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Joseph H. Hotchkiss for the degree of Doctor of Philosophy

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Title: Analysis of N-nitrosamines and Nitramines in Foods and Herbicides

Abstract approved: _____
(R. A. Scanlan)

A method to both estimate the volatile N-nitrosamine content of foods and to confirm the presence of volatile N-nitrosamines was developed. Quantitation was achieved by combined gas chromatography and thermal energy analysis. The identity of the N-nitrosamine was confirmed by combined gas chromatography and low resolution mass spectrometry. The method was demonstrated to be effective for N-nitrosodimethylamine and N-nitrosopyrrolidine added to fried bacon, fried pork, raw fish, and cheese at one microgram per kilogram (ppb). In addition, the method was employed to quantitate and confirm dimethylnitrosamine and N-nitrosopyrrolidine in fried commercial bacon at levels of less than ten micrograms per kilogram.

The analytical scheme consisted of a vacuum distillation of the food in mineral oil, a dichloromethane extraction, acid and

base extractions for cleanup, and concentration by solvent distillation. Extracts which gave positive responses when analyzed by gas chromatography-thermal energy analysis were further cleaned up on activated alumina and reconcentrated. This extract was re-injected into the gas chromatograph-thermal energy analyzer and positive peaks trapped as they exited the chromatographic column by means of a valve and a trap consisting of one-sixteenth inch nickel tubing cooled in dry ice-methoxyethanol. The traps were sealed and later injected into the gas chromatograph-mass spectrometer for confirmation.

This work has demonstrated that low resolution mass spectrometry can be successfully utilized to confirm the identity of volatile N-nitrosamines at one microgram per kilogram in complex environmental samples such as foodstuffs.

It was also demonstrated that at least five nitramines can elicit a false positive response from the thermal energy analyzer. Dipropylnitramine was responsible for a false positive response from an herbicide containing trifluralin, and butylethylnitramine was responsible for a false positive peak from an herbicide containing benefin. The presence of dipropylnitrosamine and butylethylnitrosamine in the herbicides respectively, was also confirmed by both thermal energy analysis and mass spectrometry.

Dipropylnitramine and N-nitropyrrolidine were synthesized and their molar response ratios determined by comparing their response to the response of the corresponding N-nitrosamine. The values were 0.50 and 0.10 for dipropylnitramine and N-nitropyrrolidine, respectively. Further, dipropylnitramine was Greiss reagent positive, UV sensitive and had a mass spectrum which was very similar in the lower mass region to dipropylnitrosamine.

A mixture of five diaklyl and heterocyclic nitramines was synthesized by peroxytrifluoroacetic acid oxidation of the corresponding N-nitrosamine. All five nitramines gave a false positive response when analyzed by the thermal energy analyzer, and when the five nitramines were added to a solution of the corresponding N-nitrosamines certain nitramine-nitrosamine combinations co-eluted from the gas chromatograph.

This has shown the thermal energy analyzer to be subject to false positive responses from a group of compounds which are present in certain widely used herbicides and may be present in other environmental samples. These compounds may be misidentified as N-nitrosamines if mass spectrometry is not used to confirm the N-nitrosamine, or if only high resolution mass spectrometry of the m/e 30 ion is employed as both types of compound are likely to contain this ion.

Analysis of N-nitrosamines and Nitramines in Foods and Herbicides

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Joseph H. Hotchkiss

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Head Department of Food Science and Technology

Dean of Graduate School

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To Kris whose unyielding support and belief makes all things seem possible.

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ANALYSIS OF N-NITROSAMINES AND NITRAMINES IN FOODS AND HERBICIDES

I. INTRODUCTION

N-nitrosamines (NAs) have biological properties which have generated a great deal of interest especially in the area of cancer research. At least 80% of the more than 100 N-nitroso compounds tested were carcinogenic in laboratory animals (Preussman, 1974) and some NAs can induce cancer after a single dose (Terracini and Magee, 1964). In addition to being potent carcinogens, NAs are organ specific, acutely toxic, mutagenic, teratogenic, and transplacental (Magee et al., 1976). While the occurrence of these compounds is undesireable, the significance of the low levels to which humans are exposed is not yet known.

Concern that NAs might be found in human foods resulted from the discovery that ruminants fed herring meal preserved with high levels of the food additive sodium nitrite developed severe liver disorders. Dimethylnitrosamine (DMN) was isolated and shown to be the causitive agent (Ender et al., 1964). It was further proposed that the DMN was formed from the added nitrite and endogenous di- and trimethylamines.

It is now well established that NAs may be present in low levels in certain foods (Scanlan, 1975). Since nitrite is used as a

color fixative, anti-microbial agent, and flavor enhancer in cured meats, the majority of the work on NAs in foods has concentrated on those products. Fried bacon is the most unique of the cured meats in that the highest levels of NAs are consistently found in this product (Scanlan, 1975).

The human population may be exposed to NAs through sources other than foods (Fine, 1978). N-nitrosodiethanolamine has been found in cosmetics (Fan et al., 1977); DMN in air and water in certain industrial environments (Fine et al., 1977); and at least five volatile NAs have been identified in cigarette smoke (Brunnemann et al., 1977). In addition some pesticides contain relatively large amounts of volatile NAs (Ross et al., 1977).

Early analytical methodology was in many cases nonspecific and some early reports on the occurrence of NAs in food are open to question (Crosby and Sawyer, 1976). The use of gas chromatography and nitrogen specific detectors has increased the reliability of the analysis for volatile NAs and within the last five years the Thermal Energy Analyzer (TEA) detector has become available (Fine et al., 1975). This detector is highly specific for the N-nitroso group but may be subject to false positive responses (Gough and Webb, 1978). Because of the possible significance of finding NAs in human food only mass spectrometry (MS) is generally accepted as reliable confirmation.

The use of gas chromatography-mass spectrometry (GC-MS) is not without problems, however. The detection limit of the TEA is 100x to 1000x lower than the mass spectrometer and MS may be subject to both positive and negative false responses (Gough, 1978). Some reviewers have questioned the adequacy of low resolution GC-MS for the confirmation of trace amounts of NAs in foods (Foreman and Goodhead, 1975). In addition, certain authors have stated that the most reliable (Stephany, 1977) or the only reliable (Sen et al., 1976c) method for the unambiguous confirmation of NAs is high resolution GC-MS. Other workers have produced acceptable confirmations by low resolution GC-MS (Fazio et al., 1973; Wasserman et al., 1972). In the only comparison of the two methods on a quantitative basis it was concluded that low resolution GC-MS was satisfactory but less consistent than high resolution GC-MS (Gough et al., 1977a). The GC conditions used may have favored high over low resolution MS. This has little bearing on the use of low resolution GC-MS as a qualitative tool. A subcommittee on NA analysis concluded at a meeting of the International Agency for Research on Cancer that both high and low resolution GC-MS are the best available tools for positive identification of NAs (Bogovski et al., 1972).

The objective of this work was to develop an analytical scheme in which volatile NAs could be quantitated by combined

GC-TEA and their qualitative identity confirmed by full-spectrum low resolution GC-MS. Spectra are presented which demonstrate the reliability of this technique down to a concentration of one μg/kg in a variety of foods. In addition, a second objective was to describe a group of structurally related compounds which occur in some herbicides and elicit a false positive response from the thermal energy analyzer (TEA) detector.

II. REVIEW OF THE LITERATURE

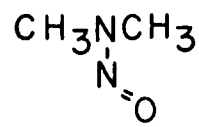
Chemical and Physical Properties

The structures of some N-nitrosamines are given in Figure 1. NAs have one common feature, that being the presence of the N-N=O group. The large number of possible substituents on the amine nitrogen results in a broad range of physical and chemical properties for NAs as a class of compounds. The chemistry of NAs has been reviewed by Fridman et al. (1971).

Fan and Tannenbaum (1972) studied the decomposition of NAs of different structural characteristics; dialkyl, heterocyclic, and α-carboxylic substituted. They concluded the compounds were quite stable but that the introduction of a carboxylic group decreases stability at acidic pHs.

The solubilities and partition coefficients of NAs vary greatly depending on the nature of the substituents. Water solubility for DMN is infinite while dibutylnitrosamine (DBN) is only soluble at 8×10^{-3} moles/liter at 24^{o} (Druckrey et al., 1967). The hexane/water partition coefficients for DMN, DBN, and N-nitrosopyrrolidine (NPyr) are 3×10^{-2}, 117, and 3.6×10^{-2}, respectively. Dichloromethane (DCM) has been the most widely used solvent for the extraction of volatile NAs because of favorable partition

Figure 1. Structure of certain N-nitrosamines: a, dimethlnitrosamine; b, x=1 diethylnitrosamine, x=2 dipropylnitrosamine, x=3 dibutylnitrosamine; c, nitrosopiperidine; d, nitrosomorpholine; e, nitrosoproline, f, nitrosopyrrolidine.



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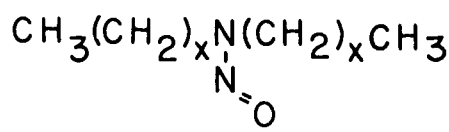

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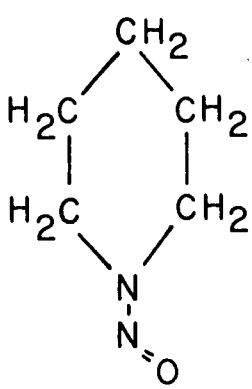

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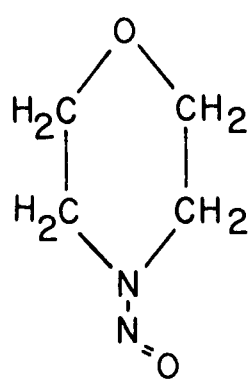

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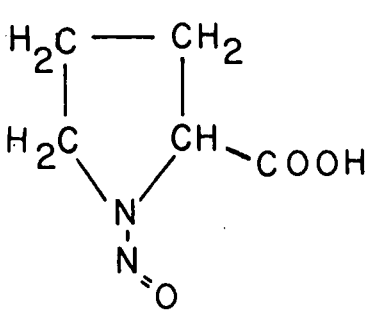

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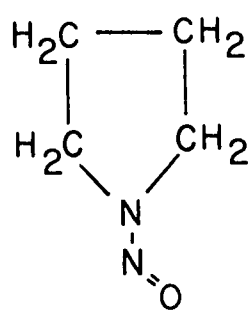


(f)

coefficients. The K values for DMN, DBN and NPyr in DCM/water are 3.5, 150, and 7.6 respectively (Singer et al., 1977). These same authors attempted to correlate structure and biological activity with liposolubility. They found no correlation for non-cyclics but certain cyclic NAs did show correlation.

The infrared absorption spectrum for NAs depends in part on the substituents, but two characteristic bands due to the N-N=O group are seen. In the liquid state NAs absorb near 1448 cm^{-1} due to unassociated N=O stretching and 1065 to 1015 cm^{-1} due to N-N stretching (Colthrup, 1964).

NAs produce characteristic UV absorptions at 230-235 nm and 332-374 nm in water (Fridman et al., 1971). The exact wavelength depends upon the substituents and the solvent (D'Agostino and Jaffe', 1969). These absorptions are not useful for environmental analysis but are useful to determine reaction kinetics (Mirvish et al., 1973).

The partial double bond character of the N-N bond in NAs restricts rotation around this bond. This restricted rotation produces syn and anti geometrical isomerism (March, 1968). This makes protons on the substituent groups magnetically non-equivalent. Syn α-methyl and α-methylene protons are shifted up field by 0.3 to 0.8 ppm (Karabotos and Taller, 1964). In some instances the geometrical isomers are stable enough to allow

separation by liquid chromatography (Iwaoka and Tannenbaum, 1976).

The specific fragments produced by electron impact mass spectrometry of NAs depends to a large extent on the substituents. However, if the NAs are divided into aliphatic and cyclic groups, some generalizations can be made: aliphatic NAs generally show significant molecular ions (M^+); ions at $M-OH^+$ may be present; cleavage between carbons α and β to the N-N=O group and subsequent loss of HNO produces alkyl radicals ($M-RHNO^+$); ions at m/e 30 and 42 are common (Saxby, 1972). Cyclic NAs also show significant molecular ions (M^+); ions at M-17 ($M-OH^+$) may be present but vary widely in abundance; M-30 ($M-NO^+$) is common in higher molecular weight cyclics; ions at m/e 30, 41, 42 are common and of diagnostic value (ApSimon and Cooney, 1971). NPyr fragments somewhat differently than other cyclic NAs; $M-17^+$ is very minor or absent and rather than m/e 70 ($M-30^+$) being significant, m/e 68 ($M-H_2NO^+$) is of greater diagnostic value. Lijinsky et al. (1973), Rainey et al. (1976) and Pensabene et al. (1972) have published complete low resolution spectra of nearly 150 N-nitroso compounds along with tables of relative ion intensities and some physical data. In addition to electron impact spectra, chemical ionization spectra have been published (Fish et al., 1976). Most NAs give intense $M+1^+$ ions using methane

as the reagent gas.

NAs decompose to amine hydrochloride and nitrous acid when treated with boiling HCl (Jones and Urbanski, 1949).

$$R_2NNO \underset{H_2O}{\overset{HCl}{\rightleftharpoons}} R_2NH \cdot HCl + HNO_2$$

Hydrogen chloride is a stronger denitrosating agent than H_2SO_4 (Jones and Kenner, 1933) and hydrogen bromide in acetic acid denitrosates under mild conditions (Eisenbrand and Preussman, 1970). NAs with at least one aromatic substituent undergo a Fisher-Hepp rearrangement when treated with acids producing ring substituted C-nitroso compounds.

NAs also undergo redox reactions. Several reducing agents such as zinc in acetic acid reduce NAs to hydrazines (Fridman et al., 1971). NAs can also be oxidized to the corresponding nitramine (Emmons, 1954).

Formation of Nitrosamines

Several authors have presented detailed discussions of the chemistry of the nitrosation reaction (Ridd, 1961; Ingold, 1969; Challis and Butler, 1968; Mirvish, 1975, 1977).

The products formed by the reaction of an amine $R_XN(H)$ with a nitrosating species NO-X depend upon the degree of subsitution of the amine. The overall reaction can be described

by the following equation:

$$\underset{(H)R}{\overset{R}{\diagdown}}\!\!>N\text{-}H + NO\text{-}X \xrightarrow{a} \underset{(H)R}{\overset{R}{\diagdown}}\!\!>N\text{-}N{=}O \xrightarrow{b} \underset{(I)}{} \underset{(II)}{RN_2^+} \xrightarrow{c} \underset{(III)}{R^+} \xrightarrow{d} \text{deamination products}$$

Secondary amines form stable N-nitrosamines (I) while primary aromatic amines form stable diazonium salts (II). Primary aliphatic amines deaminate, forming a carbonium ion (III) which may eliminate, react with solvent or other nucleophiles, or rearrange (March, 1968). Tertiary amines also form stable NAs but only after the formation of a secondary amine which then reacts as shown above.

Except at very high acidities the nitrosation step (a) has been demonstrated to be rate controlling. Kalatzis and Ridd (1966) have demonstrated that the primary amine aniline and the secondary amine N-methylaniline have the same kinetic equation and very similar reaction rates. Similarities in reaction rates can also be demonstrated for diazotisation and deamination (Challis and Butler, 1968).

Hughes et al. (1958) have demonstrated the overall rate equation for aqueous nitrosation (diazotisation) to be third order overall at moderate acidities.

$$\text{rate} = k_1 [R_2NH] [HNO_2]^2 \qquad (1)$$

The rate is second order in nitrous acid because it is not the direct nitrosating species but must first be converted to one or more of the following nitrosating species.

$ON\text{-}NO_2$	nitrous anhydride
$ON\text{-}X$	nitrosyl halide
$ON\text{-}OH_2^+$	nitrous acidium ion
ON^+	nitrosonium ion

In the absence of high acidities and halide ions, nitrous anhydride is the major nitrosating species (Ridd, 1961). The overall nitrosation reaction can be described by the following equations:

$$2(HNO_2) \overset{\text{fast}}{\rightleftharpoons} N_2O_3 + H_2O \qquad (2)$$

$$N_2O_3 + R_2NH \overset{\text{slow}}{\rightarrow} R_2NN = O \qquad (3)$$

As is suggested by the rate equation given above (1) nitrosation rate is pH dependent. Mirvish (1970) demonstrated that the pH optimum for nitrosation of dimethylamine was 3.4 and the rate decreased as the pH was changed in either direction. Two factors operate with opposing effects on the nitrosation rate:

i) the pH dependent equilibrium between NO_2^- and HNO_2 (pka = 3.35) and the formation of the nitrosating species which is favored by high acidities (equation 2).

ii) the concentration of free unprotonated amine which is favored by low acidities.

For a given amine the rate will decrease as the pH is raised above the maximum rate pH due to a decrease in HNO_2 concentration. The rate will also decrease as the pH is lowered below the maximum rate pH due to protonation of the amine. Amine basicity can also effect the rate of product formation at a given pH; the concentration of free amine being greater for weakly basic amines (diphenylamine $Pk_a = 0.79$) than for strongly basic amines (dipropylamine, $Pk_a = 10.9$).

Primary amines can also form NAs in very low yield through the attack of the carbonium ion formed by deamination on an unreacted amine (Ender and Ceh, 1971). In the case of primary diamines, heterocyclic nitrosamines can be formed (Warthesen et al., 1975).

Tertiary amines can also form NAs in the presence of nitrous acid but with less efficiency than secondary amines. Ohshima and Kawabata (1978) found the mechanism for the formation of DMN from trimethylamine and trimethylamineoxide to be temperature dependent. They suggested that nitrosation at 100°C involves a dealkylation of the tertiary amine and results in a secondary amine which is in turn nitrosated. At lower temperatures a different mechanism may be involved.

In a series of papers, Challis and coworkers have shown that dialkylamines can be nitrosated by at least two oxides of nitrogen in aqueous alkaline medium. It has been known for sometime that nitrous anhydride (N_2O_3) and dinitrogen tetroxide (N_2O_4) will nitrosate amines in organic solvent (White and Feldman, 1957). Challis and Kyrtopoulos (1977) reacted piperidine (2×10^{-3}M) dissolved in 0.1 M NaOH with both N_2O_4 and N_2O_3 at 25°C. The concentration of oxides varied between 0.82×10^{-2} and 4.82×10^{-2} M for N_2O_4 and 1.94×10^{-2} and 7.20×10^{-2} for N_2O_3. Yields of N-nitrosopiperidine ranged from 8.3 to 44% for N_2O_4 and 25.8 to 68.2% for N_2O_3 after 5 min. The N-nitro derivative of the amine was also formed along with but in lower yields than the nitrosamine. The reaction was also attempted in pH 6.85 buffer but neither the N-nitroso nor N-nitro derivatives were formed. This implies that only unprotonated amine reacts.

The nature of the nitrosating species is less clear. The authors point out that 2×10^{-3} M amine competes effectively as a nucleophile with 55.5 M H_2O and 0.1 M OH^-. This suggests that it is not the anhydride form of the nitrogen oxides that nitrosates but rather some other form which is less susceptable to base hydrolysis. Also, the mechanism must explain formation of both the N-nitroso and N-nitro products. In earlier work, the authors suggest a free radical mechanism in which an amino radical is

formed and further reacts with either an $NO^{\cdot}$ or $NO_2^{\cdot}$ radical (Challis and Kyrtopoulos, 1976). Recently the authors have suggested that tautomeric forms of the nitrogen oxides ($ON\text{-}NO_2 \rightleftharpoons ON\text{-}ONO$; $O_2N\text{-}NO_2 \rightleftharpoons ON\text{-}NO_3$) react with amines via a one-step four-center transition state to form both products (Challis et al., 1978). Neither mechanism has been confirmed.

Nitrogen oxides are end products of combustion and can be produced from sulphuric acid and aqueous sodium nitrite or by the reduction of nitric acid (Haworth and Hey, 1940). Thus, the formation of N-nitroso compounds under alkaline conditions might explain the occurrence of N-nitrosamines in some foods and herbicides.

Anions of certain acids can catalyze the nitrosation reaction by forming the more reactive NO-X species. Fan and Tannenbaum (1973) have shown thiocyanate anion, which is present in saliva, to effectively increase the rate of nitrosation of morpholine at pH 2.0. Halides and certain other anions are also catalytic but to a lesser extent than thiocyanate (Mirvish, 1975). This catalysis is pH dependent; being more pronounced at low pH values. Hildrum et al., (1975) has demonstrated that Cl^- is catalytic at low pH but is inhibitory above pH 2.5.

Keefer and Roller (1973) have shown that formaldehyde can catalytically nitrosate amines in the pH range 6.4 to 11.0. The

authors suggested this method for synthesis of NAs containing acid labile groups. The catalytic nature of aldehydes in general might explain some environmental occurrences of NAs.

Keefer (1976) has also investigated the role of metal complexes in NA formation. Biological iron is capable of forming nitrosyl derivatives which, it was suggested, might act as nitrosating agents. The role, if any, of metals in forming NAs environmentally is not known.

Inhibition of nitrosation has generated a great deal of interest. The majority of interest has focused on substances which effectively compete with amines for nitrosating agent through redox reactions.

Ascorbate and isoascorbate have been the most widely studied inhibitors (Douglass et al., 1978). Under acidic conditions ascorbate reduces nitrite to nitric oxide (NO) which can in turn be air-oxidized to the nitrosating species N_2O_4, hence, the need for excess ascorbate to affect inhibition (Dahn et al., 1960). Mirvish et al. (1972) reacted five amines and one amide with nitrite at various pHs, with and without ascorbate. Morpholine and piperazine nitrosation was better than 98% inhibited at a 2:1 ratio ascorbate:nitrite. The authors recommended that ascorbate be incorporated in certain drugs and foods, a recommendation which has been adopted for bacon (Federal Register 43:20992, 1978).

Mottram and Patterson (1977) have shown ascorbate to be catalytic in a biphasic system of aqueous buffer and nonpolar solvent. Sodium ascorbate increased the formation of DPN and NPyr 5 to 25x over ascorbate free controls. The authors theorized that nitrogen oxides were formed from the reaction of ascorbate and nitrite in the aqueous phase and transferred to the lipid phase where nitrosation took place. The inhibition seen in aqueous systems was due to hydrolysis of the nitrogen oxides by water. The lipophilic substance ascorbyl palmitate greatly reduced nitrosation, presumably by not producing nitrogen oxides and by reacting with any that did migrate to the lipid phase. Sen et al. (1976b) also found lipophilic inhibitors to be more effective than sodium ascorbate. Propyl gallate and ascorbyl palmitate were more inhibitory when sprayed on bacon just prior to frying than was sodium ascorbate.

Inhibition by ascorbate has been demonstrated by in vivo carcinogenicity tests. Concentrations of ascorbate in the food of 11.5 or 23.0 g/kg reduced the incidence of lung adenomas in mice fed piperazine, morpholine, or methylurea with nitrite by 89 to 98% (Mirvish et al., 1975). This effect was attributed to a blocking of NA formation rather than a direct inhibition of the action of NAs in vivo.

Primarily in model systems, other compounds have been shown to inhibit NA formation. Several of these compounds are naturally present in certain foods. Phenolic compounds act as inhibitors of nitrosation but under some conditions may be catalytic (Challis and Bartlett, 1975). Gray and Dugan (1975) found that the thiols cysteine and glutathione inhibited nitrosation to varying degrees depending on the pH of the aqueous system. Massey et al. (1978) compared the effects of ascorbic acid, cysteine and p-cresol on the rate of pyrrolidine nitrosation in both aqueous and protein-based pH 5.25 model systems. All three compounds reduced the rate of nitrosation; ascorbic acid showed the greatest effect and p-cresol the least. The effects were attributed to competitive formation of C- and S-nitroso compounds, reducing the available nitrite.

The role of other antioxidants in NA formation has been studied. Pensabene et al. (1978) concluded that α-tocopherol can reduce NPyr formation in model food systems. Astill (1978) studied the role of phenolic antioxidants on NA formation in foods as well as in the stomach. Experiments with rats showed ascorbate and gallic acid inhibited DMN formation in vivo while butylated hydroxytoluene and butylated hydroxyanisole had no effect. A small inhibition was seen with tertbutylhydroquinone.

Other compounds have been investigated as NA formation inhibitors. Williams (1978) studied the effectiveness of sulfamic acid and ascorbic acid as nitrite traps in sulphuric acid catalyzed denitrosation of N-nitroso-N-methylaniline. Ascorbic acid was a more effective trap, increasing in effectiveness as acidity decreased. The food additive sorbic acid demonstrated an inhibitory effect equal to ascorbic acid for inhibition of DMN formation in vitro (Tanaka et al., 1978). However, inhibition by sorbic acid was considerably less than by ascorbic acid for nitrosomorpholine formation and totally absent for N-methylaniline nitrosation.

