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Determination of Volatile N-Nitrosamines in the Vapour Phase of the Smoke from Various Tobacco Products*

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1. INTRODUCTION

Most N-nitrosamines are potent carcinogens for various animal species and presumably also for man. According to *Druckrey* and *Preußmann* (1) their presence may be expected wherever nitrosatable amines come into contact with N_2O_3 vapours under appropriate conditions. They anticipated that such conditions were to be found in tobacco smoke, and also in the human body when nitrogen oxides are inhaled. Later it was proved or suggested that the conditions for nitrosamine formation were also fulfilled in feedstuffs and food products treated or prepared with nitrite (herring meal, cured meat products, some fish specialities) or nitrate (some cheese varieties), in flour dried with exhaust gases and in the human stomach from ingested nitrite and nitrosatable amines. Also Maillard reaction products, some alcoholic beverages, mushrooms, spinach and other products came under suspicion. These findings caused widespread alarm. However, there followed a period of uncertainty about the results obtained till then, as it became apparent that some of the methods used for the identification of the nitrosamines were questionable, mostly because of insufficient specificity. Thorough reviews on all these matters have been given by *Sebranek* and *Cassens* (2), *Eisenbrand* (3) and *Schuller* (in Dutch) (4) and need not be repeated here. The yearly number of papers on the analysis and formation of volatile nitrosamines has steadily increased since the beginning of the seventies and appears to have reached a peak in 1973, which presumably will not be exceeded in 1974. Only a small fraction of these papers deal with tobacco smoke:

Year	1954	55	62	63	64	65	66	67	68	69	70	71	72	73	74
Number of papers	1	1	1	4	5	2	4	18	7	6	23	49	54	84	25
the following of which are on tobacco smoke	1	2	2	1	2	4	1	1	1	2	7	3	2		

To date, i.e. November 1974, the authors are aware of 284 papers, 29 of which are on tobacco smoke.** Articles on non-volatile nitroso compounds and on carcinogenesis aspects are not included. The direct assessment with gas-liquid chromatography (GLC) in combination with mass spectrometry (MS) and nitrogen-specific detection, without formation of derivatives, seems to have become the method preferred for the determination of volatile nitrosamines (2 – 4). However, many characteristic and useful derivatives have been prepared: Oxidation to nitramines, reduction to hydrazines, photolysis or acidolysis to amines and subsequent formation of coloured, UV absorbing or electron capturing compounds (2 – 4).

In the determination of nitrosamines in tobacco smoke one has to deal with an additional difficulty which is not prominent in the analysis of food products, viz the possibility of artifact formation during the collection and storage of smoke condensates. This has been demonstrated in an impressive study by *Neurath* et al. (5). This difficulty probably explains the relatively low number of subsequent papers on the subject. Some of the observations by *Neurath* et al., however, are not easily comprehensible – as the authors also pointed out themselves – for instance the almost exclusive formation of methylbutylnitrosamine as an artifact and the fact that it is formed from the vapour phase alone and is not present in condensates collected on a cotton wool filter or in a cold trap. It is regrettable that these details from the experiments have never been checked and corroborated by others. To prevent artifact formation, smoke is now mostly trapped in a NaOH solution.

Recent determinations of nitrosamines in tobacco smoke have shown amounts of up to 3400 ng dimethylnitrosamine in the smoke of one cigarette (6). Earlier estimates for nitrosopiperidine were 1000–1500 ng per cigarette (7). We investigated the problem from a different starting point, taking into consideration that, if nitrosamines are really present in such large amounts in tobacco smoke, at least part of them must inevitably show up in the vapour phase of the smoke. We analyzed the vapour phase by GLC-MS and by GLC with nitrogen-specific detection after accumulation of large volumes of it on

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** At the time of proof-reading (June 1975) the total numbers of papers for 1972, 1973 and 1974 have become 50, 99 and 55 respectively. The number of papers on nitrosamines in tobacco smoke in those years remains as given.

cooled GLC columns. Nitrosatable amines are mostly deposited in protonated form on the Cambridge filter and, as far as they are present in the vapour phase, are not in contact with a nitrosating mixture of nitrogen oxides very long since at least one of these, the extremely volatile nitric oxide, is swept away with the carrier gas immediately. Likewise, the oxygen required for oxidation of NO to NO_2 is carried away immediately.
The products investigated were commercial cigarettes, hand-made cigarettes, the 1R1 reference cigarette and cigars.

2. EXPERIMENTAL

Tobacco Products

The commercial cigarettes, smoking tobaccos and cigars were purchased in local stores. The 1R1 reference cigarettes were kindly made available by the Tobacco and Health Research Institute of the University of Kentucky, U.S.A. Before smoking, the products were conditioned over saturated salt solutions as follows:

Manufactured cigarettes
NH_4NO_3, relative humidity about 60 %,

Hand-made cigarettes
NaCl, relative humidity about 75 %,

Cigars $Ca(NO_3)_2$, relative humidity about 50 %.

Smoking tobaccos for roll-your-own cigarettes are packed and smoked at a higher humidity than commercial cigarettes. When taken from the package they rapidly lose moisture over a saturated NH_4NO_3 solution and attract moisture over a saturated KCl solution (relative humidity about 85 %). Over NaCl solution the moisture content is stable. Cigars taken from their package retain their weight over a saturated $Ca(NO_3)_2$ solution, whereas NH_4NO_3 solution induces a too high moisture content.
Roll-your-own cigarettes were made by a group of laboratory personnel who habitually smoke such cigarettes; they were selected from a larger group from which those who produced extremely heavy-weight or light-weight cigarettes were excluded.
All cigarettes were plain and 70 mm long, except the 1R1 reference cigarettes, which were 85 mm long.

