FORMATION OF CARCINOGENIC NITROSAMINES IN SOIL TREATED WITH PESTICIDES, AND IN SEWAGE AMENDED WITH NITROGEN COMPOUNDS*

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Soil samples treated with thiram, eptam or vernam yielded secondary amines, but no nitrosamines. Nitrosamines appeared in soil samples treated with any one of these pesticides and potassium nitrite. The addition of glucose generally reduced formation of secondary amines and nitrosamines.

Dimethylnitrosamine was not detected in sewage, sewage effluent, or well water. Sewage amended with dimethylamine and potassium nitrite produced more nitrosamine than sewage effluent and well water. A small amount of nitrosamine was detected in samples treated with dimethylamine alone.

INTRODUCTION

Since Magee and Barnes (1) first demonstrated liver damage induced by dimethylnitrosamine (DMNA) in animals, considerable concern has been expressed about the formation and occurrence of nitrosamines in the environment. About 80 out of 100 nitrosamines so far tested have proved highly toxic and carcinogenic at μg/g level, having caused malignant tumors in laboratory animals (2).

A number of nitrosamine compounds have been detected in foodstuffs, such as fish, meat and bacon treated with nitrite, and alcoholic beverages (3, 4, 5, 6). Nitrosamines have been shown to form in soil in presence of secondary amines and nitrite at acid pH values (7, 8). Much of the concern regarding the formation of nitrosamines in the environment is related to the widespread occurrence of nitrosamine precursors (nitrite and secondary or tertiary amines) in the environment and their possible interactions to form *N*-nitroso compounds. Many agricultural chemicals contain structures that can be degraded to secondary amines (9). Excessive and widespread use of these pesticides may result in accumulation of secondary amines in localized environments, and may contribute to the formation of nitrosamines.

The objectives of the present investigation were to determine if nitrosamines could be formed in soil in presence of certain pesticides and if DMNA could be formed and detected in sewage and water treated with dimethylamine (DMA).

MATERIALS AND METHODS

Three pesticides were used: a fungicide, thiram (tetramethylthiuram disulfide); and two herbicides, vernam (*S*-propyl dipropylthiocarbamate) and eptam (*S*-ethyl dipropylthiocarbamate).

Ten-gram portions of air-dried and sieved sandy soil (pH 4.8, organic matter content 2.15%) were introduced into clean dry test tubes (15 × 1.5 cm). Individual sets of soil tubes received one of the following treatments: I, none (control); II, pesticide; III, pesticide plus KNO_2; IV, pesticide plus KNO_3; V, pesticide plus KNO_2 plus glucose; and VI, pesticide plus KNO_3 plus glucose. The pesticides were dissolved in organic solvents (thiram in dichloromethane, and vernam and eptam in ethanol), and a one-m*l* portion of the pesticide solution was added to the soil tube to obtain a final concentration of 500 μg/g soil. Solutions of KNO_2 or KNO_3 and glucose in distilled water were added to obtain 100 and 5000 μg/g soil, respectively. The control soil tubes received equal amounts of organic solvent and distilled water. The contents of the tubes were thoroughly mixed after addition of sufficient distilled water to bring the moisture level to field capacity. The soil tubes were covered with aluminum foil and incubated in the dark at 30 C up to 30 days. Duplicate tubes from each treatment were withdrawn at 0, 5,

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10, 15, 20, and 30 days of incubation for analyses. The contents of the tubes were mixed with 15 m*l* distilled water and the suspension centrifuged. An aliquot of the supernatant was used for the determination of nitrite by a colorimetric method (10), The remaining portion of the supernatant was steam distilled (11). The distillate was analyzed for nitrosamines by a photochemical method (12), and for secondary amines by the method of Pribyl and Nedbalkova (13), with DMA as the standard.

