two susceptible lines, it was conceivable that seed coats of this particular line had a weakened polysaccharide network which facilitated the diffusion of soluble material to the testa surface. The monosaccharide patterns found in six experimental peanut lines also appeared to be unique to the testa. Large amounts of glucose and fructose over those of arabinose and galactose, which is characteristic of the cotyledon (Amaya-F. and Young, 1976), contrasted with the sugars present in the testa.

The amino acid pattern of hydrolyzed water extracts from the peanut testa was unique to this organ and was characterized by a large proportion of ammonia. Most of these diffusable nitrogenous compounds seemed to be implicated in the growth of germinating fungal (*A. flavus*) spores assumed to be randomly distributed over the seed surface. Soluble monosaccharides found in testae did not appear to be correlated with susceptibility or resistance of the seed. Lower contents of arabinose in the acid hydrolysate of a susceptible line, however, was an indication of debilitated polysaccharide structures which could be important in maintaining the physical barrier characteristics of the testa.

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A Comparison of Various Mass Spectrometric and a Chemiluminescent Method for the Estimation of Volatile Nitrosamines

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Extracts of a variety of commodities have been analyzed for the presence of volatile nitrosamines using high- and low-resolution mass spectrometry and chemiluminescent detection. The quantitative results obtained by these methods have been compared to assess their reliability. The high-resolution peak matching and chemiluminescent data are in agreement with each other for all the analyses, but inconsistencies were observed using the other mass spectrometric procedures.

It has been established that many nitrosamines are carcinogenic (Magee and Barnes, 1967; Wolff and Wasserman, 1972), and their formation is possible between secondary or tertiary amines and nitrite (Mirvish, 1970; Fan and Tannenbaum, 1971). The need to detect trace amounts of nitrosamines in foodstuffs, biological fluids, vegetation, and other matter has been met by using a variety of analytical procedures. Volatile nitrosamines, after gas chromatographic (GC) separation, have been detected down to mg/L amounts, using nitrogen selective detectors such as the flame thermionic and the Coulson electrolytic conductivity detector. Extracts of biological origin are still complex mixtures even after extensive cleanup and frequently contain nitrogen-containing compounds. Some of these will have identical retention characteristics to nitrosamines, so that nitrogen selective detectors can give rise to false positive results (Goodhead and Gough, 1975). Some means of confirming the presence of nitrosamines tentatively observed using GC detectors is necessary, and mass spectrometry (MS) offers the most reliable means of achieving this. Several workers have developed methods based on mass spectrometry (Fazio et al., 1971; Gough and Webb, 1972; Telling et al., 1971), all of which involve a prior separation of the nitrosamines from each other and from extraneous material, using combined gas chromatography and mass spectrometry (GC–MS). The degree of sophistication of the GC apparatus varies from single isothermal packed columns to systems incorporating solvent-venting pressure programming (Gough and Webb, 1973) and high-efficiency narrow bore columns (Essigmann and Issenberg, 1972; Gough and Sugden, 1975). The identification of a nitrosamine is based both on its GC retention time and some characteristic of its mass spectral fragmentation. Quantitation is normally based on the intensity of selected ions in the spectrum, after calibration using standard nitrosamine solutions. Using GC–MS several groups have reported the presence of nitrosamines in food such as cured meat, fish, and cheese (Crosby et al., 1972; Fazio et al., 1971, 1973; Fong and Chan, 1976; Gough et al., 1976; Sen et al., 1973; Wasserman et al., 1972).

The only other technique available at the present time which is likely to offer the specificity necessary for the unequivocal detection of nitrosamines is that based on chemiluminescence, and such a system has been described by Fine et al. (1975). Nitrosamines are catalytically cleaved

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to produce nitric oxide, which on reaction with ozone produces a chemiluminescent emission in the infrared region. No data have yet been reported using this procedure for the determination of nitrosamines in food, although traces of nitrosamines in air have been detected by chemiluminescence (Fine et al., 1976).

