STABILITY OF CARCINOGENIC DIMETHYLNITROSAMINE IN SOIL

By

KESETE TESFAI

Bachelor of Science

Oklahoma Panhandle State University

Goodwell, Oklahoma

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1977

STABILITY OF CARCINOGENIC

DIMETHYLNITROSAMINE

IN SOIL

Thesis Approved:

Lester W. Reed

Thesis Advisor

J. O. Lynd

Billy L. Tucker

Norman N. Durham

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my adviser, Dr. L. W. Reed, for his guidance and patience throughout this thesis preparation. I would also like to express my appreciation to the other members of my Graduate Committee, Dr. B. B. Tucker and Dr. J. Q. Lynd.

I wish to express my sincere thanks to Dr. S. K. Pancholy and Dr. M. A. B. Mallik for their invaluable assistance in this study.

This research was supported by a grant from Cooperative Science and Education Service, U. S. Department of Agriculture, Washington, D. C., and is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Occurence and distribution of nitrosamine compounds in the natural environment has aroused great concern, because several members of the nitrosamines have been shown to be carcinogenic, mutagenic, teratogenic and toxic in very small doses. It has been demonstrated that nitrosamine can be formed in the ecosystem provided secondary amines and nitrites are present in sufficient quantity (1). Most soils are known to contain secondary amines; they may be formed by demethylation of tertiary amines which are produced by degradation of plant and animal residues. Certain pesticides after decomposition may give rise to secondary amines. Nitrate, the other precursor in the nitrosation reaction, is being added to the soil. Therefore, higher concentrations of pesticides as well as nitrate and nitrite can lead to the formation of nitrosamines in soil.

Sander first pointed out that bacteria can catalyze nitrosation reactions at neutral pH values (29). Ayanaba and Alexander (2) stated that micro-organisms can contribute to the production of nitrosamine by demethylation of tertiary amines and by reducing nitrate to nitrite.

The stability and decomposition of dimethylnitrosamine rather than any other nitrosamine was investigated because it was considered to be highly potent carcinogenic and mutagenic agent, highly water soluble

and most frequently found in analysis of natural products. Thus, dimethylnitrosamine once produced in soil, can leach down into the ground water, and may pose a health hazard to wild animals, livestock and human beings.

The objective of this study was to investigate the role of temperature, soil moisture content, nutrition and microbial influence on stability and decomposition of dimethylnitrosamine in soil.

CHAPTER II

LITERATURE REVIEW

Nitrosamines have recently received considerable attention because of their possible carcinogenic, teratogenic, and mutagenic properties. These compounds depending on their chemical structure, mode of application and dosage are known to produce malignant tumors selectively in the liver, lungs, fore-stomach, esophagus, bladder, kidney and nasal sinuses in several animal species. Localization and histological structure of tumors in human pathology were found to correspond closely to nitrosamines (9). Death and abnormalities of the fetus in aminals have also been reported due to an injection of nitrosamines in late pregancy (9). Thus N-nitrosamines have been described as the most formidable and versatile groups of carcinogens yet discovered.

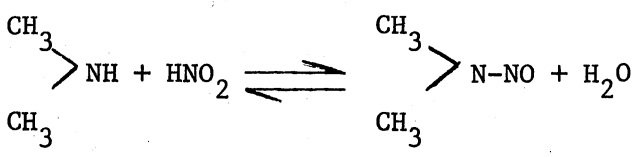
Nitrosamines can be formed by the reaction of secondary amines with nitrous acid, nitrite, or oxides of nitrogen at relatively acid pH values. Much of the concerns by scientists is related to the widespread occurrence of the nitrosamine precursors (nitrate, nitrites and secondary or tertiary amines) in the environment and their possible interactions to form the N-nitroso compounds. Although nitrosamines are found in some plants and nonprocessed food products, they occur more frequently in processed food. These include smoked fish, raw and cured meat, flour, milk, tobacco smoke and spirits. In particular, efforts have made to

find nitrosamine in cured meats where, theoretically naturally occuring secondary amines might react with nitrite (which is used for preservation of meats) to form nitrosamines. But so far little information is available regarding these compounds, because their determination is a tedious analysis and requires the use of sophisticated instruments.

The National Research Council Committee on Nitrate Accumulation raised the question of the possibility of the formation of nitrosamines in soil and natural bodies of water in which nitrate or nitrite appears in significant ammounts (3).

One of the precursors of nitrosamines may be nitrate which is known to be present in soils at variable amounts at all times, chiefly because of high nitrogen fertilization, organic matter decomposition, poor drainage and a rising water table. Nitrite can be formed by denitrification of nitrate as well as by nitrification of ammonium compounds in soils. Thus it seems that nitrite may be readily available in sufficient amounts in the soil for the formation of nitrosamines under varying pH conditions.

Nitrosamines, as exemplified by dimethylnitrosamine (DMNA), is chemically stable under physiological conditions, although they undergo photochemical decomposition in ultraviolet light. The chemical reaction which leads to the formation of dimethylnitrosamine (DMNA) from dimethylamine (DMA) and nitrous acid is shown as follows:



The limiting factor, then, in the formation of nitrosamines in soil seems to be the availability of "nitrostable" secondary amines.

Soils are known to contain amine precusors such as proteins, amino acids, phospholipids and quaternary ammonium compounds; and other substances may be available to form nitrosamines.