Smoking and Sampling Procedure

The cigarettes and cigars were smoked on a Borgwaldt RM 30/65 smoking machine according to the international standard conditions for cigarettes: puffs of 35 ml in two seconds, once a minute, until a butt of 23 mm was left with the cigarettes and a butt of 30 mm with the cigars. Thirty cigarettes at a time can be smoked on the smoking machine. The cigarettes (for the sake of brevity only cigarettes will be mentioned hereafter) are placed before the central suction orifice one after the other by the quasi-continuous rotation of the manifold cigarette holder and a stream of puffs is generated in the main smoke duct. From this duct the vapour phase sample is slowly taken with a manually operated graduated syringe through a rubber cap in the duct behind the central Cambridge filter. A 50 ml Hamilton syringe with poly tetrafluoro ethylene plunger is used. A preliminary version of this sampling method has been applied in earlier work on sulphur compounds in cigarette smoke (8). The method will be discussed in more detail elsewhere. The sample collected is the average puff from the cigarettes according to the concept of *Norman* et al. (9) and of *Millar* et al. (10). The cigarettes are not lit in one rotation of the machine, but with intervals of one minute, i.e. one rotation. Cigars are lit with intervals of three minutes in view of the large number of puffs required, so that every third puff contributes to the average puff sample. Some cigarettes or cigars precede the series for equilibration of the syringe.
There is a bypass route parallel to the main smoke duct to divert all the smoke from puffs generated before the average puff, in order to provide a fresh Cambridge filter for the average puff. This ensures that even with cigars the filter does not become overloaded. Aging of smoke as such does not occur, as the first and last puffs of the cigarettes or the cigars are generated during one and the same rotation of the machine. Besides, the vapour phase is separated from the particulate phase immediately.
Samples of 2.5 ml were withdrawn from every passing puff. If the cigarette delivered fewer than ten puffs, the number of cigarettes put on to the machine was twice its number of puffs, so that twice the number of cigarettes could contribute to the average puff. If the number of puffs was above ten, only one series was put on to the machine. The number of cigars smoked was one third of their number of puffs. The smoking machine used here was not designed for cigar smoking and the distance between the cigars was rather short. This may have had some influence on their burning behaviour. In Table 1 a characterization is given of the products investigated and of the vapour samples drawn for analysis. For the GLC-AFID determinations (gas-liquid chromatography with alkali flame ionization detection), six separate analyses were carried out per product. As blank determinations, 35 ml of room air and of vapour phase from unlit cigarettes were analyzed.
For the GLC-MS determinations (gas-liquid chromatography in combination with mass spectrometry), similar samples were analyzed, now of cigarettes 2–1, 2–4, 2–6, 1R1 and cigars S III only. Still larger volumes of vapour samples were injected in an additional GLC-MS determination, viz 2×40 ml from cigarettes 2–1, 2×35 ml from cigarettes 2–4 and 5×35 ml (175 ml!) from cigarettes 2–6. For this last experiment the smoking and sampling procedure was carried out five times in succession, which took more than an hour. As will be discussed later, the GLC separation was not influenced by this long column loading time.
Withdrawal of larger volumes per puff than 2.5 ml would have disturbed the smoking parameters too much.

Table 1. Characterization of the products investigated and of the samples taken.

Products	Code	Type	Number of standard puffs	Sample taken (ml)	Fraction of vapour phase of whole cigarette/ cigar
Commercial cigarette	2—1	Bright Virginia	8	16 × 2.5 = 40	1/7*
Commercial cigarette	2—4	Dark	7	14 × 2.5 = 35	1/7*
Commercial cigarette	2—6	American blend	7	14 × 2.5 = 35	1/7*
Commercial cigarette	2—7	Oriental	8	16 × 2.5 = 40	1/7
Reference cigarette	1R1	American blend	11	11 × 2.5 = 27.5	1/14
Hand-made cigarette	Sh 1	"Medium strength", blend	12	12 × 2.5 = 30	1/14
Hand-made cigarette	Sh 11	"Full strength", dark	11	11 × 2.5 = 27.5	1/14
Medium size cigar	S I	102 × 12 mm	24	8 × 2.5 = 20	1/42
Cigar	S III	109 × 19/10 mm	39	13 × 2.5 = 32.5	1/42

* For GLC-MS even larger volumes were accumulated on the GLC column, so that 2/7 (cigarettes 2—1 and 2—4) or even 5/7 (cigarettes 2—6) of the whole vapour phase of the smoke from one cigarette came under investigation.

Gas-Liquid Chromatography — Alkali Flame Ionization Detection (GLC-AFID)

The column was a 150 m × 0.75 mm i. d. stainless steel wide-bore capillary, coated with Ucon 50 HB 280X. It was fitted without inlet splitter into a Hewlett-Packard 5750G gas chromatograph with nitrogen-specific alkali flame ionization detector. Carrier gas: helium 10 ml/min. Flame gases: hydrogen 30 ml/min, auxiliary helium 90 ml/min, air 175 ml/min. Flame temperature: 365° C. Temperature of the injection port: 140° C. The RbBr crystal was not used in its maximum ionization current position, but in a lower one, closer to the burner tip, where the ionization current is two thirds of its maximum. In this position the selectivity and sensitivity of the detector seemed optimal under the experimental conditions used. The detector alternates periods of good stability with periods of anomalous and erratic behaviour. One of the error sources is the intense corrosion of the stainless-steel burner jet caused by the hot RbBr or HBr vapours, which may be aggravated by halogenated solvents. The jets have to be replaced after three months of continual use. The cylindrical collector electrode which contains the RbBr crystal is made of platinum and is resistant to attack.

Gas-Liquid Chromatography — Mass Spectrometry (GLC-MS)

A column of the same kind as the previous one was fitted in a Perkin-Elmer 881 gas chromatograph. The carrier gas flow and the temperature of the injection port were the same as before, and again there was no inlet splitter. The column was coupled to an Atlas CH_4 low resolution mass spectrometer through a silicone rubber membrane separator. Electron energy was 70 eV. Spectra were recorded on magnetic tape approximately every seven seconds, starting after the elution of toluene. The first part of the chromatograms was not admitted into the ion source; the bulk of the air injected plus water and the most volatile vapour phase constituents were vented. The air and water would only have deteriorated the mass spectrometer vacuum and the most volatile compounds were of no importance for the present study. Tapes were searched for nitrosamines with a Varian SS 100 Data System by use of mass spectrometry. Dedicated mass plots could initially be made for one mass at a time, and for eight masses simultaneously at the time of the final evaluation of the results. The masses scanned were generally the molecular mass, the mass fragments 42 and 30 and other characteristic masses e.g. 56/57 or 73/74. Whenever the presence of a nitrosamine was suspected, full mass spectra were printed and spectra were refined by a background subtraction routine. The dedicated mass plots are a guide to deciding which background spectrum is to be subtracted for an optimal result. For the present study the tapes were screened for nitrosamines only, but of course they will be studied further.

Vapour Sample Injection Procedure

The large vapour samples were injected directly on to the column at a slow rate. About 20 cm of the column near the entrance was cooled in dry ice. In the meantime the column oven was at room temperature. Five minutes after the injection the dry ice coolant was removed, the oven closed and brought to 80° C. After twenty minutes the oven temperature was programmed linearly up to 150° C at 1°/min. For the GLC-MS determinations the oven was brought to 40° C after removal of the coolant, then programmed after twenty minutes at 0.5°/min up to 125° C or at 1°/min up to 150° C. No heating was needed for transfer of the sample from a pre-column to the analytical column, as the vapours were accumulated directly on the main column.

Reference Nitrosamines

For the determination of retention times by co-chromatography in both the GLC-AFID and the GLC-MS analysis systems, a 100 ppm nitrosamine standard

Figure 1. Chromatogram (first part) of 0.35 ml vapour phase of the average puff from the American blend cigarette 2–6. 150 m × 0.75 mm i. d. Ucon 50 HB 280X column. Nitrogen detection. Retention times of known smoke constituents determined by co-chromatography.