Samples of municipal sewage and sewage treatment plant effluent were collected from the Oklahoma City Sewage treatment plant, and well water was collected from a nearby rural area; these were adjusted to pH 5 or 7 with 0.1 *M* phosphate buffer and 1 *N* HCl. The samples were amended with DMA at concentration of 0 or 500 μg DMA-N, and KNO_3 at 0 or 100 μg NO_3-N/g, and mixed thoroughly. They were incubated on a rotary shaker at 110 rpm in the dark at 30 C. Subsamples were removed after 0, 5, 10 or 15 days incubation, steam distilled, and the distillate was analyzed for DMNA as before (12).

To confirm the identity of the nitrosamines, the distillate was extracted with dichloromethane and concentrated in a Kuderna-Danish evaporator and finally with a stream of nitrogen gas (14). This fraction was analyzed for nitrosamines by thin-layer chromatography (TLC) (15).

RESULTS AND DISCUSSION

In the unamended soil after 5-day incubation 2.5 μg NO_2-N and 0.24 μg DMA-N/g soil were recorded (Table 1). DMA was found in the thiram-treated soil at 0 day; the possibility of breakdown of a very small quantity of thiram during distillation, producing DMA, could not be ruled out. A small amount of DMA was detected by Ayanaba and Alexander (16) in sterilized soil treated with thiram, indicating that DMA was produced from the fungicide by nonmicrobial degradation. The concentration of DMA continued to rise during the 30-day incubation period suggesting that the degradation of thiram is a slow process. After 30 days incubation, 9.4 μg DMA-N/g had been produced in soil treated with thiram alone. This value amounted to 16.5% of the thiram-N. DMA formation and accumulation was higher in soil treated

TABLE 1. *Production of NO_2-N, DMA-N, and DMNA-N in soil (μg/g) treated with thiram, glucose, nitrate, or nitrite during various incubation periods*

Treatment	Incubation (days)																	
	0			5			10			15			20			30		
	NO_2	DMA	DMNA[a]	NO_2	DMA	DMNA	NO_2	DMA	DMNA	NO_2	DMA	DMNA	NO_2	DMA	DMNA	NO_2	DMA	DMNA
None	0.4	0.0	.00	2.5	0.2	.00	0.6	0.0	.00	0.8	0.0	.00	0.0	0.0	.00	0.0	0.0	.00
Thiram	0.4	0.5	.00	0.0	3.4	.00	1.2	4.8	.00	0.6	7.4	.00	0.7	6.3	.00	0.0	9.4	.00
Thiram + NO_2^-	105.2	0.4	.00	103.7	4.9	0.62	93.8	8.1	.70	82.5	9.3	0.77	62.5	13.3	1.02	46.2	16.1	1.41
Thiram + NO_2^- + Gl.	106.7	0.5	.00	103.7	5.5	0.46	93.7	6.3	.38	83.0	4.8	0.56	62.5	8.4	0.70	41.2	14.5	0.70
Thiram + NO_3^-	3.5	0.6	.00	3.0	3.5	.00	9.8	4.9	.00	2.5	2.7	.00	1.2	5.9	.00	0.9	9.7	.00
Thiram + NO_3^- + Gl.	3.0	0.5	.00	2.4	3.4	.00	4.4	2.7	.00	0.5	4.3	.00	0.4	5.2	.00	0.4	7.5	.00

[a]Means of duplicate analyses

TABLE 2. *Production of NO_2-N, DPA-N, and DPNA-N in soil ($\mu g/g$) treated with vernam, glucose, nitrate, or nitrite during various incubation periods*

Treatment	Incubation (days)																	
	0			5			10			15			20			30		
	NO_2	DPA	DPNA[a]	NO_2	DPA	DPNA	NO_2	DPA	DPNA	NO_2	DPA	DPNA	NO_2	DPA	DPNA	NO_2	DPA	DPNA
Vernam	1.2	0.2	.00	0.2	0.4	.00	0.0	0.6	.00	0.4	0.9	.00	0.3	0.4	.00	0.6	0.1	.00
Vernam + NO_2^-	108.0	0.2	.00	102.0	0.4	tr[b]	96.2	0.2	tr	91.2	1.4	tr	62.5	0.3	.00	30.0	0.4	.00
Vernam + NO_2^- + Gl.	108.0	0.3	.00	101.0	0.3	tr	99.0	0.3	tr	92.5	N.D.[c]	tr	58.7	0.7	.00	55.0	0.1	.00
Vernam + NO_3^-	4.0	0.0	.00	3.1	0.3	.00	2.2	0.4	.00	1.0	N.D.	.00	2.5	0.5	.00	2.1	0.4	.00
Vernam + NO_3^- + Gl.	1.5	0.2	.00	1.2	0.2	.00	1.0	0.3	.00	1.8	0.6	.00	1.0	0.3	.00	0.8	0.0	.00