In many biological systems, significance amounts of N-nitrosamines have been formed from nitrite and chlorine, acetyl choline , carnitine and betaine. The reaction rate to form nitrosamine has been found to be most rapid for dimethylnitrosamine. Dimethylnitrosamine is not only a potent carcinogen but it is also highly soluble in water as are some of the other nitrosamines. Therefore there is a good possibility that such water soluble nitrosamines, after they are formed in soils, and can leach down through the soil along with nitrate into the ground water.

Although research on nitrosamine toxicity and carcinogenesis was initiated after an indication of human toxicity arising from the industrial use of dimethylnitrosamine (8), it was not until considerably later that the industrial environment was emphasized rather dramatically in Norway by an outbreak of serious liver disease in 1960 (13). This incident involved number of sheep which became seriously ill, sometimes fatally, and a postmortem examination revealed severe necrosis of the liver as the main pathological feature. All of the affected sheep were found to have been fed on a diet containing fish meal preserved with nitrite. This finding suggested that the formation of nitrosamine might have occurred, since fish contain relatively large amounts of secondary and tertiary amines. The nature of the toxic principle in this meal was studied by Ender, et al. (13) who showed that dimethylnitrosamine was present, sometimes at levels as high as 30-100 ppm.

The information on the toxicity of nitrosamine is recent, after the work of Barnes (17) who demonstrated what liver damage was induced by

dimethylnitrosamine. Dimethylnitrosamine was first studied with laboratory animals because of its acute or subacute effects on exposed personnel in industrial laboratories (6). Druckery et al. (9) reported that nitrosamines varied in their carcinogenic and organ-specificity, and most of them produced tumors in rats. Magee and Barnes (18) observed that generally, asymmetical nitrosamines caused tumors in the kidney, bladder, nasal sinuses, lungs and bronchia, alimentary canal, the nervous system and the skin of rats.

Druckery et al. (10) and Magee and Barnes (18) reported that many of the nitrosamines were also potent teratogen and mutagens in laboratory animals. Thus, the toxicity and carcinogenicity of nitrosamines has been well established.

Greenblatt et al. (14) reported that simultaneous feeding of nitrite and secondary amines yielded as many tumors in rats as by feeding the formed nitrosamines. Nitrosamines have been shown to be present in smoked fish (13), raw and cured meat (20), spirits (19), tobacco smoke (24), flour, milk and a few plants.

It is well known that nitrosamine formation from secondary amines and nitrite is strongly pH dependent and is favored by the acid condition prevailing in the mammalian stomach. There is now substantial evidence that nitrosamines can be formed under these conditions. Sander et al. (29) demostrated the formation of the corresponding N-nitroso compound by incubating several amines with nitrite in the presence of gastric juice under various conditions.

The yield of nitrosamine was much greater with weakly basic than strongly basic secondary amines and the optimum pH was in the range 1-3. There has been much recent interest in the geographical pathology

of cancer (11), and it appears highly probable that the observed differences in tumor incidence in different geographical areas may be related to aetiological factors in the environment. A notable example of such localized distribution of tumors are the remarkably high incidence of liver cancer in parts of East Africa and of the esophagus in the Transkei area of South Africa. In the other areas there appears to have been a marked increase in esophageal cancer among the Bantu population since about 1940 and it has been suggested that a nitrosamine occurance in the environment might be responsible, since these carcinogens, especially the unsymmetical dialkynitrosamines (9), are among the very few which induce tumors of the oesophagus in experimental animals. It was therefore of interest that the presence of nitrosamines has been reported in food and drink consumed by the local population. In Zambia, McGlashan et al. (22) found a link between cancer of the esophagus and the drinking of locally distilled spirits that contained 1-3 ppm of methyl-nitrosamino-benzaldehyde.

Although at present no information is available regarding the distribution of nitrosamines in soil, water, and crop plants they seem to be definitely more widespread in nature than thought earlier because of widespread presence of their precursors. Now, it has been found that these precursors taken up separately by man or aminals can combine in the stomach and cause toxicity (18). Greenblatt et al. (14) reported that simultaneous feeding of nitrite and secondary amines yielded as many tumors in rats as by feeding the formed nitrosamines. Sen et al. (31) obtained DMNA by incubating sodium nitrate and dimethylamine in a simulated stomach.

It seems that soil would provide an ideal environment for the

formation of nitrosamines because it contains many precursors for nitrite and amines under varying conditions of pH, moisture, temperature and many other factors. Recently, Ayanaba and Alexander (4) did report the formation of DMNA in soil by addition of nitrite and dimethylamine separately.

Nitrates in soil have been found to be present at all times varying from 5-10 ppm for irrigated land to several hundred ppm in animal feedlots (30). High nitrite content in water could induce methemoglobinemia in man and animals (1) and nitrites can be readily formed from nitrates and ammonium compounds in the soil. Nitrite is one of the precursors of nitrosamines.

Many precursors for amines have also been reported from soil. These included amino acids, proteins, phospholipids, trimethylamine, choline, creatinine etc. (6). Also, some of the pesticides such as phosphamidon (15), Thiram, Ziram, and Tillam which are basically amine compounds could serve as amine precursors after their breakdown in soil.

The model systems, the rate of formation of low molecular weight nitrosamines has been found to be greater than for heavier ones. These low molecular weight compounds were found to be highly toxic and soluble in water in all proportions. Therefore, the possibility has been raised that such compounds could leach down through the soil to the ground water in significant amounts after their formation in soil (1). Thus, the reaction of nitrite with some classes of amines in soil is a matter of public health interest at this time and requires immediate attention.

