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Degradation of Herbicide-Related Nitrosamines in Aerobic Soils

James E. Oliver,* Philip C. Kearney, and Arnold Kontson

Four ^{14}C-labeled nitrosamines were incorporated into aerobic soils, and the rate of $^{14}CO_2$ production was measured. Rates of $^{14}CO_2$ evolution were similar for *N*-nitrosodipropyl-, diethyl-, and dimethylamines. Samples of nitrosodipropylamine individually labeled with ^{14}C at carbons 1, 2, and 3 were synthesized. The rate of $^{14}CO_2$ production was independent of the position of the label. The reaction is believed to be microbiological because sterilization of the soil inhibited CO_2 production. For a few days after application, volatilization of unchanged nitrosamine from soil also occurred. It is estimated that the half-life of these nitrosamines in aerobic soils is on the order of three weeks. *N*-Nitrosopendimethalin [*N*-(1-ethylpropyl)-*N*-nitroso-3,4-dimethyl-2,6-dinitrobenzenamine] was relatively stable in soil and significant quantities could be recovered unchanged after several months.

N-Nitrosamines have been of agricultural interest for several years, in part because it has been postulated that various nitrogen-containing pesticides and fertilizers might be nitrosamine precursors (Ayanaba et al., 1973). Interest in the environmental chemistry of nitrosamines was considerably enhanced by the announcement (Fan et al., 1976; Ross et al., 1977) that four commercially acquired herbicides had contained nitrosamines, and by the subsequent investigation by the Environmental Protection Agency that revealed that several additional pesticides were also contaminated with nitrosamines (Pesticide and Toxic Chemical News, 1977). One of the obvious questions arising in assessing whether the compounds present an environmental hazard is, what are their fates in soil?

Tate and Alexander (1975, 1976) reported that *N*-nitrosodipropylamine (NDPA, **1**), *N*-nitrosodiethylamine (NDEA, **2**), and *N*-nitrosodimethylamine (NDMA, **3**) were resistant to microbiological degradation (all three of these nitrosamines have since been detected as impurities in herbicides; Pesticide and Toxic Chemical News, 1977). Kearney et al. (1977) found, in contrast, that *N*-nitrosoatrazine was rapidly degraded in Metapeake loam; only 8% of the added nitrosoatrazine could be recovered after 1 month. We recently studied the formation of *N*-nitrosobutralin in soil (Oliver and Kontson, 1978). Nitrosation of butralin was observed only when the soil was heavily amended with sodium nitrite; however, the limited amount of nitrosobutralin that did form proved to be quite persistent, and a small amount was recovered after 6 months. Fan et al. (1976) were unable to detect NDPA in soil following trifluralin application, and Elanco (Eli Lily and Co.) researchers examined soils and well water in areas with histories of high trifluralin usage, but did not detect NDPA in either medium (Amundson, 1978). Recently, Saunders et al. (1979) reported that NDPA was, in fact, degraded in both aerobic and anaerobic soils.

Because of the importance of some of the nitrosamine-containing herbicides, we have examined the degradation of four ^{14}C-labeled nitrosamines (**1–4**, Figure 1) in aerobic soils. To the extent that the experiments can be compared, our results seem to be in good agreement with those of Saunders et al. (1979).

EXPERIMENTAL SECTION

Caution. Many nitrosamines are potent carcinogens and must be handled and disposed of accordingly.

Analytical Procedures. Gas chromatography was performed on a Hewlett-Packard Model 5310 instrument equipped with a flame ionization detector. Thin-layer chromatography (TLC) was performed with 0.25-mm precoated silica gel plates, F-254, E. Merck, Darmstadt. The most common TLC solvents were CH_2Cl_2 (for **1–3**) and toluene (for **4**). No-screen medical X-ray film (NS-54 T, Kodak) was used to autoradiograph TLC plates.