In addition to the chemistry discussed above, three lines of evidence indicate that NAs may be formed in vivo when appropriate precursors are ingested. Sen et al. (1969) incubated gastric juice from rats, rabbits, cats, dogs and man containing added diethylamine and sodium nitrite at 37°C for 20 to 60 min in vitro. The amount of diethylnitrosamine (DEN) produced varied with pH; the lower pH gastric juices produced more DEN. However, all gastric juices produced some DEN. Sander et al. (1968) demonstrated the synthesis of NAs in vivo in rats when treated with nitrite and methylbenzylamine. The amount of NA formed was proportional to the basicity of the amine. The third and perhaps strongest line of evidence comes from the observation of metabolic, toxic and

carcinogenic effects of NAs when nitrite and amines are concurrently administered to laboratory animals (Magee et al., 1976).

Formation in Foods

While it is firmly established that NAs do occur at low levels in certain foods (Scanlan, 1975; Crosby, 1976), their exact origin is not known. Because NAs are not endogeneous to living matter and not intentionally added to foods, evidence indicates that in most cases they are formed either in processing or preparation of the food for consumption. An exception to this might be the curing of meats with premixed curing salts and spices which may contain preformed NAs (Sen et al., 1973a). A great deal of work has been directed towards the occurrence of precursor amines and nitrites and the mechanism of NA formation in food systems.

Nitrate is widely dispersed in nature, used as a fertilizer and is concentrated by certain edible plants. Nitrate is of low toxicity and forms NAs only indirectly. Nitrite is an intermediate in the biological reduction of nitrate to ammonia and hence is in very low concentration in biological materials (Walker, 1975). Nitrites oxidize hemoglobin from ferrous to ferric (methemaglobin) in vivo and are acutely toxic because methemaglobin will not reversably bind oxygen. Nitrite can react with amines to form NAs as discussed above and is considered a direct precursor of NAs in foods.

In addition, nitrite itself may be a carcinogen (Food and Drug Administration, 1978).

The use of nitrate and nitrite as intentional food additives has been practiced for many centuries (Binkerd and Kolari, 1975). It is now known that nitrite is the active biostatic ingredient and that nitrate serves only as a reservoir of nitrite through bacterial reduction. Nitrite serves three functions in meats: color fixation; cured-flavor development; anti-microbial (Engle, 1977). Nitrite is an effective inhibitor of outgrowth and toxin production by Clostridium botulinum under proper conditions (Collins-Thompson et al., 1974; Ingram, 1974).

Nitrite intentionally added to food may account for only a fraction of mans exposure to nitrite (White, 1976). The major human exposure to nitrite appears to come from the reduction of endogeneous salivary nitrate by oral microflora (Tannenbaum et al., 1974). Human exposure to nitrate and nitrite may be greater than the dietary intake due to synthesis in the human digestive tract (Tannenbaum et al., 1978).

Amines are ubiquitous in biological materials and are involved in metabolism (Crosby, 1976). Foodstuffs have been surveyed for volatile amines and free amino acids to determine the extent to which these possible NA precursors occur. The majority, but not all, of this work has been with meat and fish products.

Patterson and Mottram (1974) followed the concentration of volatile amines in pork from immediately post-slaughter through curing and cold storage. The dimethylamine (DMA) content of the tissue was 135 μg/kg fresh and increased to 348 μg/kg after 43 days storage at 1 °C. Except for methylamine, the other volatile amines also increased in concentration. Miller et al., (1973) found up to 150 mg/kg DMA in fish protein concentrate and Gruger (1972) found 1.1 to 6.6 mg/kg DMA in stored frozen salmon. Trimethylamine (TMA) values were slightly higher than DMA values. Neurath and Schreiber (1974) surveyed a number of products including fish products, cheeses, barley, malt, hops, coffee products, tea, wine, and water for several volatile amines. Thirty-seven amines were tentatively identified, most in the 0.1 to 1.0 mg/kg range. Singer and Lijinsky (1976) have analyzed foodstuffs for secondary amines by GC-MS after forming the tosylamide derivatives. Nitrosatable secondary amines were found in fish, meats, and beverages. The concentration of DMA in fish was highest at 740 mg/kg with most other amines in the range of 0.1 to 10 mg/kg. As pointed out by the authors, finding morpholine in nearly all samples is surprising in light of the fact that morpholine is thought not to be found in nature.

Certain naturally occurring free amino acids and polyamines can also be precursors of NA formation. Lijinsky et al. (1970)

synthesized the N-nitroso derivative of several amino acids and Mirvish et al. (1973) demonstrated through kinetic studies that amino acids having a secondary amino group can be nitrosated under conditions similar to food storage or preparation. Warthesen et al. (1975) demonstrated low yields of NAs from primary diamines and others (Hildrum, 1975; Hotchkiss et al., 1977) have identified at least nine volatile and nonvolatile products resulting from nitrosation of the polyamine spermidine.

Nitrite cured fried bacon is unique in that it consistently contains appreciable amounts of the carcinogen N-nitrosopyrrolidine (NPyr). Consequently, most studies concerning the mechanism of formation of NAs in foods have either used bacon directly or have used model systems intended to simulate bacon. The formation of NAs in bacon has been reviewed (Gray, 1976).

The amount of NPyr formed in fried bacon correlates with the amount of added nitrite. Sen, et al. (1974) prepared bacon with 0, 50, 100, 150, and 200 mg/kg levels of nitrite and analyzed for DMN and NPyr after frying. NPyr values increased with increasing initial nitrite concentration. The NPyr values did not, however, correlate to residual nitrite concentrations at the end of six weeks storage at -20°C. The authors suggest that the precursor to NPyr is an intermediate nitroso compound which is produced early in the curing of bacon. Gough and Walters (1976) have also

found that increasing initial nitrate/nitrite levels produced increasing amounts of NPyr in bacon. They did not report nitrite levels at the time of frying, however.

Other workers have focused on the organic precursor(s) of NPyr. Several amines naturally present in tissue have been shown to produce small amounts of NPyr when treated with nitrite in model systems. Bills et al. (1973) demonstrated the formation of NPyr from N-nitrosoproline (NPro), pyrrolidine, spermidine, proline, putrescine. The yields were 2.6, 1.0, 1.0, 0.4, and 0.04% respectively. Glutamic acid, glutamine, and hydroxyproline did not produce NPyr. Huxel et al. (1974) showed collagen to be a potential precursor, but only after heating for 2 hr. Others have discounted the role of collagen (Patterson et al., 1976).

Current work indicates that free proline is the most likely precursor of NPyr in fried bacon (Gray, 1977). Hwang and Rosen (1976) using ^{14}C-labeled amines found that proline produced greater yields of NPyr than did spermidine or putrescine in fried bacon. NPro produced the largest yields and the authors suggest that proline is nitrosated then decarboxylated during frying. Gray and Collins (1977) found the free proline content of whole green pork bellies was 11.8 μM/100g tissue and increased to 35.6 μM/100g tissue after 28 days storage at 2°C. Coleman (1978), working with rendered bacon fat and with bacon, found that the addition of free

proline greatly increased the yield of NPyr upon heating to 170°C for 10 min.

The mechanism by which NPyr is formed from proline is not clear. Because NPyr is not found in raw bacon but is consistently found in fried bacon, a thermally induced mechanism is likely. Two mechanisms have been proposed (Gray, 1976). Lijinsky and Epstein (1970) proposed that proline may be first nitrosated then subsequently decarboxylated. The observation of Sen et al. (1974) that NPyr formation correlates to initial but not residual nitrite would support a mechanism where NPro is formed during curing then decarboxylated during frying. Model system work has indicated NPro is thermally decarboxylated in systems similar to frying bacon (Pensabene et al., 1974). Hwang and Rosen (1976) added ^{14}C-NPro to bacon and recovered ^{14}C-NPyr after frying. The yield of NPyr was greater from NPro than from proline and nitrite. Gray and Collins (1978) rehydrated freeze-dried bacon with water containing nitrite and proline or NPro. After heating for 12 minutes at temperatures between 100 and 200°C., they found 0 to 0.33% of the proline and 0 to 2.18% NPro converted to NPyr. Using a 0.33% conversion of proline to NPyr and a free proline content of 23.8 μM these authors concluded that 90 μg/kg NPyr would be produced in fried bacon.

Evidence has been presented in support of a nitrosation followed by decarboxylation mechanism. Kushnir et al. (1975) assayed NPro in raw bacon and found values between 0.38 and 1.18 mg/kg. Gray and Collins (1978) found similar levels, but only in bacon to which 1000 mg/kg nitrite had been added. These authors concluded that at the conversion rates they demonstrated (see above) this was an insufficient amount of NPro to account for the NPyr found.

Hansen et al. (1977) disputed the role of preformed NPro in NPyr formation. They assayed raw bacon for NPro by two independent chromatographic methods and found concentrations of 10 to 80 μg/kg. As they point out, these values are too low to account for the total amount of NPyr in bacon, the fried-out fat, and the vapors. Baker and Ma (1978) also assayed cured meats for NPro and also concluded the level of preformed NPro was too low to account for all NPyr formed. Janzowski et al. (1978a) also found NPro in raw bacon but at a lower concentration than reported by Kushnir et al. (1975). Eight samples contained an average of 30 and a high of 88 μg/kg. These authors concluded that decarboxylation of nitrosoamino acids is not mandatory in the formation of NPyr during frying. The fact that cured meats do not contain sufficient amounts of preformed NPro does not rule out the possibility that both the nitrosation of proline and the subsequent

decarboxylation occur during frying.

Gray (1976) mistakenly reports a pK_b value of 12.0 for proline in support of this mechanism. The pK_b = 12.0 would be for the -COOH group; the pK_b for the amine group is 3.36 (Mirvish et al., 1973). This makes the amino group of proline only slightly less basic than pyrrolidine (pK_a = 11.3).

The second mechanism for the formation of NPyr from proline holds that proline is first decarboxylated during frying, then subsequently nitrosated. Less study of this mechanism has been undertaken but evidence for it does exist. Ender and Ceh (1971) reacted proline with nitrite in a dry starch matrix for two hours at 130°, 150° and 170°C. NPyr was produced at 3.47, 18.7 and 72.5 mg/g proline. They suggested that the amino acid is first decarboxylated then nitrosated but offer no evidence for this mechanism. Nakamura et al. (1976) could not isolate NPro from raw bacon at ten μg/kg but did find pyrrolidine in the range of 30 to 110 μg/kg. After frying at 200°C for 10 minutes the level of pyrrolidine in the bacon increased more than ten fold. They also demonstrated a yield of 2.01% NPyr from pyrrolidine and nitrite added to raw bacon and subsequently fried at 175°C. After repeating the experiments with NPro, the authors concluded that in the temperature range of 175 to 200°C formation of NPyr is via decarboxylation of proline to pyrrolidine which is subsequently

nitrosated. Below 150°C the authors suggest that both mechanisms may be operative but with very low yields. Recently Coleman (1978) has supported this mechanism. Rendered pork fat was heated to 170°C for 1.5 h and assayed for pyrrolidine. The heated fat increased five fold in pyrrolidine content over the unheated fat. In addition, NPro when heated in aqueous methanol to 170°C in a sealed tube gave 10 to 100x greater yields of Pro and pyrrolidine than NPyr.

Whatever the exact mechanism, NPyr appears to occur only in the fat portion of fried bacon. Fazio et al. (1973) found NPyr in fried bacon and the cooked out fat but not in fried ham. They speculated that NPyr was held in the fat due to its fat solubility while it was volatilized from the fried ham. Fiddler et al. (1974) could not detect NPyr in the lean portion of bacon when fried separately from the fat. NPyr was found in the fried fat solids and fried-out fat. Mottram et al. (1977) and Coleman (1978) observed a similar preference for formation in the adipose portion. Different authors offer different explanations. Fiddler et al. (1974) suggested the chemical precursors exist in the fat but not the lean portions. Mottram et al. (1977) suggested the precursors are in both lean and fat but the nonpolar lipid environment is more condusive to NPyr formation. They found freeze-dried lean did produce NPyr when rapidly heated in corn oil. Coleman (1978)

has found that pyrrolidine is not produced upon heating proline in aqueous solution, but that when heated in the less polar solvent tetralin (1, 2, 3, 4 tetrahydronaphthalene) decarboxylation does occur. Scanlan (1975) suggested that the preferential formation in the fat may be due to the fat reaching higher internal temperatures than the lean. Using thermocouples both Mottram et al. (1977) and Coleman (1978) have confirmed that fat may reach an internal temperature of 50°C greater than lean.

Dimethylnitrosamine (DMN) also occurs in fried bacon but its mechanism has been less studied. Gray et al. (1978) studied several possible precursors including DMA, TMA, sarcosine, and choline chloride. Sarcosine, in their system, proved to be the more likely precursor.

Occurrence of Nitrosamines in Foods

NAs have been shown to occur sporadically in a variety of foodstuffs in low μg/kg amounts. With the exception of fried bacon, the presence of NAs in food is neither consistent nor always associated with the use of nitrite as a food additive. Scanlan (1975) has reviewed much of the literature on the occurrence of NAs in foods through 1974 and Crosby (1976) has reviewed foods by product type.

When perusing the literature on the occurrence of NAs in foods, changes in specificity and sensitivity of analytical procedure must be understood. Lack of specificity prior to 1970 has brought some positive reports into question and recent improvements in sensitivity may bring some negative findings into question.

While the occurrence of NAs has been overall unpredictable, certain generalizations can be made. The first is that DMN followed by NPyr are the most commonly found NAs in foods. Gough et al. (1977b) examined nearly 500 foods for volatile NAs at a sensitivity of one μg/kg. Only four samples contained volatile NAs other than DMN or NPyr while more than 80 samples contained one or both of these NAs.

A second generalization can be made concerning the levels of NAs in foods. Eisenbrand et al. (1978) examined a number of cured meat products for volatile NAs and found NAs to occur in one-third of the samples. DMN, NPyr, and N-nitroso piperidine were found in concentrations of 1-10, 2-17, and 2-66 μg/kg, respectively. Values of one to six μg/kg were found in 20 of 168 cheeses analyzed. Groenen et al. (1977) analyzed several cooked and raw meat products for volatile NAs and found DMN to be the most widely occurring NA at 0.1 to 15.5 μg/kg. Their sensitivity for NPyr was 10 μg/kg, 100x less than for DMN. A survey of the literature shows that with the exception of fried bacon, the

volatile NA content of foods is usually in the low μg/kg range.

While volatile NAs have been reported in a variety of foods, the majority of positive findings have been in cured meat products (Sen et al., 1976). Havery et al. (1976) surveyed 121 products including meat, dairy, and fish products. Only fried bacon contained NAs at a level greater than 10 μg/kg. Using a less specific but more sensitive method of analysis Gough et al. (1978) concluded, after analyzing a wide variety of foodstuffs, that cured meats represented the major source of volatile NAs in foods.

Some fish samples, both fresh and cured/cooked, have been shown to contain low levels of volatile NAs. Iyengar et al. (1976) surveyed 29 raw and cooked fish products for volatile NAs. Fifteen were positive, although some results were too low a level to confirm by other means. Havery and Fazio (1977) surveyed 78 fish samples and found none to contain volatile NAs above the 10 μg/kg detection limit.

Some cheeses have been shown to contain low μg/kg amounts of volatile NAs (Sen et al. 1978). It does not appear that the occurrence is related to the practice of adding nitrate to certain cheeses (Stephany et al., 1978).

Volatile NAs have also been reported in other foods such as fruits, soups and pasteries (Gough, 1978). The levels reported are too low (< 1 μg/kg) for confirmation and are unsubstantiated.

Because of difficulties in methodology, nonvolatile NAs in foods have not been studied in detail. Sen et al. (1978) and Eisenbrand et al. (1978) recently described methodology for nitroso amino acids in foods. Lee et al. (1978); Sen et al. (1977); Eisenbrand et al. (1978) reported the occurrence of N-nitroso-3-hydroxypyrrolidine in fried bacon.

Occurrence of Nitrosamines in Pesticides

Several workers have postulated that under proper conditions certain pesticides might form or be associated with N-nitroso compounds. Elespuru and Lijinsky (1973) reacted several agricultural type alkylureas and alkylcarbamates with nitrite at pH 3.5 and 37°C. Both dialkylnitrosamines and alkylnitrosamides were produced in significant yields. Eisenbrand et al. (1974) demonstrated the in vivo formation of DMN when rats were given a dithiocarbamate type fungicide and nitrite by stomach tube. Yields were as high as 0.9% of theoretical after 15 minutes. Sen et al. (1974a) has also demonstrated in vivo nitrosation of pesticides in the guinea-pig. The dinitroaniline based herbicide butralin (4-(1,1-dimethylethyl)-N-(1-methylpropyl)-2,6-dinitrobenzenamine) has been converted into its N-nitrosamine derivative in a soil high in nitrite and shown to persist for up to 6 months (Oliver and Kontson, 1978).

Recently, certain commercially available herbicides were shown to contain large amounts of NAs (Ross et al., 1977). The largest amounts of NA were present in samples of 2,3,6-trichlorobenzoic acid which was formulated as the dimethylamine salt. This product contained DMN up to a concentration of 640 mg/L (0.06%). The dinitroaniline type herbicide trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) contained 154 mg/L dipropylnitrosamine (DPN). Fan et al. (1978) demonstrated that a wettable powder formulation of atrazine contains the nonvolatile NA N-nitrosodiethanolamine.

A more comprehensive study by the U.S. Environmental Protection Agency (Cohen et al., 1978) revealed that NAs may be present in commercially available formulations of several pesticides. Each of 25 sample pesticides was analyzed by two independent laboratories for six NAs; both volatile and nonvolatile. Eight samples were shown to contain NAs. A trifluralin sample taken before a manufacturing process adjustment contained 188 mg/L DPN. After the adjustment, another sample contained 22 mg/L, demonstrating that the NA content of this herbicide can be lowered through manufacturing changes. Some samples were reported to contain extraneous peaks in the chromatograms which might indicate the presence of other NAs.

Analysis of Foods for Nitrosamines

The fact that NAs occur in foods at concentrations in the low μg/kg range makes their analysis difficult. This is further complicated by the heterogeneous nature of most foods. The problems encountered in the analysis have necessitated a multi-step approach: isolation from the food; cleanup and concentration; separation; detection and quantification; confirmation. The analysis of foods for NAs has been reviewed (Scanlan, 1975; Crosby and Sawyer, 1976; Eisenbrand, 1974).

Isolation

Distillation and direct extraction are the two most common means of isolating NAs from foods; the former is most often employed for volatile NAs while the later for nonvolatile NAs. Direct extraction has also been used for analysis of liquids such as water (Fine et al., 1977) and alcoholic beverages (Castegnaro et al., 1974). Dichloromethane (DCM) has been the most widely used solvent for direct extraction by blending (Sen, 1972) or by Soxhlet extraction (Eisenbrand, 1972). Hot water has also been employed as a direct extracting solvent (Ceh and Ender, 1978).

Direct solvent extraction has found widest use in analyzing nonvolatile NAs. Lee et al. (1978) extracted 3-hydroxy-N-

nitrosopyrrolidine from bacon with water:methanol (3:2) while Sen et al. (1978) used acetonitrile. NPro has been isolated from raw bacon by extracting with cold water (Kushnir et al., 1975; Hansen et al., 1977; Baker and Ma, 1978) and acetone:water (7:3) mixtures (Janzowski et al., 1978). Fan et al. (1978) used both DCM and acetone:water (2:1) as direct extractants in an analytical scheme designed to encompass several classes of NAs. Fine et al. (1976) extracted foods low in lipids directly with DCM and lipid containing foods with acetonitrile to analyze for nonionic nonvolatile NAs.

Distillation, both atmospheric and vacuum, has been widely employed in the analysis of foods for volatile NAs. Eisenbrand et al. (1970) distilled 16 volatile NAs from acidic, neutral, and basic solutions, under atmospheric and reduced pressures. Their results indicate comparable recoveries by all methods, however, acidic conditions may lead to artifact formation. Telling et al. (1971) found vacuum distillation of NA spiked ham more efficient than atmospheric distillation and later reported improvements (Telling et al., 1974) in their procedure giving recoveries ranging from 55 to 90% for five dialkyl NAs.

Fazio et al. (1972) described a method for the simultaneous isolation of nine volatile NAs from fish and meat products. The method included a predigestion of the food in methanolic potassium

hydroxide and a continuous DCM extraction of the digestant. Aqueous sodium hydroxide is added to the DCM and the solvent is distilled off. The remaining aqueous phase is then distilled. This multidetection method has been widely used and is currently used by the U.S. Food and Drug Administration.

Another distillation method which has been adopted by some laboratories was first described by Goodhead and Gough (1975). The sample to be analyzed is slurried with water and salt and subsequently distilled at atmospheric pressure. The distillate is then subjected to extraction, cleanup and concentration. Recoveries from meat for six NAs (ten μg/kg) ranged from 65 to 81% for NPyr and DPN, respectively. The standard deviation for the recovery of NPyr in seven determinations was 32.9, indicating a lack of precision for this NA.

Fine et al. (1975b) described a rapid and simple distillation technique which has found wide application in the analysis of foods for volatile NAs. Distillation is carried out by placing a small sample (20 g) in a rb flask along with an equal amount of mineral oil. Vacuum (< 1 mm Hg) is applied and the contents slowly heated to 110°C. The distillate is trapped in a vapor type trap immersed in liquid N_2. After reaching temperature the vacuum is discontinued, the distillate thawed, extracted and concentrated. The authors report recoveries of 71 to 75 and 100% for DMN and NPyr

in tuna fish at the 5 μg/kg level, respectively. Sensitivity of 10 to 50 ng/kg was claimed but no work was presented to support this extreme sensitivity.

Havery et al. (1978) compared the multidetection method (Fazio et al., 1972) with a modified mineral oil distillation method for the recovery of 14 volatile NAs. The multidetection method averaged 75% recovery while the mineral oil distillation averaged 92%. Additionally, 106 meat samples were analyzed by both methods. Both methods were in general agreement, with the mineral oil distillation giving slightly higher results.

Cleanup and Concentration

The lack of a detector which responds exclusively to NAs and the complex nature of most foods has necessitated the use of cleanup procedures to remove interfering compounds from the food extracts. Most often cleanup has been by extraction and/or column chromatography. Other procedures such as distillation from acidic and basic solutions (Eisenbrand, 1972) and derivatisation of the NAs (Sen et al., 1978; Sen, 1970) have had limited use.

Nearly all methods use extraction at some point for cleanup. In the multidetection method (Fazio et al., 1972) the aqueous distillate was acidified and extracted with DCM, the DCM was then extracted with aqueous base to remove basic and acidic components,

respectively. Goodhead and Gough (1975) also extracted NAs from the acidic distillate with DCM. The DCM was then washed with aqueous base. Eisenbrand et al. (1969) partitioned the NAs between acetonitrile and heptane as a method of separating NAs from neutral lipids. Nonvolatile NAs have been cleaned up by extracting with various solvents after pH adjustment (Eisenbrand et al., 1978; Fan et al., 1978).

Column chromatography of both aqueous and solvent extracts has been widely used. Fazio et al. (1972) used silica gel and eluted the NAs with DCM. Others (Eisenbrand et al., 1978) have used basic alumina and still others have used both adsorbents in the same procedure (Essigmann and Issenberg, 1972). Other adsorbents such as polyamide resins (Sen, 1972) and Celite 545 (Howard et al., 1970) have been used. N-nitroso amino acids have been cleaned up on cellulose (Eisenbrand et al., 1978), acidic alumina (Sen et al., 1978) and anion-exchange resin (Hansen et al., 1977).

Because of the very low concentrations in which NAs have occurred, concentration is necessary. Most often concentrations of 100 to 1000x can be achieved for volatile NAs by evaporating the volatile solvent in a Kuderna-Danish evaporator fitted with a Snyder column. Final concentration is usually under a stream of N_2. Nonvolatile NAs may be concentrated by removing water under vacuum (Hansen et al., 1977).

Separation of Nitrosamines

Several types of chromatographic procedures have been employed in the separation of the food extract after cleanup and concentration. One of the simplest but less sensitive methods is thin-layer chromatography (TLC). Sen and Dalpe' (1972) described a system for the separation of volatile NAs by TLC and Gray et al. (1977) used TLC to purify hydroxy-heterocyclic NAs.

