Table IV

Solvent	Temp. of Extraction	% Extracted	% Nicotine in Tobacco			Nicotine in Smoke (mg/cigt)			Total tar in smoke (mg/cigt)		
			before extraction	After extraction	% reduction	before Extraction	After extraction	% reduction	before extraction	After extraction	% reduction
n-hexane	Room Temp.	1.2	1.81	1.73	4.4	2.43	2.3	3.7	28.1	27.2	3.2
toluene*	80-85°C	3.3	1.95	1.86	46.6	2.43	1.4	39.6	28.9	21.6	25.6
"	Room Temp.	2.1	1.81	1.59	12.0	2.43	2.1	11.2	28.1	25.8	6.2
Acetone	50°C	6.0	--	--	--	--	--	--	--	--	--

* Hot toluene cigarettes had a high pressure drop.

1
extract no. 15

Dissolved in n-hexane
Extracted with 60 - 82% alcohol



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