The IARC Collaborative Studies (Walker and Castegnaro, 1976) cannot be used for the comparison of the methods discussed in this paper, as each participating laboratory was responsible for the extraction and concentration of the food under study and used only one mass spectrometric technique. In the present work we have set out to make such a comparison by analyzing a range of food extracts using three mass spectrometric procedures and chemiluminescence.

DISCUSSION OF DETECTION METHODS

The mass spectrometric procedures which have been used for nitrosamine determination can be divided conveniently into those based on low and high resolution. Low-resolution determinations using the complete spectrum have been used but are usually based on the detection of several predominent ions characteristic of the nitrosamine. In the case of *N*-nitrosodimethylamine, the GC effluent is normally monitored for fragments of *m/e* 30, 42, and 74, the last mentioned being the base peak and parent ion. Confirmation of the presence of the nitrosamine is based on the simultaneous occurrence of these ions both at the appropriate GC retention time and in the appropriate relative abundance. It has, however, been observed by Fazio et al. (1971) that the relative intensities obtained using standard nitrosodimethylamine solutions do not always match those observed in spiked fish extracts, where presumably coeluted material also gave rise to at least one of these fragments. Indeed, *m/e* 42 is one of the most commonly encountered fragments in mass spectrometry. A similar procedure is used for other nitrosamines and for *N*-nitrosopyrrolidine, for example, fragments of *m/e* 30, 41, 43, 69, and 100 (parent ion) have been used.

High-resolution mass spectrometry has been used less commonly, although in principle it is more likely to give rise to unambiguous results than low-resolution mass spectrometry. Two procedures have been described: one monitoring the NO^+ ion of *m/e* 29.9980, which is common to all the nitrosamines, and the other monitoring the parent ion of each nitrosamine in turn. The latter procedure, while requiring greater operator skill, is inherently more sensitive for the steam volatile nitrosamines, as the parent ion is either the base peak or at least of substantial relative abundance. In this laboratory we have used the parent ion technique for a number of years and have only encountered one potential interferant. This is $^{29}SiMe_3$ and arises from the use of antifoam tablets during the preparation of extracts and is eluted from the GC with a retention time close to *N*-nitrosodimethylamine. It is distinguishable from the nitrosamine on this basis and is just sufficiently resolved from the nitrosamine parent ion even at a resolution of 7000 to avoid ambiguity. These observations have been reported in more detail both by Gough and Webb (1973) and by Dooley et al. (1973).

We have observed other potentially undesirable effects using high-resolution mass spectrometry, and these result not from compounds of the same nominal mass of the nitrosamine, but from the means of displaying the high-resolution signal. Bryce and Telling (1972), Gough and Webb (1972), and Pensabene et al. (1975) describe specifically the use of peak matching in their techniques. Using this procedure the mass region in the vicinity of the reference fragment (usually derived from a fluorinated hydrocarbon) and the nitrosamine fragment, of the same nominal mass, are alternately scanned every few seconds and displayed on an oscilloscope. This enables the analyst to observe not only the rise and fall of the nitrosamine peak, but also to observe peaks of the same nominal mass and the reference peak. Coeluted material of *any* mass will tend to suppress the mass spectrometer response and hence affect quantitation. In an extreme case, complete suppression resulting in a false negative for the nitrosamine could result. By peak matching any suppression is immediately apparent, as the reference peak is also suppressed. Some workers, while using high-resolution techniques, monitor only the precise mass of the parent ion, and the safeguard from peak matching is thus lost. Precise ion monitoring demands a very stable instrument and has the added danger that the tails of large peaks of the same nominal mass could be detected as if they were nitrosamines, thus giving rise to false positive results. Consequently, when the mass spectrometer is operated in the high-resolution mode, peak matching should always be used, as this provides a higher specificity than precise ion monitoring.

It follows that similar effects could occur using low-resolution mass spectrometry, and thus the possibility of suppressed or enhanced signals can occur under these conditions. Suppression would be difficult to observe, although some safeguard against enhancement is achieved provided the interferant does not contribute fragments at all the same values as the major nitrosamine fragments.

