POSSIBLE FORMATION OF A CARCINOGENIC NITROSAMINE FROM AN INSECTICIDE, PHOSPHAMIDON, AND NITRATE IN SOIL

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Nitrosamines are of potential ecological concern because of their carcinogenic, mutagenic, and teratogenic nature (1). These compounds are known to be formed in samples of soil, sewage, and lake water which have received certain nitrogenous compounds (nitrite or nitrate and secondary amines). Many agricultural chemicals (e.g. Ziram, Thiram, Eptam, Vernam and Phosphamidon) also contain structures that can be *N*-nitrosated in soil and water. Ayanaba *et al.* (2) have shown that dimethylnitrosamine can be formed in soil from the fungicide, Thiram, and nitrite.

Phosphamidon (2-chloro-2- (*N*,*N*-diethylcarbamoyl)-l-methylvinyl dimethyl phosphate), a systemic insecticide, has been used to control aphids, leafhoppers, certain beetles, plant bugs and other insects. It has been used on a wide variety of crops. The purpose of this study was to investigate the possibility of formation of nitrosamines in soil which received applications of Phosphamidon and nitrite or nitrate.

A sandy loam soil of pH 4.75, organic carbon content 0.56%, and 5.60 μg NO_3-N/g was used in this study. The soil samples were obtained at a depth of 0-15 cm. Air-dry soil samples (10 g each) were treated to contain 0 or 100 μg/g of Phosphamidon, nitrate or nitrite, and glucose. Then the samples received 10 ml of distilled water so that the soil was flooded to minimize the conversion of nitrite to nitrate. These soil samples were incubated at 30 C and analyzed for nitrosamines after 10 and 20 days. Nitrosamines were isolated by steam distilling the soil samples and collecting 80 ml of the distillate. The distillate was extracted three times in a separatory funnel with 30-ml portions of dichloromethane. The combined extract was dried over anhydrous Na_2SO_4. Then it was concentrated to approximately 5 ml in a Kudrena-Danish evaporator (3). A stream of nitrogen was used to further reduce the volume to exactly one ml. Nitrosamines were measured in a 0.1 ml fraction by the procedure of Preussman *et al.* (4). The remainder of the fraction (0.9 ml) was equally divided and spotted on two silica gel 60 F-254 thin layer plates along with various known compounds. The plates were developed in a 4:3:2 solvent system (v/v/v) of *n*-hexane-ether-dichloromethane. After air-drying one plate was sprayed with 0.3% ninhydrin and heated in an oven at 80 C for 5 min. The second plate was exposed to intense short wave UV light for 5 min and then sprayed with Griess reagent (0.1% α - naphthylamine in 30% acetic acid and 0.1% sulfanilic acid, 1:1). R_f values and colors of the spots observed were recorded and compared with those for the knowns.

In the 20-day incubation study no nitrosamines were detected in soil samples receiving nitrate, nitrite, and glucose. However, carcinogenic nitrosamines were produced in soil samples containing Phosphamidon or Phosphamidon plus nitrate or nitrite (Table 1). Maximum concentrations of nitrosamines were detected after 10 days

TABLE 1. *Maximum concentrations of nitrosamines formed in a flooded sandy loam soil.*

Compounds added[a]	Nitrosamines formed (μg/ml) 10 days	20 days
None	0	0
Nitrite	0	0
Nitrate	0	0
Glucose	0	0
Phosphamidon	0.25	0.15
Phosphamidon + nitrite	0.60	0.40
Phosphamidon + nitrate	0.45	0.35
Phosphamidon + nitrite + glucose	0.40	0.20
Phosphamidon + nitrate + glucose	0.35	0.15

a Phosphamidon, nitrate, nitrite, and glucose added to make final concentration 100 μg of each per g soil.

TABLE 2. *R_f values and spot colors of various compounds*

Compounds		R_f	Color	
			Ninhydrin	Griess
Known Phosphamidon		0.02	purple	no color
Known dimethylnitrosamine		0.25	purple	grey
Known diethylnitrosamine		0.52	purple	grey
Known dipropylnitrosamine		0.70	purple	grey
Known dibutylnitrosamine		0.78	purple	grey
Concentrated fractions	*a.*	0.02	purple	no color
from soil treatments	*b.*	0.50	purple	grey
(10 and 20 days)	*c.*	0.87	yellow	pink

of incubation and the highest amount was observed when Phosphamidon and nitrite were present in the soil. Lower concentrations of nitrosamines were observed in each insecticide-treated sample after 20 days of incubation. The presence of nitrite or nitrate in soil enhanced the formation of nitrosamines, but addition of glucose produced about a 25% reduction in nitrosamines at 10 days and 50% reduction at 20 days.

The results of thin layer chromatography are shown in Table 2. Four known nitrosamines had distinct R_f values and color reactions with ninhydrin and Griess reagent. Exposure to intense short UV light causes the breakdown of nitrosamines and the production of nitrite (4) which can be identified by using Griess reagent. The concentrated fractions from soil treatments yielded three distinct spots (Table 2). Spot *a* with a R_f of 0.02 was identified as due to Phosphamidon, spot *b* was identified as representing diethylnitrosamine, and *c* remained unidentified. Further co-chromatography work confirmed the identity of spot *b* as due to diethylnitrosamine.

In order to assess the possibility of formation of nitrosamines in the soil, one should look at the availability of the precursors. Nitrate and nitrite are readily available in the soil, mainly from nitrification and denitrification processes. Secondary amines are not very abundant in soil. However, they can be produced from the breakdown of pesticides (2), by bacteria (5) and algae (6). It can be argued that the concentrations of pesticides used in this study do not exist in the soil, but it may not be uncommon to find fairly high concentrations as a result of pesticide treatments. Also, one has to keep in mind that concentrations in soil may be sufficient to produce nitrosamines higher than the "permissible" dose which has been established at 5-10 μ g/kg (7). Finally, even traces of these compounds in soil would be of great concern because of the hazard they present and their high solubility in water.

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