sample requires 50 ml of the reagent to dissolve the matrix. A 50-gram nickel sample was analyzed by separately dissolving five 10-gram portions of the sample and then concentrating the residue on a single filter. Tartaric acid is added to the reagent to prevent hydrolysis of titanium, etc. when high purity superalloys are analyzed by this method. The method has been evaluated for a few alloys only to illustrate its future applicability to alloys which may have very low sulfur levels. There are no standard materials available to check the validity of the method at the 1-ppm level; however, the precision (σ value) is given for several materials, each was analyzed 20 times.

The time required for the dissolution of a sample in the Meineke Reagent depends upon the type of material and its surface area. Finely divided nickel or iron powders dissolve in a few hours, whereas nickel pellets 0.6 cm in diameter require about 20 hours. No attempt has been made to accelerate the dissolution step by applying heat or changing the ratio of copper chloride, hydrochloric acid, or potassium chloride in the reagent. Meineke (*6*) dissolved 5 grams of iron powder in 1 to 1.5 hours by gentle warming of the reagent. During the dissolution of finely powdered nickel, the temperature of the reagent increases a few degrees Centigrade and aluminum powder reacts violently.

A 10-ml buret is used for the titration. One full buret represents 200 μg of sulfur. The buret can be read to within ± 1 μg of sulfur. The accuracy of the photometric end point is ± 1.7 μg or 0.17 ppm for a 10-gram sample. Aliquots of a sodium thiosulfate solution were added to the titration vessel to ascertain precision of the photometric end point. The precision of the method is not limited by the titration. No attempt has been made to complete the determination with a weaker titrant or spectrophotometrically (*13*).

The proposed procedure is designed for the determination of 0.2 to 5 ppm sulfur using a sample weight of at least 10 grams. The sulfur residue obtained by dissolving nickel in the Meineke Reagent contains other insoluble materials. Carbon is present and it produces a gray to black deposit on the filter. The presence of sufficient particulate matter may be necessary to collect fine or colloidal particles containing the sulfur residue. The proposed procedure is not recommended, or required, for the analysis of materials with high sulfur levels wherein a sample weight of less than 0.5 gram would be required. Application of the Meineke separation for high levels of sulfur, using small sample weights, requires the addition of some sulfur-free asbestos or metal powder to ensure retention of the sulfur-containing precipitate. The filtration rate for a sample dissolved by 500 ml of the reagent is very rapid, but the rate decreases after a liter of the spent reagent has been passed through a single filter.

Table IV. Application of the Meineke Separation to the Determination of Sulfur in Various Materials by the Combustion–Titration Method

Material	Sample weight, g.	Sulfur, ppm, $\pm \sigma$
Nickel powder Type 123-A	25	0.6, 0.8
Nickel pellets	10	3.3 $\pm$ 0.6
Nickel powder Type 123-B	50	0.5
Nickel powder Type 123-C	25	2.6, 2.1
Nickel 270	10	1.7 $\pm$ 0.7
ORC[a] copper wire bar	10	1.7 $\pm$ 0.3
Iron, electrolytic–H_2 annealed	10	<0.2
Cobalt sponge	10	<0.2
Aluminum Super Pure (BCS 198d)	10	<0.2, <0.2
INCONEL alloy 600[a]	10	3.7, 3.6
Maraging steel	5	14, 14

[a] INCONEL and ORC are trademark products of the International Nickel Company, Inc.

Received for review December 14, 1973. Accepted February 28, 1974. Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 1974.