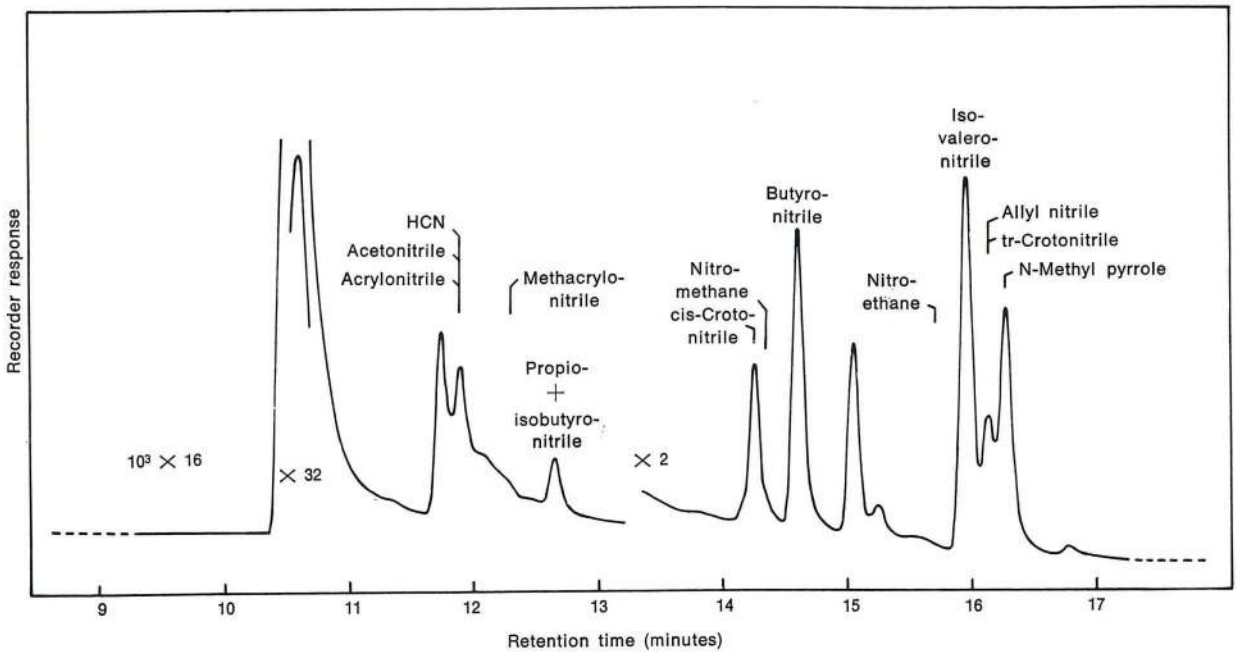
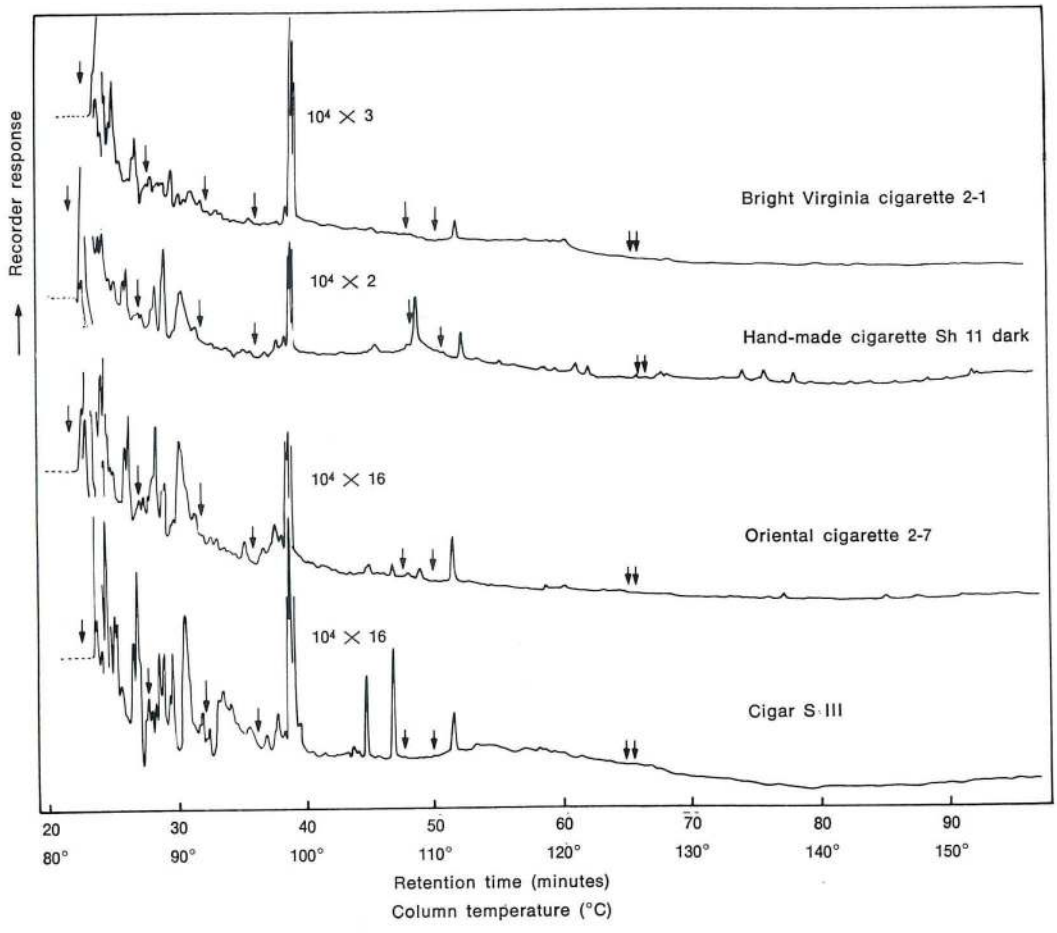


Figure 3. Chromatograms of the vapour phase of the average puff from various tobacco products. 150 m × 0.75 mm i. d. Ucon 50 HB 280X column. On-column trapping of 27.5–40 ml samples. Nitrogen detection. Arrows indicate retention times of DMNA, MENA, DENA, MPNA, MBNA, DPNA, PipNA and PyrrNA successively.



solution was injected on to the cooled column immediately after the introduction of a smoke vapour sample. The column temperature was then programmed as described. For the determination of detection limits and the preparation of reference mass spectra the standard solutions were injected separately. The nitrosamines, dissolved in dichloromethane, were stored at room temperature in Jena Duran glass volumetric flasks with protection against direct illumination. The solutions were stable for months, contrary to what is sometimes thought. The nitrosamine search was restricted to dimethyl- [DMNA], methylethyl- [MENA], diethyl- [DENA], methyl-n-propyl- [MPNA], methyl-n-butyl- [MBNA], and di-n-propylnitrosamine [DPNA], and the heterocyclic nitrosamines nitroso-piperidine [PipNA] and -pyrrolidine [PyrrNA], but isomers of the aliphatic series and other volatile nitrosamines would certainly not have passed unnoticed in the MS runs.

