(distilled in glass). A 0.3 m × 4 mm glass column containing Gas Chrom Q 80–100 mesh support coated with 3% Silicone SE-30 (Applied Science Laboratories) was used. Carbaryl, supplied by Union Carbide Corporation (99.23% purity), was further purified by recrystallization from ethyl ether.

RESULTS AND DISCUSSION

Considerable conditioning of the columns was necessary before the optimum sensitivity could be obtained. New columns were heated for 48 hr at an oven temperature of 225°, with a nitrogen gas flow through the column of 120 cm^3/min. Columns were then cooled and optimum conditions as stated below were established. 20–50 μl of a concentrated solution containing 100 ng/μl of carbaryl in 2,2,4-trimethylpentane was injected for conditioning new columns. Columns were additionally conditioned with 50–100 ng of carbaryl immediately prior to making a series of carbaryl determinations.

All samples of high-purity carbaryl which we received contained amounts of α-naphthol detectable under the conditions stated below. Careful recrystallization of carbaryl in ethyl ether removed these detectable quantities of α-naphthol.

At an optimum column temperature of 145°, carbaryl, injected in benzene and 2,2,4-trimethylpentane solutions, eluted as a single symmetrical peak with a retention time of 1.4 min. Mass spectrometry and thin-layer chromatography confirmed the identification of the peak. Under the same conditions, α-naphthol was detected with a retention time of 0.4 min.

Optimum analysis conditions were: column temperature, 145–150°; detector temperature, 225°; inlet temperature, 170°; carrier gas flow (N_2), 120 ml/min.

Sensitivity to carbaryl decreased rapidly with an increase or decrease in column temperature from the listed conditions. The minimum quantity of carbaryl capable of producing a measurable response was 0.2–0.5 ng, and the minimum detectable quantity of α-naphthol was approximately 1.3 times that of carbaryl. Thermal decomposition of carbaryl during chromatography has been observed elsewhere (Fishbein and Zielinski, 1965; Krishna *et al.*, 1962). In order to avoid errors resulting from decomposition, a very short column with a low liquid phase load was used as suggested by Riva and Carisano (1969) and no decomposition was detected except at column temperatures exceeding 160°.

No compounds interfering with carbaryl analysis were detected in 2,2,4-trimethylpentane extracts, even from water containing high microbial populations and organic nutrients; extracts were therefore not cleaned up prior to chromatographic analysis.

Instability of carbaryl in stored organic solvents was a problem when excess water was not completely removed from the extracts. However, samples stored in dry chloroform, methylene chloride, 2,2,4-trimethylpentane, and benzene stored in the dark were found to be stable over a 1-week period.

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Received for review May 9, 1973. Accepted October 5, 1973. Mention of commercial products does not necessarily constitute endorsement by the Environmental Protection Agency.

Formation of Dimethylnitrosamine from Dimethylamine and Trimethylamine at Elevated Temperatures

Varying concentrations of dimethylamine (DMA) and trimethylamine (TMA) were reacted with sodium nitrite at pH 6.4, 100°, for 2.5 hr. In the presence of equimolar concentrations of amines and nitrite, more dimethylnitrosamine (DMNA) was produced from DMA rather than TMA. When the molar ratio of amine to nitrite was increased, the amounts of DMNA from the two amines became nearly equal, and at very high amine to nitrite ratios, more DMNA was formed from TMA than from an equimolar amount of DMA. The optimum pH for the conversion of TMA to DMNA at 100° was 3.2–3.3.