Chemicals. NDMA-^{14}C (**3a**), NDEA-*1*-^{14}C (**2a**), and sodium propionate-*1*-^{14}C, -*2*-^{14}C, and -*3*-^{14}C (**5a**, **5b**, **5c**) were purchased from New England Nuclear. Pendimethalin-*1-ethyl*-^{14}C (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine-*1-ethyl*-^{14}C) was supplied by American Cyanamid.

***N*-Nitrosodipropylamine-^{14}C (1a, 1b, 1c).** Sodium propionate-*1*-^{14}C (**5a**, 50 μCi, 2.5 mg) was suspended in dichloromethane (200 μL). The mixture was stirred and cooled, then thionyl chloride (55 μL) was added. After warming gently 0.5 h, the mixture was cooled and a solution of *n*-propylamine (150 μL) in dichloromethane (500 μL) was added dropwise. After 15 min the mixture was partitioned between dichloromethane and 2 N KOH; the organic phase was washed successively with 1 N HCl, 2% K_2CO_3, and saturated NaCl. The solution was dried ($MgSO_4$) and concentrated just to dryness. Dry tetrahydrofuran (1 mL) was added and also evaporated just to dryness, then a borane–tetrahydrofuran solution (0.5 mL, 1 M) was added to the residue and the resulting mixture was warmed under N_2 for 6 h. After cooling, concentrated HCl (5 drops) was added, then the mixture was evaporated

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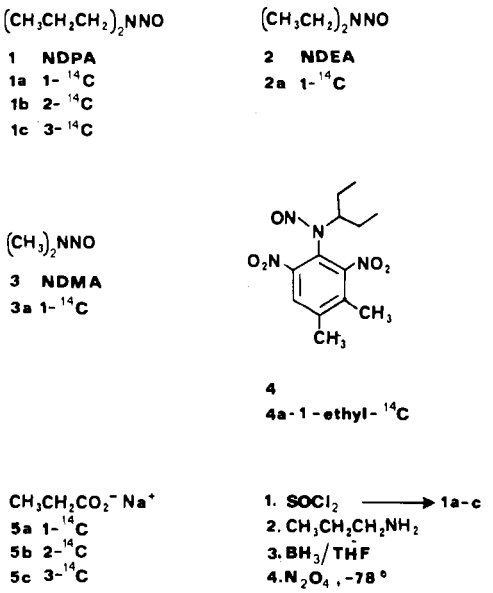

Figure 1. Nitrosamines studied and synthesis of ^{14}C-labeled NDPA.

to dryness. Anhydrous sodium acetate (0.4 g) and dichloromethane (0.5 mL) were added, the mixture was cooled to −78 °C and excess dinitrogen tetroxide (0.7 mL of a 1 M solution in dichloromethane) was added. The mixture was allowed to warm to room temperature, then was partitioned between dichloromethane and water. The organic phase was washed successively with 1 N HCl, 5% K_2CO_3, and saturated NaCl, then was dried ($MgSO_4$), filtered, and made up to 25.0 mL with dichloromethane. Only a single spot (R_f 0.24, CH_2Cl_2) was observed after TLC and autoradiography. Gas chromatography (1.8 m × 3 mm 5% OV-17 on Chromosorb W, 100 °C isothermal 4 min, then programmed 8 °C/min to 190 °C) indicated that the material consisted of approximately 97% **1a** (7.25 min) and 3% of the corresponding *N*-nitramine (White and Feldman, 1957). *N*-Nitrodipropylamine had retention time 9.75 min; an authentic sample, bp 118–121 °C, 20 mm (literature bp 105–106 °C, 10 mm, Robson, 1955), was prepared by trifluoroperacetic acid oxidation of **1** (Emmons, 1954). The overall yield of **1a** was 31 μCi (62%). Identical procedures were followed with sodium propionate-2-^{14}C and -3-^{14}C (**5b** and **5c**) to provide **1b** and **1c**, respectively. Yields and purities were essentially the same as those of **1a**.