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Chemical Studies on Tobacco Smoke

Quantitative Analysis of Hydrazine in Tobacco and Cigarette Smoke

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Hydrazine (H) is trapped from tobacco smoke by reaction with pentafluorobenzaldehyde. The formation of decafluorobenzaldehyde azine (DFBA) prevents the loss of H by artificial reaction with other smoke constituents. DFBA is enriched by chromatography and subsequently determined by gas chromatography with FID and ECD (detection limit for H, 0.1 ng/cig.). From the mainstream and sidestream smoke of a popular U.S. nonfilter cigarette were isolated 31.5 and 94.2 ng of H, respectively. The tobacco of the cigarette contained 30 ng of H. The widely used sucker growth inhibitor maleic hydrazide contains trace impurities of H and with it contributes to H in tobacco and tobacco smoke. More detailed studies are needed for the identification of the major precursor of H in tobacco smoke. This communication reports the first identification of hydrazine in a nonoccupational respiratory environment.

Tobacco smoke contains a variety of toxic compounds (*1, 2*). Most of them are unaltered constituents of tobacco, or they are pyrosynthesized from tobacco. However, mate-

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(2) E. L. Wynder and D. Hoffmann, "Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis," Academic Press, New York, N.Y., 1967.

rials which may also contribute to the overall toxicity of tobacco smoke are agricultural chemicals, flavoring agents, and other additives. One of these, maleic hydrazide (MH), is widely used in the cultivation of tobacco (*3*). Recently, it was demonstrated that 4–10% of the MH transfers as such from tobacco into mainstream smoke (*4*). Although some MH will be found in the sidestream smoke, one can assume that most of MH will serve as precursor for a large number of decomposition products. Pyrolysis experiments with MH support this concept (*5–7*). Until now, hydrazine has not been found in tobacco smoke. Recently, it has been identified in a 650 °C pyrolysis product of MH although not in one obtained at 900 °C (*7*). Hydrazine induces adenomas and adenocarcinomas in the lungs of mice (*8*).

This study reports a quantitative method for the determination of hydrazine in tobacco and tobacco smoke. Pentafluorobenzaldehyde traps hydrazine under formation of decafluorobenzaldehyde azine (DFBA), preventing artifactual reaction of hydrazine with other smoke constituents. The DFBA is enriched by absorption chromatography and is subsequently determined by gas chromatography. Using a ^{3}H-electron capture detector, one can determine as little as 0.1 ng of hydrazine in the form of DFBA. The method was used for the determination of hydrazine in technical MH, tobacco, tobacco smoke, and pyrolysis products.

EXPERIMENTAL

Apparatus. A 20-channel automatic smoker RM 20/68 was used for the analysis of the mainstream smoke and a 20-port automated Phipps and Bird machine for sidestream smoke (*9, 10*). The apparatus for impregnating cigarettes with potassium nitrate solution, a syringe type applicator, was developed and made available by W. L. Maddox and M. R. Guerin from Oak Ridge National Laboratories, Oak Ridge, Tenn.

A Perkin-Elmer gas chromatograph Model 800 with dual-flame ionization detector and a 4:1 splitter was used for the isolation of unknowns, and a Varian Aerograph Model 1200 with an electron capture detector (tritiated titanium as β-source) for the quantitative analysis. Ultraviolet absorption spectra were obtained with a Cary Model 118 and infrared spectra with a Perkin-Elmer Model 21. The mass spectra were determined with a Hitachi/Perkin-Elmer RMU-6D instrument by the Morgan Schaffer Corp. (Montreal, Canada) at 70 eV or with a Hitachi/Perkin-Elmer RMU-6D GC-MS instrument (courtesy of K. Biemann, Mass Spectrometry Laboratory, MIT). The elemental analysis was performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. A horizontal vycor tube heated by a Lindberg heavy-duty furnace was used for the pyrolysis experiments (*11*). The laboratories were illuminated with yellow light (Sylvania Electric Tubes F-40 GO) which excludes radiation below 450 mμ.