A comparison of the quantitative results for the presence of nitrosamines, based on a technique other than mass spectrometry, would be very useful, and the most suitable technique available at the present time is chemiluminescence. Chemiluminescent detectors for nitrosamine analysis have been described by Fine and Rufeh (1974) and Gough and Woollam (1976), the former equipment being much more sensitive than mass spectrometry but rather sophisticated, and the latter a simple laboratory constructed apparatus and, although having similar specificity, somewhat less sensitive and suitable for screening purposes.

EXPERIMENTAL SECTION

1. Low Resolution Mass Spectrometry. A Pye 104 chromatograph fitted with a flame ionization detector was interfaced to an AE1 MS 30 mass spectrometer using a silicone membrane separator (Gough and Webb, 1972). The mass spectrometer was fitted with an AE1 multiion monitor and was operated at a resolution of 1000 (10% valley). The signal was displayed on a multichannel UV recorder, each channel monitoring a single nominal mass once every second. For *N*-nitrosodimethylamine, the ions monitored were *m/e* 30 and 74 and for *N*-nitrosopyrrolidine, *m/e* 69 and 100. Quantitative data were based on the intensities of these ions after calibration of the mass spectrometer using 1, 10, and 100 mg/L solutions of the nitrosamine in hexane. The GC was fitted with a solvent-venting valve to minimize suppression effects arising from the solvent and other extraneous material entering the mass spectrometer. Even so, other ions common to the nitrosamines, such as *m/e* 41, 42, and 43, were generally present to varying extents in the background signal during a GC–MS run and were found to be of little diagnostic value. The GC column was 2 m × 4 mm i.d. glass, containing 5% Carbowax 20 M on Chromosorb W AW. DMCS, with a helium gas flow rate of 15 mL/min. Column temperatures were 100 °C for *N*-nitrosodimethylamine and 150 °C for *N*-nitrosopyrrolidine.

2. High-Resolution Mass Spectrometry with Peak Matching. The GC system was similar to that described above and was interfaced to an AE1 MS 902 mass spectrometer with the same type of separator. The mass spectrometer was set to a resolution of 7000, and, using the peak matching unit, the mass range in the vicinity of the appropriate parent ion (74.0480 or 100.0637) was continuously scanned. The output was displayed on the oscilloscope attached to the peak matching unit of the spectrometer, thus enabling the nitrosamine and reference fragment regions to be alternatively monitored every 4 s throughout the analysis. The concentrations of the nitrosamines in the extracts were estimated from the oscilloscope peak height readings after calibration using standard nitrosamine solutions.

3. High-Resolution Mass Spectrometry with Precise Ion Monitoring. The GC–MS 902 system was used under identical conditions to those described above, except that the mass spectrometer was focused precisely on the appropriate parent ion instead of scanning through this region. The peak matching unit cannot be used under these conditions, which were designed to simulate the procedure adopted by workers who do not have this facility. The signal was displayed on a storage oscilloscope, although in this mode of operation the signal can equally well be displayed on a potentiometric recorder to obtain a permanent record. Concentrations of nitrosamines were obtained from the peak heights.

4. Chemiluminescence. A Pye 104 chromatograph was connected in series with a Thermal Energy Analyser (TEA) Model 502. GC effluent was passed into a catalytic chamber at 400 °C and then into a cold trap at −130 °C to remove organic compounds from any nitric oxide generated from the nitrosamines. The nitric oxide was then reacted with 0.3% ozone in oxygen in a chamber held at 1 Torr. The resulting chemiluminescence was detected by a photomultiplier tube behind a 600-nm filter in this chamber and the signal displayed on a potentiometric recorder. Concentrations of nitrosamines were calculated from peak heights after calibration.