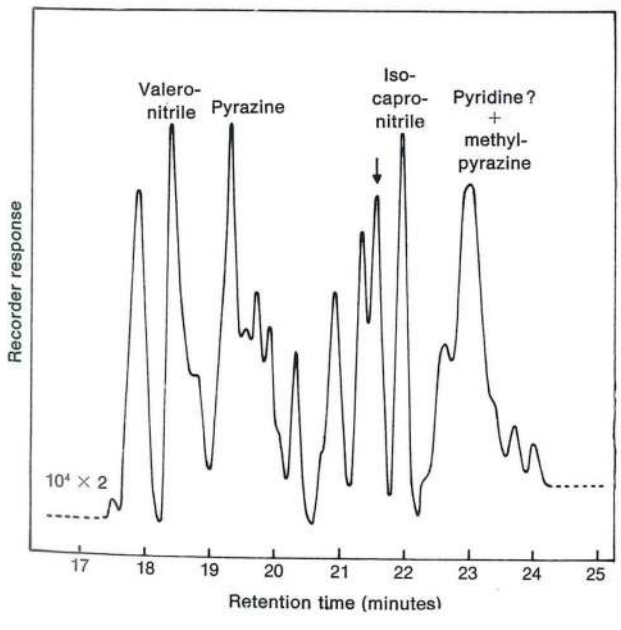
Caution: N-nitrosamines are potent carcinogens for various animal species and most probably also for man. The lowest members of the series are rather volatile.

3. RESULTS AND DISCUSSION

Gas-Liquid Chromatography — Alkali Flame Ionization Detection (GLC-AFID)

Six analyses were carried out for each of the nine tobacco products. Chromatograms representing the average result for each product were selected. The chromatograms, though far less complex than with normal flame ionization detection, still contained a considerable number of peaks. Since the sensitivity of the detector for non-nitrogen compounds is quenched to one thousandth or less of the sensitivity for nitrogen compounds — detection limits being of the order of one microgram and one nanogram respectively — non-nitrogen peaks will be rare.

Figure 2. Chromatogram (DMNA area only) of 35 ml vapour phase of the average puff from the American blend cigarette 2–6. 150 m × 0.75 mm i. d. Ucon 50 HB 280X column. On-column trapping. Nitrogen detection. Arrow indicates retention time of DMNA.



The first part of the chromatograms contains many large and close peaks, mainly nitriles, some nitroalkanes and N-methyl pyrrole, but no nitrosamines. This area can easily be studied from much smaller injection volumes. Figure 1 shows the first part of the chromatogram for the blend cigarette 2–6 (0.35 ml injected). The compounds indicated are known smoke constituents; here they have been localized by co-chromatography. The detector does not respond to NH_3 and NO_x. It is conceivable that the lower aliphatic hydrocarbons and some aldehydes give a response in this area, since they are known to be present in large quantities in the vapour phase of smoke, but this was not further studied.

The next part of the chromatogram should contain DMNA, if present; it is shown in Figure 2. This time 35 ml of vapour phase from blend cigarette 2–6 was injected.

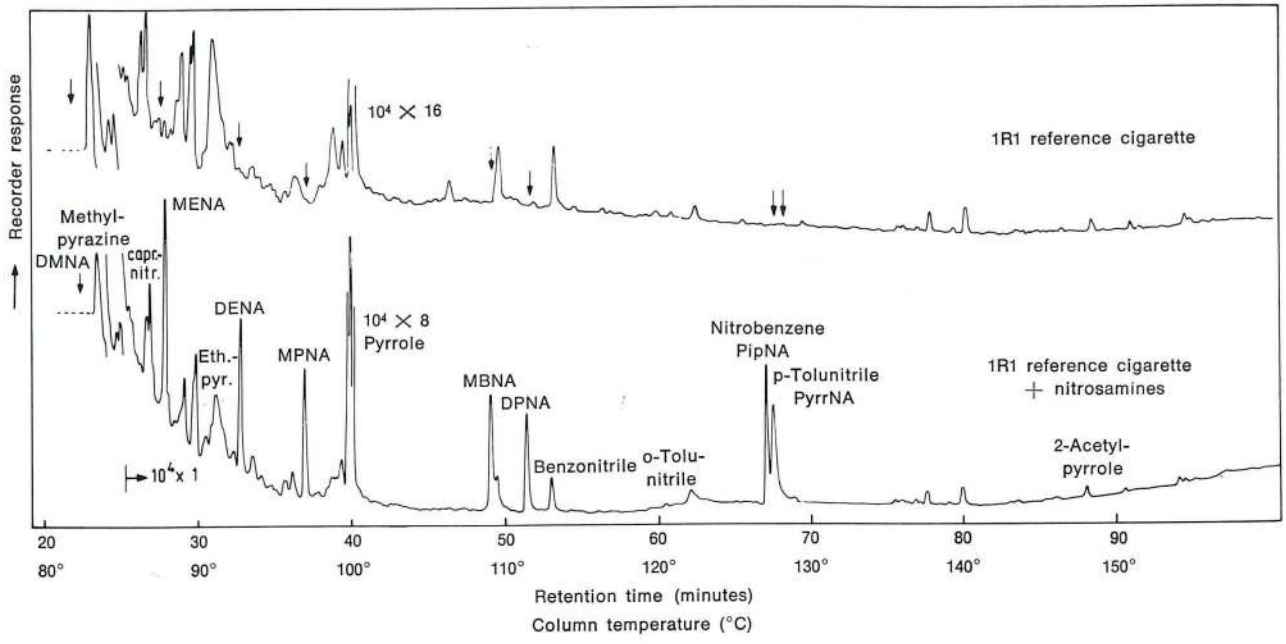
Figure 3 shows the chromatograms from MENA upwards, as obtained for four of the eight commercial products. For space reasons and in view of the impossibility of further reduction in size, the other chromatograms have been omitted. The chromatograms of the blend cigarette 2–6 and the "medium strength" smoking tobacco Sh 1 were very similar to the chromatogram of the Virginia cigarette 2–1, shown in the figure. The chromatogram of the dark cigarette strongly resembled that of the dark "full strength" smoking tobacco, whereas the chromatogram of the medium size cigars was very much like that of the large cigars.

The chromatogram of the 1R1 reference cigarette and the elution behaviour of eight nitrosamines as determined by co-chromatography are shown in Figure 4. Some additional nitrogen compounds are indicated as well.