Reagents. All organic solvents were spectrograde, the other chemicals of analytical reagent grade. Pentafluorobenzaldehyde from PCR Inc., Gainesville, Fla. was purified by column chromatography on silica gel, which was pre-washed with *n*-hexane. Silica gel powder was purchased from J.T. Baker Chemical Co. (mesh 80/100); precoated glass plates with fluorescent indicator (silica gel and aluminum oxide) from Brinkmann Instruments; 11% OV-17 plus QF-1 (mixed phase) on Gas Chrom Q (mesh 80/100) from Applied Science Laboratories; and 10% UC-W98, 80-100S, packed in a 3-mm × 1.8-m column, from Hewlett-Packard. Commercial MH, designated as MH-30, consists of the MH-diethanolamine salt in water (MH 30%). These samples were obtained from T.C. Tso, Plant Genetics and Germplasm Institute, USDA, Beltsville, Md.

Cigarettes. Cigarettes without filter tips (85 mm) were purchased on the open market in July 1972 for use in establishing the analytical method. The experimental cigarettes (85 mm without filters) were obtained from T.C. Tso, Plant Genetics and Germplasm Institute, USDA, Beltsville, Md., and stored before use in a cold room at 4 °C. The cigarettes were selected (±20 mg within the average weight of 200 cigarettes) and stored for 24 hours in a chamber at 60% rel. humidity (22°) before smoking.

Reference Compounds. Benzaldehyde azine was synthesized according to H. H. Hatt, mp 92 °C (lit. (*12*) mp 92–93 °C).

Decafluorobenzaldehyde azine: 1.37 g (7m*M*) of pentafluorobenzaldehyde was added to 0.18 g (85% in water; 3m*M*) of hydrazine hydrate. A yellow precipitate was formed immediately. After the mixture had been stirred overnight, the precipitated decafluorobenzaldehyde azine was filtered and washed with water. The product in quantitative yield was recrystallized from ethanol, as yellow needles melting at 137 °C (lit. (*13*) mp 138 °C); uv λ_{max} (cyclohexane) 294 nm (ϵ 46550); ir, (KBr) 1645, 1610 (aromatic ring stretch), and 966 cm^{-1} mass spectrum (70 eV) *m/e* (rel. intensity) 388 (52), 369 (45), 360 (10), 207 (39), 201 (34), 194 (88), 193 (39), 180 (83), 174 (84), 167 (53), 161 (44), 124 (66), 117 (99), 93 (43). *Anal.* Calcd for $C_{14}H_2F_{10}N_2$: C, 43.30; H, 0.52; F, 48.97; N, 7.22. Found: C, 42.53; H, 0.70; N, 7.35.

Pentafluorobenzaldehyde azine: To a solution of 208 mg (1m*M*) of benzalazine and 196 mg (1m*M*) of pentafluorobenzaldehyde in 50 ml methanol, 200 ml phosphate buffer (pH 5.2) was added (prepared by mixing 98 ml of solution A with 2 ml of solution B; solution A = $\frac{1}{15}$ molar KH_2PO_4 in methanol/water, 1:4; solution B = $\frac{1}{15}$ molar Na_2HPO_4 in methanol/water, 1:4). The mixture was stirred at room temperature overnight and then extracted with ether (4 × 50 ml); the dried ether extract (Na_2SO_4) was concentrated and purified on a preparative silica gel thin-layer plate and was developed with benzene/hexane (1:1), the middle band (R_f 0.28) was extracted with ether and evaporated to dryness. The residue was recrystallized twice from ethanol yielding yellow needles, mp 116 °C (lit. (*14*) mp 108 °C); uv λ_{max} (cyclohexane) 299 nm (ϵ 41350); ir (KBr) 1630, 1530, 1500 (ring stretch), 1160, 1140, 1036 (ring bending), 966, 767, and 698 cm^{-1} (monosubstituted aromatic ring); mass spectrum (70 eV) *m/e* (rel. intensity) 298 (56.7), 279 (42.3), 270 (10.0), 167 (7.5), 131 (44.3), 109 (43.8), 104 (43.3), 103 (11.4), 77 (100), 51 (44.8). *Anal.* Calcd for $C_{14}H_7N_2F_5$: C, 56.39; H, 2.37; N, 9.39; F, 31.85. Found: C, 56.26; H, 2.41; N, 9.27.