SELECTION AND ANALYSIS OF SAMPLES

The nitrosamines selected for this study were *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine, as these are the most commonly encountered in foodstuffs. Luncheon meat was chosen to represent a typical canned cured meat. Bacon has been the commodity most consistently reported to contain volatile nitrosamines, and cooked bacon and bacon fat have been studied in the present work. Bacon-frying vapors have been included since, although relatively free from organic matter, they do contain volatile nitrosamines. Urine was included as the most commonly studied biological fluid for pollutants. Vegetation gives rise to fairly clean extracts by comparison with animal matter and is thus useful for comparing the techniques for realistic but simple extracts. Previous experience in the laboratory with salted fish, sauces, and soya bean foods of Hong Kong Chinese origin has shown that they are among the most difficult commodities to clean up satisfactorily, and a comparison of the techniques using these foods represents a fairly stringent test of their reliability.

The samples for examination were prepared by the standard laboratory method (Goodhead and Gough, 1975), the concentration factor being 1000 in all cases. The range of nitrosamine levels encountered in the extracts was from 0.03 mg/L to 100 mg/L and were, in most cases, naturally present in the commodities prior to extraction and concentration. At the beginning of each day and twice during the day each instrument was calibrated using the same standard nitrosamine solutions, and the same extracts were examined by each technique in the intervening period where possible. In all cases the extracts were analyzed by each technique within an 8-h period, and the results are presented in Table I for *N*-nitrosodimethylamine and Table II for *N*-nitrosopyrrolidine. In instances where there were substantial discrepancies between the concentrations derived from the various techniques, the samples were reanalyzed simultaneously by at least two of the techniques. The detection limit using the mass spectrometric techniques is 1 mg/L and using the chemiluminescent detector 0.02 mg/L for *N*-nitrosodimethylamine and 0.04 mg/L for *N*-nitrosopyrrolidine. In the Tables, ND indicates not detected above these limits.

Safety Note: Since the majority of extracts were expected to contain nitrosamines, they were stored in septum-sealed vials in the dark at 10 °C until analysis when they were handled with great care.

Table I. Comparison of *N*-Nitrosodimethylamine Concentrations in Extracts (mg/L)[a]

Commodity and extract no.		Method: Low resolution MS (1)	High resolution MS (2)	High Resolution MS (3)	Chemiluminescence TEA (4)
Luncheon meat	1	2	2	2	1.8
	2	1.7	1.6	2	1.9
	3	10	13	9	11.4
Bacon, cooked rasher	4	4	3	4	3.3
	5	2	3	4	1.3
	6	2	2	3	1.5
	7	1.5	2	3	1.1
	8	2	2	3	1.0
	9	1.7	1	2	0.83
Bacon, cooked fat	10	2	3	4	1.3
	11	ND	ND	1	1.1
	12	ND	ND	4	0.03
Bacon, frying vapor	13	10	10	9	9.4
	14	5	5	5	4.9
	15	25	50	53	55
Urine	16	1.5	2	2	2.3
	17	>100	2	15	1.2
	18	21	13	14	12.2
	19	45	29	28	27.2
	20	50	43	39	36
	21	115	100	100	114
Vegetation	22	2	1	2	1.5
	23	1	1	2	1
	24	1	1	1	1
Chinese foods	25	ND	ND	1	ND
	26	16	20	16	24.0
	27	65	64	40	62.1
	28	13	13	9	18
	29	33	31	50	34.8
	30	30	9	38	10.9
	31	100	8	80	9.4
	32	75	30	56	33.2

[a] Mg/L in extract ≡ μg/kg in original sample.

RESULTS

The estimated concentrations of the nitrosamines in the extracts using the different methods are listed in Tables I and II. For the luncheon meat and cooked bacon (extracts 1–9) in which the level of *N*-nitrosodimethylamine ranged from 1–13 mg/L, all four analytical methods gave compatible results. In the bacon fats (extracts 10–12) where the levels were near the MS detection limit, there was no discrepancy between the MS methods 1 and 2 (low resolution and peak matching) and the TEA (method 4).