Gas chromatography has been used extensively in the separation of volatile NAs. Fazio et al. (1972) separated nine volatile NAs on a glass 9 ft. x 4 mm i.d. 10% Carbowax 1540 + 5% KOH column, programmed 80 to 180°C at 5°C/min and later Havery et al. (1978a) extended this to 14 volatile NAs. Gough and Sugden (1975) described a Carbowax 20M system in which a 1.6 M x 1.8 mm i.d. packed column was connected in series with a 30M x 0.5 mm i.d. support coated open tubular (SCOT) column. Microvalving allowed the solvent to be vented when large column injections (five μL) were made, while still allowing for optimum performance of the SCOT column. Essigmann and Issenberg (1972) described a similar Carbowax 20M system except that a liquid N_2 trap was used to collect the NAs from the precolumn after the solvent was vented. The trap was then warmed and the NAs reinjected onto a 0.5 mm i.d. x 160M open tubular column.

While Carbowax has been the most popular liquid phase for volatile NAs, others have been used. Fine and Rounbehler (1975) used packed 15% FFAP columns and Eisenbrand et al. (1978) used the similar liquid phase Carbowax 20M-terephthalic acid terminated at the same loading. Pensabene et al. (1972) reported retention times for volatile NAs on this phase. Other liquid phases have been reported: Ucon 50 HB (Groenen et al., 1976); OV-101 (Stephany et al., 1976); Chromosorb 103 (Freed and Mujsce, 1977). Derivatisation of volatile and nonvolatile NAs has necessitated the use of less reactive liquid phases such as silicone oils (Janzowski et al., 1978).

High-performance liquid chromatography (LC) has been increasing in use since it was first reported for separating NAs (Cox, 1973). Iwaoka and Tannenbaum (1976) described a system which was capable of separating syn and anti conformers of NPro. Both Hansen et al. (1977) and Baker and Ma (1978) analyzed raw bacon by LC for the nitrosoamino acid NPro. Both groups used reverse-phase chromatography on μ-Bondapack-C_{18} columns. Fan et al. (1978) described an analytical procedure involving LC of both volatile and nonvolatile NAs and Fine et al. (1976) separated nonionic nonvolatile NAs on a μ-Porasil column.

Detection and Quantification

The methods available for detection and quantification and their degree of specificity are in part determined by the procedure used to separate the NAs. Sen and Dalpé (1972) described the use of Griess and ninhydrin reagents with TLC to detect nitrite and amine, respectively. UV irradiation photolytically cleaved the N-N bond of the N-nitroso group after separation on the TLC plate. Young (1978) quantitated the fluorescence formed when irradiated NAs, which had been separated by TLC, were reacted with fluorescamine. Detection limits of 10 to 15 ng were claimed. Cross et al. (1978) have presented a similar procedure.

Photolylic cleavage and reaction of the nitrite formed with Griess reagent has been used in an automatic colorimetric device for the determination of N-nitroso compounds (Fan and Tannenbaum, 1971). Walters et al. (1974) used this colorimetric reaction to determine the total nonvolatile NA content of foods after nitroso cleavage by thionylchloride and flushing the nitrosyl chloride into a caustic trap. Dikun (1976) has detailed a colorimetric procedure intended for total NA analysis and Ceh and Ender (1978) have quantitated the chromophore produced when dialkyl NAs are reduced to alkylhydrazine and then reacted with p-dimethylaminobenzaldehyde.

The FID-GC detector has found limited use in NA analysis because of its low specificity. Several types of more specific GC detectors have been used. Fazio et al. (1972) used a modified potassium chloride thermionic detector in the multidetection method and Goodhead and Gough (1975) used a Coulson electrolytic conductivity detector (reduction mode) as a screening device. Palframan et al. (1973) compared these detectors.

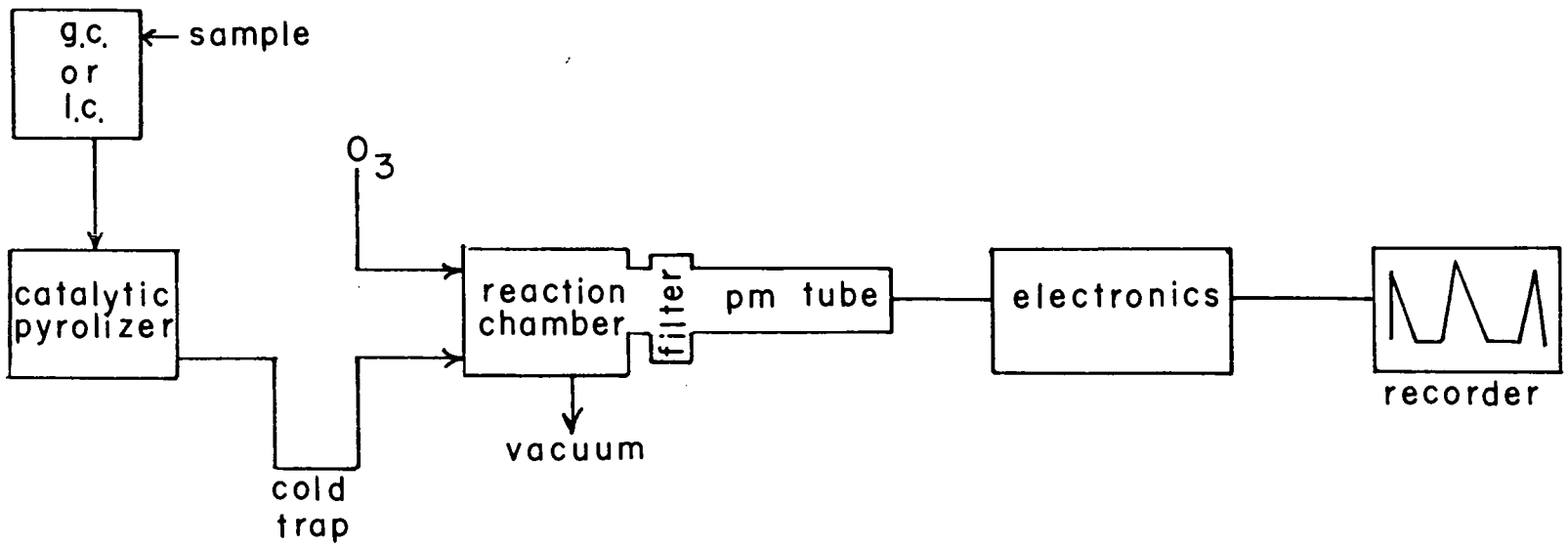
The formation of derivatives of NAs has been employed to increase both specificity and sensitivity. The oxidation of NAs to the electron capturing nitramine and subsequent detection by EC detectors has been described (Sen, 1970; Althorpe et al., 1970). This method was more sensitive than either the thermionic or Coulson detectors (Telling, 1972). Gough et al. (1975) reacted NAs directly with heptafluorobutyric anhydride to form an electron capturing species. Nonvolatile NAs have been derivatized making them amenable to GC (Lee et al., 1978; Janzowski et al., 1978).

The thermal energy analyzer (TEA) is a new instrument which is employed as a group specific detector for the analysis of NAs in a variety of substances. The TEA was first used as a GC detector (Fine and Rounbehler, 1975) and more recently as an LC detector (Fine et al., 1977b). As the NA exits the chromatograph it enters a flash heater and catalytic pyrolysis chamber where the N-N bond is catalytically cleaved. The nitric oxide

produced is then swept by argon gas through a cold trap (-150°C) where organic materials are frozen out. The nitric oxide then reacts with ozone to produce an energy emission in the near-infrared. The emission is quantitated and is proportional to the concentration of NA. A flow diagram of the TEA is given in Figure 2. The methods by which the TEA attains its selectivity have been detailed (Fine et al., 1975) and its operation described in detail (Fine et al., 1975a). Gough et al. (1977) have described a similar non-commercial detector and Drescher and Frank (1978) used chemiluminescence to determine total N-nitroso concentrations in environmental samples.

The high selectivity of the TEA detector over more conventional detectors is well established (Fine et al., 1975), but recent work indicates that certain samples contain non-NAs which can elicit a positive TEA response. Stephany and Schuller (1977) have shown that certain tertiary C-nitroso compounds are TEA positive and Fiddler et al. (1978) reported unidentified TEA positive peaks in chromatograms of tobacco smoke condensate, cheese, and fish products. They also identified three pyrrole compounds which gave relative response ratios of 0.0015 to 0.0559. As pointed out by the authors, concentrations of these compounds would have to be large to produce a significant peak. Fan et al. (1978a) identified a TEA positive organic dinitrate

Figure 2. Diagram of the thermal energy analyzer.


g.c.
or
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sample
O_3
catalytic
pyrolizer
reaction
chamber
filter
pm tube
electronics
recorder
vacuum
cold
trap

(dinitroethyleneglycol) in drinking water and Gough and Webb (1978) found an unidentified non-NA which was TEA positive and had a retention time on Carbowax 20M which coincided with DPN. Krull et al. (1978) have shown saccharin to be TEA positive at a response ratio 0.0001. This very weak response appears to result from thermal decomposition of saccharin in the pyrolizer. The instrument manufacturer (Thermo Electron Corporation) reported that certain organic nitrites and nitrates, plus some inorganic nitrites produce a positive TEA response, particularly when the instrument is operated in the direct injection mode. The response ratios of seven alkyl nitrates and nitrites ranged from 0.2 to 1.0 (Fan et al., 1978a). More extensive lists of both positive and negative responding compounds have been published (Fine et al., 1975). To date, no false negative N-nitroso compounds have been reported, however, signal repression was reported when DMN and dimethylamine were co-injected into the GC-TEA (Gough, 1977).

Confirmation

The potential implications that low levels of NAs in human food have in terms of public health mandates that positive results be confirmed by the best means available. Mass spectral (MS) analysis is considered the most reliable means of confirming the

presence of NAs in foods (Walker et al., 1976) but other workers have proposed a set of criteria which do not include MS (Fine et al., 1977a). This method depends on the coincidence of retention times of unknown peaks with known standards on both GC-TEA and LC-TEA columns. This procedure has been used to identify NAs in human blood (Fine et al., 1977a) and feces (Wang et al., 1978). The subcommittee on the analysis of NAs (International Agency for Research on Cancer) has recommended that findings which are not confirmed by MS be labeled "apparent" (Walker et al., 1978).

The TEA instrument is 100 to 1000x more sensitive than most mass spectrometers and hence has lead to reporting NAs in foods at 10 ng/kg; a level well below the minimum level confirmable by MS (Gough, 1978). At the present time the USDA considers 10 μg/kg the minimum confirmable level for NAs in foods (Federal Register 43:20992, 1978). Doerr and Fiddler (1977) have suggested that samples containing TEA positive peaks be photolyzed after TEA analysis and reanalyzed with the assumption that NAs decompose under UV irradiation and disappear from the chromatogram while false positive compounds will not.

Mass spectrometry has become obligatory for the confirmation of NAs in foods and other environmental samples. However, a wide variety of MS methods have been used; the number of methods is nearly as great as the number of groups using MS.

Several of these techniques have been reviewed by Gough (1978a).

One of the simplest low resolution MS techniques is a single-ion monitoring of the GC effluent. Eisenbrand et al. (1975) used single-ion monitoring to detect the TMS (trimethylsilyl) derivatives of N-nitrosoamino acids in solution. This technique is of limited value for complex samples, however, because any fragment of the same nominal mass will produce a response. The use of multi-ion mass fragmentography is an improvement over the single-ion method. Essigmann and Issenberg (1972) plotted the intensity of ions at m/e 30, 42 and 74 in the GC-MS analysis of chopped ham spiked with DMN. Tentative confirmation was made if all three ions maximized at the correct retention time for DMN. Gadbois et al. (1975) used mass fragmentography at m/e 30, 42 and 74 and coincidence of retention time to identify DMN in smoked fish. Hwang and Rosen (1976) monitored m/e 69 and 100 to confirm NPyr in fried bacon when studying the mechanism by which NPyr is formed. Nakamura et al. (1976) also reported on the mechanism of NPyr formation using mass fragmentography.

As pointed out by Gough (1978a), positive results for NAs in complex media, such as foods, based on mass fragmentography must be interpreted with caution. Often, the absence of certain ions is as important as the presence of other ions in the spectrum. For example, ethyl lactate, if in large concentration, will produce

significant ions at m/e 74, 42, and 30. With full spectrum scanning, large ions at m/e 29, 45, 75 would clearly differentiate this compound from DMN. Mass fragmentography does have the advantage of increased sensitivity over full spectrum MS.

Both volatile and nonvolatile NAs have been confirmed in foods by full spectrum MS. Sen et al. (1973) published a spectrum of NPyr found in fried bacon and later (Sen et al., 1974) used low resolution MS to further confirm this finding. Havery et al. (1976) confirmed three different NAs in foods and spice premixes. Wasserman et al. (1972) studied the occurrence of DMN in frankfurters and confirmed three samples. Lee et al. (1978) confirmed the nonvolatile NA N-nitroso-3-hydroxypyrrolidine as the TMS derivative by low resolution MS. Gray et al. (1977) studied the formation of this NA in model systems and published its full spectrum.

High resolution MS can also give unambiguous confirmation but sufficient resolution must be employed to differentiate the NAs from other ions of the same nominal mass. Telling et al. (1971) developed a procedure for monitoring NO^+ at a resolution of 15,000 which distinguishes it from all other species except $C^{18}O^+$. Some workers prefer monitoring the molecular ion due to the increased sensitivity gained by using lower resolution. Sen et al. (1976a) monitored both the M^+ and NO^+ ions at resolutions of

5,000 and 10,000 respectively to confirm DMN and NPyr in fried bacon fumes and later used this technique to confirm NAs in other foods (Sen et al., 1978). Compson et al. (1977) determined that a minimum resolution of 10,000 was necessary to identify DMN in tobacco smoke condensate and Dooley et al. (1973) have identified $^{29}SiMe_3$ as a potential false positive species at a resolution of less than 70,000. One of the suggestions of the authors was to obtain full spectrum low resolution MS in questionable cases. Despite the need for caution, some workers have reported more decimal places for the NO^+ ion than was justified by the reported resolution (Sen et al., 1977).

Several workers have confirmed by high resolution MS that certain foods contain NAs. In a comprehensive study Gough et al. (1977b) confirmed NAs in some foods after screening the samples by GC-Coulson electrolytic conductivity detector and Iyengar et al., (1976) reported the NA content of several fish products which were confirmed by high resolution MS. Janzowski et al. (1978) confirmed N-nitroso-3-hydroxypyrrolidine in fried cured meats as the trifluoroacetyl (TFA) derivative. Fiddler et al. (1974) and Mottram et al., (1977) used high resolution MS to study the formation of NAs in the fat and lean portions of bacon.

While several authors have reported confirming NAs by various MS techniques, very little hard data has been presented on

the sensitivity of these methods. Stephany et al. (1976) reported a detection limit of 0.1 to 0.2 μg/kg using single-ion monitoring at a resolution of 4,000, however, they do not present any data to support this sensitivity. These same workers, in a later paper, demonstrated that single-ion monitoring can give false positive results under certain conditions (Stephany, 1977). Gough and Webb (1972) monitored the molecular ion of DMN at resolutions of 7,000 and 12,000 and reported detection limits of one and five μg/kg, respectively. Fazio et al. (1972) reported a ten μg/kg detection limit for volatile NAs in foods. The present minimum detectable level, in foods, for most high resolution methods is approximately one μg/kg and for low resolution MS ten μg/kg. This difference may not be inherent to the instruments, but may be due to different degrees of method refinement.

The TEA and low and high resolution MS methods of quantitating NAs in foods have been compared (Gough et al., 1977a). Thirty-two samples of meats, vegetables, other foods, and urine were quantitatively compared for DMN and NPyr content by low resolution MS, high resolution MS with peak matching, high resolution MS with precise ion monitoring, and TEA analysis. The GC column used for all MS techniques was a 2M x 4mm i.d. glass column containing 5% Carbowax 20M. Low resolution MS quantitation was based on mass fragmentography at m/e 30 and 74 for

DMN and m/e 69 and 100 for NPyr. High resolution quantitation was based on monitoring the M^+ ion at a resolution of 7,000. All MS techniques had a detection limit of one μg/kg and the TEA had a lower limit of 20 to 40 ng/kg. The authors concluded that for all 64 determinations the TEA and high resolution peak matching MS gave consistent quantitative results and that low resolution MS gave enhanced or suppressed results in 7 of 32 DMN assays and 13 of 32 NPyr assays. High resolution precise ion monitoring MS gave erroneous results in 7 of 32 and 18 of 32 assays for DMN and NPyr, respectively. As pointed out by the authors, a more efficient GC system would probably improve the results from the later two methods. Also, it must be noted that this study does not relate directly to the ability of any one technique to qualitatively confirm the presence of NAs in foods.

Fine et al. (1976a) compared the TEA detector, the Coulson electrolytic conductivity detector, high resolution MS, and TLC for the quantification of volatile NAs. The data presented consisted of only three samples but the authors concluded that all four techniques gave essentially similar results. The TEA required considerably less cleanup, however.

The International Agency for Research on Cancer (IARC) has sponsored collaborative studies on the analysis of volatile NAs. Samples of NA spiked water and DCM (Walker and Castegnaro,

1976), spiced canned luncheon meat (Castegnaro and Walker, 1978) and canned cheese (Castegnaro, 1978) were sent to 15 to 20 laboratories throughout the world. The method of analysis was left to the participants and at least five different procedures were used. Statistical analyses of the more recent studies (Castegnaro and Walker, 1978) showed that for three of the four NAs tested the TEA method gave the lowest standard deviations and the lowest coefficients of variation. On the average, all methods underestimated the amount of NA added to spiced luncheon meats. The spiced luncheon meat study also included samples of non-volatile N-nitrosoamino acids in vials from which the solvent had been removed by freeze-drying. Direct LC methods were slightly more precise than derivatization.

III. EXPERIMENTAL

Reagents

Dimethylnitrosamine (DMN) and nitrosopyrrolidine (NPyr) were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin); diethylnitrosamine (DEN), dipropylnitrosamine (DPN), dibutylnitrosamine (DBN), nitrosopiperidine (NPip) were obtained from Eastman Organic Chemicals (Rochester, New York) and nitrosomorpholine (NMor) from Fluka AG (Switzerland). Standard solutions were made gravimetrically in hexane. Dichloromethane (DCM) was glass distilled (Burdick and Jackson, Muskegon, Michigan) and hexane was nanograde (Mallinckrodt, St. Louis, Missouri). Standards and concentrated solvents were checked for purity by GC-FID and GC-TEA, respectively. Certain nanograde DCM lots contained extraneous TEA peaks which were not seen in the glass distilled DCM used in this work. Activity II alumina (Merck, Rahway, N.J.) was prepared in the manner of Bobbitt et al. (1968). Water used in the procedures was distilled and not purified through ion-exchange resin. Mineral oil (Squibb, Princeton, N.J.) was pharmaceutical grade and purchased locally. All other reagents were analytical reagent grade.

Reagents used to synthesize authentic compounds were as follows: fuming nitric acid (Mallinckrodt) was purified with urea

and N_2 flushing; acetic anhydride, acetone cyanohydrin, dipropylamine, trifluoroacetic anhydride were purchased from Eastman Organic Chemicals; 50% hydrogen peroxide was obtained from Fisher Scientific (Fair Lawn, N.J.).

Synthesis of Nitramines

Dipropylnitramine ($DPNO_2$) was synthesized by two independent methods. The first method was a modification of the method given for the synthesis of N-nitromorpholine (Baumgarten, 1973). In this method, the nitrating agent acetone cyanohydrin nitrate is first synthesized and then reacted with the secondary amine yielding the corresponding nitramine. The modifications were as follows: all reagents were used in one-half molar amounts; dipropylamine was substituted for morpholine; yield of acetone cyanohydrin nitrate was 77%; the final product was distilled over a 20 cm Vigreux column. GC-TEA and GC-MS analyses of this product showed it to be contaminated with 1-5% DPN.

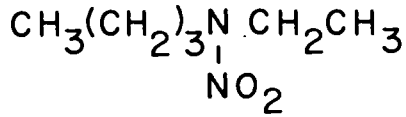
The second method used to synthesize $DPNO_2$, N-nitropyrrolidine ($PyrNO_2$) and a mixture of nitramines was a modification of the method of Emmons (1954). In this method, secondary N-nitrosamines are oxidized to their corresponding nitramines by peroxytrifluoroacetic acid. The modifications were as follows: an equal molar amount of 50% H_2O_2 was substituted for 90% H_2O_2;

one μL aliquots of the reaction mixture were taken during heating and analyzed by GC-FID until the nitrosamine peak was less than 1% of the nitramine peak; the total heating time was 2.5 h. The $DPNO_2$ was distilled at 92-94°C (4mm; literature value, 105-106°C, 10mm; Robson, 1955) and the yield was 7.6 g of colorless liquid (52% theoretical). The $PyrNO_2$ was synthesized in a similar manner except that the final product was recrystallized twice from 10% (v/v) ethanol in water. This yielded white crystals at 59% of theoretical (mp 55-57°C; literature value, 55-57°C; Emmons and Freeman, 1955). The structure of $DPNO_2$ was confirmed by IR spectrophotometry and both $DPNO_2$ and $PyrNO_2$ were analyzed by GC-MS. Figure 3 represents the structures of three nitramines discussed in this paper.

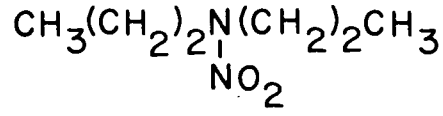
A mixture of five nitramines was synthesized by treating ten mg each of five NAs in ten mL hexane with peroxytrifluoroacetic acid. The resulting solution was analyzed by GC-TEA. The five nitramines were, in order of elution on Carbowax 20M: dimethylnitramine, diethylnitramine, $DPNO_2$, dibutylnitramine, $PyrNO_2$.

The molar response ratio, r, of $DPNO_2$ and $PyrNO_2$ to their corresponding NAs was determined by injecting a solution of NA and nitramine in hexane into the GC-TEA and dividing the area of the nitramine peak by the area of the NA peak and correcting for mole content of the solutions. To determine the stability of hexane

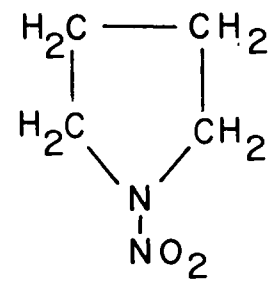
Figure 3. Structure of three nitramines: a, butylethylnitramine; b, dipropylnitramine; c, N-nitropyrrolidine.



a



b



c

solutions of $DPNO_2$ and DPN to UV light, irradiation was carried out in one cm^2 fused silica stoppered cuvets (Beckman) placed 40 cm from a sunlamp (General Electric, RSK/3).

Thin-layer chromatography (TLC) was accomplished on 0.25 mm thick Silica Gel G plates (Macherey-Nagel and Company) using hexane:ethylether:DCM, 4:3:2 (v:v:v) as the developing solvent. Griess reagent, as modified by Fan and Tannenbaum (1971), and UV irradiation were used for visualization.

Analysis of Herbicides

One-half milliliter aliquots of the herbicide as an emulsifiable concentrate formulation were cleaned up prior to analysis on 15 g of activity II alumina in a 14 mm i.d. glass column. The column was slurry packed with hexane and the herbicide applied. The column was then washed with 100 mL of hexane, followed by 50 mL of 10% (v/v) DCM in hexane. The compounds of interest were eluted from the column by 100 mL of DCM; the eluate was collected in a Kuderna-Danish apparatus fitted with a three-ball Snyder column and concentrated to ca. three mL. The DCM was further concentrated to 0.5 mL under a stream of N_2 and then analyzed by GC-TEA. One-tenth-milliliter aliquots were further concentrated to ca. ten μL for GC-MS analysis. Recovery studies for DPN and $DPNO_2$ in hexane gave essentially quantitative

recovery. The concentrated eluent was analyzed by GC-TEA on a Model 502 TEA analyzer (Thermo Electron Corporation) coupled to a Varian Model 1400 GC. Instrument parameters were as follows: (GC) injection port, 200°C; column, 0.125 in. o.d. x 20 ft stainless steel packed with 10% Carbowax 20M on 60/120 Chromosorb G, isothermal at 200°C; (TEA) furnace, 400°C; trap, isopentane and liquid N_2 slurry (-160°C).

Quantification of the $DPNO_2$ and DPN content in the herbicide was accomplished by adding diethylnitrosamine (DEN) as an internal standard after concentration. Previously it had been determined that the herbicide did not contain detectable levels of DEN.

Mass spectral data were obtained with a Finnigan Model 1015C quadrupole mass spectrometer coupled to a Varian 1400 GC. Instrument parameters were as follows: (GC) injection port, 200°C; column, 0.03 in. i.d. x 500 ft stainless steel open tubular wall-coated with SF-96, isothermal at 130°C; (MS) filament current, 350 μA; electron voltage, 70 eV; analyzer pressure, 10^{-6} Torr. Data were collected by a Systems Industries System 250 computerized data system.