Gas Chromatography. The most satisfactory separation of DFBA from a concentrate of tobacco "tar" was obtained at 145 °C on a 3-mm × 1.8-m column, packed with 11% OV-17 plus QF-1 (mixed phase) on Gas Chrom Q (mesh 80/100). Nitrogen was used as carrier gas with a flow rate of 40 ml/min. The retention time for DFBA was 18 minutes. With a titanium tritide-ECD, the lower limit of detection of hydrazine as DFBA was 0.1 ng (Figure 1). The linear range extended from 0.1–3.0 ng. A 1:4 effluent splitter was installed in conjunction with FID to isolate DFBA from a concentrate of tobacco smoke. In this way, 80% of the effluent was collected in a glass capillary and subsequently purified by rechromatography on a 10% UC-W98,80-100S, 3-mm × 1.8-m column at 160 °C, helium flow rate 40 ml/min before spectral analysis.

For the separation of benzalazine, decafluorobenzaldehyde azine (DFBA) and pentafluorobenzaldehyde azine, we used the following glc system: 3-mm × 1.3-m column, packed with 11% OV-17 and QF-1 on Gas Chrom Q; FID; helium flow rate 40 ml/min; column temperature 180 °C; retention times: DFBA, 2.7 min; pentafluorobenzalazine, 4.4 min; and benzalazine, 9.0 min.

Procedure. *Isolation and Quantitative Analysis of Hydrazine from Cigarette Smoke (Mainstream or Sidestream).* Twenty cigarettes without filter tips and selected by weight were smoked under standard conditions as reported earlier (*15*). The main-

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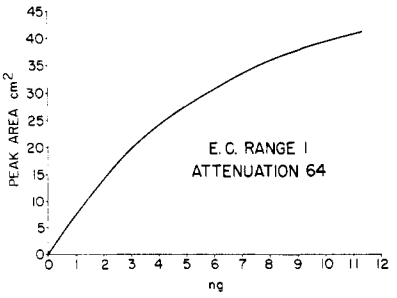

Figure 1. Linearity curve for decafluorobenzaldehyde azine

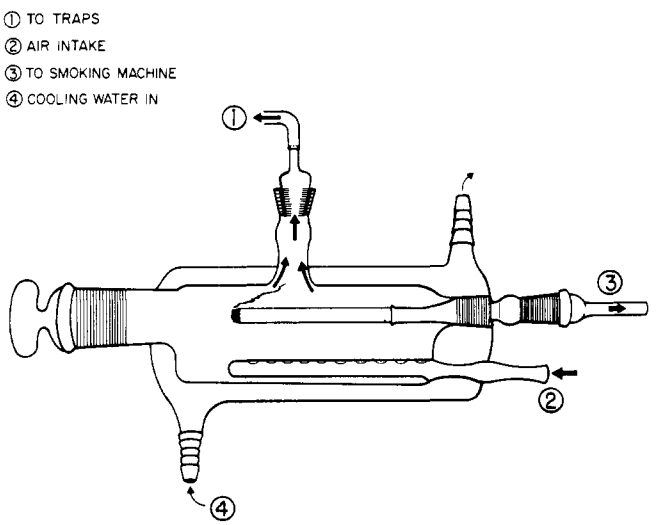

Figure 2. Smoking chamber for sidestream smoke (modified chamber of Neurath and Ehmke (*16*), manufactured by R. C. Ewald, Queens, N.Y.)