Table II. Comparison of *N*-Nitrosopyrrolidine Concentrations in Extracts (mg/L)[a]

Commodity and extract no.		Method			
		Low resolution MS (1)	High resolution MS (2)	High resolution MS (3)	Chemiluminescence TEA (4)
Luncheon meat	1	3	ND	5	ND
	2	5	3	4	2.3
	3	1.5	ND		
Bacon, cooked rasher	4	9	10	14	11
	5	20	11	20	13.6
	6	10	19	16	18.2
	7	13	16	17	20.0
	8	9	19	18	21.6
	9	10	ND	6	1.0
Bacon, cooked fat	10	30	10	10	7.8
	11	6	1	1	0.79
	12	12	1	2	1.5
Bacon, frying vapor	13	30	14	20	14.1
	14	39	7	8	12
	15	>100	100	63	98
Urine	16	ND	ND	2	0.2
	17	ND	ND	3	ND
	18	ND	ND	1	0.04
	19	ND	ND	2	0.4
	20	ND	ND	2	0.08
	21	5	3	6	1.5
Vegetation	22	ND	ND	ND	ND
	23	ND	ND	2	0.10
	24	ND	ND	4	0.14
Chinese foods	25	ND	ND	1	ND
	26	ND	ND	2	ND
	27	ND	ND	5	ND
	28	2	ND	3	ND
	29	ND	ND	3	ND
	30	70	ND	3	ND
	31	ND	ND		ND
	32	6	ND	3	ND

[a] Mg/L in extract ≡ μg/kg in original sample.

MS method 3 (high-resolution precise ion monitoring) gave a much higher value than the other methods for extract 12. The bacon-frying vapor results (extracts 13–15) are all in excellent agreement, except for sample 15 using low-resolution MS (1), which was the result of signal suppression by an interferant. This effect was not observed directly, but only became apparent when the same sample was examined by high-resolution peak matching. The results for *N*-nitrosopyrrolidine on the same commodities, covering the range 1–100 mg/L, are in excellent agreement by MS peak matching (2) and TEA (4), but less satisfactory by the other MS methods. Low resolution (1) gives enhanced results for 8 out of 15 extracts, including one case where *N*-nitrosopyrrolidine was not detected even at the 0.04 mg/L level by TEA (4). Precise ion monitoring (3) also gave a false positive result on the same extract, but was in fair agreement with peak matching for the remainder of the meat samples.

N-Nitrosodimethylamine levels in urine, ranging between 1 and 100 mg/L, were in agreement by both high-resolution MS methods (2 and 3) and TEA (4), except extract 17 which gave a high value by precise ion monitoring. Analysis of this sample by peak matching showed a relatively large impurity of the same retention time as the nitrosamine and having a fragment of the same nominal mass. Mass measurement of this fragment gave a value of m/e 74.0606, corresponding to an empirical formula of C_3H_8NO. The same sample gave an exceptionally high nitrosamine level by low-resolution MS (1). Low resolution also gave rise to high values in extracts 18 and 19. *N*-Nitrosopyrrolidine levels in the urines 16–20 were very low by TEA (4), which was consistent with the MS techniques (1 and 2) by which no nitrosamine was detected. Precise ion monitoring, however, gave positive values, which must be considered false.

All the methods were satisfactory for the determination of *N*-nitrosodimethylamine in vegetation and for *N*-nitrosopyrrolidine, except precise ion monitoring.

Some difficulty was experienced in analyzing the Chinese samples by all techniques, and GC retention times increased with consecutive analyses of a given sample. The extracts were clearly causing column deterioration, which, although temporary, resulted in minor disagreement between the peak matching and TEA results. Using freshly conditioned GC columns, the results agreed satisfactorily, and it is these results which are quoted in the Tables. However, for both *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine, the other two techniques gave unrealistically high values with some of these extracts.