It is surprising to note the overall similarity of the nitrogen chromatograms for such diverging tobacco products as shown in Figures 3 and 4. Only few qualitative differences are present and even the quantitative differences, after conversion to comparable injection volumes, are relatively small.

As to the localization of the nitrosamines in the chromatograms, it should be emphasized that, despite the use of an element-specific detector and a high-resolution capillary column, other nitrogen compounds may elute at the retention times of nitrosamines and thus may erroneously be taken for nitrosamines. Under the conditions applied in this study and on the polypropylene glycol type stationary phase used, DMNA is eluted in the overlap region of the higher aliphatic nitriles and the lower pyrazines and pyridines, viz in the close vicinity of isocapronitrile and some unknown compounds, and in the region where methyl pyrazine and, presumably, pyridine elute (Figure 2). Figure 4 shows that MENA elutes close to capronitrile;

Figure 4. Chromatograms of 55 ml vapour phase of the average puff from the 1R1 reference cigarette. 150 m × 0.75 mm i. d. Ucon 50 HB 280X column. On-column trapping. Nitrogen detection. The lower chromatogram shows response to about 100 ng of each of the nitrosamines. The identity of some other nitrogen compounds, known to be present in smoke, is indicated as determined by co-chromatography (methyl pyrazine, capronitrile, ethyl pyrazine, pyrrole, benzonitrile, o-tolunitrile, nitrobenzene, p-tolunitrile, 2-acetyl pyrrole).



DENA is near to ethyl pyrazine and (not shown) 3-methyl pyridine. Unlike the nitriles, the pyrazines and especially the pyridines showed tailing on the columns and their exact retention maxima varied with the amounts injected. MBNA, if present in smoke from dark tobacco cigarettes such as 2–4 and Sh 11, would almost coincide with some unknown compound. We further observed that PyrrNA and PipNA would elute almost simultaneously with p-tolunitrile and nitrobenzene respectively and are very close to each other; PyrrNA and PipNA can even be made to change places or to co-elute by a change of column temperature. All the non-nitrosamine nitrogen compounds mentioned are known to be present in tobacco smoke and, together with some unknown compounds, could interfere with nitrosamine determinations, particularly on columns of lower resolution. The most serious interference in GLC-AFID determinations, however, is to be expected with the determination of DMNA.

With these facts in mind we have estimated the amounts of apparent nitrosamines maximally present in the vapour phase of the smoke from the various products, assuming for a moment that the peaks present at the relevant retention times are really and entirely nitrosamines. The results, which for DMNA were remarkably high, will be given in full below together with the GLC-MS results.

When room air was injected or when cigarettes 2–6 were "smoked" without being lit, there were no detectable peaks at nitrosamine retention times. For simplicity's sake, the detection limit for the whole nitrosamine series may be put at approximately 2 ng per injection (see Table 1 for volumes injected). Actually the response of the nitrogen detector to the individual aliphatic nitrosamines decreases with increasing carbon content, i.e. with decreasing nitrogen content, contrary to the behaviour of the normal flame ionization detector towards these nitrosamines.

Gas-Liquid Chromatography — Mass Spectrometry (GLC-MS)

The GLC-AFID results were cross-examined by means of a separate set of analyses with GLC-MS. Firstly, cigarettes 2–1, 2–4, 2–6 and 1R1 and cigars S III were studied as indicated in Table 1. Total ion current chromatograms and individual mass spectrograms were prepared for masses 30 and 42, and for the molecular masses 74, 88, 100, 102, 114, 116 and 130, and these were thoroughly searched for nitrosamines. In the end it turned out — flagrantly contradicting the GLC-AFID results — that none of the eight reference nitrosamines nor any of their isomers were present in the vapour phase of the smoke from the five products (see Table 2). As to DMNA, there was not the slightest indication that this compound occurred in any of the samples. Sometimes, the individual mass fragmentograms gave the impression of the higher nitrosamines' [parent masses 88, 102, 116, 130] and the heterocyclic ones' [100 and 114] being present, but a check of the complete mass spectrum always ruled them out. The detection limit for the GLC-MS runs was about 2 ng per injection, which is the same as for the AFID.

A compound that was present in the dedicated mass plots for mass 116 of cigarettes 2–1, 2–4, 2–6, 1R1 and the cigars S III was hydroxyacetone acetate. In a superficial inspection by mass fragmentography, this

Table 2. Amounts of N-nitrosamines detected in the vapour phase of the smoke from various tobacco products. Analysis with a high-resolution capillary GLC column and 1) nitrogen-specific detection, 2) mass spectrometry.

Note: For the five products studied with GLC-MS, all the positive results obtained with nitrogen-specific detection (on the assumption that peaks eluting at nitrosamine retention times really are entirely nitrosamines) are disproved and are shown to be false-positives.

Tobacco product	Amounts of N-nitrosamines detected in the vapour phase of the smoke of a whole cigarette/cigar (ng)[a) b)]							
	DMNA	MENA	DENA	MPNA	MBNA	DPNA	PipNA[d)]	PyrrNA
Virginia cigarette 2—1	750 ;--	80 ;--	14 ;--	10 ;--	--;--	--;--	--;--	--;--
Dark cigarette 2—4	950 ;--	42 ;--	28 ;--	--;--	49 ;--	21 ;--	21 ;--	--;--
Blend cigarette 2—6	685 ;--	35 ;--	21 ;--	--;--	--;--	--;--	--;--[d)]	--;--
Oriental cigarette 2—7[c)]	1110	91	46	--	32	--	--	--
Reference cigarette 1R1	1620 ;--	60 ;--	28 ;--	28 ;--	49 ;--	32 ;--	--;--	--;--
Hand-made cigarette Sh 1[c)]	810	77	42	--	63	--	--	--
Hand-made cigarette Sh 11[c)]	1220	84	42	--	98	42	63	84
Medium size cigar S I[c)]	5630	335	270	63	--	125	--	190
Cigar S III	11100 ;--	860 ;--	460 ;--	84 ;--	84 ;--	84 ;--	--;--	190 ;--
Unlit cigarette 2—6	--	--	--	--	--	--	--	--

a) -- = not detectable; detection limit is about 2 ng per injection, both for AFID and MS (for volumes injected see Table 1).
b) Where two values are given (x; y), x is the GLC-AFID result and y is the GLC-MS result. All positive AFID results cross-examined with GLC-MS are disproved.
c) These products were not studied with GLC-MS; positive results presumably wrong.
d) Nitrosopiperidine was the only nitrosamine that was positively and unambiguously identified by GLC-MS. For this, the sample volume analyzed had to be increased from 35 ml to 175 ml, i. e. 5/7 of the vapour phase of a whole cigarette (blend cigarette 2—6). See the text.

compound might suggest the presence of nitrosomorpholine or a methylbutylnitrosamine isomer. The mass spectra contain the following masses (not all fragments are shown):

Hydroxyacetone acetate	116	86	73	57		43	42
Methylbutylnitrosamines	116		73	57	56	43	42
Nitrosomorpholine	116	86			56		42

It is evident that mass fragmentography of a few selected masses is only the first step towards a positive identification. Inspection of the full mass spectrum is imperative and in this special case easily prevents mistakes. The retention times of hydroxyacetone acetate and nitrosomorpholine were not known to us.