Analysis of Foods

Preparation

Fresh ground pork was obtained through a local merchant and divided into 125 g portions. Each portion was flattened into a ca. one-half-centimeter thick patty and fried four min per side in a home-style electric griddle (Sunbeam Model RC) at 170°C. Sixty percent of the fresh weight was recovered as fried solids which were stored in plastic bags at -23°C. Subsequent analyses of random samples failed to detect volatile NAs in the fried pork.

Nitrite-free bacon was manufactured by soaking fresh pork bellies in a pickling solution containing only NaCl and sucrose (3:1) and subsequent heating to an internal temperature of 63°C in a smoke house. The bellies were cut into ca. two lb pieces, wrapped and stored at -23°C. This product is described as "nitrite-free bacon" in this report, however, nitrate is endogeneous to many biological tissues (Swann, 1975) and bacon produced without added nitrite may still contain traces of nitrite through reduction of nitrate. Immediately before frying, the bellies were thawed and sliced into ca. two mm thick slices. Analysis of a random sample indicated that the fried bacon contained NPyr at a level of approximately 50 ng/kg.

Raw Pacific Hake was used to determine the recovery of volatile NAs from fish. Unspiked samples did not contain volatile NAs above the detection limit.

Cheese (cheddar) was obtained locally and stored at -23°C until analysis. Analyses of unspiked samples were negative for volatile NAs.

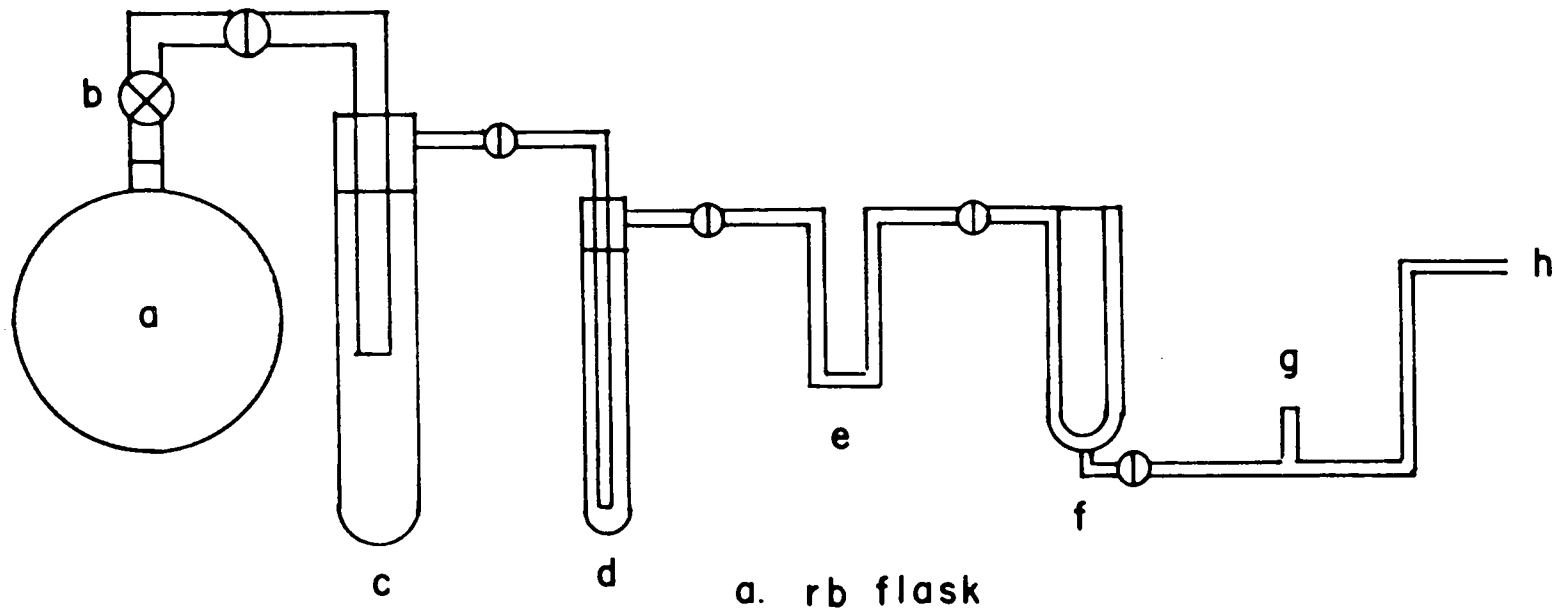
Both commercial and nitrite-free bacons were fried at 170°C for three min per side, drained of fat, and stored at -23°C until time of analysis. Either a commercial-type griddle (Hotpoint Model HG55) or home-type griddle (Sunbeam Model RC) were used to fry the bacon and the temperature was checked with surface thermometers.

Food samples were frozen in liquid N_2 and ground to a fine powder in a commercial blender (Waring Model 32 BL39) immediately prior to analysis.

Isolation of N-nitrosamines

The procedure of Fine et al. (1975b) was modified and employed to remove the volatile NAs from the food. The distillation apparatus (Figure 4) consisted of a 1000 mL single neck round bottom flask fitted with a thermometer well. The flask was connected to a series of liquid N_2 cooled traps by glass tubing interrupted by a high vacuum stopcock. The first two traps were

Figure 4. Apparatus for distilling volatile nitrosamines from foods.



a. rb flask
b. high vacuum valve
c. large vapor trap
d. small vapor trap
e. u-tube trap
f. cylindrical safety trap
g. to vacuum guage
h. to vacuum pump

vacuum vapor traps fitted with tapered joints; the second trap being smaller than the first. The third trap was an 18 mm o.d. U-shaped tube and used only to insure that artifacts could not reach the vapor traps. A dry ice and acetone Dewar-type trap was installed between the distillation apparatus and the pump as a safety precaution and a glass tee was used to fit a McLeod gauge into the system. A vacuum of more than 20 μM Hg was achieved in the system by the vacuum pump. All traps were inter-connected by 28/15 ball and socket joints. The glassware is described in greater detail in the Appendix.

The NAs were distilled by placing 250 g of ground sample, five g ammonium sulfamate in ten mL H_2O, 20 mL 6 N H_2SO_4, and 100 mL mineral oil in the flask and connecting it to the system. One drop of SF-96 was added to some bacon samples which had a tendency to foam. Vacuum was applied to the system with the valve above the flask closed and the traps were cooled by liquid N_2. When the vacuum was more than 100 μM Hg the valve was slowly opened and the system allowed to pump down for five min. Heat was then applied to the flask by an electric heating mantle. Heating was continued until the contents of the flask reached 100°C and the vacuum was greater than 20 μM Hg. This took between 45 and 90 min, depending on the water content of the sample. Heat was then removed and vacuum applied for 15 additional min; the

vacuum value was shut and the vacuum broken.

Extraction and Cleanup

The distillate in the first two traps was allowed to melt and transferred to a 500 mL separatory funnel. Each trap was rinsed with 3 x 25 mL H_2O followed by 1 x 25 mL DCM; all rinses were combined with the distillate. The aqueous phase was saturated with Na_2SO_4 and the DCM transferred to a second funnel. The aqueous phase was further extracted with DCM (3 x 25 mL) and the combined extracts washed with 25 mL 1:4 (w:v) $NaHSO_3$ in water, 25 mL 3 N HCl, and 25 mL 1.5 N NaOH. The $NaHSO_3$ and HCl washes were backwashed with DCM (1 x 10 mL). The DCM was dried over Na_2SO_4 and one mL hexane added. The Na_2SO_4 was washed with DCM (1 x 25 mL) and the combined DCM reduced to ca. two mL in a Kuderna-Danish concentrator (125 mL) fitted with a three-ball Snyder column and one mL concentrator tube. The concentrator tube was fitted with a micro Snyder column and the volume of solvent reduced to one mL under a stream of N_2 at ambient temperature. Eight to ten μL were injected into the GC-TEA and any volatile NAs present quantitated.

Positive samples were further cleaned up for GC-MS confirmation on ca. 11 g activity II alumina which had been slurried in hexane and packed into a 14 mm i.d. glass column. The

sample was pipetted onto the column and the concentrator tube rinsed with hexane (3 x 1 mL). The column was then washed with 100 mL 10% (v/v) DCM in hexane, and the NAs eluted with 100 mL DCM. The DCM was concentrated to ca. 250 μL as described above.

Separation and Detection

Separation and detection of volatile NAs was accomplished by combined GC-TEA analysis. The instrument parameters were as follows: (GC) Varian 1400; injection port, 160°C; column, 9 M x 0.125 in. o.d. stainless steel packed with 11% Carbowax 20M on 60/120 Chromosorb G and fitted with a 20 cm x 0.125 in. o.d. precolumn containing the same packing material and extended into the injection port for on-column injection, isothermal at 150°C; carrier gas, helium at 25 mL/min; (TEA) furnace, 400°C; trap isopentaneliquid N_2 slurry (-160°C).

Quantification and Recovery

Quantification of NAs was accomplished by comparing the peak heights or areas of unknowns to standards on the GC-TEA. Each value was the average of two injections of both standard and unknown. Recoveries were determined by spiking the food with a known amount of NA and comparing the final extract to the original

standard. These recovery values were used to correct the NA content of unknowns for loss during work-up.

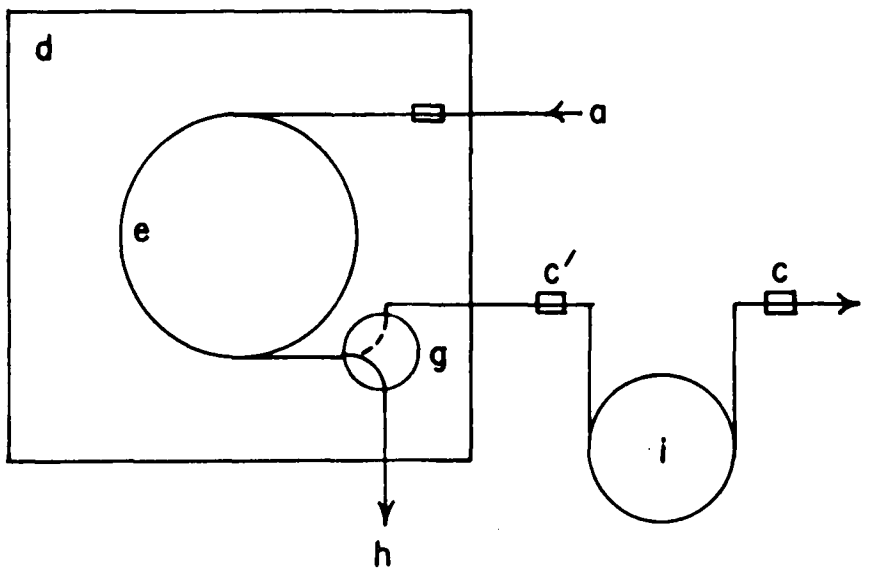
Confirmation by Mass Spectrum

TEA positive peaks were trapped onto one-sixteenth inch o. d. x 10 in. nickel tubing bent into a small loop and reinjected into the GC-MS. Figure 5(A) details the gas flow in the trapping system. The end of the GC column was fitted with a low dead-volume GC valve (Carle Instruments Inc.) which was capable of directing the column effluent to either the TEA or to the nickel trap outside the oven. As the recorder began to respond to the TEA positive compound, the valve (g) was rotated to direct the flow to the nickel trap (i) which was cooled in a dry ice and methoxyethanol bath. After approximately two minutes the valve was turned back to the TEA. All tubing external to the oven was heated with heat tape to 180°C. As many as five injections of 50 μL each were trapped on a single loop when the NA content was less than five μg/kg in the food. The GC oven temperature was adjusted to give a retention time between 8 and 16 min for any particular peak (100 to 150°C). After trapping, the traps were sealed with one-sixteenth in caps and stored in dry ice for up to two weeks.

The compounds trapped were injected into the GC-MS by the system diagrammed in Figure 5(B). With valve b' closed and valve

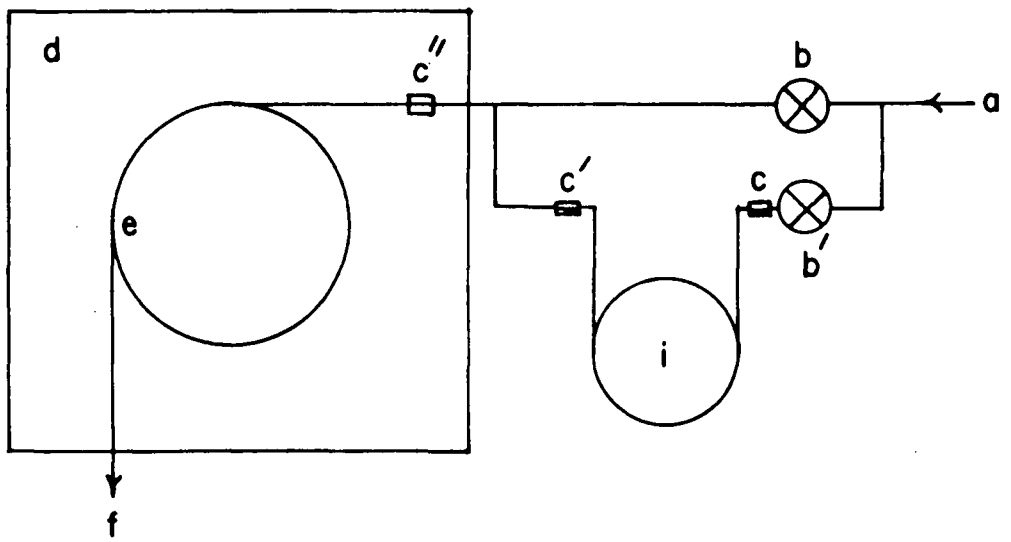
Figure 5. Flow diagram for (A) trapping nitrosamines from gas chromatographic column and (B) reinjecting trapped nitrosamines into the gas chromatograph-mass spectrometer.

A



a. gas in
b. toggle valve
c. union
d. g.c.oven
e. g.c. column
f. to m.s.
g. g.c. valve
h. to t.e.a.
i. sample loop

B



b open the trap was connected between unions c and c' while being kept cold in crushed dry ice. Valve b was then closed, valve b' opened and the trap heated by a forced air heat gun. Heating was continued for ten sec to transfer the compounds to the analytical GC column.

Mass spectra were obtained by a Finnigan Model 1015C quadrupole mass spectrometer interfaced to a Varian 1400 GC by an all glass jet-type separator. The GC column was a 180 M x 0.5 mm i.d. Carbowax 20M glass support coated open tubular (SCOT) column. GC parameters were as follows: carrier gas, helium at 8 cc/min; temperature, isothermal, 120 to 150°C depending on the NA. Mass spectrometer parameters were as follows: filament current, 450 μA; electron voltage, 70 eV; analyzer pressure, 10^{-6} Torr. Rapid scans with minimal delay between scans were taken to increase the chromatographic resolution. Data was collected by a Systems Industries System 250 data system. In most cases the spectrum of the NA was located by limited mass searches for the M^+ ion and corrected for background by computer. All analyses of unknown samples were preceded by standards in the same concentration range to determine both retention times and instrument sensitivity.

Retention indices (I_E) were determined for five volatile NAs on both Carbowax 20 and SF-96 capillary columns in the same manner

as van Den Dool and Kratz (1963) using ethyl esters of fatty acids. These values are given in Table 1.

Artifacts

As noted by Krull et al. (1978a), NAs can occur as artifacts of some analytical schemes unless precautions are taken. When 50 mg/kg pyrrolidine and 50 mg/kg DMA were added to nitrite-free bacon and 120 mg/kg nitrite added to the distillation flask, 1.0 and 1.3 mg/kg DMN and NPyr were formed respectively. When the bacon was acidified with 20 mL 6 N HCl (pH< 2.5) and 5 g ammonium sulfamate added prior to distillation, the amount of DMN and NPyr formed was less than one μg/kg. This is the amount of NA that might be expected from using amines that were contaminated with the NA derivatives (Spiegelhalder et al., 1978). If the acid was not added and only ammonium sulfamate added NAs were formed in the range of 1 to 100 μg/kg.

Safety

Extreme caution was exercised in handling NAs as they may be potent human carcinogens. Protective clothing, gloves and respirators were worn when the possibility of exposure was present. Concentration of solutions and all weighings were done in fume hoods.

TABLE I. I_E VALUES

N-nitrosamine	Liquid Phase	
	20M	SF-96
DMN	765	400
DEN	781	500
DPN	926	670
DBN	1094	858
NPyr	1161	672

IV. RESULTS AND DISCUSSION

Herbicide Analyses

Analyses of two separate lots of an emulsifiable concentrate formulation of the herbicide trifluralin showed both to contain $DPNO_2$. Figure 6 is a GC-TEA chromatogram of the concentrated eluent derived from the herbicide. Ross et al. (1977) in their report on the DPN content of a formulated trifluralin sample did not report the occurrence of extraneous TEA positive peaks. In a recent report (Cohen et al., 1978) unknown peaks were not reported in a trifluralin sample, but were reported in other similar dinitroaniline herbicides. Analysis of a different dinitroaniline herbicide formulation containing benefin (α, α, α-trifluoro-2,6-dinitro-N-butyl-N-ethyl-p-toluidine), in the same manner as described above, indicated that this herbicide contained more than one TEA positive peak (Figure 7). The mass spectrum of the smaller peak (Figure 8) was very similar to a standard spectrum of ethylbutylnitramine (Stenhagen et al., 1974) and the unknown spectrum was identified by computer search as being ethylbutylnitramine. The larger TEA positive peak was identified as being ethylbutylnitrosamine, which confirms the work of Cohen et al. (1978) (Figure 8). This indicates that the occurrence of nitramines in this type of herbicide may be general and due to the manufacturing process.

Figure 6. Chromatogram from the GC-TEA analysis of an herbicide containing trifluralin after cleanup (DPN, dipropylnitrosamine; $DPNO_2$, dipropylnitramine).

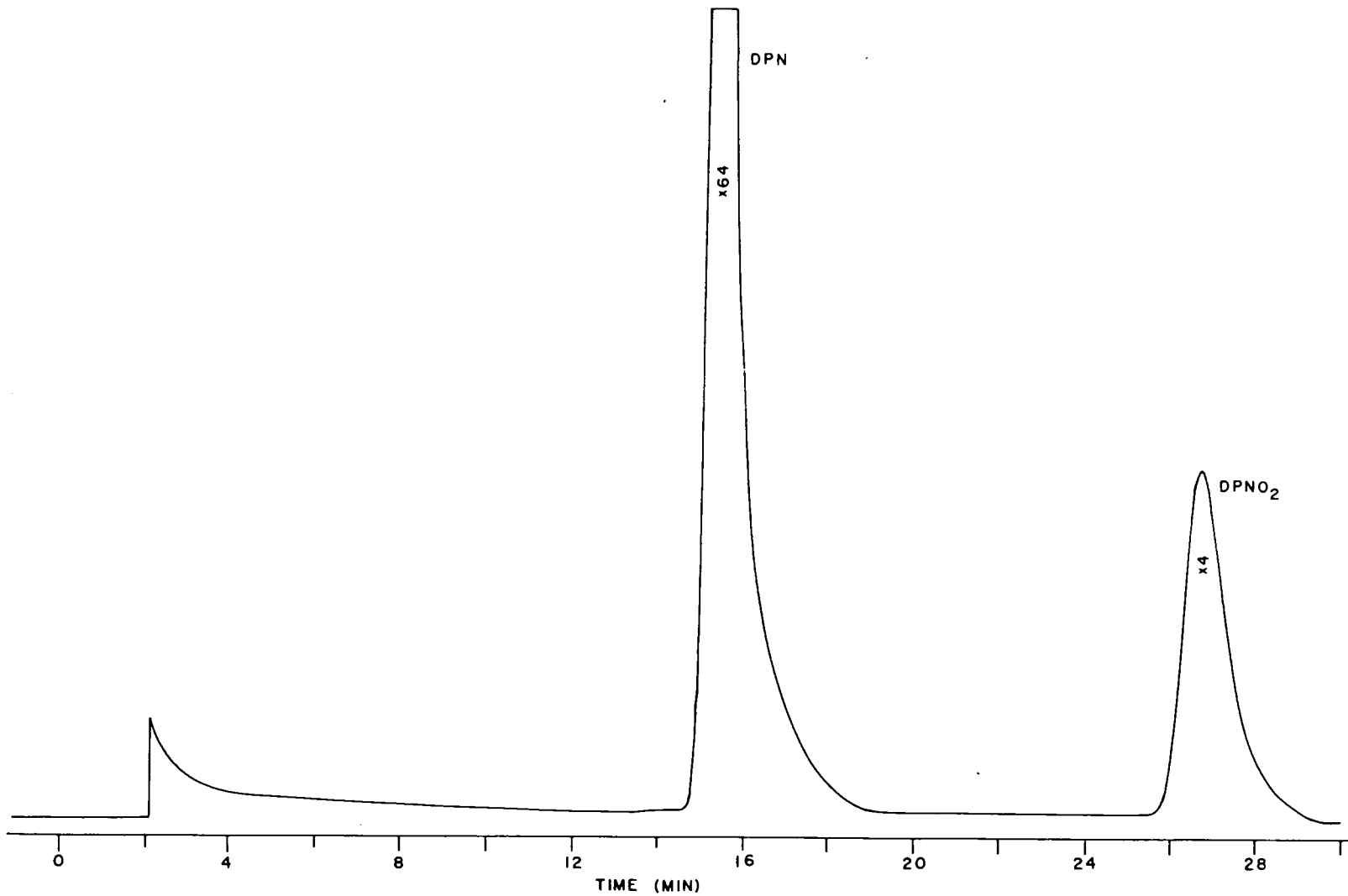

DPN
x64
DPNO2
x4
0
4
8
12
16
20
24
28
TIME (MIN)

Figure 7. Chromatogram from the GC-TEA analysis of a benefin containing herbicide after cleanup (BEN, butylethylnitrosamine; $BENO_2$, butylethylnitramine).