[a]Means of duplicate analyses
[b]Trace
[c]Not determined

TABLE 3. *Production of NO_2-N, DPA-N and DPNA-N in soil ($\mu g/g$) treated with eptam, glucose, nitrate, or nitrite during various incubation periods*

Treatment	Incubation (days)																	
	0			5			10			15			20			30		
	NO_2	DPA	DPNA[a]	NO_2	DPA	DPNA	NO_2	DPA	DPNA	NO_2	DPA	DPNA	NO_2	DPA	DPNA	NO_2	DPA	DPNA
Eptam	0.8	0.4	.00	0.8	0.0	.00	0.2	0.2	.00	0.4	0.3	.00	0.0	0.5	.00	0.0	0.6	.00
Eptam + NO_2^-	106.0	0.2	.00	104.0	0.2	tr[b]	52.5	0.3	tr	56.2	0.3	tr	21.2	1.1	.00	22.6	0.8	.00
Eptam + NO_2^- + Gl.	106.0	0.3	.00	96.0	0.1	tr	43.8	0.3	tr	47.2	0.4	tr	21.2	0.6	.00	15.5	0.7	.00
Eptam + NO_3^-	4.0	0.0	.00	2.5	0.2	.00	0.5	0.4	.0	0.9	0.2	.00	0.0	0.8	.00	0.4	0.8	.00
Eptam + NO_3^- + Gl.	1.1	0.0	.00	1.5	0.2	.00	0.2	0.4	.0	0.4	0.3	.00	0.0	0.3	.00	0.4	0.6	.00

[a]Means of duplicate analyses
[b]Trace

with both thiram and NO_2; the maximum amount recorded was 16.1 μg DMA-N/g, or 28.1% of thiram-N, at 30-day incubation. Our data are in agreement with the findings that secondary amines can accumulate in soil treated with thiram (16) and with phosphamidon (17).

No DMNA appeared in soil treated with thiram alone, thiram and nitrate, or in unamended soil. However, a maximum of 1.4 μg DMNA-N/g was recorded in soil treated with thiram and nitrite at 30-day incubation. The amount of DMNA produced appeared to be related to the amount of DMA available for nitrosation. Tate and Alexander (9) could detect a small amount of DMNA in soil treated with dimethyldithiocarbamate alone; the nitrogen moiety could have been provided from the degradation of the fungicide or from a nitrogen compound already present in the soil.

The TLC of the dichloromethane fraction showed a spot with R_f of 0.26, which was identical to that of authentic DMNA.

The degradation of vernam and eptam leading to the formation of dipropylamine (DPA) reached a peak between 15-30 days incubation in all treatment combinations (Tables 2 and 3). The highest yield, 1.4 μg DPA-N/g, was obtained in soil treated with vernam and nitrite. Soil treated with eptam and nitrite produced less DPA than soil amended with nitrite and thiram or vernam. It appears that eptam undergoes degradation very slowly; the level of DPA increased until the termination of the experiment after 30 days incubation. The loss of DPA from vernam-treated soil after 15 days incubation cannot be explained. Detectable amounts of DPNA were found in soil treated with nitrite and vernam or eptam.