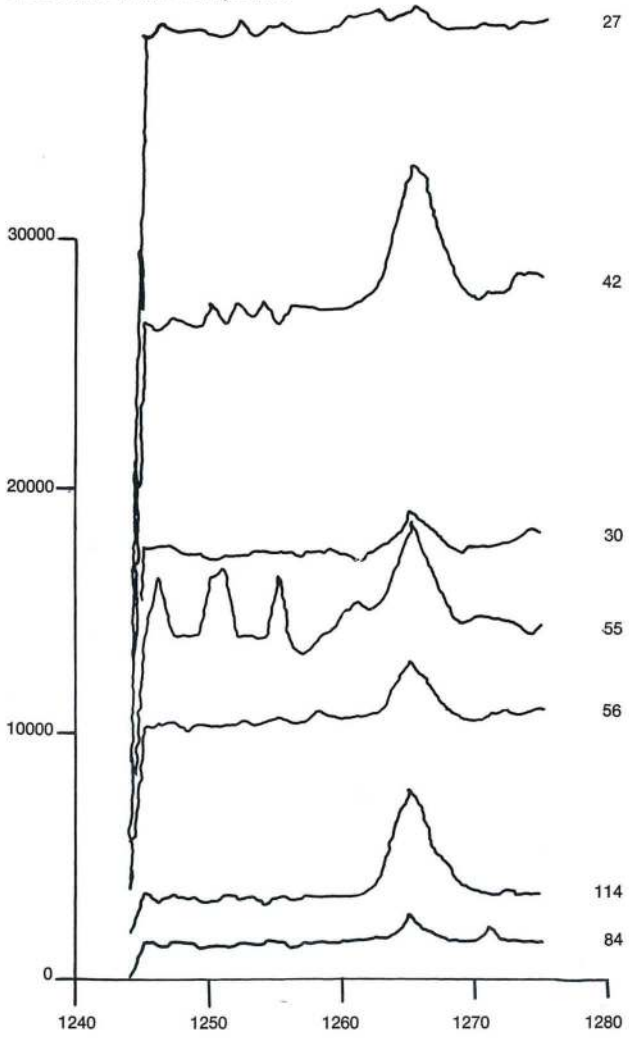
After the above negative results had been obtained, a study was made of vapour phase volumes which were twice as high from cigarettes 2—1 and 2—4. The results were again negative. It was only when a volume five times as high was injected — 5×35 ml of vapour phase from cigarette 2—6 — that we were able to positively identify a nitrosamine, viz nitrosopiperidine. The other nitrosamines remained absent. The detection limit was again about 2 ng per injection, which would this time mean about 7 ng in the whole vapour phase of the smoke from cigarettes 2—1 and 2—4, and about 2.8 ng in the vapour phase from one cigarette 2—6.

Figure 5 shows mass fragmentograms for a region of the chromatogram where mass 114 had been seen exactly at the expected retention time of nitrosopiperidine. Mass fragments were present as required and coincided in spectrum 1265, which was then printed (Figure 6). It contained mass 114, buried in a coherent series of polypropylene glycol bleeding fragments 59-73-87-89-101-103-115-117-131-133-145-147-159-161-175 ..... However, this contaminated spectrum could be refined to a high degree by background subtraction. Figure 7 shows the result: the mass spectrum corresponded almost completely to the reference spectrum. Mass fragmentography showed that some remaining mass fragments [80, 117, 124 and 145], not present in the reference spectrum, did not belong to the spectrum of the smoke constituent either. They are residual bleeding fragments [145], or belong to neighbouring peaks [80 and 124] or both [117]. Mass 68 does belong to the spectrum of the smoke constituent and some of it is present in the reference spectrum, too.

We conclude from the mass spectral evidence obtained and on the basis of the correctness of the retention time, that nitrosopiperidine is present in the vapour phase of the smoke from the American blend cigarette. The exact amount of it can only be assessed semi-quantitatively; we estimate that about 4 ng was present in the 175 ml vapour sample. In an injection volume a fifth of the size the compound would indeed remain undetected. About 5.6 ng of the compound is present in the vapour phase of the smoke of the whole cigarette 2—6.

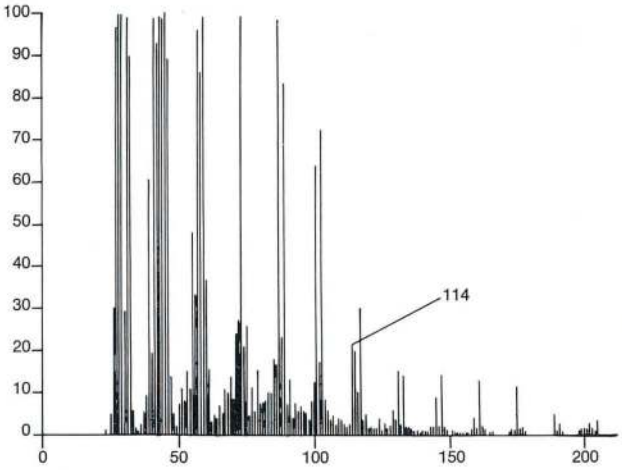
The analysis was performed on this large scale for cigarettes 2—6 only. It would be interesting to know whether nitrosopiperidine (or other nitrosamines) also become detectable in the vapour phase of the other products by a similar increase of the injection volume. However, any further lowering of the detection limit will then be precluded since the 175 ml vapour injection was about the largest possible. When larger volumes are injected without removal of the water vapour in the sample, the cooled column becomes blocked. It is remarkable that under these rather drastic circumstances — five vapour injections of 35 ml in the space of about one hour — a peak width of only about seven spectra (about 50 seconds) can be attained (see Figure 5).

Figure 5. Mass fragmentograms for a region of the GLC-MS chromatogram of American blend cigarettes 2–6. X-axis: serial number of successive mass spectra, registered on tape. Y-axis: ion current. Spectrum number 1265, at the expected retention time of N-nitrosopiperidine, contains the prominent mass fragments required by the reference spectrum of this compound.