stream smoke was drawn through three traps each containing a solution of pentafluorobenzaldehyde in 10 ml methanol and 50 ml phosphate buffer at pH 5.2. The first trap contained 0.8 g of pentafluorobenzaldehyde, the second 0.2 g, and the third another 0.2 g. The first two traps were at ambient temperature, and the last trap was cooled in an ice water bath. The residue from the ether washings of the traps and filters was combined with the suspension of smoke condensate and kept overnight. This suspension was saturated with sodium chloride and extracted with ether (6 × 50 ml); the ether layer was extracted with 20% sodium bisulfite (4 × 50 ml) to remove excess pentafluorobenzaldehyde. The sodium bisulfite solution was saturated with sodium chloride and back extracted with ether (2 × 50 ml). The combined ether solutions were dried (Na_2SO_4), concentrated, and chromatographed consecutively on two silica gel plates (2 mm) with benzene as developing solvent. The band corresponding to the reference compound was scraped off the two plates, extracted with ether, and purified further by chromatography on one aluminum oxide tlc plate (1 mm) with hexane/benzene 1:1 as developing solvent. The band corresponding to DFBA was again scraped off the plate and extracted with ether. DFBA was then determined by injection of an aliquot into a gas chromatograph equipped with an ECD and by measurement of the corresponding peak area. The recovery rate was determined by adding 1 mg of hydrazine sulfate to the first trap in the above procedure, smoking the mainstream smoke through three traps, and analyzing for hydrazine. The sidestream smoke, collected in the three traps by passing air through a specially designed smoking chamber at a rate of 25 ml/sec. (Figure 2), was analyzed as described above for mainstream smoke.

From Tobacco. Tobacco from 20 cigarettes was ground in a blender and mixed with 1.2 g pentafluorobenzaldehyde in 300 ml phosphate buffer and 50 ml methanol. This mixture was stirred overnight, filtered and the filtrate analyzed for hydrazine in the same way as described above.

From Commercial MH-30. Five ml of an MH-30 sample was dissolved in 50 ml phosphate buffer and adjusted to pH 5 by adding hydrochloric acid; 125 mg of pentafluorobenzaldehyde was added to this mixture. After standing overnight, the mixture was saturated with sodium chloride, was extracted with ether (4 × 50 ml). The dried ether solution (Na_2SO_4) was concentrated to 3-5 ml and an aliquot injected into a gas chromatograph equipped with a flame ionization detector. The peak corresponding to DFBA was then used to determine hydrazine.

Pyrolysis Experiments. These were conducted in a vycor tube at 870 or 400 °C, respectively, while the vycor tube was continuously flushed with nitrogen at a flow rate of 30 ml/min (*11*). The volatile pyrolysis products were collected in traps containing pentafluorobenzaldehyde and analyzed for hydrazine as described above.