CHAPTER III

MATERIALS AND METHODS

The three soil types used were sandy, sandy loam and clay loam: Eufaula loamy fine sand, siliceous, thermic Psammentic Palenstalf sample taken in the SE ¼ Section 25 T 18N R2E; Minco fine sandy loam, silty, mixed, thermic Udic Haplustoll sample taken in SE ¼ Section 35 T 8N R 3W; and Bethany clay loam, fine, mixed, thermic Pachic Paleustoll sample taken in SE ¼ Section 14 T 9N R3W, respectively. Prior to treatment each soil sample was air-dried, ground and screened through a 2 mm screen. Ten grams of each soil sample were transferred to test tubes and were treated as follows:

1. 1µl of 10µCi/ml of ^{14}C-dimethylnitrosamine (New England Nuclear Co.) plus 1 ml of 1000 ppm unlabeled dimethylnitrosamine was added to 4 ml of distilled water and transferred to 10 gm soil samples and incubated at 4, 25, and 37C.
2. 1µl of 10µCi/ml of ^{14}C-dimethylnitrosamine plus 1 ml of 1000 ppm unlabeled dimethylnitrosamine was added to 4 ml of 0.05% glucose, 500 ppm each of nitrite-nitrogen and nitrate-nitrogen solution and then transferred to 10 gm soil samples and were incubated at 30C.
3. 1µl of 10µCi/ml of ^{14}C-dimethylnitrosamine plus 1 ml of unlabeled dimethylnitrosamine was added to 4 ml of 0.1% glucose, 1000 ppm each of nitrite-nitrogen and nitrate-nitrogen

solution and then transferred to 10 gm soil samples and were incubated at 30C.

4. 1µl of 10µCi/ml of ^{14}C-dimethylnitrosamine plus 1 ml of 1000 ppm unlabeled dimethylnitrosamine was added to 4 ml of distilled water and were transferred to 10 gm soil samples and were incubated at 30 C at a moisture level of about 50% (wt/vol).
5. 1µl of 10µCi/ml of ^{14}C-dimethylnitrosamine plus 1 ml of 1000 ppm unlabeled dimethylnitrosamine was added to 14 ml of distilled water and were transferred to 10 gm soil samples and were incubated at 30C at a moisture level of about 150% (wt/vol).
6. 1µl of 10µCi/ml of ^{14}C-dimethylnitrosamine plus 1 ml of 1000 ppm unlabeled dimethylnitrosamine was added to 4 ml of distilled water and were transferred to autoclaved 10 gram soil samples and were incubated at 30C.
7. 1µl of 10µCi/ml of ^{14}C-dimethylnitrosamine plus 1 ml of 1000 ppm unlabeled dimethylnitrosamine was added to 4 ml of distilled water and were transferred to nonautoclaved 10 gm soil samples and were incubated at 30C.
8. All 10 gm soil samples were incubated for 0[1], 5, 10, 20, 30, 45 and 60 days.

The soil samples were transferred to a micro-kjeldahl flask, 5 ml of 20% K_2CO_3 were added to each flask (this prevents the decomposition of some of the nitrosamine) and were steam distilled. The distillation was continued until 80 ml of distillate was collected. The distillate

---

[1]Three hours incubation.

was transferred to a separatory funnel and 10 ml of dichloromethane was added. The mixture was throughly shaken and the bottom or dichloromethane layer was drained into a beaker. The top layer was further extracted three times with a total of 30 ml dichloromethane (20); fractions were pooled and saved for counting the radioactivity of ^{14}C-dimethylamine.

One ml of each sample was added to 10 ml of Ready-Solv EP scintillation cocktail (Beckman) and radioactivity was counted on a Beckman model LS-3150T liquid scintillation counter.

$^{14}CO_2$ was trapped in 5 ml of hydroxide of hyamine 10-X (Beckman) and 1 ml of each sample was transferred to 10 ml of a scintillation cocktail. The cocktail solution contained 6 gram of 2, 5-diphenyloxazole, 100 gram of naphthalene, and 1 liter of 1, 4-dioxane. The radioactivity was counted on a Beckman model LS-3150T liquid scintillation counter.

Physical Analysis

A 1:1 ratio of soil and water was used to measure the pH as described by Peech, and mechanical analyses were determined by the hydrometer method (7).

Organic Matter Determination

Organic matter was determined by the modified Schollenberger method (25).

Thin Layer Chromatography

A flash evaporator was used to reduce further the volume of water extract to about one ml. Thirty µl was spotted on silica gel 60 F-254

thin layer plate along with known dimethylamine compounds. The plate was developed in a 4:1:1 solvent system (v/v/v) of butanol-acetic acid-water. After air-drying, the plate was sprayed with 0.3% ninhydrin and heated in an oven at 80 C for 15 minutes. Rf values of the spots observed were recorded and compared with the known compound.

CHAPTER IV

RESULTS AND DISCUSSION

Dimethylnitrosamine (DMNA) was added to three soils of different texture, and incubated at three different temperatures. The soil tubes were analyzed after 0, 5, 10, 20, 30, 45, and 60 days incubation periods to determine the decomposition of dimethylnitrosamine in soil. At the 4C incubation, the concentration of dimethylnitrosamine in the sandy soil and sandy clay loam soil decreased by about 20-30 percent after 0 to 20 days, but after 20 to 30 days, the nitrosomine was about 30 to 50 percent for both the sandy and sandy clay loam soil, and no additional decline was noted in the next 30 days.