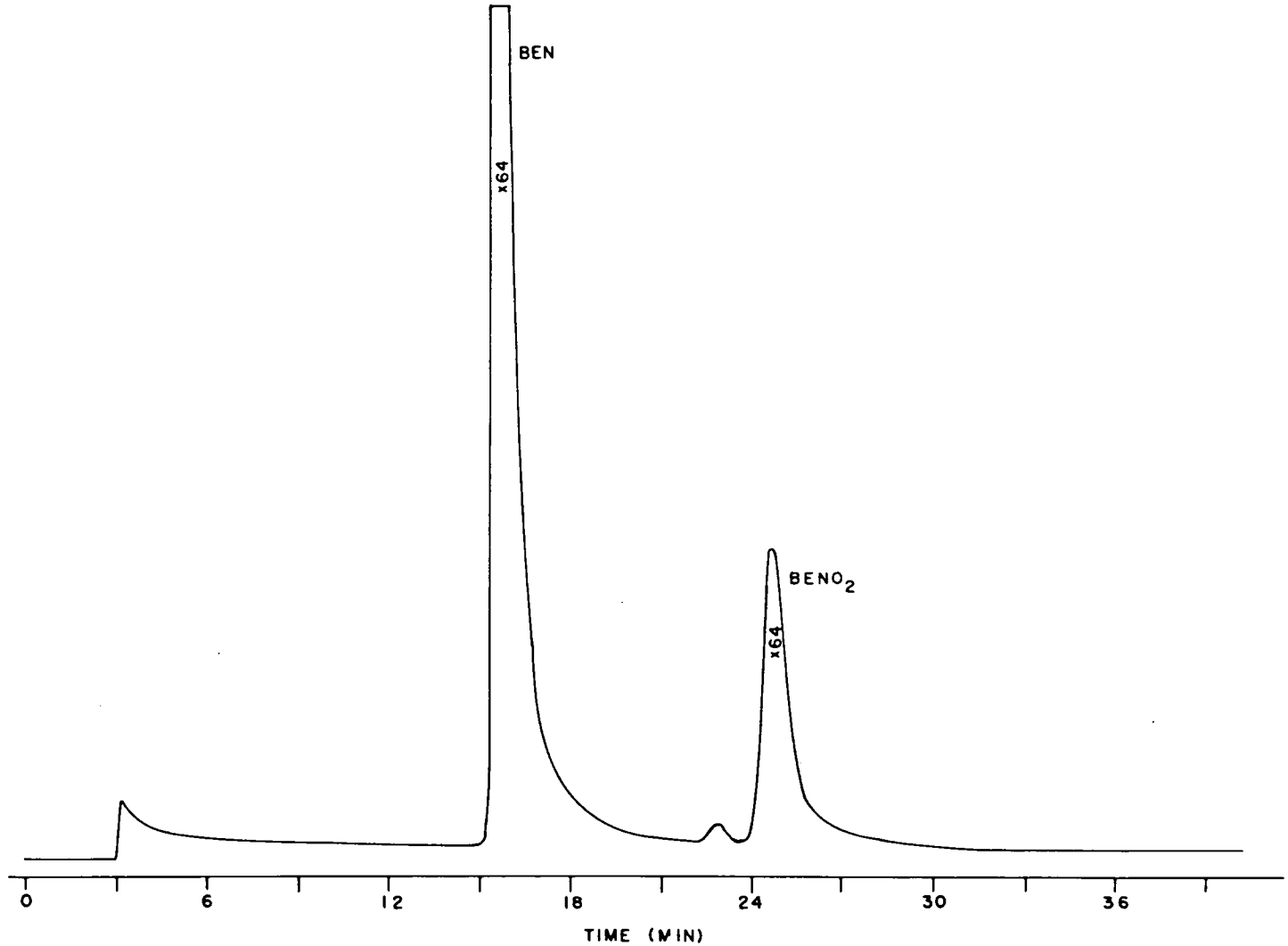
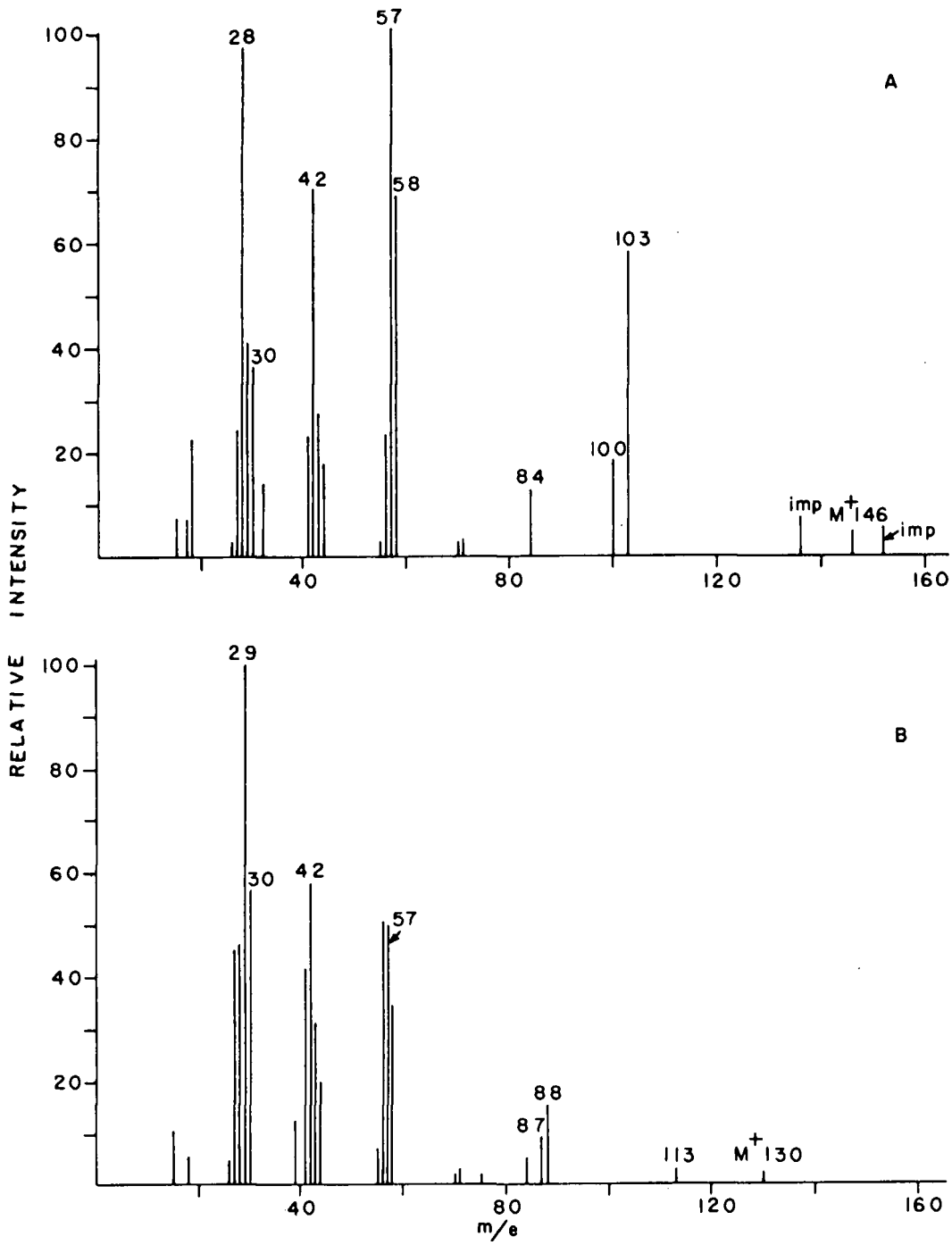

BEN
x64
BENO2
x64
0
6
12
18
24
30
36
TIME (MIN)

Figure 8. Mass spectra of (A) butylethylnitramine and (B) butylethylnitrosamine from an herbicide containing benefin.

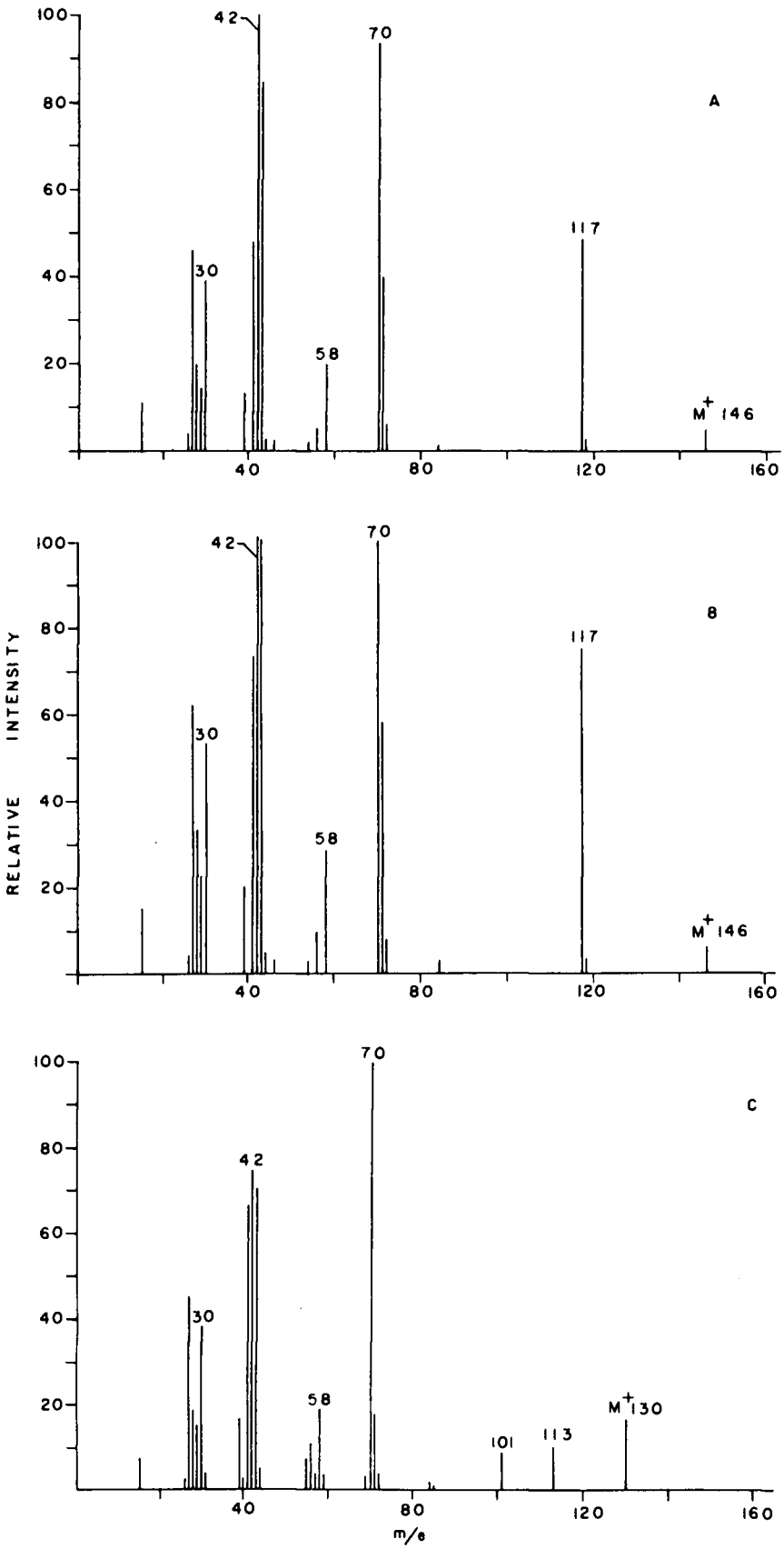

A
100
80
60
40
20
28
57
42
58
103
30
100
84
imp
M+146
imp
40
80
120
160
RELATIVE INTENSITY
B
100
80
60
40
20
29
30
42
57
88
87
113
M+130
40
80
120
160
m/e

The structure of the authentic $DPNO_2$ was confirmed by both IR and MS analyses. The IR spectrum of $DPNO_2$ showed characteristic nitramine absorptions at 1280 and 1520 cm^{-1}. The mass spectrum of authentic $DPNO_2$ was nearly identical to the mass spectrum of $DPNO_2$ found in both lots of the trifluralin containing herbicide (Figure 9). The authentic and the herbicide $DPNO_2$ mass spectra both matched closely to a standard spectrum (Stenhagen et al., 1974). In addition, the presence of DPN in both lots of herbicide was confirmed by mass spectrometry (Figure 9).

Comparison of peak areas of the unknowns to the internal standard DEN gave concentrations of 130 and 10 mg/kg for DPN and $DPNO_2$ in the trifluralin formulation, respectively. It must be noted that these values may not reflect concentrations produced by current manufacturing practices. This is further indicated by the fact that a DPN concentration of 154 mg/kg was first reported by Ross et al. (1977) but more recent EPA work on registrant supplied herbicide gave DPN levels of 121 mg/kg for one sample and 13 mg/kg for another, reflecting changes in manufacturing processes (Cohen et al., 1978). Eizember et al. (1978) have reported that the DPN content of dinitroaniline type herbicides can be reduced by 95% through process changes.

The TLC R_f values of DPN and $DPNO_2$ standards were 0.43 and 0.45, respectively. The nitramine was Griess reagent positive

Figure 9. Mass spectra of (A) dipropylnitramine from an herbicide containing trifluralin, (B) authentic dipropylnitramine, and (C) dipropylnitrosamine from an herbicide containing trifluralin.


A
B
C
RELATIVE INTENSITY
m/e
42
70
30
58
117
M+ 146
M+ 130
101
113
100
80
60
40
20
40
80
120
160

although increased amounts of compound or increased irradiation times were necessary.

The possibility that $DPNO_2$ was formed as an artifact of the analysis was considered. First, DPN in hexane at the same order of magnitude concentration as in the herbicide formulation was subjected to the same analytical procedure and analyzed by GC-TEA for $DPNO_2$. Second, DPN was added to the herbicide formulation in sufficient amount to double the DPN content of the herbicide. The sample was then subjected to the analytical procedure and analyzed by GC-TEA to determine if the $DPNO_2$ content had increased. Both procedures indicated that $DPNO_2$ was not an artifact.

The molar response ratios, r, of $DPNO_2$ and $PyrNO_2$ over five trials gave average values of 0.50 and 0.10, respectively, on our TEA (compared to the corresponding NA where r=1). Fiddler et al. (1978) have reported that a peak with the same retention as diethylnitramine ($DENO_2$) was found in smoked salmon and that $DENO_2$ had an r of 0.35 compared to DEN. To determine if these differences were due to changes in instrument parameters $DPNO_2$ and $PyrNO_2$ were repeatedly injected on the same day. It appears that the differences in r values are due to the intrinsic nature of the compounds and not due to instrumental variables.

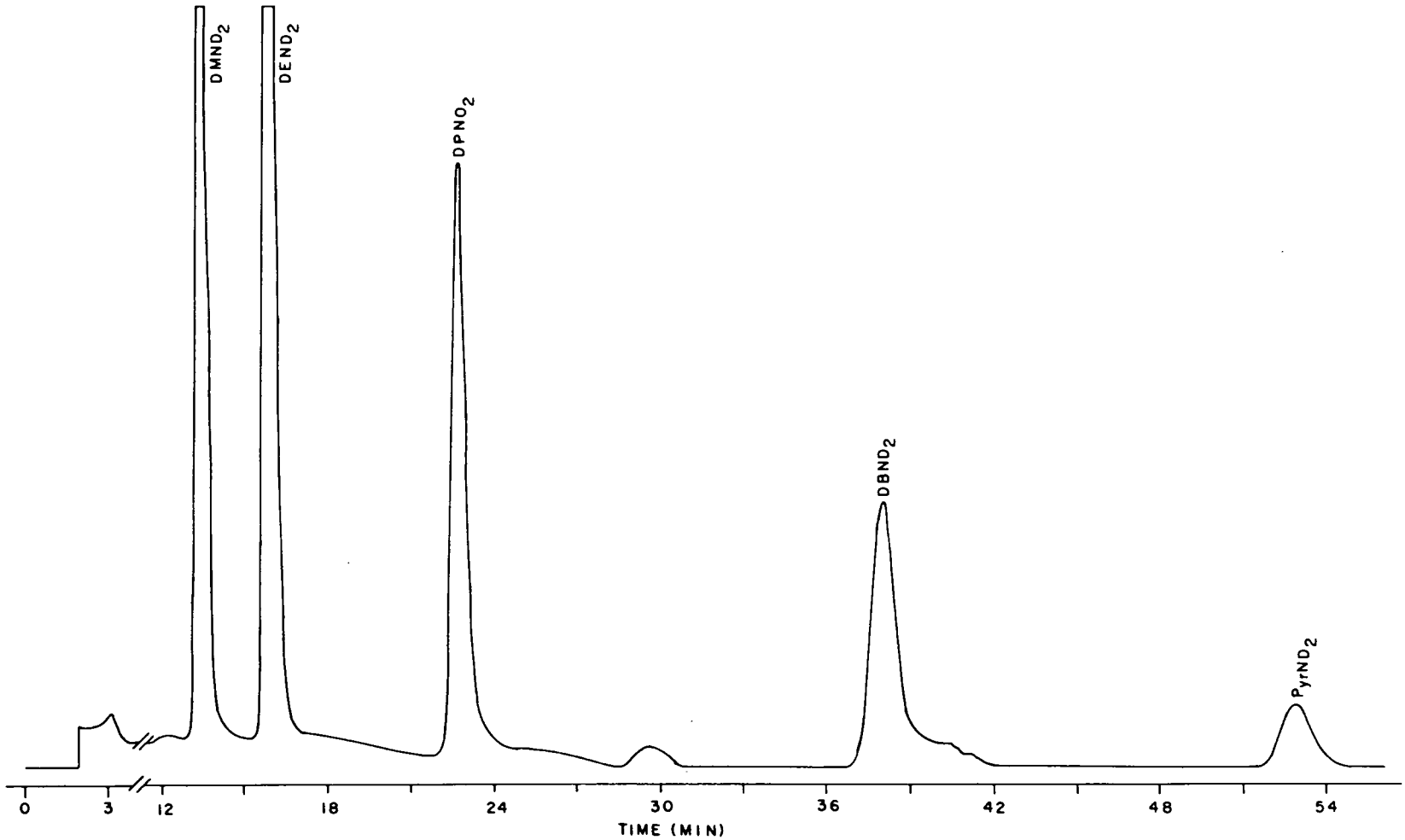
To determine if the TEA response to nitramines was more general, a mixture of five NAs were oxidized to the nitramine

derivatives. Figure 10 is the GC-TEA chromatogram of the resulting mixture of five nitramines. The five peaks observed indicates that the TEA may respond to nitramines in general.

Trifluralin is manufactured industrially in several steps (von Rumker et al., 1975). First, p-chloro-α,α,α-trifluorotoluene is nitrated with nitric and sulfuric acids. The product is dissolved in chloroform and the acids recovered for reuse. Amination is then carried out under alkaline conditions after adding water, sodium carbonate and dipropylamine.

Ross et al. (1977) speculated that all the nitric acid may not be removed after nitration and that some of the amine may then be nitrosated producing the DPN. Presumably, Ross et al. (1977) assumed nitric acid is first converted to nitrous acid which in turn forms a nitrosating species. The fact that amination is under alkaline conditions would seem to preclude this mechanism because the nitrous acid would be dissociated and not able to form nitrous anhydride. A more likely explanation might be that nitrogen oxide(s) (N_2O_4 and/or N_2O_3) are formed during the nitration reaction and these oxides carried through to the amination step where they react with the amine. Two facts lend credence to this explanation. Nitrogen oxides can be produced by the reduction of nitric acid or solutions of nitrites and nitrates with sulfuric acid (Cotton and Wilkinson, 1966). Also, Challis et al. (1978) have shown that certain

Figure 10. Chromatogram from the GC-TEA analysis of a mixture of five nitramines ($DMNO_2$, dimethylnitramine; $DENO_2$, diethylnitramine; $DPNO_2$, dipropylnitramine; $DBNO_2$, dibutylnitramine; $PyrNO_2$, N-nitropyrrolidine).

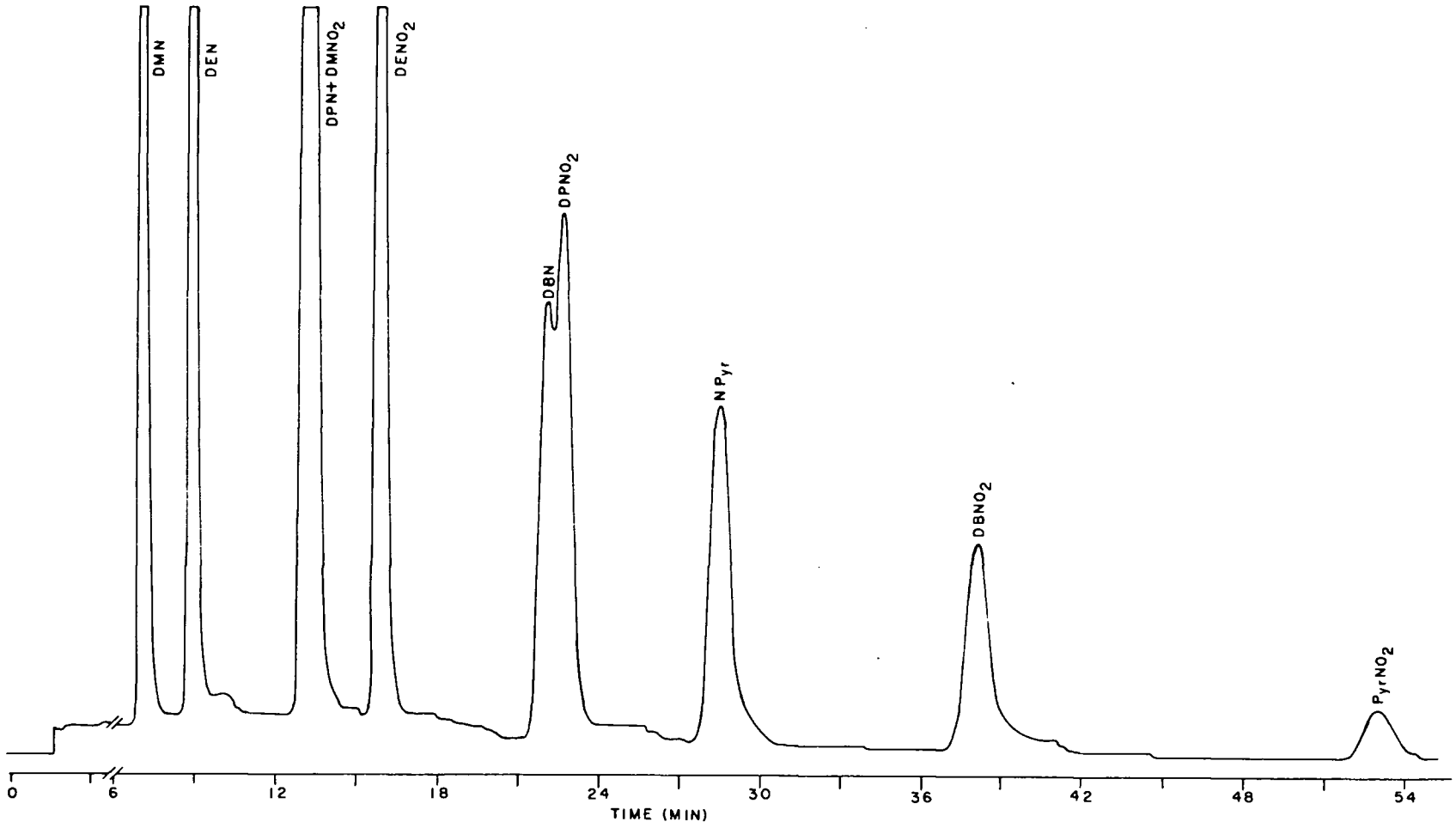

DMND2
DEND2
DPND2
DBND2
PyrND2
0
3
12
18
24
30
36
42
48
54
TIME (MIN)

nitrogen oxides react with amines under alkaline conditions to produce both the NA and the nitramine derivatives. The fact that both derivatives are found in the herbicide further supports this route of formation.

While the above mechanism seems likely, it has not been confirmed experimentally. Other mechanisms for the formation of NAs in alkaline media have also been demonstrated. Keefer and Roller (1973) demonstrated alkaline nitrosation when formaldehyde was used as a catalyst and Keefer (1976) described the role of metal complexes in the nitrosation reaction.

While it should be noted that at least one dialkylnitramine (dimethylnitramine) has been reported carcinogenic in rodents (Goodall and Kennedy, 1976), the main concern is misidentification of a nitramine as a NA. This possibility becomes real when one considers that these compounds are TEA positive, Griess reagent positive, and have ions at the low end of the mass spectrum that are similar to the corresponding NA ions. In addition, the retention times of certain NAs are similar to those of some nitramines. On the 20ft Carbowax column described above, $DPNO_2$ and dibutylnitrosamine had retention times of 26.25 and 25.50 min, respectively, and dimethylnitramine and DPN co-eluted. Figure 11 is a GC-TEA chromatogram of a mixture of five NAs and the corresponding five nitramines. With less chromatographic

Figure 11. Chromatogram from the GC-TEA analysis of a mixture of five nitrosamines and the five corresponding nitramines (DMN, dimethylnitrosamine; DEN, diethylnitrosamine; DPN, dipropylnitrosamine; $DMNO_2$, dimethylnitramine; $DENO_2$, diethylnitramine; DBN, dibutylnitrosamine; $DPNO_2$, dipropylnitramine; NPyr, N-nitrosopyrrolidine; $DBNO_2$, dibutylnitramine; NO_2Pyr, N-nitropyrrolidine).


DMN
DEN
DPN+DMNO2
DENO2
DBN
DPNO2
NPyr
DBNO2
PyrNO2
0
6
12
18
24
30
36
42
48
54
TIME (MIN)

efficiency, results from the TEA based on GC retention times might be ambiguous for certain nitrosamine and nitramine combinations. Althorpe et al. (1970) have also reported close retention times for certain NA and nitramine combinations on Carbowax 20M.

Fiddler et al. (1978) and Doerr and Fiddler (1977) have suggested that samples containing TEA positive peaks be photolyzed after TEA analysis and reanalyzed with the assumption that NAs will decompose under UV irradiation and disappear from the chromatogram while false positive compounds will not. To test this procedure with regard to nitramines hexane solutions of DPN and $DPNO_2$ in separate cuvets were exposed to artificial sunlight. The solution was sampled periodically and analyzed by GC-TEA. In as little as 15 min a decrease in the DPN peak and the appearance of a $DPNO_2$ peak was seen in the solution containing only DPN. In five h the DPN peak had disappeared. This indicates $DPNO_2$ is a product of the UV decomposition of DPN, which is in agreement with the findings of Althorpe et al. (1970). The appearance of new peaks after photolysis has also been noted by Fiddler et al. (1978). The $DPNO_2$ containing solution was more stable but did show signs of decreasing concentration after 8 h. At the end of 24 h the $DPNO_2$ peak was ca. 20% of the original value. It appears, that nitramines may also be degraded by UV, but at a much slower rate than NAs. The most reliable method for confirmation of NAs continues to be

high resolution mass spectral analysis of not only the NO^+ ion but also the M^+ ion, or complete low resolution spectra.

Food Analyses

Recovery

Table II compares the efficiency of trapping five volatile NAs from the GC-TEA column to directly injecting the compounds by syringe. The "trap injection" values were obtained by injecting ten ng of each NA into the cold trap by syringe and then purging them into the GC-TEA. The five NAs were also trapped off the GC column and reinjected into the GC-TEA to give the "reinjection" values. This data indicates that volatile NAs can be trapped from and reinjected into a GC column with reasonable efficiency. However, if any portion of the trapping system was heated over 205°C the efficiency of NPyr fell off due to its thermal instability.