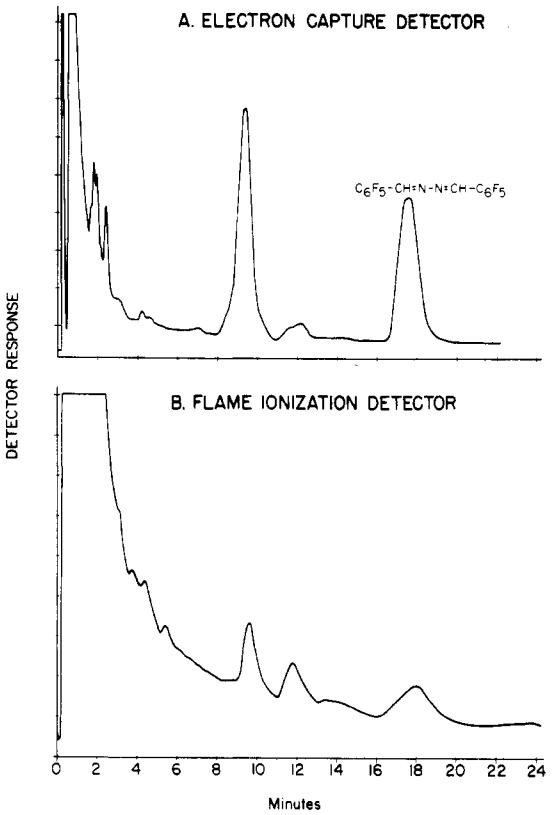

Figure 3. Gas chromatograms of a concentrate of decafluorobenzaldehyde azine

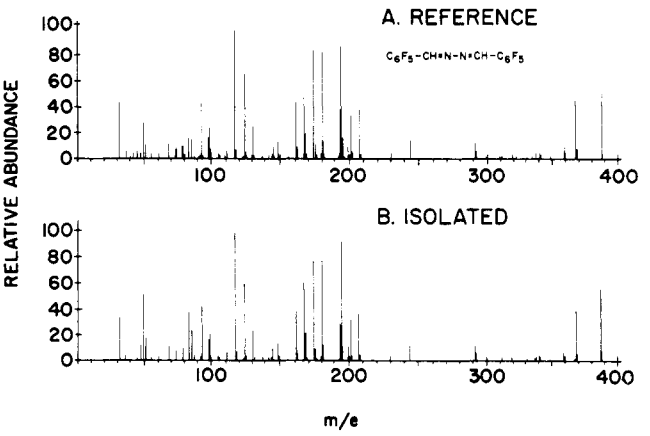

Figure 4. Mass spectra of decafluorobenzaldehyde azine

RESULTS AND DISCUSSION

Gas chromatograms obtained from instruments equipped with FID and ECD are shown in Figure 3. The traces represent a concentrate of cigarette smoke condensate which has been reacted with pentafluorobenzal-

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(16) G. Neurath and H. Ehmke, *Beitr. Tabakforsch.*, **2**, 117 (1964).

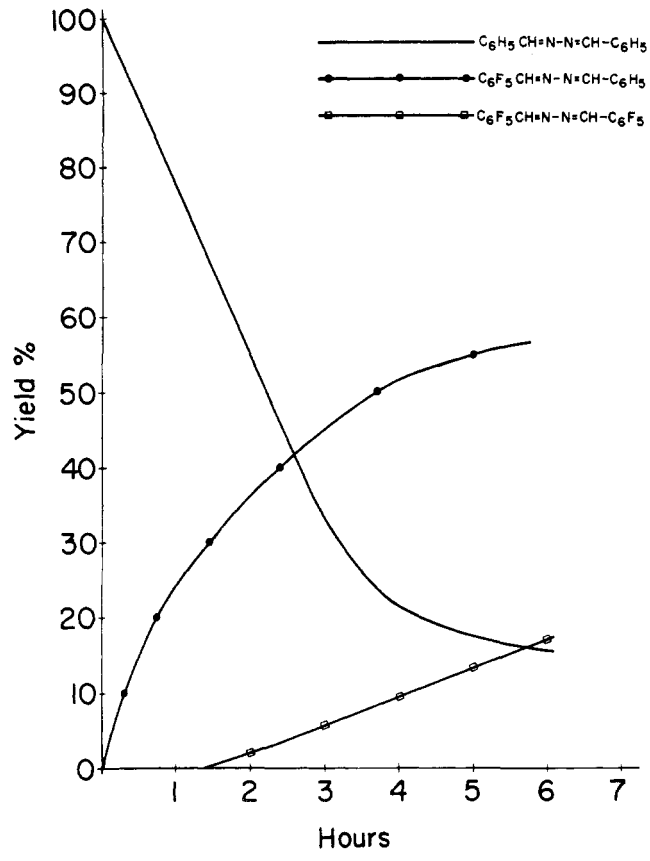

Figure 5. Exchange reaction between benzaldehyde azine and pentafluorobenzaldehyde (molar ratio 1:20, pH 5.2) to pentafluorobenzaldehyde azine and decafluorobenzaldehyde azine

dehyde, and show a peak corresponding to DFBA. The effluent with the retention time of DFBA was collected and identified by UV and mass spectra (Figure 4).

In order to trap hydrazine quantitatively from tobacco smoke before it can react with aldehydes, ketones, and other reactive smoke constituents, we had to add an excess of the highly reactive pentafluorobenzaldehyde to the traps. The amounts isolated from the mainstream and sidestream smoke of one 85-mm U.S. blended cigarette without filtertip were 31.5 ng and 94.2 ng, respectively (Table I). The recovery rate varied between 81 and 87%.