The rate of decomposition of dimethylnitrosamine added to the sandy loam soil incubated at 4C for 0 to 30 days was about 30 to 55 percent. After 30 days, no further decrease or decomposition was detected. The patterns of dimethylnitrosamine decomposition was essentially identical in the three soils incubated at 4 C.

The decomposition of dimethylnitrosamine at the 25C incubation temperature was higher than the 4C temperature by about 10% in sandy soil and sandy clay loam than in the sandy loam soil. The rate of decomposition in sandy loam soil was quite slow especially after 5 days incubation period (Table II).

Decomposition of dimethylnitrosamine was slow at the 37C incubation temperature. The percent decomposition at this temperature

was between 30-50 percent. The results show, moreover, that dimethylnitrosamine decomposition in soils of different texture was not affected by temperature.

The percent yields of dimethylamine of each sample at different temperatures is shown in tables I, II, and III. There was no definite pattern in the time courses of dimethylamine decomposition attained at 25C in sandy soil and obtained at the 37C incubation in sandy loam soil. Therefore, there were no consistent differences due to temperature.

The rates of release of CO_2 from dimethylamine at three different temperatures were identical in all three soils.

In order to identify dimethylamine for the water extract of the soils the water extract was spotted on silica gel 60F-254 thin layer plates along with known compounds. The Rf value was 0.0875. This Rf value was identical to that of known dimethylamine.

In order to study the effect of different concentration of carbon and nitrogen on dimethylnitrosamine decomposition, 10 gm samples of soils of different textures were incubated at 30C. (The organic matter content of each sample is given in Table XIV). When sandy soils were amended with 0.05% glucose and 500 ppm of NO_3^--N and NO_2^--N and incubated at 30C, about 30-60 percent of the dimethylnitrosamine was decomposed after 60 days incubation period (Table IV). The rate of breakdown of dimethylnitrosamine to dimethylamine was essentially identical for both treatments. The sandy soil sample received 0.05% of glucose and 500 ppm of NO_3^--N and NO_2^--N, the percent yield of dimethylamine was about 15.30 to 32.37 percent after the 60 day incubation. By contrast, there was no appreciable yield of dimethylamine (DMA) accumulated when soil was amended with 0.01% of glucose, 1000 ppm of NO_3^--N and NO_2^--N

TABLE I

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DIMETHYLNITROSAMINE TREATED SANDY SOIL INCUBATED AT DIFFERENT TEMPERATURES

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity								
	Unaltered DNMA			DMA			CO_2		
	4C	25C	37C	4C	25C	37C	4C	25C	37C
0	88.70	87.13	87.89	2.70	3.70	3.15	0.00	0.00	0.00
5	82.22	63.79	74.42	11.04	23.72	16.04	0.35	1.34	1.88
10	78.65	62.58	73.52	19.80	20.40	16.00	0.45	1.23	0.98
20	63.40	59.94	52.90	26.15	29.68	29.68	0.48	1.45	1.31
30	60.30	55.60	78.04	24.50	28.07	27.92	1.39	1.28	1.34
45	59.69	59.47	70.78	24.10	24.17	22.90	0.92	0.95	1.94
60	51.80	62.52	57.64	17.58	14.20	27.35	0.95	0.98	2.10

TABLE II

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DIMETHYLNITROSAMINE TREATED SANDY LOAM SOIL INCUBATED AT DIFFERENT TEMPERATURES

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity								
	Unaltered DMNA			DMA			CO_2		
	4C	25C	37C	4C	25C	37C	4C	25C	37C
0	89.40	88.70	87.15	2.15	1.17	3.15	0.00	0.00	0.00
5	73.14	78.45	77.12	22.13	14.99	18.59	0.63	0.75	0.98
10	70.66	70.95	70.08	14.90	18.60	15.97	0.65	0.85	0.87
20	66.83	69.92	76.85	24.91	27.82	22.12	0.45	1.45	0.63
30	52.08	76.79	67.66	20.61	21.05	35.73	0.61	0.75	1.78
45	52.64	75.55	66.78	21.96	20.63	22.58	1.00	1.23	1.14
60	55.50	76.14	54.14	21.80	22.01	29.35	1.25	1.30	1.33

TABLE III

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DIMETHYLNITROSAMINE TREATED SANDY CLAY LOAM SOIL INCUBATED AT DIFFERENT TEMPERATURES

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity								
	Unaltered DMNA			DMA			CO_2		
	4C	25C	37C	4C	25C	37C	4C	25C	37C
0	89.70	88.70	86.51	2.13	3.14	2.15	0.00	0.00	0.00
5	82.24	69.47	78.68	10.08	14.13	13.73	0.39	0.66	0.58
10	78.11	69.43	76.46	23.03	14.07	12.77	0.75	0.78	0.67
20	53.56	67.73	63.96	28.80	29.84	28.86	1.75	1.52	0.41
30	60.84	65.81	74.83	26.88	27.09	29.58	0.52	0.73	0.93
45	49.56	57.02	69.10	24.03	24.97	29.84	0.78	0.75	0.79
60	54.60	58.40	63.75	17.81	28.59	25.48	1.14	0.83	0.85