Table III lists the percent recovery for seven volatile NAs added to nitrite-free bacon at ten μg/kg. These values represent the efficiency of the distillation and cleanup procedures prior to quantitative analysis by GC-TEA. DMN gave the lowest percent recovery, probably due to its volatility (bp 154°C). Experiments in which a standard solution of DMN was diluted with DCM and concentrated as described in the experimental section showed that the greater part of this loss occurred during the concentration procedure.

TABLE II. PERCENT RECOVERY BY TRAPPING. [a]

N-nitrosamine	Trap Injection	Reinjection
DMN	81	88
DEN	86	86
DPN	83	81
DBN	75	70
NPyr	89	74

a. as percent of direct syringe injection

TABLE III. PERCENT RECOVERY FROM BACON SPIKED AT 10 µg/kg.

N-nitrosamine	$\bar{x}$ [a]	s
DMN	74	7.02
DEN	94	4.16
DPN	98	3.29
DBN	85	10.39
NPip	96	4.55
NPyr	92	6.36
NMor	88	6.12

a. n=5

It was also noted that concentration of the final extract to less than 0.5 mL significantly decreased the percent recovery.

Recovery of DBN was the least precise (s=10.39). The high boiling point of DBN and slight differences in the pressure of each distillation might explain the lower precision.

Few authors present the variance or number of trials used in determining recovery. These values become important when correction for recovery is made to determine actual NA content of a food. For example, the 95% confidence interval for DMN in bacon assuming an assay value of ten μg/kg and a recovery of 74% (s=7.02) would be 12 to 16 μg/kg.

Fine et al. (1975b) in their original description of the mineral oil distillation method listed the following percent recoveries; DMN 71-75%; DEN, 90%; DBN, 95-98%; NPyr, 100%. They did not indicate the precision of these values. Havery et al. (1978) in comparing the multi-detection method to the mineral oil distillation found the recoveries to average 75 and 92%, respectively. Neither values for individual NAs nor indications of precision were given. Goodhead and Gough (1975) gave detailed recovery data for six volatile NAs in water, cheese, fish and meat by atmospheric distillation. Values for recovery from meat ranged from a low of 67% for DMN to a high of 81% for DPN. Standard deviations (n=6) ranged from a low of 6.3 (DBN) to a high of 32.9 (NPyr). The large standard

deviation for NPyr in meat could make correcting for recovery of NPyr difficult. In addition, the accuracy of quantitative data based on the recovery of an internal standard depends in part on the precision of recovery for the internal standard.

The mineral oil distillation method resulted in higher overall recoveries of volatile NAs with an apparent increase in precision over other published methods. This results in greater sensitivity and more precise quantitative data.

Sample Preparation

To determine the effect of storage time and method of grinding the sample on NA content 1508 g of fried bacon were mixed and ground in a hand meat grinder. One-half of the ground meat was then frozen in liquid N_2 and shattered in a blender as described above. Both samples were stored at -23°C and portions analyzed for NPyr periodically over a two week period. Duplicate samples were also analyzed by steam distillation for comparison. As indicated in Table IV, ground and frozen samples may be stored at least two weeks without significantly altering the results. Also, hand grinding bacon samples appears to be a satisfactory method of sample preparation. The vacuum mineral oil distillation method gave slightly higher values with greater precision. Both methods of analysis were in close agreement.

TABLE IV. NITROSOPYRROLIDINE CONTENT OF STORED FRIED BACON.[a]

Day	Vacuum Distillation		Steam Distillation	
	Shattered	Ground	Shattered	Ground
1	27[b]	28	22	24
4	--	24	29	26
7	27	30	29	31
11	29	29	21	21
15	32	26	--	--
$\bar{x}$	29	27	25	26
s	2.36	2.41	4.35	4.20

a. corrected for recovery

b. µg/kg.

Spiked Foods

Figure 12 shows the total ion current (TIC) plot (solid line) from the GC-MS analysis of fried pork to which one μg/kg DMN had been added. Superimposed on the TIC plot is a limited-mass search by computer for the molecular ion (m/e 74) of DMN. This identified the spectrum containing DMN. After an appropriate spectrum was subtracted to correct for background ions, the entire spectrum of DMN was compared to published standard spectra and to standard spectra obtained prior to analysis of the unknown. This procedure was repeated for other foods spiked with NPyr as well as DMN (Table V).

Figures 13 and 14 are the spectra obtained from cheese, raw fish, fried pork and nitrite free bacon to which one μg/kg of DMN or NPyr had been added. Also included are standard spectra obtained on the same instrument. Most inconsistencies between the standards and unknowns are relatively minor. For example, the m/e 43 ion has been subtracted out of some spectra due to the high background level of this common organic fragment (McLafferty, 1963). In some spectra the ion intensities are different from the standard, depending on how much background must be subtracted. Certain ions of low diagnostic value such as m/e 68 in NPyr have also been subtracted out by the background correction. Overall,

Figure 12. Total ion current (solid line) and limited-mass search for m/e 74 (broken line) from fried pork extract.

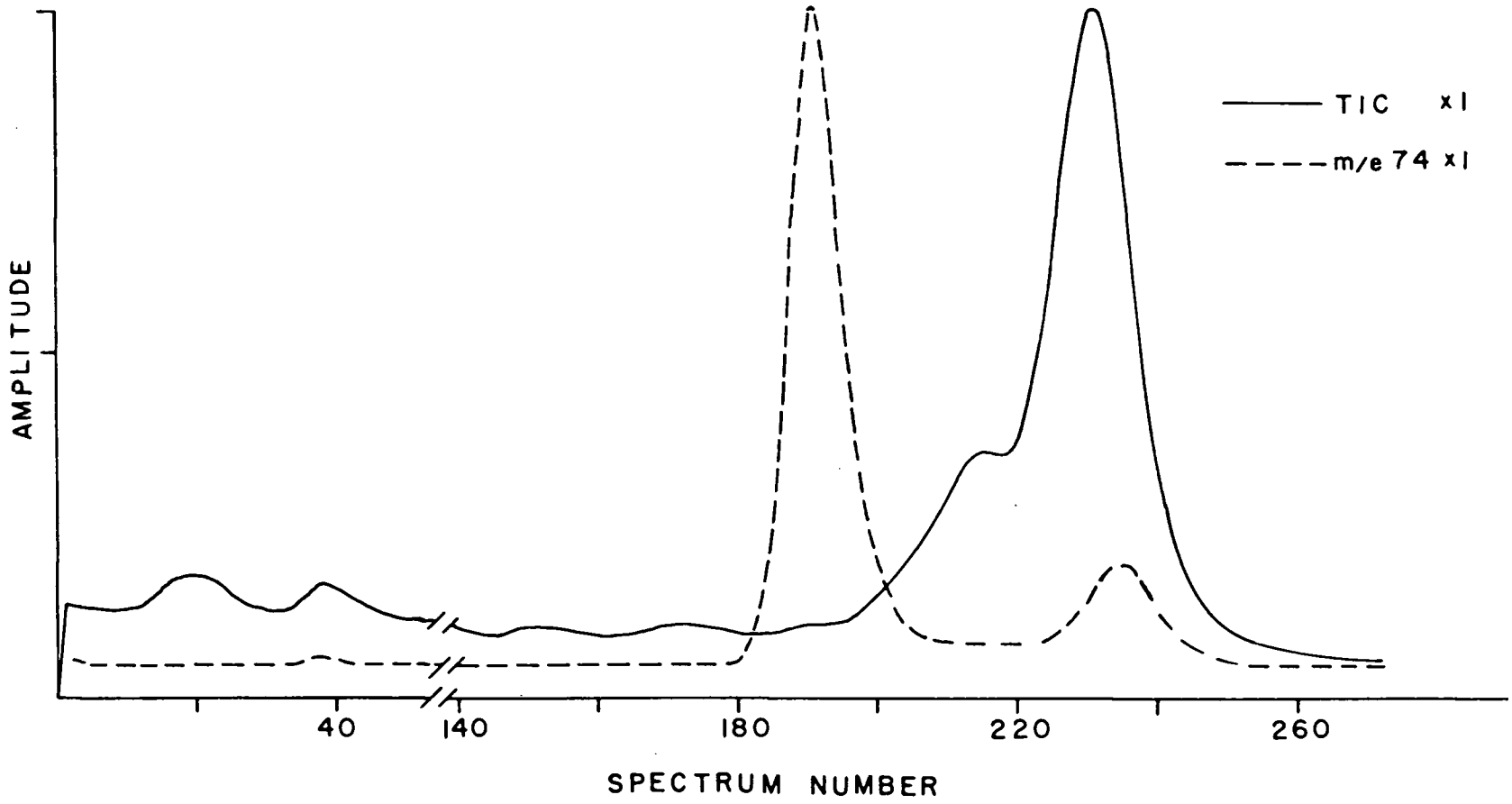

TIC x1
m/e 74 x1
AMPLITUDE
40
140
180
220
260
SPECTRUM NUMBER

TABLE V. SPIKED FOODS CONFIRMED BY MASS SPECTROMETRY

Food	Concentration [a]	
	DMN	NPyr
Bacon (NO_2 free)	--[b]	1
Fish (raw)	1	--
Pork (fried)	1	1
Cheese	1	1

a. µg/kg
b. not attempted

Figure 13. Mass spectra of dimethylnitrosamine added to (A) standard solution, (B) raw fish, (C) fried pork, and (D) cheese at one μg/kg.

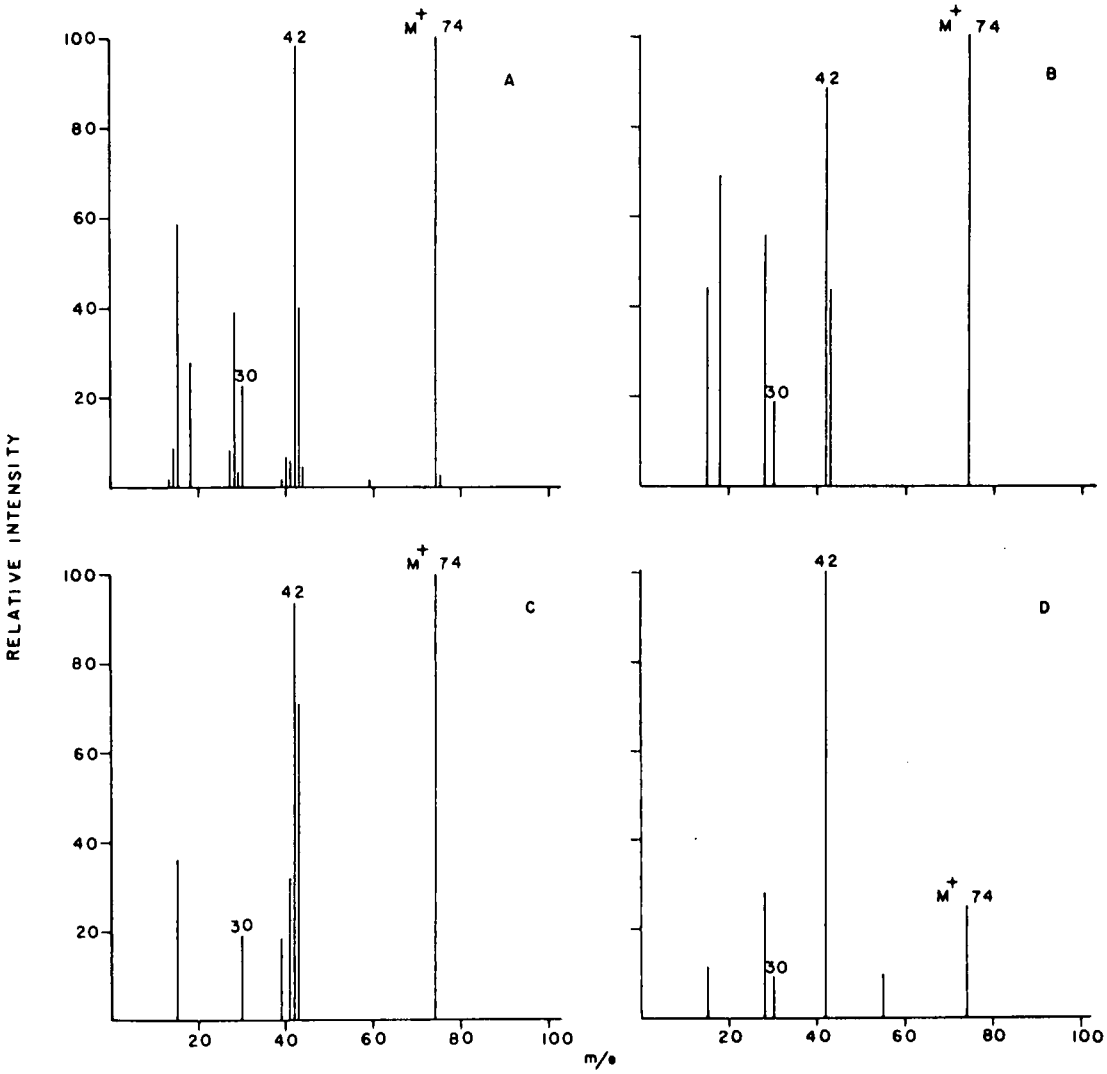
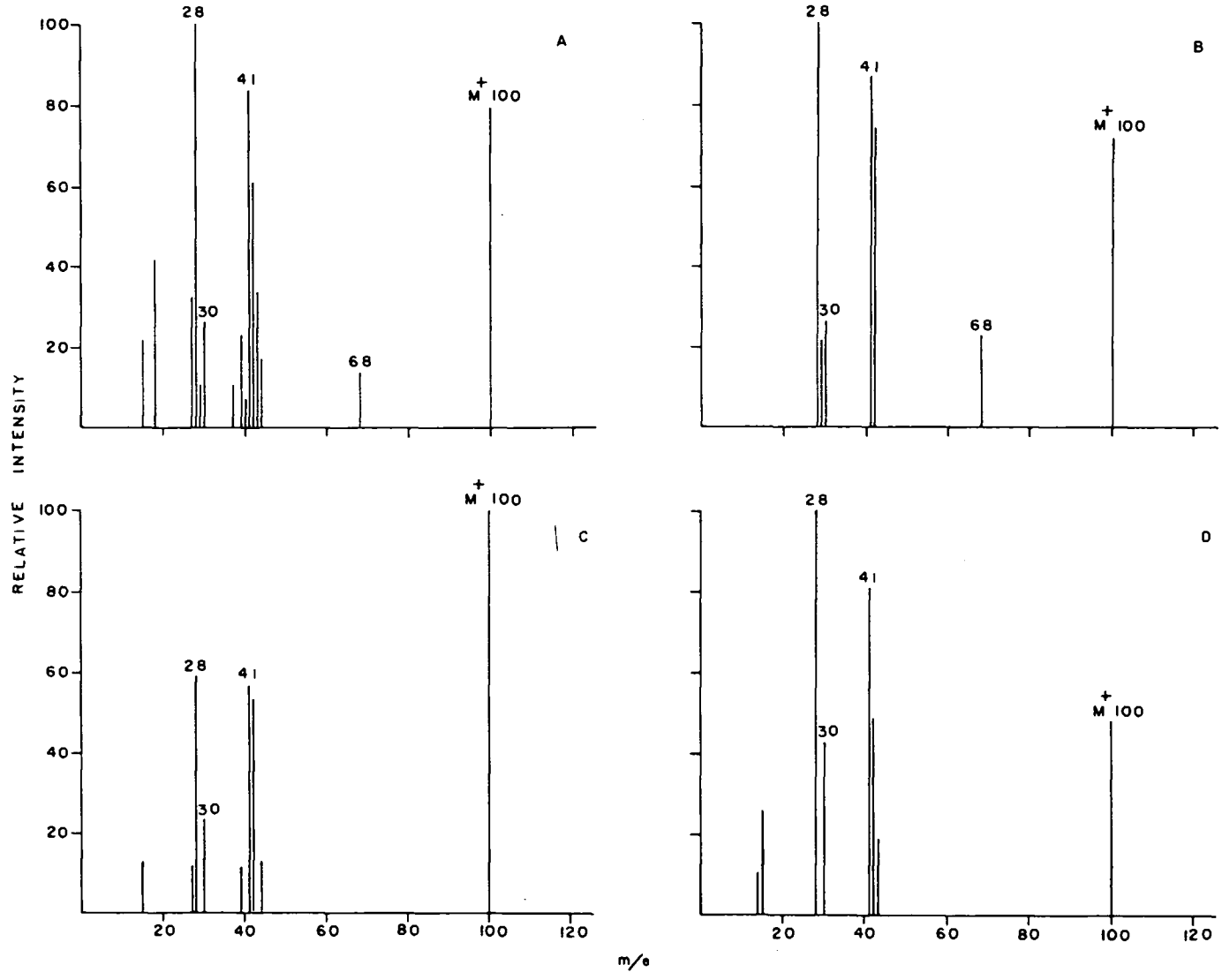

RELATIVE INTENSITY
100
80
60
40
20
42
M+ 74
30
A
B
C
D
20
40
60
80
100
m/e

Figure 14. Mass spectra of nitrosopyrrolidine added to (A) standard solution, (B) nitrite-free bacon, (C) fried pork, and (D) cheese at one μg/kg.


A
B
C
D
28
41
30
68
M+ 100
RELATIVE INTENSITY
m/e
100
80
60
40
20
20
40
60
80
100
120

these spectra contain the important diagnostic ions and can be taken as confirmatory qualitative evidence for the presence of these NAs.

The mass spectra of the lower molecular weight NAs contain relatively few diagnostic ions. For example, only the m/e 30, 42, and 74 ions are of significant value in the spectrum of DMN. Most NAs do, however, give intense molecular ions. The molecular ions are the most useful ions for locating the spectrum containing the NA. Careful attention must be paid to retention time as ions of the same mass as the M^+ ion are often seen in the chromatogram.

The maximum sensitivity of the method is not limited by the instrument but by the food sample itself. For example, ten ng NPyr in hexane will produce an acceptable mass spectrum. In food samples which contain a large number of low molecular weight compounds, injections of 50 to 100 ng may be necessary to obtain acceptable spectra. In foods which contain less contaminating compounds, such as some vegetables, the minimum level of confirmation may be lower than the one μg/kg reported here.

The TEA had a 3x signal-to-noise sensitivity of 20 to 40 ng/kg depending on the NA and column temperature. This is in general agreement with Fine et al. (1975).

Commercial Bacons

Six brands of commercial bacon were purchased locally, fried, and analyzed as described above. Quantitative data (Table VI) were obtained by GC-TEA and qualitative data confirmed by GC-MS. These values vary between brands by more than 10x. Recent surveys on trends in the NPyr content of bacons in the US also reported large variations and that the overall content was decreasing with time (Havery et al., 1978a). Brand F (Table VI) was a beef bacon and contained the lowest amount of volatile NA. Previously this product was reported as not containing volatile NAs and it was suggested that only pork bellies contain the unknown precursors to NPyr (Fiddler et al., 1974). As demonstrated here, the precursors to NPyr are not unique to pork although their concentrations may differ significantly.

Each of the bacons were analyzed by GC-MS to confirm the identity of one of the NAs present. Only those NAs below ten μg/kg were attempted as analysis of the higher concentrations would have been superfluous. Figures 15 and 16 are the spectra obtained from the bacons along with standard spectra for comparison. As with the spiked foods certain differences between standard and unknown spectra exist. The m/e 43 ion was again subtracted from some spectra and certain ion intensities vary.

TABLE VI. VOLATILE NITROSAMINES IN FRIED BACON.

Brand	Concentration[a]	
	DMN	NPyr
A	0.5	4[b]
B	7[b]	45
C	7[b]	91
D	2	4[b]
E	4[b]	19
F	0.6	2[b]

a. µg/kg (corrected for recovery)

b. confirmed by low-resolution mass spectrometry.

Figure 15. Mass spectra of dimethylnitrosamine from (S) standard solution and commercial bacons. Concentrations are given in Table VI.

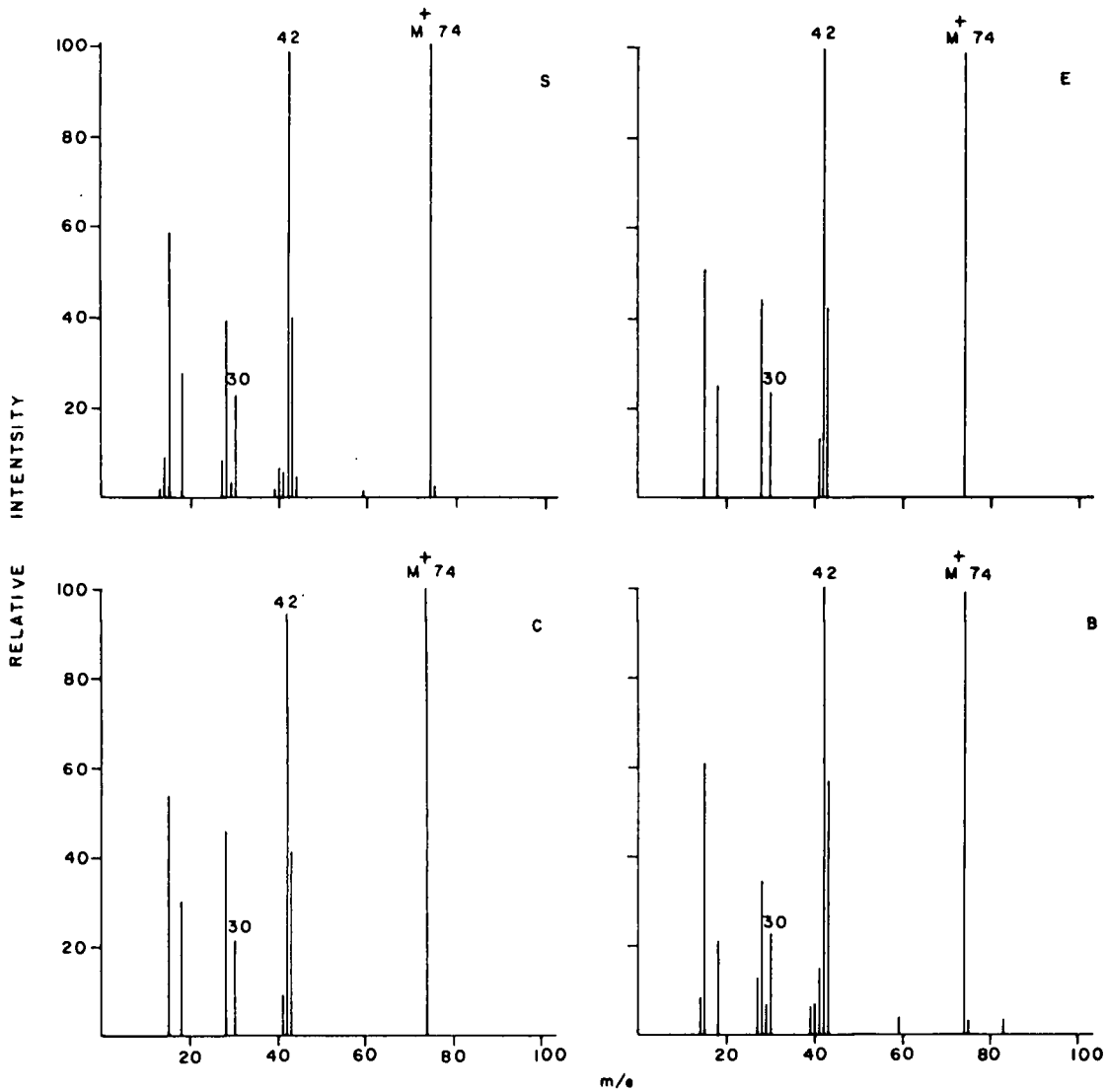
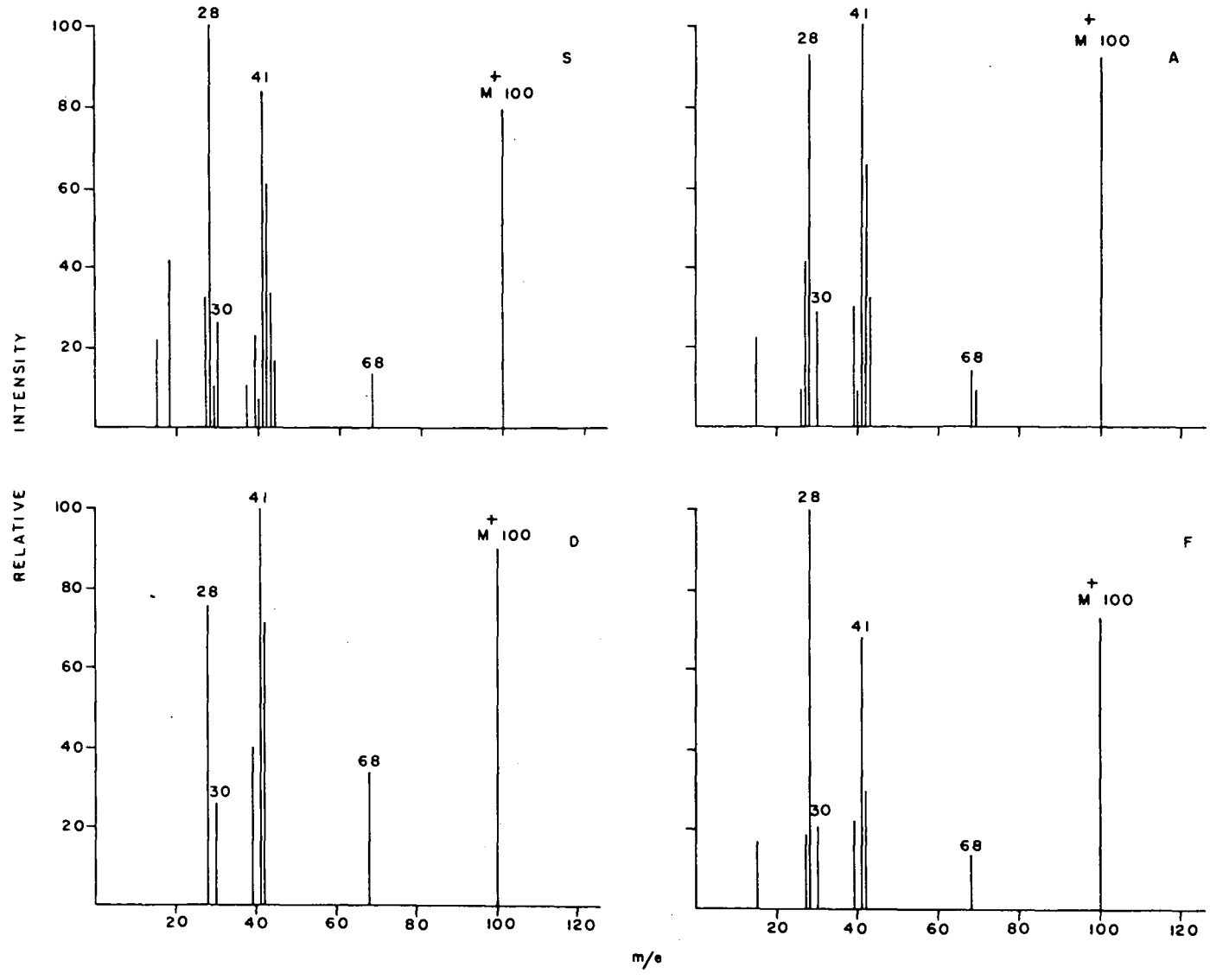

RELATIVE INTENTSITY
100
80
60
40
20
42
M+ 74
30
S
E
C
B
20
40
60
80
100
m/e

Figure 16. Mass spectra of nitrosopyrrolidine from (S) standard solution and commercial bacons. Concentrations are given in Table VI.