Since Magee and Barnes (1956) reported the carcinogenicity of dimethylnitrosamine (DMNA), much attention has been directed to the formation of *N*-nitrosamines, particularly from secondary amines. Hein (1963) and Smith and Loeppky (1967) pointed out that tertiary amines also react with nitrous acid to produce *N*-nitrosamines. Ender *et al.* (1967) demonstrated that DMNA is formed more readily from dimethylamine (DMA) than from trimethylamine (TMA) at pH 6.5 for 4 hr at 90°. Fiddler *et al.* (1972) reported approximately ten times more DMNA from DMA than from TMA when equimolar amounts of the two amines were reacted with nitrite in pH 5.6 buffer for 4 hr at 78°. Schweinsberg and Sander (1972) indicated the amount of diethylnitrosamine found in the nitrosation of triethylamine at 100° was 200 times less than in the reaction with diethylamine. Malins *et al.* (1970), however, reacted nearly equimolar amounts of TMA and DMA with nitrite at pH 6.4 for 2.5 hr at 100° and reported DMNA production from TMA but not from DMA. As recently pointed out by Lijinsky *et al.* (1972), the data of Malins *et al.* (1970) contradict the mechanism of nitrosamine formation from tertiary amines proposed

Table I. Amounts of Dimethylnitrosamine Formed from Equimolar Amounts of Dimethylamine and Trimethylamine

mmol of amine/ 100 ml	Molar ratio amine: nitrite	DMNA formed, ppm[a]	DMNA from DMA divided by DMNA from TMA
0.58 DMA	1:1	0.540	8.1
0.58 TMA	1:1	0.066	
4.44 DMA	7.6:1	12.9	2.3
4.44 TMA	7.6:1	5.6	
17.8 DMA	30.4:1	64.9	1.3
17.8 TMA	30.4:1	51.1	
44.4 DMA	76:1	133.5	0.64
44.4 TMA	76:1	208.9	
88.8 DMA	152:1	233.5	0.60
88.8 TMA	152:1	388.7	

Conditions: pH 6.4, 100° for 2.5 hr. $NaNO_2$, 0.58 mmol/100 ml (400 ppm)

[a] ppm in the reaction solution.

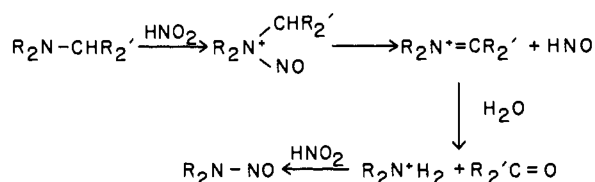

Figure 1. Proposed mechanism for the conversion of tertiary amines to *N*-nitrosamines (after Smith and Loeppky, 1967).

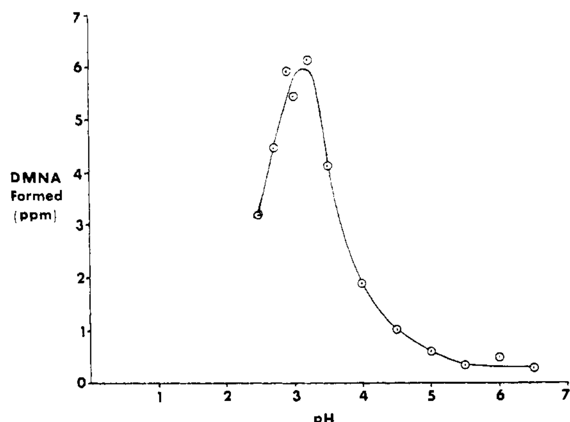

Figure 2. pH dependence of dimethylnitrosamine formation from trimethylamine. Conditions: 100° for 2.5 hr; TMA concentration, 1.7 mmol/100 ml; $NaNO_2$ concentration, 0.85 mmol/100 ml.

by Smith and Loeppky (1967). This discrepancy prompted us to study and compare the production of DMNA from DMA and TMA.