(Table IV). The rates of release of CO_2 from dimethylamine were less than 2.0 percent as determined after 60 days. For example, 0.05% of glucose, 500 ppm NO_3^--N and NO_2^--N added to sandy soil released about 0.49 to 1.97 percent of CO_2 during 60 days incubation, whereas, 0.61-1.78 percent were released when this soil was amended with 0.1% glucose, 1000 ppm of NO_3^--N and NO_2^--N. The sandy loam receiving 0.05% glucose, 500 ppm of NO_3^--N and NO_2^--N, released 84.25, 74.95, 76.04, 66.75, 64.72, and 50.26 percent after 0.5, 10, 20, 30, 45, and 60 days, respectively. When this soil was amended with 0.1% glucose, 1000 ppm of NO_3^--N and 1000 NO_2^--N, the recovery of dimethylnitrosamine was 87.67, 83.40, 74.40, 78.63, 66.48, 66.73 and 63.73% after 0, 5, 10, 20, 30, 45, and 60 days, respectively. Therefore, the patterns of dimethylnitrosamine decomposition in sandy loam soil amended with 0.05% glucose, 500 ppm of each NO_3^--N and NO_2^--N and with 0.1% glocuse, 1000 ppm of each NO_3^--N and NO_2^--N were essentially identical. The breakdown of dimethylnitrosamine to dimethylamine in sandy loam soil amended with low concentrations was increased from 7.25 - 37.36 percent after 0, 5, 10, 20, and 30 days incubation period. After a 30 day incubation period, the accumulation of dimethylamine was decreased from 24 - 37.36 percent, likewise, the breakdown of dimethylnitrosamine to dimethylamine in the same soil sample amended with high concentration of carbon and nitrogen was increased from 10.30-26.60 percent after 0 to 30 days incubation. After 30 days, the yield of dimethylamine was decreased from 24.0-26.6 percent.

CO_2 was generated from both treated sandy loam soils, but the yield was greatly influenced by different concentrations of carbon and nitrogen (Table V).

TABLE IV

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED SANDY SOIL AMENDED WITH DIFFERENT CONCENTRATIONS OF CARBON AND NITROGEN INCUBATED AT 30 C

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	500 ppm[1]			1000 ppm[2]		
	DMNA*	DMA	CO_2	DMNA	DMA	CO_2
0	88.23	6.00	0.00	87.57	2.13	0.00
5	72.50	17.30	0.97	70.10	10.82	0.64
10	66.20	15.30	0.80	60.70	19.83	0.65
20	58.42	23.51	0.49	50.25	23.13	0.61
30	68.50	30.43	0.82	68.06	28.42	0.78
45	64.75	26.21	1.84	78.17	16.93	1.30
60	42.91	32.37	1.97	65.75	32.37	1.78

* Percent recovery of DMNA

1 0.05% of glucose, 500 ppm of each NO_3^--N and NO_2^--N

2 0.10% of glucose, 1000 ppm of each NO_3^--N and NO_2^--N

TABLE V

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C- DMNA TREATED SANDY LOAM SOIL AMENDED WITH DIFFERENT CONCENTRATIONS OF CARBON AND NITROGEN INCUBATED AT 30 C.

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	500 ppm[1]			1000 ppm[2]		
	DMNA*	DMA	CO_2	DMNA	DMA	CO_2
0	88.36	2.57	0.00	87.67	3.67	0.00
5	84.25	9.37	0.82	83.40	13.72	0.73
10	74.97	16.83	0.83	74.40	15.70	0.75
20	76.04	24.92	0.84	78.63	17.47	0.61
30	66.75	37.36	1.60	66.48	26.60	1.14
45	64.72	20.12	3.14	66.73	18.16	2.46
60	50.26	24.00	3.70	63.75	24.00	2.83

* Percent recovery of DMNA

1 0.05% of glucose, 500 ppm of each NO_3^--N and NO_2^--N.

2 0.10% of glucose, 1000 ppm of each NO_3^--N and NO_2^--N.

Sandy clay loam soil was amended with carbon and nitrogen the same as before. Results are shown in Table VI. The decomposition of of dimethylnitrosamine in the first day was 13.30 percent in soil samples amended with low concentrations of carbon and nitrogen. The concentration of dimethylnitrosamine was decreased very slowly, expecially after 5 to 30 days incubation.

The breakdown of dimethylnitrosamine to dimethylamine in a soil sample amended with low concentrations of carbon and nitrogen was 2.10-26.80 percent, in higher concentration it was from 1.67 - 31.80 percent. Therefore, there is no definite pattern in the time courses of dimethylnitrosamine decomposition, in some cases the highest breakdown of dimethylnitrosamine to dimethylamine was attained during 20 days incubation period where the sandy clay loam soil was amended with 0.05% glucose, 500 ppm of NO_3^--N to NO_2^--N. By contrast, the highest breakdown of dimethylnitrosamine to dimethylamine was attained after 30 days incubation period when this sandy clay loam was amended with 0.1% glucose, 1000 ppm of NO_3^--N and NO_2^--N.

The rates of release of CO_2 from dimethylamine at low and high concentrations of glucose and nitrogen were almost identical. For example, at low concentrations of glucose, NO_3^--N and NO_2^--N, 2.05% of the dimethylamine added was degraded to CO_2, likewise 2.73 percent was released after treatment at high concentrations of carbon (glucose) and (NO_3^--N and NO_2^--N) nitrogen (Table VI).