It is conceivable that repeated injections of large vapour samples on the cooled column might lead to in situ nitrosamine formation from secondary amines and NO plus traces of NO_2, though it would not be reasonable to assume that only nitrosopiperidine and no other nitrosamines would thus be formed. (This would be as unlikely as the almost exclusive formation of MBNA in the paper of *Neurath* et al. (5), referred to in the "Introduction".) Furthermore, it can be calculated from the respective base dissociation constants that even in a smoke of alkaline character (pH about 8) amines, piperidines and pyrrolidines are present in their protonated form for more than 99.9% and are found as salts in the particulate phase. In this study the particulate phase was removed immediately after the generation of the smoke. Nevertheless, we checked this hypothetical possibility by an exaggerated control experiment with GLC-AFID, in which concentrated NO_x vapours (from an acidified nitrite solution) were injected on the cooled column immediately after the smoke

Figure 6. Mass spectrum number 1265 from the chromatogram of the American blend cigarettes 2–6, at the expected retention time of N-nitrosopiperidine. The molecular ion, mass 114, is buried in fragments of polypropylene glycol column bleeding.

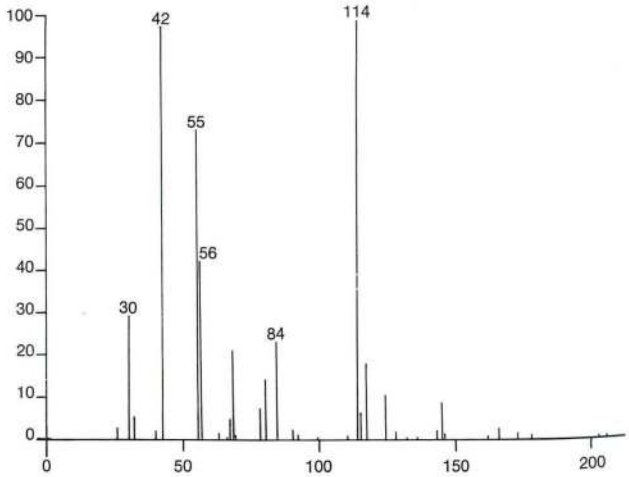


vapour phase injection. The analysis was then started as usual. No increase in peak heights at retention times of nitrosamines or of other compounds was observed.

Combined Results

Comparing the results of the GLC-AFID and the GLC-MS analyses we can draw the conclusion that even the use of high-resolution GLC could not prevent false-positive results being obtained with nitrogen-specific detection. We have, nevertheless, described the GLC-AFID investigations in some detail since we feel that they are very instructive. Especially for DMNA, high values were erroneously found. The detection limits of AFID and MS were the same under the conditions of our experiments, and negative results obtained with

Figure 7. Mass spectrum number 1265 from the chromatogram of the American blend cigarettes 2–6, at the expected retention time of N-nitrosopiperidine, after background subtraction. The spectrum now almost completely corresponds with the reference spectrum. Most remaining mass fragments, not present in the reference mass spectrum, were shown by mass fragmentography to be residual bleeding fragments or to belong to neighbouring peaks.



the nitrogen detector were corroborated by MS. However, all the AFID positive values obtained in this study for five of the products (Table 2) proved to be erroneous. Nothing can be said with certainty about the other four products of the table that were not studied with GLC-MS, but it is to be expected that the positive values given are also erroneous. During the course of this work we became more and more convinced that laboratories with GLC-MS facilities would do better to start using these immediately without trying to do preliminary screening with the nitrogen detector. It is true that at least the absence of nitrosamines can be unambiguously established with AFID, but we have never analyzed tobacco smoke or a food product with AFID for the nitrosamine series, or even for DMNA only, with a negative result. All products analyzed by us with AFID contained apparent nitrosamines and needed affirmation by MS. Of course, the alkali flame ionization detector as such remains attractive for many purposes; its high selectivity for nitrogen compounds in general and its cheapness compared with other nitrogen detectors have to be weighed against its lack of discrimination between classes of nitrogen compounds and against its often annoyingly erratic behaviour under heavy demand.

The interference of pyrazines with nitrosamine analyses, mostly in sugar/aminoacid reaction mixtures, has been observed by several authors, see e.g. *Fujimaki* et al. (11) and references given by them. The elution of pyrazines immediately before and after DMNA in GLC chromatograms of tobacco smoke condensate has been reported by *Morie* and *Sloan* (12). They determined dimethylnitrosamine with the alkali flame ionization detector and happened to observe this compound on their capillary column as a separate small peak in a crowded area of large peaks, all representing nitrogen containing compounds. Like the present authors, they too used MS to confirm this. The possible interference of aliphatic and aromatic nitriles and nitro compounds with nitrosamine determinations in tobacco smoke does not appear to have been reported before. Removal of these compounds prior to the nitrosamine assessment would certainly not be an easy task. The amine/nitrosamine detector reported by *Johnson* et al. (13), a modified Coulson electrolytic conductivity detector operated in the pyrolytic mode, is said to achieve a high selectivity for nitrosamines, but presupposes the complete removal of amine compounds. *Fine* et al. (14) have developed a thermoluminous analyzer for nitrosamine determinations which is both highly nitrosamine-specific and extremely sensitive. The analyzer has been used as a group-specific detector and has not yet been interfaced with GLC. For unambiguous direct identification of individual nitrosamines the use of mass spectrometry still is crucial.

The above measurements lead to the conclusion (taking the gradually lowered detection limit into account and certainly not suggesting differences between tobacco types) that the vapour phase of the smoke from the various products studied with GLC-MS may contain — if any — at the most the following amounts of the individual nitrosamines: cigars S III 84 ng, reference cigarettes 1R1 28 ng, Virginia cigarettes 2—1 7 ng, dark cigarettes 2—4 7 ng, and American blend cigarettes 2—6 2.8 ng per cigarette. Only one nitroso compound was positively and unambiguously identified in this study, viz nitrosopiperidine in the smoke from the blend cigarettes 2—6. It amounted to about 5.6 ng in the vapour phase of the smoke of one cigarette 2—6.

As far as we know, there are as yet hardly any publications on the partition of semi-volatile smoke constituents between vapour phase and particulate matter. *Williamson* and *Allman* (15) have given values for compounds in the region acetaldehyde to toluene. The fraction present in the vapour phase ranged from 100% to 82% while the boiling points ranged from 21° C to 111° C. The boiling point of the lowest aliphatic nitrosamine, DMNA, is about 152° C, so that presumably a smaller fraction of it will be found in the vapour phase. DPNA has a boiling point of about 205° C, PipNA and PyrrNA have boiling points of about 215° C. To give some indication, the latter values lie between those of phenol and nicotine. Apart from the boiling points, or rather the vapour pressures at room temperature, the solubility of the compounds in the aqueous particulate phase will also play a role. In the series of aliphatic nitrosamines, the water solubility decreases as the boiling point increases. The net effect on the partition of these nitrosamines between vapour phase and particulate phase is unknown. It is not possible, therefore, to estimate the amount of nitrosamines that may be present in whole smoke on the basis of the results of this study for the vapour phase of the smoke. Our measurements have to be considered as a piece of additional information fitting into existing knowledge concerning the problem of nitrosamines in tobacco smoke.