EXPERIMENTAL SECTION

All chemicals were reagent grade. DMA·HCl and TMA·HCl were dried over KOH before use. Equimolar amounts (Table I) of DMA·HCl and TMA·HCl were combined in separate tubes (Kimble No. 45066-A) with a constant concentration of $NaNO_2$ and were buffered at pH 6.4 with 19.1 ml of 0.1 *M* citric acid and 36.3 ml of 0.2 *M* dibasic sodium phosphate. A known amount of diethylnitrosamine was added to each sample as an internal standard. The volume was adjusted to 100 ml with distilled water, the tube was sealed with a Teflon-lined screw cap, and each sample was reacted in a boiling water bath for 2.5 hr. After reaction, all samples were cooled in an ice bath, saturated with anhydrous Na_2SO_4, extracted three times with dichloromethane (100 ml total), and dried over anhydrous Na_2SO_4. Samples were then evaporated to 1 ml on a Buchi Rotavapor, transferred to a Chromaflex sample tube (Kontes Glass Co.), and concentrated to 50 µl under a stream of nitrogen. Ten microliters of this concentrate was injected into a Varian Aerograph 1200 series gas chromatograph equipped with a flame ionization detector. A 12 ft × 0.085 in. i.d. stainless steel column packed with 20% Carbowax 20M on 120–140 mesh Celite 545 was used for the separation and quantitative analysis of DMNA. The flow rate was 30 ml of nitrogen/min and the injector, column, and detector temperatures were 200°, 140°, and 250°, respectively. The identity of DMNA was confirmed by combined gas chromatographic–mass spectral analysis (Scanlan and Libbey, 1971); however, the gas chromatographic operating conditions were the same as described above.

Experiments designed to determine the optimum pH for conversion of TMA to DMNA at 100° for 2.5 hr were carried out employing the procedure described above. Citrate-phosphate buffers covering the pH range 2.5 to 6.5 were prepared and diluted to a final volume of 100 ml as described by Gomori (1955).

DMNA formation from equimolar amounts of DMA and TMA was also determined at pH 6.4 for 2.5 hr at 100° and 24° and at pH 3.2 for 2.5 hr at 37°.

Table II. Amount of Dimethylnitrosamine Formed from Equimolar Amounts of Trimethylamine and Dimethylamine at Different Acidities and Temperatures

pH	Temperature, °C	DMNA from DMA, ppm[a]	DMNA from TMA, ppm[a]
6.4	100	37.1	3.61
6.4	24	0.5	0.010
3.2	37	8.6	0.209

Concentrations: amines, 4.44 mmol/100 ml; $NaNO_2$, 2.22 mmol/100 ml
Reaction time: 2.5 hr

[a] ppm in the reaction solution.

RESULTS AND DISCUSSION

The mass spectral fragmentation pattern of DMNA obtained under the experimental conditions described in this study was identical to that obtained for authentic DMNA. In most cases, each DMNA value reported represents the mean of three sample determinations and the maximum difference between a sample and its mean value was less than ±25%.

The amounts of DMNA formed from equimolar amounts of DMA and TMA are shown in Table I. When the molar ratio of amine to nitrite was 1:1, considerably more DMNA was formed from DMA than from TMA. These observations agree with the reports of Fiddler *et al.* (1972) and Ender *et al.* (1967) in which the amine to nitrite ratios were approximately 1:5 and 1:1, respectively. As the ratio of amine to nitrite was increased, nearly equal amounts of DMNA were produced from both amines, and when the ratio was further increased, slightly more DMNA was produced from TMA than from DMA. When the amine to nitrite ratio was 76:1 for reaction times of 1, 2.5, 4, and 5.5 hr at 100°, more DMNA was formed from TMA than from DMA in each case. These results suggest that as the TMA to nitrite ratio increases,

a mechanism other than the one proposed by Smith and Loeppky (1967) becomes operative. In the latter mechanism (Figure 1), the tertiary amine undergoes nitrosative dealkylation to the corresponding secondary amine which reacts with nitrite to form the *N*-nitrosamine. If this mechanism were the only operative one, it would be difficult to rationalize the production of more DMNA from TMA than from an equimolar amount of DMA. In this regard, Lijinsky *et al.* (1972) suggested and Keefer and Roller (1973) demonstrated that the *N,N*-dimethylformaldiammonium ion initially produced can undergo nucleophilic attack by free nitrite with the adduct thus formed, collapsing to DMNA and formaldehyde. Keefer and Roller also demonstrated the catalytic effect of formaldehyde in the conversion of various secondary amines to nitrosamines in the pH range 6.4 to 11.0. Perhaps the catalytic effect of formaldehyde produced by nitrosative dealkylation of TMA (Figure 1) explains the increased yields of DMNA at the higher TMA levels considered in this study.