In order to assess the effect of moisture on dimethylnitrosamine decomposition in three soils of different texture, samples treated at different soil moisture contents were used. When sandy soil, sandy loam and sandy clay loam were incubated aerobically and anaerobically,

TABLE VI

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED SANDY CLAY LOAM SOIL AMENDED WITH DIFFERENT CONCENTRATION OF CARBON AND NITROGEN INCUBATED AT 30 C

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	500 ppm[1]			1000 ppm[2]		
	DMNA*	DMA	CO_2	DMNA	DMA	CO_2
0	86.70	2.10	0.00	89.07	1.67	0.00
5	73.15	10.92	0.71	84.02	9.78	0.98
10	64.37	16.80	0.75	72.48	10.66	1.23
20	65.48	27.66	0.79	55.06	20.66	0.86
30	63.19	24.20	1.12	67.75	31.80	1.46
45	53.20	25.82	1.90	75.59	22.29	2.01
60	47.70	26.80	2.05	60.16	23.60	2.73

* Percent recovery of DMNA

1 0.05% of glucose, 500 ppm of each NO_3^--N and NO_2^--N

2 0.10% of glucose, 1000 ppm of each NO_3^--N and NO_2^--N

the results were basically the same. For example, dimethylnitrosamine was found to be stable under both anaerobic and aerobic incubation conditions. More than 50 percent of the added dimethylnitrosamine remained after 60 days (Table VII, VIII, IX). These data are similar to those presented by A.R. Moiser and S. Torbil (1976) for dimethylnitrosamine systhesis and stability in cattle manure (5).

The breakdown of dimethylnitrosamine to dimethylamine in all treated samples with different soil moisture content was identical. Thus, 60 days, after incubation, 2.40-30.71 percent of the added dimethylnitrosamine was connected to dimethylamine (Table VII).

The rates of release of CO_2 from dimethylamine treated soils of different textures with different soil moisture contents incubated at 30C for 60 days was the same,and was quite similar to results obtained from soils treated at different temperatures, different concentrations of carbon (glucose) and nitrogen, as well as those soil samples both autoclaved and nonautoclaved.

In order to establish that microorganisms contribute to the decomposition of dimethylnitrosamine, soils with different textures were sterilized by autoclaving and after treatment were incubated for 60 days.

The patterns of dimethylnitrosamine decomposition in the non-sterile and sterile soil were essentially identical. After 60 days of treatment , no consistent differences between the sterile and non-sterile treatment at any sampling time were evident. The decomposition of dimethylnitrosamine in three soils of different textures was less than 50 percent, a result similar to that reported by Rober and Alexander (26). For example, in both autoclaved and

TABLE VII

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED SANDY SOIL WITH DIFFERENT SOIL MOISTURE CONTENTS INCUBATED AT 30 C

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	Field Capacity			Flooding Condition		
	DMNA*	DNA	CO_2	DMNA	DMA	CO_2
0	88.11	3.17	0.00	89.40	3.58	0.00
5	74.25	13.92	1.70	60.23	15.03	1.58
10	73.17	14.50	1.62	80.91	21.70	1.62
20	60.11	32.93	0.73	79.92	22.44	1.64
30	41.08	27.13	0.65	70.76	22.33	2.05
45	49.09	27.22	1.70	66.83	23.56	4.84
60	53.86	27.99	1.85	65.40	28.38	4.75

* Percent recovery of DMNA

TABLE VIII

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED SANDY LOAM SOIL WITH DIFFERENT SOIL MOISTURE CONTENTS INCUBATED AT 30 C

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	Field Capacity			Flooding Condition		
	DMNA*	DNA	CO_2	DMNA	DMA	CO_2
0	88.87	2.40	0.00	91.42	4.80	0.00
5	68.19	11.06	1.37	83.31	9.80	1.11
10	64.05	13.50	1.22	76.80	12.17	1.15
20	60.26	25.66	0.69	57.71	23.12	1.21
30	52.40	25.37	1.01	60.73	25.72	1.81
45	68.00	20.32	1.90	62.80	23.29	2.63
60	68.98	20.32	1.90	68.00	30.71	3.05

* Percent recovery of DMNA

TABLE IX

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED SANDY CLAY LOAM SOIL WITH DIFFERENT SOIL MOISTURE CONTENTS INCUBATED AT 30 C

Days of Incubations	Percent of Total Extractable ^{14}C-radioactivity					
	Field Capacity			Flooding Condition		
	DMNA*	DMA	CO_2	DMNA	DMA	CO_2
0	87.77	5.10	0.00	89.17	3.50	0.00
5	71.15	10.22	0.31	58.31	17.23	0.74
10	70.63	12.83	0.57	55.48	14.06	0.82
20	70.60	27.24	0.77	46.67	18.94	0.51
30	51.10	25.08	0.94	59.17	24.20	1.09
45	75.83	16.89	2.66	43.20	23.11	3.27
60	70.12	24.95	2.70	63.00	25.19	3.80

* Percent recovery of DMNA

nonsterile sandy soil incubated at 30C for 60 days, dimethylnitrosamine was degraded by about 30 percent after a 5 day incubation period. On day 10 and thereafter for a total of 50 days, decomposition was very slow, and more than 50 percent of the dimethylnitrosamine was still present in both autoclaved and non-autoclaved soil samples at day 60 (Table X).

The breakdown of dimethylnitrosamine to dimethylamine in both autoclave and nonautoclaved sandy soil was about 1.57 to 39.93 percent. The decomposition of dimethylnitrosamine to dimethylamine was not a biological reaction, since the results were almost identical from both autoclaved and non-autoclaved samples. Therefore, the presence of microorganisms in soil had no effect on decomposition of dimethylnitrosamine in soil. The percent yield of dimethylamine from dimethylnitrosamine in sandy loam and clay loam soil are shown in Table XI and XII. The results from this clay loam were similar to those obtained from a sandy soil.