RELATIVE INTENSITY
100
80
60
40
20
28
41
30
68
M+ 100
S
A
D
F
20
40
60
80
100
120
m/e

The identity of each NA was not only confirmed by the similarity of its mass spectrum to a standard, but was further substantiated by the fact that it was TEA positive and had chromatographed on both packed and capillary GC columns with the proper retention times. Taken together these three data confirm the presence of the NA in the food sample.

The quantitative amounts of the NAs were determined by GC-TEA and were corrected for recovery. As discussed above there are certain statistical limitations to any quantitative analysis. The world-wide collaborative studies and the studies which have compared the GC-TEA to other quantitative methods have indicated that this method is probably as precise and accurate as any quantitative method available at present. However, increased attention to the variability of all methods needs to be made.

A number of workers have reported NAs in foods (Scanlan, 1975) and in recent years the majority have confirmed their results by MS. Few authors have presented actual MS data in support of such findings (Kushnir et al., 1975; Gough et al., 1977b; Iyengar et al., 1976; Janzowski et al., 1978a). The International Agency for Research on Cancer subcommittee on NA analysis has recommended that all essential details of MS methods be presented (Walker et al., 1978). This should be extended to the publication of spectra and/or mass fragmentograms, especially in the cases of NAs below ten

μg/kg and new findings. Confirmation of very low levels of NAs in foods is not a dichotomy and in some cases judgments must be made.

At least a portion of the m/e 30 ion produced in the spectra of nitramines is probably due to NO^+. When one considers the similarity of retention times for certain NAs and nitramines, the possibility of false positives when monitoring the NO^+ by high resolution MS becomes apparent. Foreman and Goodhead (1975) recognized this for other nitro compounds. However reports based on this technique persist (Iyengar et al., 1976; Sen et al., 1976; Sen et al., 1977).

SUMMARY AND CONCLUSIONS

Two dinitroaniline based herbicides were analyzed and found to contain TEA positive peaks which were not NAs. These compounds were identified as nitramines and their relative response ratios, compared to NAs, were determined. The TEA responded to other nitramines which indicates that this response is probably general. The similarity of retention times of certain nitramines and NAs was demonstrated. There is a possibility that nitramines can be interpreted as being NAs if only high resolution MS of the NO^+ ion and retention time data are collected. Nitramines are responsible for TEA positive peaks reported in certain herbicides. In addition, previous work demonstrating that an herbicide containing trifluralin also contained the carcinogen dipropylnitrosamine was confirmed.

An analytical method was described for the quantitation and qualitative confirmation of volatile NAs in foods. Quantitation was achieved by combined gas chromatography-thermal energy analysis and qualitative confirmation made by gas chromatography-low resolution mass spectrometry. The recovery of seven volatile NAs from fried bacon averaged 90% with a high of 98 and a low of 74%. The method was more precise and gave slightly higher values than a steam distillation method used in some laboratories. A

maximum sensitivity of one μg/kg for DMN and NPyr in several different foods was realized. The NA content of several commercial bacon samples was determined and confirmed by low resolution mass spectrometry.

This work demonstrates that compounds other than NAs can elicit a response from the TEA and that these compounds do occur in conjunction with NAs in certain herbicides. This verifies the need to confirm the qualitative nature of compounds which give a TEA response by an independent method.

It was also demonstrated that unambiguous confirmation of the identity of volatile NAs in foods at one μg/kg can be achieved by low resolution mass spectrometry.

BIBLIOGRAPHY

Althorpe, J., D. A. Goddard, D. J. Sissons, and G. M. Telling. 1970. The gas chromatographic determination of nitrosamines at the picogram level by conversion to their corresponding nitramines. Journal of Chromatography 53:371.

ApSimon, J. W. and J. D. Cooney. 1971. Some aspects of the mass spectra of N-nitrosamines. Canadian Journal of Chemistry 49:1367.

Astill, B. D. 1978. Antioxidants and N-nitrosamine formation. Journal of the American Oil Chemists Society 55:248A.

Baker, J. K. and C. Y. Ma. 1978. Determination of N-nitrosoproline in meat samples. Journal of Agricultural and Food Chemistry 26:1253.

Baumgarten, H. E. 1973. Organic Syntheses Collective Volume Five. John Wiley & Sons, New York. p. 839.

Bills, D. D., K. I. Hildrum, R. A. Scanlan, and L. M. Libbey. 1973. Potential precursors of N-nitrosopyrrolidine in bacon and other fried foods. Journal of Agricultural and Food Chemistry 21:876.

Binkerd, E. F. and O. E. Kolari. 1975. The history and use of nitrate and nitrite in the curing of meat. Food and Cosmetics Toxicology 13:655.

Bobbitt, J. M., A. E. Schwarting, and R. J. Gritter. Introduction to Chromatography. D. Van Nostrand Co., New York. p. 90.

Bogovski, P., R. Preussmann, and E. A. Walker, Eds. 1972. N-nitroso Compounds Analysis and Formation. International Agency for Research on Cancer, Lyon. p. 92.

Brunnemann, K. D., L. Yu, and D. Hoffmann. 1977. Assessment of carcinogenic volatile N-nitrosamines in tobacco and in mainstream and sidestream smoke of cigarettes. Cancer Research 37:3218.

Castegnaro, M., B. Pignatelli, and E. A. Walker. 1974. A rapid method for the semi-quantitative determination of volatile N-nitrosamines in alcoholic beverages. Analyst 99:156.

Castegnaro, M. and E. A. Walker. 1978. New data from collaborative studies on analyses of nitrosamines. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 53.

Castegnaro, M. 1978. Personal communication to R. A. Scanlan.

Ceh, L. and F. Ender. 1978. A sensitive method for the colormetric determination of volatile nitrosamines in food products and air. Food and Cosmetics Toxicology 16:117.

Challis, B. C. and R. A. Butler. 1968. Substitution at an amino nitrogen. In: The Chemistry of the Amino Group. Patai, S. Ed. Interscience, New York. p. 277.

Challis, B. C. and C. D. Bartlett. 1975. Possible carcinogenic effects of coffee constituents. Nature 254:532.

Challis, B. C. and S. A. Kyptopoulos. 1976. Nitrosation under alkaline conditions. Journal of the Chemical Society Chemical Communications 1976: 877.

Challis, B. C. and S. A. Kyrtopoulos. 1977. Rapid formation of carcinogenic N-nitrosamines in aqueous alkaline solutions. British Journal of Cancer 35:693.

Challis, B. C., A. Edwards, R. R. Hunma, S. A. Kyrtopoulos, and J. R. Outram. 1978. Rapid formation of N-nitrosamines from nitrogen oxides under neutral and alkaline conditions. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 127.

Cohen, S. Z., G. Zweig, M. Law, D. Wright, and W. R. Bontoyan. 1978. Analytical determination of N-nitroso compounds in pesticides by the United States Environmental Protection Agency--a preliminary study. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 333.

Coleman, M. H. 1978. A model system for the formation of N-nitrosopyrrolidine in grilled or fried bacon. Journal of Food Technology 13:55.

Collins-Thompson, D. L., P. C. Chang, C. M. Davidson, E. Larmond, and H. Pivnick. 1974. Effect of nitrite and storage temperature on the organoleptic quality and toxinogenesis by Clostridium botulinum in vacuum-packed side bacon. Journal of Food Science 39:607.

Colthup, B., L. H. Daly, and S. E. Wiberley. 1964. Introduction to Infrared and Raman Spectroscopy. Academic Press, New York. p. 273.

Cotton, F. A. and G. Wilkinson. 1966. Advanced Inorganic Chemistry. Interscience, New York. p. 342.

Compson, K. R., S. Evans, D. Hazelby, and L. E. Moore. 1977. Advantages of high resolution mass spectrometry for GC/MS work. 25th Annual Conference on Mass Spectrometry and Allied Topics. May 29-June 3, 1977. Washington, D.C.

Cox, G. B. 1973. Estimation of volatile N-nitrosamines by high performance liquid chromatography. Journal of Chromatography 83:471.

Crosby, N. T. 1976. Nitrosamines in foodstuffs. Residue Reviews 64:77.

Crosby, N. T. and R. Sawyer. 1976. N-nitrosamines: Review of chemical and biological properties and their estimation in foodstuffs. Advances in Food Research 22:1.

Cross, C. K., K. R. Bharucha, and G. M. Telling. 1978. Determination of volatile N-nitrosamines in bacon cook-out fat by nitrite release and thin-layer chromatography of fluorescent amine derivatives. Journal of Agricultural and Food Chemistry 26:657.

D'Agostino, J. T. and H. H. Jaffe'. 1969. A pure intramolecular charge-transfer transition. Substituted N-alkyl-N-nitrosoanilines. Journal of the American Chemical Society 91:3383.

Dahn, H., L. Loewe, and C. A. Bunton. 1960. Uber die oxydation von ascorbinsaure durch salpetrige saure Teil VI: uebersicht und diskussion der ergebnesse. Helvetica Chimica Acta 43:320.

Dikun, P. P. 1976. Group evaluation of volatile and nonvolatile N-nitroso compounds in foodstuffs. In: Environmental N-nitroso Compounds Analysis and Formation. Walker, E. A., P. Bogovski, and L. Griciute, Eds. International Agency for Research on Cancer, Lyon. p. 57.

Doerr, R. C. and W. Fiddler. 1977. Photolysis of volatile nitrosamines at picogram levels as an aid to confirmation. Journal of Chromatography 140:284.

Dooley, C. J., A. E. Wasserman, and S. Osman. 1973. A contaminant in N-nitrosodimethylamine confirmation by high resolution mass spectrometry. Journal of Food Science 38:1096.

Douglass, M. L., B. L. Kabacoff, G. A. Anderson, and M. C. Cheng. 1978. The chemistry of nitrosamine formation, inhibition and destruction. Journal of the Society of Cosmetic Chemists 29:581.

Drescher, G. S. and G. W. Frank. 1978. Estimation of the extractable N-nitroso compounds at the parts-per-billion level. Analytical Chemistry 50:2118.

Druckrey, H., R. Preussmann, S. Ivankovic, and D. Schmahl. 1967. Organotrope carcinogene wirkungen bei 65 verschiedenen N-nitroso-verbindungen an BD-ratten. Zeitschrift fur Krebsforschung 69:103.

Eisenbrand, G., P. Marquardt, and R. Preussmann. 1969. Trace analysis of N-nitroso compounds. I. Liquid-liquid distribution in acetonitrile/heptane as a cleanup method. Zeitschrift fur Analytische Chemie 247:54.

Eisenbrand, G. and R. Preussmann. 1970. Eine neue method zur kolorimetrischen bestimmung von nitrosaminen rach spaltung der N-nitrosogruppe mit bromowasserstoff in Eisessig. Arzneim -- Forsch 20:1515.

Eisenbrand, G., A. von Hodenberg, and R. Preussmann. 1970. Trace analysis of N-nitroso compounds. II. Steam distillation at neutral, alkaline and acid pH under reduced and atmospheric pressure. Zeitschrift fur Analytische Chemie 251:22.

Eisenbrand, G. 1972. Determination of volatile nitrosamines at low levels in food by acid catalyzed denitrosation and formation of derivatives from the resulting amines. In: N-nitroso Compounds Analysis and Formation. Bogovski, P., R. Preussmann, and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 64.

Eisenbrand, G. 1974. Determination of volatile nitrosamines: A review. In: Proceedings of the International Symposium on Nitrite in Meat Products. Krol B. and B. J. Tinbergen, Eds. Center for Agricultural Publishing and Documentation, Wageningen. p. 45.

Eisenbrand, G., O. Ungerer, and R. Preussmann. 1974. Formation of N-nitroso compounds from agricultural chemicals and nitrite. In: N-nitroso Compounds in the Environment. Bogovski, P. and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 71.

Eisenbrand, G., C. Janzowski, and R. Preussmann. 1975. Gas chromatographic determination of N-nitrosoamino acids by trimethylsilylation and single-ion fragmentography. Journal of Chromatography 115:602..

Eisenbrand, G., B. Spiegelhalder, C. Janzowski, J. Kann, and R. Preussmann. 1978. Volatile and nonvolatile N-nitroso compounds in foods and other environmental media. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 311.

Eizember, R. F., K. R. Volger, R. W. Souter, W. N. Cannon, and P. M. Wege. 1978. Reduction of nitrosamine levels in dinitroaniline pesticides. Presented at the semi-annual meeting of the American Chemical Society, March 1978.

Elespuru, R. K., and W. Lijinsky. 1973. The formation of carcinogenic nitroso compounds from nitrite and some types of agricultural chemicals. Food and Cosmetics Toxicology 11:807.

Emmons, W. D. 1954. Peroxytrifluoroacetic acid. I. The oxidation of nitrosamines to nitramines. Journal of the American Chemical Society 76:3468.

Emmons, W. D. and J. P. Freeman. 1955. Alkaline nitration. I. The nitration of amines with cyanohydrin nitrates. Journal of the American Chemical Society 77:4387.

Ender, F., G. Harve, A. Helgebostad, N. Koppang, R. Madsen, and L. Ceh. 1964. Isolation and identification of a hepatotoxic factor in herring meal produced from sodium nitrite preserved herring. Naturwissenschaften 51:637.

Ender, F. and L. Ceh. 1971. Conditions and chemical reactions mechanisms by which nitrosamines may be formed in biological products with reference to their possible occurrence in food products. Zeitschrift fur Lebensmittel-Untersuchung und Forschung 145:133.

Engel, R. E. 1977. Nitrites, nitrosamines, and meat. Journal of the American Veterinary Medicine Association 171:1157.

Essigmann, J. M. and P. Issenberg. 1972. Gas chromatographic determination of volatile nitrosamines in foods. Journal of Food Science 37:684.

Fan, T. Y. and S. R. Tannenbaum. 1971. Automatic colorimetric determination of N-nitroso compounds. Journal of Agricultural and Food Chemistry 19:1267.

Fan, T. Y. and S. R. Tannenbaum. 1972. Stability of N-nitroso compounds. Journal of Food Science 37:274.

Fan, T. Y. and S. R. Tannenbaum. 1973. Factors influencing the rate of formation of nitrosomorpholine from morpholine and nitrite: Acceleration by thiocyanate and other anions. Journal of Agricultural and Food Chemistry 21:237.

Fan, T. Y., U. Goff, L. Song, and D. H. Fine. 1977. N-nitrosodiethanolamine in cosmetics, lotions, and shampoos. Food and Cosmetics Toxicology 15:423.

Fan, T. Y., I. S. Krull, R. D. Ross, M. H. Wolf, and D. H. Fine. 1978. Comprehensive analytical procedures for the determination of volatile and non-volatile, polar and non-polar N-nitroso compounds. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle Eds. International Agency for Research on Cancer, Lyon. p. 3.

Fan, T. Y., R. Ross, and D. H. Fine. 1978a. Isolation and identification of some thermal energy analyzer (TEA) responsive substances in drinking water. Environmental Science and Technology 12:692.

Fazio, T., J. W. Howard, and R. White. 1972. Multidetection method for determining volatile nitrosamines in foods. In: N-nitroso Compounds Analysis and Formation. Bogovski, P., R. Preussmann, and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 16.

Fazio, T., R. H. White, R. Dusold, and J. W. Howard. 1973. Nitrosopyrrolidine in cooked bacon. Journal of the Association of Official Analytical Chemists 56:919.

Fiddler, W., J. W. Pensabene, J. C. Fagan, E. J. Thorn, E. G. Piotrowski, and A. E. Wasserman. 1974. The role of lean and adipose tissue on the formation of nitrosopyrrolidine in fried bacon. Journal of Food Science 39:1070.

Fiddler, W., R. C. Doerr, and E. G. Piotrowski. 1978. Observations on the use of the thermal energy analyzer as a specific detector for nitrosamines. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 33.

Fine, D. H. and D. P. Rounbehler. 1975. Trace analysis of volatile N-nitroso compounds by combined gas chromatography and thermal energy analysis. Journal of Chromatography 109:2.

Fine, D. H., F. Rufeh, D. Lieb, and D. P. Rounbehler. 1975. Description of the thermal energy analyzer (TEA) for trace determination of volatile and nonvolatile N-nitroso compounds. Analytical Chemistry 47:1188.

Fine, D. H., D. Lieb, and F. Rufeh. 1975a. Principle of operation of the thermal energy analyzer for the trace analysis of volatile and nonvolatile N-nitroso compounds. Journal of Chromatography 107:351.

Fine, D. H., D. P. Rounbehler, and P. E. Oettinger. 1975b. A rapid method for the determination of sub-part per billion amounts of N-nitroso compounds in foodstuffs. Analytica Chimica Acta 78:383.

Fine, D. H., R. Ross, D. P. Rounbehler, A. Silvergleid, and L. Song. 1976. Analysis of nonionic nonvolatile N-nitroso compounds in foodstuffs. Journal of Agricultural and Food Chemistry 24:10.

Fine, D. H., D. P. Rounbehler, and N. P. Sen. 1976a. A comparison of some chromatographic detectors for the analysis of volatile N-nitrosamines. Journal of Agricultural and Food Chemistry 24:980.

Fine, D. H., D. P. Rounbehler, E. Sawicki, and K. Krost. 1977. Determination of dimethylnitrosamine in air and water by thermal energy analysis: Validation of analytical procedures. Environmental Science and Technology 11:577.

Fine, D. H., R. Ross, D. P. Rounbehler, A. Silvergleid, and L. Song. 1977a. Formation in vivo of volatile N-nitrosamines in man after ingestion of cooked bacon and spinach. Nature 265:753.

Fine, D. H., D. P. Rounbehler, A. Silvergleid, and R. Ross. 1977b. Trace analysis of polar and apolar N-nitroso compounds by combined high-performance liquid chromatography and thermal energy analysis. In: Proceedings of the Second International Symposium on Nitrite in Meats. Tinbergen, B. J. and B. Krol, Eds. Centre for Agricultural Publishing and Documentation, Wageningen. p. 191.

Fine, D. H. 1978. An assessment of human exposure to N-nitroso compounds. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaio, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 267.

Fish, R. H., R. L. Holmstead, and W. Gaffield. 1976. Gas phase protonalysis reactions: Chemical ionization mass spectrometry of N-nitrosamines. Tetrahedron 32:2689.

Food and Drug Administration. 1978. FDA's and USDA's action regarding nitrite. Joint press release, August 1978.

Foreman, J. K. and K. Goodhead. 1975. The formation and analysis of N-nitrosamines. Journal of the Science of Food and Agriculture 26:1771.

Freed, D. J. and A. M. Mujsce. 1977. Generation of nitrosamines for gas chromatographic analysis via direct injection. Analytical Chemistry 49:1544.

Fridman, A. L., F. M. Mukhametshin, and S. S. Novikov. 1971. Advances in the chemistry of aliphatic N-nitrosamines. Russian Chemical Review 40:34.

Gadbois, D. F., E. M. Ravesi, R. C. Lundstrom, and R. S. Maney. 1975. N-nitrosodimethylamine in cold-smoked sablefish. Journal of Agricultural and Food Chemistry 23:665.

Goodall, C. M. and T. H. Kennedy. 1976. Carcinogenicity of dimethylnitramine in NZR rats and NZO mice. Cancer Letters 1:295.

Goodhead, K. and T. A. Gough. 1975. The reliability of a procedure for the determination of nitrosamines in food. Food and Cosmetics Toxicology 13:307.

Gough, T. A. and K. S. Webb. 1972. The use of a molecular separator in the determination of trace constituents by combined gas chromatography and mass spectrometry. Journal of Chromatography 64:201.

Gough, T. A. and K. Sugden. 1975. Dual column gas chromatographic system for use in mass spectral determination of nitrosamines. Journal of Chromatography 109:265.

Gough, T. A., K. Sugden, and K. S. Webb. 1975. Pyridine catalyzed reaction of volatile N-nitrosamines with heptafluorobutyric anhydride. Analytical Chemistry 47:509.

Gough, T. A. and C. L. Walters. 1976. Volatile nitrosamines in fried bacon. In: Environmental N-nitroso Compounds Analysis and Formation. Walker, E. A., P. Bogovski, and L. Griciute, Eds. International Agency for Research on Cancer, Lyon. p. 195.

Gough, T. A. 1977. Report of a Meeting Held at Thermo Electron Corporation, Waltham, Massachusetts. August 27, 1977.

Gough, T. A., K. S. Webb, and R. F. Eaton. 1977. Simple chemiluminescent detector for the screening of foodstuffs for the presence of volatile nitrosamines. Journal of Chromatography 137:293.

Gough, T. A., K. S. Webb, M. A. Pringuer, and B. J. Wood. 1977a. A comparison of various mass spectrometric and a chemiluminescent method for the estimation of volatile nitrosamines. Journal of Agricultural and Food Chemistry 25:663.

Gough, T. A., M. F. McPhail, K. S. Webb, B. J. Wood and R. F. Coleman. 1977b. An examination of some foodstuffs for the presence of volatile nitrosamines. Journal of the Science of Food and Agriculture 28:345.

Gough, T. A., K. S. Webb, and R. F. Coleman. 1978. Estimation of volatile nitrosamine content of UK food. Nature 272:161.

Gough, T. A. and K. S. Webb. 1978. Positive interferant in the chemiluminescent detection of nitrosamines. Journal of Chromatography 154:234.

Gough, T. A. 1978. An examination of some foodstuffs for trace amounts of volatile nitrosamines using the thermal energy analyzer. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 297.

Gough, T. A. 1978a. Determination of N-nitroso compounds by mass-spectrometry. The Analyst 103:785.

Gray, J. I. and L. R. Dugan. 1975. Inhibition of N-nitrosamine formation in model food systems. Journal of Food Science 40:981.

Gray, J. I. 1976. N-nitrosamines and their precursors in bacon: A review. Journal of Milk and Food Technology 39:686.