Despite the uncertainties surrounding the partition of the nitrosamines between vapour phase and particulate matter, we think it rather remarkable that of all nitrosamines nitrosopiperidine has been detected and not dimethylnitrosamine. The latter compound has been found in a considerable number of cigarette smoke studies. The estimates range from 3 to 3400 ng per cigarette (see Table 3). If the high results of *Pailer* and *Klus* (20) and *Barkemeyer* (6) were correct, some of the DMNA should unquestionably have been found in the vapour phase of the smoke in our analyses. Likewise, as has also been remarked by *Neurath* (22), the nitrosopiperidine values (1000—1500 ng in the smoke of one cigarette) reported by *Serfontein* and *Hurter* (7) are improbably high.

The nitrosamine contents found in food products as well as in tobacco smoke have become lower and lower over the years as analytical methods improve. One anonymous scientific commentator (23) has spoken of the case of the disappearing nitrosamine. We feel that research into the presence of nitrosamines in food

Table 3. Recent estimates of dimethylnitrosamine in cigarette smoke.

Authors	Cigarette type	Amount of DMNA (ng in the smoke of one cigarette)
Neurath et al. (5)	High-nitrate cigarettes	3
Barkemeyer (6)	Virginia, Air-cured	800 3400
Morie and Sloan (12)	Two commercial cigarette brands, High-nitrate cigarettes	<10 50—95
Hoffmann (16)	U.S. blend	84
McCormick et al. (17)	Flue-cured commercial brand, Commercial French brand, Experimental cigarettes	5 143 9—180
Johnson and Rhoades (18)	Experimental cigarettes, Commercial U.S. blend	0—140 8
Kadar and Devik (19)	Blend	<50*
Pailer and Klus (20)	American blend	700
Michelson and Rathkamp (21)	American blend	118

* The summary of this paper appears to contain an inaccuracy; the results do not apply to whole smoke but to the vapour phase of smoke.

products and especially in tobacco smoke should be continued intensively in the near future. As far as tobacco smoke is concerned we plan to concentrate further investigations on whole smoke.

SUMMARY

Volatile N-nitrosamines were determined in the vapour phase of tobacco smoke after accumulation of large vapour samples on to a cooled capillary GLC column. Detection was effected by computerized mass spectrometry and by a nitrogen-specific detector. Five different tobacco products were studied with mass spectrometry for the presence of eight individual nitrosamines. The result was negative thirty-nine times and positive only once. Initially, the detection limit for the individual nitrosamines was 2 ng per 20—55 ml vapour injection; during this study the effective detection limit was lowered for some of the compounds by a further twofold to fivefold increase of the vapour volume analyzed. The one nitroso compound identified positively and unambiguously was N-nitrosopiperidine in the smoke from the American blend cigarettes. It amounted to about 5.6 ng in the vapour phase of the smoke of one cigarette. The partition of nitrosamines between vapour phase and particulate phase is not known, but amounts of more than 1 µg of dimethylnitrosamine or nitrosopiperidine in the whole smoke of one cigarette as found by some authors, seem improbably high.
It is shown in this paper that exclusive use of the alkali flame ionization detector for nitrosamine determinations would have led to false-positive results, especially for dimethylnitrosamine.

ZUSAMMENFASSUNG

Nach Ansammlung umfangreicher Dampfphase-Proben in einer gekühlten Kapillarsäule eines Gaschromatographen (GLC) wurden die flüchtigen N-Nitrosamine in der Dampfphase von Tabakrauch bestimmt. Der Nachweis wurde unter Einsatz eines mit einem elektronischen Rechner gekoppelten Massenspektrometers und eines stickstoffspezifischen Detektors durchgeführt. Fünf verschiedene Tabakprodukte wurden mittels Massenspektrometrie auf das Vorkommen von acht einzelnen Nitrosaminen untersucht. Der Befund war 39mal negativ und nur einmal positiv. Anfänglich belief sich die Nachweisgrenze für die einzelnen Nitrosamine auf 2 ng je 20—55 ml Dampfinjektion; im Laufe dieser Untersuchungen wurde die tatsächliche Nachweisgrenze bei einigen der Verbindungen durch eine weitere zwei- bis fünffache Vergrößerung des zu untersuchenden Dampfvolumens gesenkt. Die eine Nitrosoverbindung, die positiv und eindeutig im Rauch von Cigaretten einer amerikanischen Mischung identifiziert wurde, war N-Nitrosopiperidin; die in der Dampfphase des Rauches einer Cigarette aufgefundene Menge betrug etwa 5,6 ng. Die Verteilung der Nitrosamine zwischen Dampf- und Partikelphase ist unbekannt; Mengen von über 1 µg Dimethylnitrosamin oder Nitrosopiperidin in dem gesamten Rauch einer einzigen Cigarette — wie sie von einigen Autoren gefunden wurden — scheinen jedoch unwahrscheinlich hoch zu sein.
Es wird in dieser Arbeit gezeigt, daß die ausschließliche Verwendung des Alkali-Flammen-Ionisationsdetektors bei der Bestimmung von Nitrosaminen, besonders bei Dimethylnitrosamin, zu falschen positiven Ergebnissen geführt haben könnte.

RESUME

On a déterminé les N-nitrosamines volatiles dans la phase vapeur de la fumée de tabac après accumulation d'importants échantillons de vapeur dans une colonne chromatographique gaz/liquide (GLC) refroidie. La détection a été effectuée par spectrographie de masse couplée à un ordinateur et un détecteur spécifique de l'azote. On a étudié la présence de 8 nitrosamines différentes dans cinq produits de tabac différents. Les résultats ont été négatifs dans 39 cas, et positifs dans un seul cas. Au début, la limite de détection pour les

nitrosamines se situait à 2 ng par injection de 20—55 ml de vapeur; au cours de cette étude, on a abaissé cette limite pour certains composants grâce à un volume de vapeur analysée allant du double au quintuple. Le seul composé nitroso identifié positivement et sans équivoque est la N-nitroso-pipéridine dans la fumée de cigarettes du mélange américain. On en a trouvé à peu près 5,6 ng dans la phase vapeur de la fumée d'une seule cigarette. La distribution des nitrosamines entre la phase vapeur et la phase particulaire n'est pas connue, mais des quantités de plus d'un µg de diméthylnitrosamine ou de nitrosopipéridine dans la fumée d'une seule cigarette, comme certains auteurs l'ont écrit, semblent très improbables.

On démontre dans cette communication que l'utilisation exclusive d'un détecteur à ion de flamme à l'alcali pour la détermination des nitrosamines aurait amené de fausses conclusions positives, particulièrement en ce qui concerne la diméthylnitrosamine.

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