Dimethylnitrosamine was investigated from the viewpoint of microbial influence, temperature, soil moisture content and nutrition on stability and decomposition in three different soils. The results show that dimethylnitrosamine is a stable compound, since more than 50 percent of the added dimethylnitrosamine remained in the soil after 60 days.

TABLE X

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED AUTOCLAVED AND NON-AUTOCLAVED SANDY SOIL INCUBATED AT 30 C

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	Autoclaved			Non-autoclaved		
	DMNA*	DMA	CO_2	DMNA	DMA	CO_2
0	87.35	1.57	0.00	88.25	2.58	0.00
5	70.65	10.94	1.16	74.25	13.92	1.70
10	75.66	17.76	1.15	73.17	14.50	1.62
20	76.06	18.50	1.64	60.11	32.93	0.73
30	63.79	29.45	1.45	41.08	27.13	0.65
45	56.85	19.50	2.63	49.09	27.22	1.70
60	47.10	26.15	2.70	53.86	27.99	1.85

* Percent recovery of DMNA

TABLE XI

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED AUTOCLAVED AND NON-AUTOCLAVED SANDY LOAM SOIL INCUBATED AT 30 C

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	Autoclaved			Non-autoclaved		
	DMNA*	DMA	CO_2	DMNA	DMA	CO_2
0	88.59	3.89	0.00	85.58	1.77	0.00
5	72.10	11.20	1.21	68.19	11.06	1.37
10	70.02	17.30	1.32	64.05	13.50	1.22
20	69.69	20.12	2.17	60.26	25.66	0.69
30	63.96	24.44	3.16	52.40	25.37	1.01
45	63.54	26.97	3.01	68.00	20.32	1.90
60	52.36	27.84	3.70	68.98	20.32	1.90

* Percent recovery of DMNA

TABLE XII

^{14}C-LABELED COMPOUNDS EXTRACTED FROM ^{14}C-DMNA TREATED AUTOCLAVED AND NON-AUTOCLAVED SANDY CLAY LOAM SOIL INCUBATED AT 30 C

Days of Incubation	Percent of Total Extractable ^{14}C-radioactivity					
	Autoclaved			Non-autoclaved		
	DMNA*	DMA	CO_2	DMNA	DMA	CO_2
0	86.78	3.74	0.00	89.40	2.33	0.00
5	65.86	10.30	0.50	71.15	10.22	0.31
10	65.02	22.83	0.75	70.63	12.83	0.57
20	57.08	20.52	1.60	70.60	27.24	0.77
30	54.42	22.18	1.89	51.10	25.08	0.94
45	59.68	19.83	1.31	75.83	16.89	2.66
60	55.16	24.16	1.57	70.12	24.95	2.70

* Percent recovery of DMNA

TABLE XIII

PHYSICAL ANALYSES OF SOIL SAMPLES

Texture	pH	Sand (%)	Silt (%)	Clay (%)	Organic Matter (%)
Sandy	4.8	70	20	10	2.16
Loam	7.2	32.5	42.5	25	7.18
Clay loam	6.8	22.5	25	52.5	7.40

CHAPTER V

SUMMARY AND CONCLUSIONS

Three soil types were used to study the stability and decomposition of carcinogenic ^{14}C-dimethylnitrosamine. ^{14}C-dimethylnitrosamine was investigated from the viewpoint of temperature, nutrition, soil moisture content and microbial influence.

The data shows that there was no obvious effect of temperature on decomposition of ^{14}C-dimethylnitrosamine. More than 50% of the added ^{14}C-dimethylnitrosamine was retained in the soil after 60 days at all of the three different temperatures. Neither was there any effect of soil texture and soil organic matter content in ^{14}C-dimethylnitrosamine loss.

The rates of loss of ^{14}C-dimethylnitrosamine from soil amended with different concentration of glucose and nitrogenous compounds was also similar in all soils.

One set of soils was incubated under anaerobic condition and the other at aerobic condition, under both conditions the rate of loss of ^{14}C-dimethylnitrosamine was similar.

In both autoclaved and non-autoclaved soil, about 30 percent of the ^{14}C-dimethylnitrosamine was degraded in the first 5 days of incubation, thereafter the rate of decomposition was slow and similar for both autoclaved and non-autoclaved soil.

It appears that dimethylnitrosamine is a highly stable compound,

highly resistant to microbial attack; and soil physical factors have very little or no effect on breakdown of dimethylnitrosamine. Therefore, whatever amount of breakdown noted in the investigation was a slow auto-degradation.

LITERATURE CITED

1. Alexander, M. and Mills, A.L. (1976). Factors Affecting Dimethylnitrosamine Formation in Samples of Soil and Water. J. Environ. Qual. 5(4):437-440.

2. Ayanaba, A. and Alexander, M. (1974). Transformation of Meltylamines and Formation of Hazordous Products, DMNA, in Samples of Treated Sewage and Lake Water. J. Environ. Qual. 3(1):83-89.

3. Anon. (1972). Hazard of Nitrate, Nitrite and Nitrosamines to Man and Livestock. Accumulation of Nitrate. National Academy of Sciences. Washington D.C.

4. Ayanaba, A. and Alexander, M. (1972). Formation of Dimethylnitrosamine in Soil, a Carcinogen and Mutagen. Agronomy Abstract.

5. Bremner, J.M. (1965). Inorganic forms of Nitrogen. In Methods of Soil Analysis. Part 2. American Society of Agronomy. 1179-1232.