N-Nitrosopendimethalin-^{14}C **4a**, *N*-(1-ethylpropyl)-*N*-nitroso-3,4-dimethyl-2,6-dinitrobenzenamine-*1-ethyl*-^{14}C). A solution of pendimethalin-*1-ethyl*-^{14}C (28.8 μCi, 2.03 mg) in dichloromethane (3 mL) was treated with anhydrous sodium acetate (0.5 g); the mixture was stirred and cooled to −78 °C, then treated with 0.5 mL of a 1.5 M solution of dinitrogen tetroxide in dichloromethane. The mixture was allowed to warm to −20 °C, then a mixture of aqueous $NaHCO_3$ and ice was added. After the ice melted, the organic phase was separated, washed with water, dried ($MgSO_4$), filtered, and made up to 25.0 mL with dichloromethane. The yield was 24.7 μCi (86%) of *N*-nitrosopendimethalin that was pure according to TLC–autoradiography (R_f, toulene 0.25; R_f, dichloromethane 0.45). A sample of unlabeled *N*-nitrosopendimethalin (**4**) was similarly prepared on a somewhat larger scale and was recrystallized from acetic acid–water, mp 67.5–69 °C. Mass spectrum, electron impact, 70 eV: *m/e* 310, $M^{+}\cdot$, 4.8%; 280, $M^{+}\cdot$ − NO, 100%. Mass spectrum, chemical ionization (isobutane): *m/e* 311, M + H^+, 96%; 282, M + H^+ +− NO^+ + H^+, 100%.

Table I. Properties of Soils

soil[a]	% sand	% silt	% clay	% org. matter	pH
A	40	38	22	1	7.6
B	9.5	60.2	30.3	4.1	7.1
C	38.4	49.4	12.2	1.5	5.5
D	3.6	28.8	67.6	2.9	6.7

[a] A, Avondale loam (Torrifluventic Haplustoll); B, Drummer silty clay (Typic Haplaquoll); C, Matapeake loam (Typic Hapludult); D, Sharkey clay (Vertic Haplaquepts).

Soils. Selected properties of the soils used in these experiments are presented in Table I.

Soil metabolism studies were conducted in duplicate with 250-mL biometer flasks, and averaged results are presented here. Total $^{14}CO_2$ production rarely varied more than 5% in duplicate runs, and the results from a number of related experiments over a considerable period of time were similarly consistent. Soils (50 g of dry weight/flask) were initially adjusted to 70% of the 0.33 bar tension moisture content, and $^{14}CO_2$ was collected in 10-mL portions of 0.1 N NaOH. In all but the two earliest experiments, the sampling procedure for compounds **1–3** was as follows: at 2–3-day intervals the NaOH trapping solution was withdrawn and replaced. Duplicate 1.0-mL aliquots were transferred to scintillation vials for counting, the remaining 8 mL was extracted 2 × 2 mL with dichloromethane, and duplicate 1.0-mL portions of the extracted NaOH solution were withdrawn for scintillation counting. Spot checks (counting solutions before and after precipitation of $^{14}CO_3^{2-}$ from the NaOH solutions as $Ba^{14}CO_3$, and TLC and autoradiography of carefully concentrated dichloromethane extracts) indicated that within experimental error, all of the radioactivity was accounted for by CO_2 or unchanged nitrosamine that had volatilized from the soil and had been trapped in the NaOH solution.

Nitrosamines **1a–3a** were applied to soils as aqueous solutions; **4b** was administered by adding a dichloromethane solution to about 10 g of the soil, allowing the solvent to evaporate, then mixing the treated soil with the remaining soil in the biometer flask. Rates of nitrosamine applications to soils varied from experiment to experiment. Treatments of NDPA-^{14}C ranged from as low as 5 ppb to a high of 10 ppm. NDMA-^{14}C (**3a**) was studied at 10 ppm; **2b** was applied at about 8 ppb, and **4a** was applied at about 23 ppb.

Steam-sterilized soils were prepared by autoclaving the biometer flasks containing the soils for 90 min (120 °C, 20 psi), allowing them to stand 3 days, and repeating the process for 45 min. Because of the volatility of **1–3**, and possible thermal instability of **4**, nitrosamines were added after sterilization. Ethylene oxide sterilizations were achieved by placing soil-containing biometer flasks in a vacuum desiccator, evacuating with an aspirator, then admitting ethylene oxide until atmospheric pressure was attained. The desiccator was closed, stored over a weekend, then reevacuated and refilled with ethylene oxide. After standing overnight, the flasks were removed, soils were treated, trapping solutions were added, and the experiments were conducted as usual.