Gray, J. I. 1977. N-nitrosamine precursors in bacon: Review. Journal of the Canadian Institute of Food Science and Technology 10:A15.

Gray, J. I. and M. E. Collins. 1977. The development of free proline during the storage of green pork bellies. Journal of the Canadian Institute of Food Science and Technology 10:97.

Gray, J. H., M. E. Collins, and L. R. Russel. 1977. Formation of N-nitrosohydroxypyrrolidine in model and cured meat systems. Journal of the Canadian Institute of Food Science and Technology 10:36.

Gray, J. I. and M. E. Collins. 1978. Formation of N-nitroso-pyrrolidine in fried bacon. Journal of Food Protection 41:36.

Gray, J. I., M. E. Collins, and B. McDonald. 1978. Precursors of dimethylnitrosamine in fried bacon. Journal of Food Protection 41:31.

Groenen, P. J., R. J. G. Jonk, C. van Ingen, and E. N. de Braun. 1976. Determination of eight volatile nitrosamines in thirty cured meat products with capillary gas chromatography-high resolution mass spectrometry: The presence of nitrosodiethylamine and the absence of nitrosopyrrolidine. In: Environmental N-nitroso Compounds Analysis and Formation. Walker, E. A., P. Bogovski, and L. Griciute, Eds. International Agency for Research on Cancer, Lyon. p. 321.

Groenen, P. J., M. W. de Cock-Bethebeder, R. J. G. Jonk, and C. van Ingen. 1977. Further studies on the occurrence of volatile N-nitrosamines in meat products by combined gas chromatography and mass spectrometry. In: Proceedings of the Second International Symposium on Nitrite in Meat Products. Tinbergen, B. J. and B. Krol. Eds. Center for Agricultural Publishing and Documentation, Wageningen. p. 227.

Gruger, E. H. 1972. Chromatographic analyses of volatile amines in marine fish. Journal of Agricultural and Food Chemistry 20:781.

Hansen, T., W. Iwoka, L. Green, and S. R. Tannenbaum. 1977. Analysis of normal nitrosoproline in raw bacon--Further evidence that nitrosoproline is not a major precursor of nitrosopyrrolidine. Journal of Agricultural and Food Chemistry 25:1423.

Havery, D. C., D. A. Kline, E. M. Miletta, F. L. Joe Jr., and T. Fazio. 1976. Survey of food products for volatile N-nitrosamines. Journal of the Association of Official Analytical Chemists 59:540.

Havery, D. C. and T. Fazio. 1977. Survey of finfish and shellfish for volatile nitrosamines. Journal of the Association of Official Analytical Chemists 60:517.

Havery, D. C., T. Fazio, and J. W. Howard. 1978. Survey of cured meat products for volatile N-nitrosamines: Comparison of two methods. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 41.

Havery, D. C., T. Fazio, and J. W. Howard. 1978a. Trends in levels of N-nitrosopyrrolidine in fried bacon. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 305.

Hildrum, K. I. 1975. Formation of N-nitrosamines from the nitrosation of spermidine and spermine. PhD. Thesis, Oregon State University, Corvallis.

Hildrum, K. I., J. L. Williams, and R. A. Scanlan. 1975. Effect of sodium chloride concentration on the nitrosation of proline at different pH levels. Journal of Agricultural and Food Chemistry 23:439.

Hotchkiss, J. H., R. A. Scanlan, and L. M. Libbey. 1977. Formation of bis(hydroxyalkyl)-N-nitrosamines as products of the nitrosation of spermidine. Journal of Agricultural and Food Chemistry 25:1183.

Howard, J. W., T. Fazio, and J. O. Watts. 1970. Extraction and gas chromatographic determination of N-nitrosodimethylamine in smoked fish: Application to smoked nitrite-treated chub. Journal of the Association of Official Analytical Chemists 53:269.

Hughes, E. D., C. K. Ingold, and J. H. Ridd. 1958. Nitrosation, diazotisation, and deamination. Part II. Second-and third-order diazotisation of aniline in dilute perchloric acid. Journal of the Chemical Society 1958:65.

Huxel, E. T., R. A. Scanlan, and L. M. Libbey. 1974. Formation of N-nitrosopyrrolidine from pyrrolidine ring containing compounds at elevated temperatures. Journal of Agricultural and Food Chemistry 22:698.

Hwang, L. S. and J. D. Rosen. 1976. N-nitrosopyrrolidine formation in fried bacon. Journal of Agricultural and Food Chemistry 24:1152.

Ingold, C. K. 1969. Substitution at heteroelements. In: Structure and Mechanism in Organic Chemistry, Second Edition. Cornell University Press, Ithaca. p. 611.

Ingram, M. 1974. The microbiological effects of nitrite. In: Proceeding of the International Symposium on Nitrite in Meats. Krol, B. and B. J. Tinbergin, Eds. Center for Agricultural Publishing and Documentation, Wageningen. p. 63.

Iwaoka, W. and S. R. Tannenbaum. 1976. Liquid chromatography of N-nitrosamino acids and their syn and anti conformers. Journal of Chromatography 124:105.

Iyengar, J. R., T. Panalaks, W. F. Miles, and N. P. Sen. 1976. Survey of fish products for volatile N-nitrosamines. Journal of the Science of Food and Agriculture 27:527.

Janzowsky, C., G. Eisenbrand, and R. Preussmann. 1978. Occurrence and determination of N-nitroso-3-hydroxypyrrolidine in cured meat products. Journal of Chromatography 150:216.

Janzowski, C., G. Eisenbrand, and R. Preussmann. 1978a. Occurrence of N-nitrosoamino acids in cured meat-products and their effect on formation of N-nitrosamines during heating. Food and Cosmetics Toxicology 16:343.

Jones, E. and J. Kenner. 1933. Catalytic decomposition of nitroso-β-alkylamino-ketones. Part I. A new method of preparing diazomethane and evidence of the occurrence of diazotisation in the aliphatic series. Journal of the Chemical Society 1933:363.

Jones, J. and T. Urbanski. 1949. Reactions of nitroparaffins. Part II. The reaction of 2-nitropropane with formaldehyde and ammonia. Journal of the Chemical Society 1949:1766.

Kalatzis, E. and J. H. Ridd. 1966. Nitrosation, diazotisation and deamination. Part XII. The kinetics of N-nitrosation of N-methylaniline. Journal of the Chemical Society (B) 1966: 529.

Karabatsos, G. J. and Taller, R. A. 1964. Structural studies by nuclear magnetic resonance. IX. Configurations and conformations of N-nitrosamines. Journal of the American Chemical Society 86:4373.

Keefer, L. K. and P. P. Roller. 1973. N-nitrosation by nitrite ion in neutral and basic medium. Science 181:1245.

Keefer, L. K. 1976. Promotion of N-nitrosation reactions by metal complexes. In: Environmental N-nitroso Compounds Analysis and Formation. Walker, E. A., P. Bogovski, and L. Griciute, Eds: International Agency for Research on Cancer, Lyon. p. 153.

Krull, I. S., U. Goff, M. Wolf, A. M. Heos, D. H. Fine, and G. P. Arsenault. 1978. The thermal energy analysis of sodium saccharin. Food and Cosmetics Toxicology 16:105.

Krull, I. S., T. Y. Fan, and D. H. Fine. 1978a. Problem of artifacts in the analysis of N-nitroso compounds. Analytical Chemistry 50:698.

Kushnir, I., J. I. Feinberg, J. W. Pensabene, E. G. Piotrowski, W. F. Fiddler, and A. E. Wasserman. 1975. Isolation and identification of nitrosoproline in uncooked bacon. Journal of Food Science 40:427.

Lee, J. S., L. M. Libbey, R. A. Scanlan, and D. D. Bills. 1978. Identification and quantification of 3-hydroxy-N-nitrosopyrrolidine in fried bacon. Bulletin of Environmental Contamination and Toxicology 19:511.

Lijinsky, W. and S. S. Epstein. 1970. Nitrosamines as environmental carcinogens. Nature 225:21.

Lijinsky, W., L. Keefer, and J. Loo. 1970. The preparation and properties of some nitrosamino acids. Tetrahedron 26:5137.

Lijinsky, W., W. H. Christie, and W. T. Rainey. 1973. Mass spectra of N-nitroso compounds. Publication TM 4359. Oak Ridge National Laboratory, Tennessee.

Magee, P. N., R. Montesano, and R. Preussmann. 1976. N-nitroso compounds and related carcinogens. In: Chemical Carcinogens, ACS Monograph 173. Searle, C. E. ed. American Chemical Society, Washington. p. 491.

March, J. 1968. Advanced Organic Chemistry: Reactions, Mechanisms, and Structure. McGraw-Hill, New York. p. 94, 257.

Massey, R. C., C. Crews, R. Davies, and D. J. McWeeny. 1978. A study of the competitive nitrosations of pyrrolidine, ascorbic acid, cysteine, and p-cresol in a protein-based model system. Journal of the Science of Food and Agriculture 29:815.

McLafferty, F. W. 1963. Mass Spectral Correlations, ACS Advances in Chemistry Number 40. American Chemical Society, Washington. p. 8.

Miller, A., R. A. Scanlan, L. M. Libbey, M. Petropakis, and A. F. Anglemier. 1973. Quantitative determination of dimethyl- and trimethylamine in fish protein concentrate. Journal of Agricultural and Food Chemistry 21:451.

Mirvish, S. S. 1970. Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. Journal of the National Cancer Institute 44:633.

Mirvish, S. S., L. Wallcave, M. Eagen, and P. Shubik. 1972. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. Science 177:65.

Mirvish, S. S., J. Sams, T. Y. Fan, and S. R. Tannenbaum. 1973. Kinetics of nitrosation of the amino acids proline, hydroxyproline, and sarcosine. Journal of the National Cancer Institute 51:1833.

Mirvish, S. S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics and in vivo occurrence. Toxicology and Applied Pharmacology 31:325.

Mirvish, S. S., A. Cardesa, L. Wallcave, and P. Shubik. 1975. Induction of mouse adenomas by amines or ureas plus nitrite and by N-nitroso compounds: Effects of ascorbate, gallic acid, thiocyanate, and caffeine. Journal of the National Cancer Institute 55:633.

Mirvish, S. S. 1977. N-nitroso compounds--their chemical and in vivo formation and possible importance as environmental carcinogens. Journal of Toxicology and Environmental Health 2:1267.

Mottram, D. S. and R. L. S. Patterson. 1977. The effect of ascorbate reductants on N-nitrosamine formation in a model system resembling bacon fat. Journal of the Science of Food and Agriculture 28:352.

Mottram, D. S., R. L. S. Patterson, R. A. Edwards, and T. A. Gough. 1977. Preferential formation of volatile N-nitrosamines in fat of fried bacon. Journal of the Science of Food and Agriculture 28:1025.

Nakamura, M., N. Baba, T. Nakaoka, Y. Wada, T. Ishibash, and T. Kawabata. 1976. Pathways of formation of N-nitroso-pyrrolidine in fried bacon. Journal of Food Science 41:874.

Neurath, G. B. and O. Schreiber. 1974. Investigations of amines in the human environment. In: N-nitroso Compounds in the Environment. Bogovski, P. and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 211.

Ohshima, H. and T. Kawabata. 1978. Mechanism of N-nitrosodimethylamine formation from trimethylamine and trimethylamine oxide. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 143.

Oliver, J. E. and A. Kontson. 1978. Formation and persistence of N-nitrosobutralin in soil. Bulletin of Environmental Contamination and Toxicology 20:170.

Palframan, J. F., J. Macnab, and N. T. Crosby. 1973. An evaluation of the alkali flame ionization detector and the Coulson electrolytic conductivity detector in the analysis of N-nitrosamines in foods. Journal of Chromatography 76: 307.

Patterson, R. L. S. and D. S. Mottram. 1974. The occurrence of volatile amines in uncured and cured pork meat and their possible role in nitrosamine formation in bacon. Journal of the Science of Food and Agriculture 25:1419.

Patterson, R. L. S., A. A. Taylor, D. S. Mottram, and T. A. Gough. 1976. Localized occurrence of N-nitrosopyrrolidine in fried bacon. Journal of the Science of Food and Agriculture 27:257.

Pensabene, J. W., W. Fiddler, C. L. Dooley, R. C. Doerr, and A. E. Wassermann. 1972. Spectral and gas chromatographic characteristics of some N-nitrosamines. Journal of Agricultural and Food Chemistry 20:274.

Pensabene, J. W., W. Fiddler, R. A. Gates, and J. C. Fagan. 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. Journal of Food Science 39:314.

Pensabene, J. W., W. Fiddler, W. Mergens, and A. E. Wasserman. 1978. Effect of α-tocopherol formulations on inhibition of nitrosopyrrolidine formation in model systems. Journal of Food Science 43:801.

Preussmann, R. 1974. Toxicity of nitrite and N-nitroso compounds. In: Proceedings of the International Symposium on Nitrite in Meat Products. Krol, B. and B. J. Tinbergen, Eds. Center for Agricultural Publishing and Documentation, Wageningen. p. 217.

Rainey, W. T., W. H. Christie, C. A. Pritchard, and W. Lijinsky. 1976. Mass spectra of N-nitroso compounds. Publication TM 5500. Oak Ridge National Laboratory, Tennessee.

Ridd, J. H. 1961. Nitrosation, diazotisation, and deamination. Quarterly Reviews of the Chemical Society (London) 15:418.

Robson, J. H. 1955. The nitrolysis of N,N-dialkylformamides. Journal of the American Chemical Society 77:107.

Ross, R. D., J. Morrison, D. P. Rounbehler, T. Y. Fan, and D. H. Fine. 1977. N-nitroso compound impurities in herbicide formulations. Journal of Agricultural and Food Chemistry 25:1416.

Sander, J., F. Schweinsberg, and H. P. Menz. 1968. Untersuchungen uber die entstehung cancerogener nitrosamin im Magen. Hopp-Seyler's Zeitschriftfuer Physiologische Chemie 349: 1691.

Saxby, M. S. 1972. Mass spectrometry of volatile derivatives. V. Dialkylnitrosamines. Journal of the Association of Official Analytical Chemists 55:9.

Scanlan, R. A. 1975. N-nitrosamines in foods. CRC Critical Reviews in Food Science and Technology 5:357.

Sen, N. P., D. D. Smith, and L. Schwinghamer. 1969. Formation of N-nitrosamines from secondary amines and nitrite in human and animal gastric juice. Food and Cosmetics Toxicology 7:301.

Sen, N. P. 1970. Gas-liquid chromatographic determination of dimethylnitrosamine as dimethylnitramine at picogram levels. Journal of Chromatography 51:301.

Sen, N. P. and C. Dalpe'. 1972. A simple thin-layer chromatographic technique for the semiquantitative determination of volatile nitrosamines in alcoholic beverages. Analyst 97:216.

Sen, N. P. 1972. Multidetection methods for determining volatile nitrosamines in foods. In: N-nitroso Compounds Analysis and Formation. Bogovski, P., R. Preussmann, and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 25.

Sen, N. P. B. A. Donaldson, J. R. Iyengar, and T. Panalakis. 1973. Nitrosopyrrolidine and dimethylnitrosamine in bacon. Nature 241:473.

Sen, N. P., W. F. Miles, B. A. Donaldson, T. Panalaks, and J. R. Iyengar. 1973a. Formation of nitrosamines in a meat curring mixture. Nature 245:104.

Sen, N. P., J. R. Iyengar, B. A. Donaldson, and T. Panalaks. 1974. The effect of sodium nitrite concentration on the formation of nitrosopyrrolidine and dimethylnitrosamine in fried bacon. Journal of Agricultural and Food Chemistry 22:540.

Sen, N. P., B. A. Donaldson, and C. Charbonneau. 1974a. Formation of nitrosodimethylamine from the interaction of certain pesticides and nitrite in the environment. In: N-nitroso Compounds in the Environment. Bogovski, P. and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 75.

Sen, N. P., J. R. Iyengar, W. F. Miles, and T. Panalaks. 1976. Nitrosamines in cured meat products. In: Environmental N-nitroso Compounds Analysis and Formation. Walker, E. A., P. Bogovski, and L. Griciute, Eds. International Agency for Research on Cancer, Lyon. p. 333.

Sen, N. P., S. Seaman, and W. F. Miles. 1976a. Dimethylnitrosamine and nitrosopyrrolidine in fumes produced during frying of bacon. Food and Cosmetics Toxicology 14:167.

Sen, N. P., B. A. Donaldson, S. Seaman, J. R. Iyengar, and W. F. Miles. 1976b. Inhibition of nitrosamine formation in fried bacon by propyl gallate and L-ascorbylpalmitate. Journal of Agricultural and Food Chemistry 24:397.

Sen, N. P., W. F. Miles, S. Seaman, and J. F. Lawrence. 1976c. Trace analysis of 3-hydroxy-1-nitrosopyrrolidine, a non-volatile N-nitrosamine, by combined gas chromatographic-mass spectrometric method. Journal of Chromatography 128:169.

Sen, N. P., D. E. Coffin, S. Seaman, B. Donaldson, and W. F. Miles. 1977. Extraction, clean-up, and estimation as a methyl ether of 3-hydroxy-1-nitrosopyrrolidine, a non-volatile nitrosamine in cooked bacon at mass fractions of μg/kg. In: Proceedings of the Second International Symposium on Nitrite in Meat Products. Tinbergen, B. J. and B. Krol, Eds. Centre for Agricultural Publishing and Documentation, Wageningen. p. 179.

Sen, N. P., B. A. Donaldson, S. Seaman, J. R. Iyengar, W. F. Miles. 1978. Recent studies in Canada on the analysis and occurrence of volatile and non-volatile N-nitroso compounds in foods. In: Environmental Aspects of N-nitroso Compounds. Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. International Agency for Research on Cancer, Lyon. p. 373.

Singer, G. M. and W. Lijinsky. 1976. Naturally occurring nitrosatable compounds. I. Secondary amines in foodstuffs. Journal of Agricultural and Food Chemistry 24:550.

Singer, G. M., H. W. Taylor, and W. Lijinsky. 1977. Liposolubility as an aspect of nitrosamine carcinogenicity-quantitative correlations and qualitative observations. Chemico-Biological Interactions 19:133.

Spiegelhalder, B., G. Eisenbrand, and R. Preussmann. 1978. Contamination of amines with N-nitrosamines. Angewandte Chemie, International Edition 17:367.

Stenhagen, E., S. Abrahamsson, and F. W. McLafferty. 1974. Registry of Mass Spectral Data. J. Wiley & Sons, New York.

Stephany, R. W., J. Freudenthal, E. Egmond, L. G. Gramberg, and P. L. Schuller. 1976. Mass spectrometric quantification of traces of volatile N-nitrosamines in meat products. Journal of Agricultural and Food Chemistry 24:536.

Stephany, R. W. and P. L. Schuller. 1977. How specific and sensitive is the thermal energy analyzer? In: Proceedings of the Second International Symposium on Nitrite in Meat Products. Tinbergen, B. J. and B. Krol, Eds. Centre for Agricultural Publishing and Documentation, Wageningen. p. 249.

Stephany, R. W. 1977. Some critical remarks on the optimum resolution to use in trace analysis of nitrosamines with combined gas chromatography and mass spectrometry. In: Proceedings of the Second International Symposium on Nitrite in Meat Products. Tinbergen, B. J. and B. Krol, Eds. Centre for Agricultural Publishing and Documentation, Wageningen. p. 239.

Stephany, R. W., R. H. Elgersma, and P. L. Schuller. 1978. Nitrate, Nitrite, and N-nitrosamine contents of various types of dutch cheese. Netherlands Milk and Dairy Journal 32:143.

Swann, P. J. 1975. The toxicology of nitrate, nitrite, and N-nitroso compounds. Journal of the Science of Food and Agriculture 26:1761.

Tanaka, K., K. C. Chung, H. Hayatsu, and T. Kada. 1978. Inhibition of nitrosamine formation in vitro by sorbic acid. Food and Cosmetics Toxicology 16:209.

Tannenbaum, S. R., A. J. Sinskey, M. Weisman, and W. Bishop. 1974. Nitrite in human saliva. Its possible relationship to nitrosamine formation. Journal of the National Cancer Institute 53:79.

Tannenbaum, S. R., D. Fett, V. R. Young, P. D. Land, and W. R. Bruce. 1978. Nitrite and nitrate are formed by endogenous synthesis in the human intestine. Science 200:1487.

Telling, G. M., T. A. Bryce, and J. Althorp. 1971. Use of vacuum distillation and gas chromatography-mass spectrometry for determination of low levels of volatile nitrosamines in meat products. Journal of Agricultural and Food Chemistry 19:937.

Telling, G. M. 1972. A gas-liquid chromatographic procedure for the detection of volatile N-nitrosamines at the ten parts per billion level in foodstuffs. Journal of Chromatography 73: 79.

Telling, G. M., T. A. Bryce, D. Hoar, D. Osborne, and D. Welti. 1974. Progress in the analysis of volatile N-nitroso compounds. In: N-nitroso Compounds in the Environment. Bogovski, P. and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 12.

Terracini, B. and P. N. Magee. 1964. Renal tumors in rats following injection of dimethylnitrosamine at birth. Nature 202:502.

van Den Dool, H. and P. D. Kratz. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography 11:463.

von Rumker, R., E. W. Lawless, A. F. Meiners, K. A. Lawrence, and G. L. Kelso. 1975. Production, Distribution, Use, and Environmental Impact Potential of Selected Pesticides. Council on Environmental Quality, Environmental Protection Agency, Washington. p. 257.

Walker, R. 1975. Naturally occurring nitrate/nitrite in foods. Journal of the Science of Food and Agriculture 26:1735.

Walker, E. A., and M. Castegnaro. 1974. A report on the present status of a collaborative study of methods for the trace analysis of volatile nitrosamines. In: N-nitroso Compounds in the Environment. Bogovski, P. and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 57.

Walker, E. A., P. Bogovski, and L. Griciute, Eds. 1976. Environmental N-nitroso Compounds Analysis and Formation. International Agency for Research on Cancer, Lyon. p. 497.

Walker, E. A. and M. Castegnaro. 1976. New data on collaborative studies on analysis of volatile nitrosamines. In: Environmental N-nitroso Compounds Analysis and Formation. Walker, E. A., P. Bogovski, and L. Griciute, Eds. International Agency for Research on Cancer, Lyon. p. 77.

Walker, E. A., M. Castegnaro, L. Griciute, and R. E. Lyle, Eds. 1978. Environmental Aspects of N-nitroso Compounds. International Agency for Research on Cancer, Lyon. p. 555, 556.

Walters, C. L., D. G. Fueggle, and T. G. Hunt. 1974. The determination of total non-volatile nitrosamines in microgram amounts. In: N-nitroso Compounds in the Environment. Bogovski, P. and E. A. Walker, Eds. International Agency for Research on Cancer, Lyon. p. 22.

Wang, T., T. Kakizoe, P. Dion, R. Furrer, A. J. Varghese, and W. R. Bruce. 1978. Volatile nitrosamines in normal human feces. Nature 276:280.

Warthesen, J. J., R. A. Scanlan, D. D. Bills, and L. M. Libbey. 1975. Formation of heterocyclic N-nitrosamines from the reaction of nitrite and selected primary diamines and amino acids. Journal of Agricultural and Food Chemistry 23:898.

Wasserman, A. E., W. Fiddler, R. C. Doerr, S. F. Osman, and C. J. Dooley. 1972. Dimethylnitrosamine in frankfurters. Food and Cosmetics Toxicology 10:681.

White, E. H. and W. R. Feldman. 1957. The nitrosation and nitration of amines and alcohols with nitrogen tetroxide. Journal of the American Chemical Society 79:5832.

White, J. W., Jr. 1976. Relative significance of dietary sources of nitrate and nitrite. Journal of Agricultural and Food Chemistry 24:202.

Williams, D. L. H. 1978. Comparison of the efficiencies of ascorbic acid and sulphamic acid as nitrite traps. Food and Cosmetics Toxicology 16:365.

Young, J. C. 1978. Detection and determination of volatile and nonvolatile N-nitrosamines by thin-layer chromatography using fluorescamine. Journal of Chromatography 151:215.

APPENDIX

APPENDIX

The specific description of the vacuum distillation apparatus follows. Glassware numbers refer to VWR Scientific Inc. 1978 catalog.

1. 1000 mL round bottom boiling flask with thermometer well and tapered joint (29129-326).

2. Stopcock, high vacuum (59277-049).

3. Vacuum Traps with separable joints. Large trap, 41 x 250 mm o.d. tube, inner tube cut 100 mm below joint (55096-166). Smaller trap, 28 x 200 mm o.d. (55096-100).

4. Vacuum Trap, Dewar type (Ace Glass 8757).

5. Ball and socket joints, 28/15, (33063-163, 33063-560).