6. Barnes, J.M. and Magee, P.N. (1954). Some Toxic Properties of Dimethylnitrosamine. Brit. J. Ind. Med. 11:167-174.

7. Bouycous, G.T. (1951). Recalibration of the Hydrometer Method for Making Mechanical Analysis of Soils. Agronomy Journal. 43:434-438.

8. Druckery, H., Ivankovic, S., and Preussman, R. (1966). Teratogenic and Carcinogenic Effect in the Offspring after Single Injection of Ethylnitrosourea to Pregnant Rats. Nature 210:1378-1379.

9. _____, _____, and _____. (1967). Organotrope Carcinogenic Wirkungen Bei 65 Verschiedene N-nitrosoverbindungen an BD-Ratten. Z. Krebsforsch 69:103-201.

10. _____, _____, and _____. (1969). N-nitron compounds in Organotropic and Transplacental Carcinogenesis. Ann. N.Y. Acad. Sci. 163:676-696.

11. Doll, R. (1969). The Geographical Distribution of Cancer. Br. J. Cancer 23, 1.

12. Ender, F., and Ceh, L. (1968). Occurrence of Nitrosamines in Foodstuffs for Human and Animal Consumption. Food Cosmet. Toxicol. 6:569-571.

13. Ender, F., et al. (1964). Isolation and Indentification of a Hepatotoxic Factor in Herring Meal Produced from Sodium Nitrite Preserved Herring, Naturwissenschaften 51,637.

14. Greenblatt, M., et al. (1971). Nitrosamine Studies: Introduction of Lung Adenomas by Concurrent Adminstration of Sodium Nitrite and Secondary Amines in Swiss Mice. J. Nat. Cancer Inst. 45:1029-1034.

15. Keste, T., Mallik, M.A.B., and Pancholy, S.K. Possible Formation of Carcinogenic Nitrosamine from Insecticide, Phosphonudon and Nitrate in Soil. Oklahoma Academie of Science (In Press).

16. Masier, A.R. and Torbit, S. Synthesis and Stability of Dimethylnitrosamine in Cattle Manure. J. Environ. Qual. 5(4):465-468.

17. Magee, P.N. and Barnes, J.M. (1956). The Production of Malignant Tumors in the Rats by Feeding Dimethylnitrosamine. Brit. J. Cancer. 10:114-119.

18. _____, and _____. (1967). Carcinogenic Nitroso Compounds. Adv. Cancer Res. 10:163-246.

19. McGlashan, N.D., et al. (1968). Nitrosamine in Africa Alcoholic Spirits, and Esophogeal Cancer. Lancer. 2:1017.

20. Mohler, K, and Myrohfer, O. (1968). Nachweis and Bestimmung Von Nitrosamine in Lebensmittlen. Z. Lebensmittel-Unters. Forsch. 135:313-318.

21. Maeda, K., and Tonomura, K. (1971). Microbiol Degradation of Tetramethylthiuram Disulfide. Kogyo Gijutsuintlakke Kenkyusho Kenkyu Hokoku. 33:1-8.

22. McGlashan, N.D., et al. (1968). Nitrosamine in Africa Alcholic Spirits and Esophogeal Cancer. Food Cosmel. Toxical 9:207-218.

23. Peech, M. (1965). Hydrogen-ion activity. In C.A. Black (Ed) Methods of Soil Analysis. Agronomy Monograph 9. Amer. Soc. Agro., Madison Wisconsin.

24. Rhoades, J.W., and Thomson, D.E. (1970). Gas Chromatography and Selective Detection of N-nitrosomines. Jr. Chromat. Sci. 8:616-617.

25. Reed, L.W. (1977). Modification of Schollenberger Procedure for determination of soil Organic Matter. Private Communication.

26. Rebert, L.T., and Alexander, M. (1976) Resistance of Nitrosamines to Microbiol Attack. J. Qual. 5(2):131-133.

27. Smith, T.A. (1971). The Occurence, Metabolism and Functions of Amines in Plants. Biol. Rev. 46:201-241.

28. Serfontein, W.J. and Hurter, P. (1966). Nitrosamines as Environmental Carcinogens. Evidence for the Presence of Nitrosamine in Tobacco Smoke Condensate. Cancer Res., 26:575-579.

29. Sander, J. (1971). Formation of Carcinogenic Nitroso Compounds Under Biological Conditions. 24-Annual Symposium on Fundamental Cancer Research. The Williams and Wilkins Company. Page 476.

30. Stewart, B.A., et al. (1967). Nitrate and other water pollutants under fields and feed lots. Environ. Sci. Technol. 1:736-739.

31. Sen, et al. (1970). Formation of Nitrosomine in Nitrite-treated fish. J. Inst. Can. Technol. 3:66-69.

VITA 2

Kesete Tesfai

Candidate for the Degree of

Master of Science

Thesis: STABILITY OF CARCINOGENIC DIMETHYNITROSAMINE IN SOIL

Major Field: Agronomy

Biographical:

Personal Data: Born in Decamere, Eritrea, East Africa, on October 9, 1948, the son of Tesfai Yonom and Abrehazion Beraki.

Education: Graduate from Decamere Secondary School, Decamere, Eritrea in 1969; received the Bachelor of Science degree from Oklahoma Panhandle State University with a major in Agronomy in August, 1975; completed the requirements for the Master of Science Degree at Oklahoma State University in December, 1977.

Professional Experience: Graduate Research Assistant, Cooperative Science and Education Service, Langston University, 1976-1977.