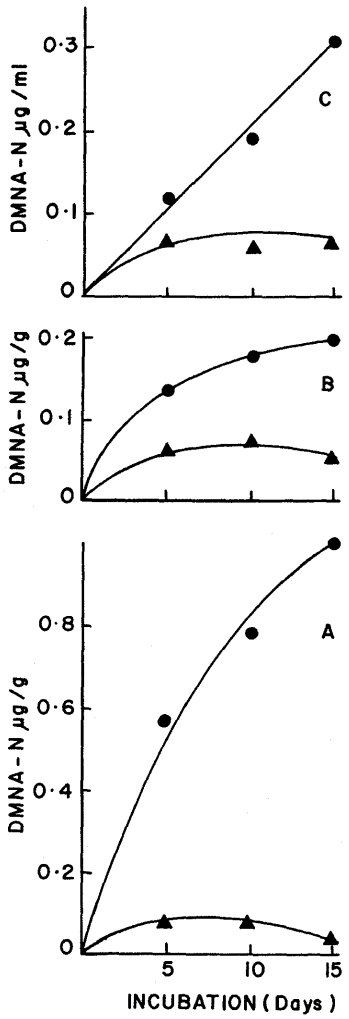

FIGURE 1. DMNA formation at different incubation times in sewage (A), sewage effluent (B) and well water (C) held at pH 5, and amended with DMA-N 500 μg/g plus NO_3-N 100 μg/g (●), and DMA-N 500 μg/g (▲).

TABLE 4. *Formation of DMNA in sewage, sewage treatment plant effluent, and well water amended with DMA and KNO_3, and held at pH 5 or 7*

Incubation (days)	pH	Yield of DMNA[a] (μg/ml)		
		Sewage	Sewage effluent	Well water
5	5	0.57	0.14	0.15
	7	tr[b]	tr	tr
10	5	0.78	0.18	0.20
	7	tr	.00	tr
15	5	1.08	0.21	0.34
	7	.00	.00	tr

[a]Means of duplicate analyses
[b]Trace

The addition of glucose and nitrate generally reduced accumulation of secondary amines and formation of nitrosamines in soil treated with thiram, vernam, or eptam. In the presence of a readily available energy source, increased microbial activities may have caused rapid metabolism of secondary amines. This suggests that there is less possibility of accumulation of secondary amines in soil high in organic matter as a readily available source of energy.

Raw sewage, sewage treatment plant effluent, and well water contained 0.7, 0.3, and 0.1 μg/g of NO_2-N respectively (not shown in the table) but no DMA.

Dimethylnitrosamine was formed in sewage, sewage effluent, and well water held at pH 5 and amended with DMA and nitrate, but not in unamended substrates (Table 4). A small quantity of DMNA was also detected in all three substrates treated with DMA only (Fig. 1). It is noteworthy that under identical conditions DMNA formation in sewage was much higher than in other two substrates. Whether high organic matter content and/ or increased microbial activity in sewage influenced the nitrosation reaction was not clear. The microbial contribution to the nitrosation reaction has been shown by Pancholy and Mallik (18) and Ayanaba and Alexander (19). DMNA formation and accumulation occurred only at acid pH values, and was negligible in neutral solution.

Our data show that small amounts of secondary amines and nitrite can be found in soil, but the quantity is not enough to effect the nitrosation reaction. Formation and accumulation of secondary amines as a result of decomposition of certain pesticides can reach high levels in soil and contribute to the formation of carcinogenic nitrosamines, particularly under acid conditions. Secondary amines are easily degradable and seldom accumulate in neutral soil (8). Therefore accumulation of secondary amines in appreciable quantity seems unlikely in neutral soil.

Since nitrosamines, particularly DMNA, are highly carcinogenic and acutely toxic even at the microgram/gram level, formation of small amounts of these compounds can be a health hazard, especially since they are highly resistant to microbial attack (20). Formation and accumulation of precursor compounds in the environment should be viewed with concern because at low pH the nitrosation reaction can occur spontaneously. It should be made clear that DMNA has not been detected in a natural ecosystem. However, excessive use of certain pesticides in acid soil could enhance accumulation of precursor compounds and contribute to formation of carcinogenic nitrosamines.

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