The results obtained using low-resolution and precise ion monitoring mass spectrometry, even on a fairly small number of samples, show serious discrepancies, and these two procedures cannot be regarded as reliable under all circumstances. No further work on these procedures was therefore undertaken, although it is appreciated that with more rigorous clean up and more efficient GC separation these techniques may be somewhat better. The agreement between high-resolution peak matching and TEA results was remarkably good in all extracts, and these two techniques were selected for a more exhaustive comparison. A total of 227 extracts of food including meat, fruit, vegetables, dairy produce, preserves, and complete meals were examined for the following nitrosamines: *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodipropylamine, *N*-nitrosodibutylamine, *N*-nitrosopiperidine, and *N*-nitrosopyrrolidine. In view of the large number of determinations involved (1362), it was not possible to carry out both MS and TEA analyses on a given sample within a short period of time, and a critical comparison between the concentrations thus obtained is not valid. However, in all instances where the MS procedure gave a positive response for any of the nitrosamines, this was always matched by a TEA response of the same order of magnitude. Conversely, in no case did the TEA give a response over 1 mg/L (the MS detection limit) which was not confirmed by mass spectrometry. Although the agreement between the two techniques was satisfactory, the TEA often gave a response at retention times which did not coincide with these nitrosamines. Since it was not the purpose of this work to examine the extracts for hitherto unknown nitrosamines, nor to demonstrate false response of the TEA outside the specified nitrosamines, no attempt was made to identify any compounds giving rise to such a response.

CONCLUSIONS

Consistent quantitative results were obtained from all extracts and both nitrosamines using high-resolution peak matching mass spectrometry and chemiluminescent methods, totalling 64 determinations. Using these two methods, qualitative agreement involving six different nitrosamines and 1362 determinations was also satisfactory. Low-resolution mass spectrometry of *N*-nitrosodimethylamine was satisfactory in a majority of cases, although wide discrepancies arising from signal suppression or enhancement were sometimes observed (7 out of 32). For *N*-nitrosopyrrolidine the results obtained by this method were frequently at variance with those obtained by peak matching MS and by TEA. Precise ion monitoring

was also more satisfactory for *N*-nitrosodimethylamine than the heterocyclic nitrosamine, for which there were deviations in 18 out of 32 determinations.

For the types of materials listed in this report, it is concluded that the most satisfactory mass spectrometric procedure is that of high-resolution parent ion monitoring with peak matching. Chemiluminescence, although not responding exclusively to the nitrosamines studied, gave results in agreement with these mass spectrometric observations in all cases.

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An Insoluble Copper Phosphate in Copper Superphosphate

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When soluble copper salts are granulated with freshly made ordinary superphosphate, a compound $NaCu_6H_3(PO_4)_5Cl{\cdot}6H_2O$ resembling the mineral sampleite is formed which is poorly soluble in water. The availability of fertilizer copper to plants growing in acid soils is probably not affected unless the compound is present as large inclusions. X-ray diffraction electron microprobe and infrared data for the compound are presented.

It is a common manufacturing practice to add micronutrients to a superphosphate carrier during granulation in order to promote uniform application of a few kilograms of micronutrients per hectare in the field (Silverberg et al., 1972). In many instances chemical reactions between the micronutrient salt and superphosphate occur, particularly when micronutrient additions are made during the granulation process when freshly made superphosphate contains considerable free acid. In some cases reactions may promote an increase in solubility of the micronutrient as in the case of zinc added as zinc oxide to concentrated superphosphate (Mortvedt and Giordano, 1969). Little is known of reactions between copper salts and superphosphate, although several possible reaction products have been proposed which are insoluble in water and may

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Table I. Solubility of Copper in Copper Ordinary Superphosphate (OSP)

	Total Cu, %	Water soluble Cu, %	Insoluble Cu, %
One 14 h water extraction[a]			
Crushed high copper OSP granules	1.2	0.6	0.6
Crushed blue-green scrapings	2.4	0.9	1.5
Crushed scraped high copper granules	1.0	0.5	0.5
Repeated water extractions[a]			
Crushed, average copper OSP granules	0.7	0.5	0.2

[a] 0.1 g in 20 mL.