The isolation of hydrazine from tobacco smoke posed several questions. First, why had earlier studies and pyrolysis experiments failed to yield the same results (5–7)? In our own preliminary investigations we used—as other chemists also had—conventional methods of trapping and isolating hydrazine, and results were negative. Even trial runs with known quantities of added hydrazine in the traps led only to low yields and irreproducible data. Similarly we were unable to apply successfully to tobacco and tobacco smoke the method of Dee because of the instability and low sensitivity of the formed pyrazoles (*17*).

Therefore, a novel approach had to be worked out and was found in the method of using a highly reactive trapping agent that prevented the interreaction of hydrazine with other freshly generated smoke constituents in the traps. The described technique afforded reproducible data for hydrazine from smoke and is presently being applied for the analysis of other environmental agents.

There could yet be a drawback in employing this technique, in that one must consider the possibility of exchange reactions between pentafluorobenzaldehyde and hydrazones and azines of aldehydes and ketones which had already formed in the smoke just prior to trapping. In order to answer this question we added benzalazine to pentafluorobenzaldehyde solution (1:20) in phosphate buffer and we took aliquots for analysis from time to time (Figure 5). We found that pentafluorobenzaldehyde azine and DFBA had been formed in exchange reactions and that, after 16 hours, more than 70% of the hydrazine moiety of benzalazine was recovered in DFBA. We concluded that the hydrazine isolated by us could have derived from the free hydrazine base, or from hydrazones and azines. Most of the hydrazones and azines of the major tobacco smoke aldehydes and ketones, however, have aliphatic moieties and are unstable. Therefore, it matters little in respect to the tumorigenicity of tobacco smoke whether the isolated hydrazine derives from the free base, or hydrazones, or azines.

Table I. Analysis of Hydrazine in U.S. Blended Cigarette

	Hydrazine isolated, ng/cig.	
Number of analysis	Mainstream smoke	Sidestream smoke
1	34.97	94.2
2	28.60	
3	33.70	
4	28.79	
Average	31.50	
St dev	3.30	
Dev coeff	10.5%	

Table II. Hydrazine in Commercial MH-30

Marketing year	Wt. analyzed	Hydrazine isolated mg	ppm
1965	6.01	0.18	100
1966	5.71	1.49	870
1969[a]	1.12	1.8×10^{-4}	0.53
1970[a]	5.51	2.29×10^{-4}	0.14
1972	6.00	0.13	73
1973	5.92	8.9×10^{-3}	5.0

[a] Determined with ECD, others with FID.

Our interest in hydrazine as tobacco smoke constituent was prompted by the theoretical possibility that the sucker growth inhibitor maleic hydrazine (MH) could give rise to hydrazine in the smoke (*4*). This could be so for two reasons—namely, because MH as applied in field treatment already contains hydrazine as impurity, and also because the reducing atmosphere of the burning cone of a cigarette favors the formation of hydrazine from MH. Table II demonstrates that, in fact, samples of MH-30 as used in cultivation of tobacco between 1965 and 1973 contain hydrazine levels of 0.16–260 μg per gram of MH.

In the tobacco of a standard cigarette, we found 30 ng of hydrazine and 29.4 μg of MH (*4*). Based on these data, the original MH used for field treatment had to contain more than 1000 ppm of hydrazine as impurity, a value similar to that found for the MH-30 sample from 1966 (Table II). Furthermore, one may hypothesize that MH gives rise to hydrazine within the treated tobacco plant, or during curing: however, data from MH-treated and hand-suckered tobacco do not support such a concept (Table III).

Finally, we attempted to determine some of the precursors for hydrazine in tobacco smoke. From Table III, we can assume that hydrazine in the smoke does not primarily derive from MH or hydrazine in tobacco. We noted,

(17) L. A. Dee, *Anal. Chem.*, **43**, 1416 (1971).