Figure 2 shows the dependence of DMNA formation from TMA on pH. The optimum appears to be pH 3.2–3.3 under the conditions of time and temperature employed and is the same as the pH optimum observed by Schweinsberg and Sander (1972) for nitrosation of TMA and triethylamine. Table II shows the amount of DMNA formed from equimolar amounts of TMA and DMA at various acidities and temperatures. It may be significant that DMNA can be formed from TMA as well as from DMA at pH 3.2, 37°, since these conditions are similar to stomach pH and body temperature. Because relatively large amounts of TMA can form in refrigerated seafood (Gulan, 1972; Keay and Hardy, 1972, Miller *et al.*, 1972), the dietary intake of TMA may have to be reevaluated if *in vivo* formation of DMNA from TMA does occur.

ACKNOWLEDGMENT

This investigation was supported by the Food and Drug Administration Grant 1R01 FD-00382.

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Received for review June 4, 1973. Accepted October 19, 1973. Technical Paper No. 3604, Oregon Agricultural Experiment Station, Oregon State University, Corvallis, Ore. 97331.

Synthesis of ^{14}C-Labeled *O,S*-Dimethyl Phosphoroamidothioate

^{14}C-Labeled *O,S*-dimethyl phosphoroamidothioate (Tamaron, Monitor) was synthesized and characterized by gas chromatography, nuclear magnetic resonance, and mass spectra data. The phosphoroamidothioate labeled in the methoxy position had a specific activity of 86 μCi/mmol.

O,S-Dimethyl phosphoroamidothioate (Tamaron, from Bayer Chemical Co., Germany; Monitor, from Chevron Chemical Co., U. S. A.) a new systemic insecticide, was earlier (Khasawinah, 1970) labeled with ^{32}P, but in order to study the mode of decomposition and residues on industrially processed fruits, a ^{14}C-labeled compound was required. Thus, [O-^{14}C]methyl *S*-methyl phosphoroamidothioate as well as the intermediates by preparing apprority 96.2%), as shown in Figure 1, by using reactions that gave few undesired side products.

EXPERIMENTAL SECTION

A high-resolution Varian XL-100 MHz spectrometer was used to study the structure of the phosphoroamidothioate as well as the intermediates by preparing appropriate solutions in $CDCl_3$. Mass spectra were taken with a Hitachi Perkin-Elmer RMU-6H mass spectrometer. Purity studies of the phosphoroamidothioate were made by glc utilizing a Hewlett-Packard 5750 gas chromatograph provided with a flame ionization detector and a 6 × 2 mm glass column (1:1 mixture of 3% Reoplex 400 on Gas Chrom Q, 80–100 mesh and 3% QF-1 on Gas Chrom Q, 80–100 mesh). Critical parameters used were: column oven, 132°; carrier gas and flow rate, nitrogen, 45 ml/min; air and hydrogen flow rates, 325 and 35 ml/min; injector temperature, 215°; detector temperature, 250°; retention time, 4.7 min.

***O*-Methyl Phosphorodichloridothioate (II).** Acridine (0.18 g, 1.0 m*M*) and pulverized calcium oxide (56.1 g, 1 mol) were added to distilled phosphorus thiochloride (84.5 g, 0.5 mol) in benzene (300 ml). Then, methanol (30 ml, 0.75 mol) was added over a period of 1 hr while stirring during the addition and for 3 hr thereafter. Solids were then filtered from the reaction mixture and washed repeatedly with benzene to remove traces of entrained II. Acridine was removed by washing the benzene phases twice with 2% HCl cooled to 2°. Evaporation of the solvent and distillation *in vacuo* gave II (67.5 g, 82.0%, bp 60° at 55 mm). The nmr spectrum of II showed a doublet centered at δ 4.00 ppm (d, 3 H, CH_3O-P, J = 19 Hz).