Table III. Maleic Hydrazide and Hydrazine in Tobacco and Tobacco Smoke

Tobacco type	MH, μg/cig.[a]		Hydrazine isolated, ng/cig.	
	Tobacco	Mainstream smoke	Tobacco	Mainstream smoke
MH treated Burley tobacco cigarettes (1970)	17.6	1.82	51.2	41.5
Hand-suckered Burley tobacco cigarettes (1970)	<0.1	<0.1	22.2	42.8
MH treated flue-cured tobacco cigarettes (1970)	25.2	1.76	12.1	23.5
Hand-suckered flue-cured tobacco cigarettes (1970)	<0.1	<0.1	13.8	33.8
Commercial cigarettes (1972)	29.4	1.16	30.0	31.5

[a] Values from Liu and Hoffman, 1973 (*4*).

Table IV. Pyrolysis Experiments[a]

Sample	Wt. pyrolyzed g (m*M*)	Pentafluoro-benzaldehyde g(m*M*) (in traps)	Hydrazine		
			Theory, mg	Isolated	
				μg	pm
Maleic Hydrazide (MH)	2.00 (17.9)	4 (20.4)	571.4	0.84	1.5
MH[b]	2.06 (18.4)	4 (20.4)	588.6	37.1	63.0
Glycine	2.04 (27.2)	5 (25.5)	435.0	2.2	5.10
Diglycyl-glycine	0.80 (4.2)	5 (25.5)	203.0	1.5	7.2
Urea	1.74 (29.0)	5 (25.5)	928.0	1.1	1.25

[a] Conducted at 870°C, under N_2, unless otherwise specified. [b] 400°C, under N_2.

however, that pyrolysis of MH at 400 °C (*vs.* 879 °C) resulted in a 40-fold increase in hydrazine yield. This is somewhat similar to the results obtained by Harke *et al.* (*7*), who were able to isolate hydrazine from pyrolysis of MH at 650 °C, although they were unable with their method to find any at a higher temperature of pyrolysis (*i.e.*, 900 °C). Pyrolysis experiments at 870 °C suggest that amino acids as well as proteins can give rise to some hydrazine (Table IV).

The ammonia delivery in tobacco smoke can be elevated by addition of nitrate to tobacco (*18*). Ammonia qualifies as a possible precursor for the formation of hydrazine (*19*). These considerations led to our investigation of smoke from potassium nitrate treated cigarettes. The results of the analyses for hydrazine in mainstream and sidestream smoke are summarized in Table V. It appears from these data that alkali nitrate may contribute to a small extent to the formation of hydrazine in the smoke, similar to contributions by MH and proteins. The hydrazine in tobacco appears to be a more likely precursor for hydrazine in smoke, although this study did not reveal such a correlation. Another group of precursors for hydrazine are *N*-nitrosamines, which by reduction give rise to hydrazines. It is planned to study this question and that of other precursors with ^{15}N-labeled tracing compounds.

Table V. Analysis of Hydrazine in Nitrated Cigarettes

	Nitrate (NO_3) mg/cig.[a]	Hydrazine isolated, ng/cig.	
		Mainstream smoke	Side-stream smoke
U.S. blended cigarette	10.4	31.5	94.2
Treated cigarette	26.4	56.7	58.0
	57.8	35.1	46.2

[a] Determined according to the method of Lipp and Dölberg (*20*).

Received for review December 28, 1973. Accepted February 19, 1974. This is Part XXVII in the Series "Chemical Studies on Tobacco Smoke." Parts of this study were presented at the 27th Tobacco Chemists Research Conference in Winston-Salem, N.C., October, 1973. It was supported in part by American Cancer Society Grant BC-56R and National Cancer Institute Contract NO1-CP-33305.

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(20) G. Lipp and U. Dölberg, *Beitr. Tabakforsch.*, **2**, 345 (1964).