RESULTS AND DISCUSSION

Figure 1 illustrates the nitrosamines studied and the synthesis of the three specifically ^{14}C-labeled *N*-nitrosodipropylamines **1a–1c**. Dipropylamine-*1*-^{14}C has been prepared in unspecified yield by alkylation of 1-aminopropane-^{14}C with 1-iodopropane (Israili et al., 1972), and **1a** and **1b** have previously been synthesized by a route

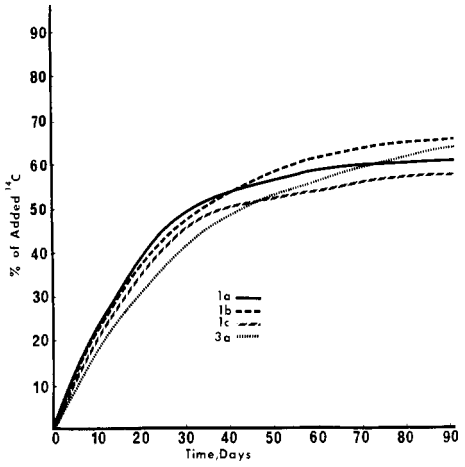

Figure 2. Dissipation of ^{14}C from Matapeake loam treated with labeled NDPA and NDMA (23 °C).

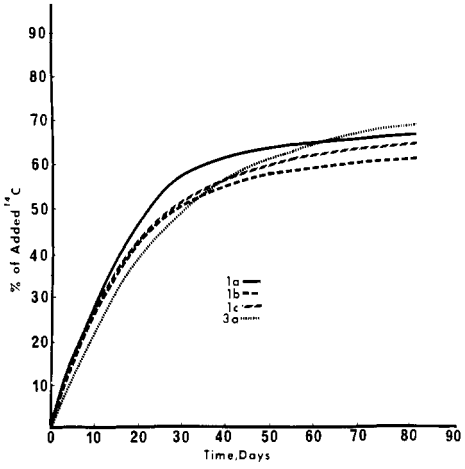

Figure 3. Dissipation of ^{14}C from Matapeake loam treated with labeled NDPA and NDMA (33 °C).

similar to ours except on a considerably larger scale (Kruger, 1971). Worthy of note is the nearly negligible yield of *N*-nitrodipropylamine from the low-temperature reaction of dipropylamine-^{14}C with dinitrogen tetroxide (N_2O_4). White and Feldman (1957) reported that conditions similar to these gave almost exclusively *N*-nitrodiethylamine from diethylamine. The fact that our reaction was run on the HCl salt of the amine, and that boron salts from the previous step were still present, may have been a factor. Indeed, we found that the reaction of pure dipropylamine with N_2O_4 in dichloromethane at −78 °C, or with N_2O_4 in the presence of various additives or other solvents, gave varying mixtures of *N*-nitrosodipropylamine and *N*-nitrodipropylamine in which the nitramine content ranged from zero to about 85%.

Our initial soil experiments consisted of incubating **1a**, **1b**, **1c**, and **3a** in Matapeake loam at 23 and 33 °C (Figures 2 and 3). These experiments were conducted before we became aware of the extent to which volatilization initially competed with nitrosamine degradation; thus what was measured, and is displayed on the vertical axes of Figures 2 and 3, were total ^{14}C from $^{14}CO_2$ and volatilized nitrosamine. We subsequently learned that volatilization of nitrosamines from nonsterile soil was significant for a few days after treatment, but was generally negligible thereafter (vida infra; Oliver, unpublished data). At both temperatures, ^{14}C evolution proceeded, within experimental error, at the same rate for all the samples. This indicates (1) that the nitrosamines were degraded in soils, (2) that degradation rates of NDPA and NDMA were not

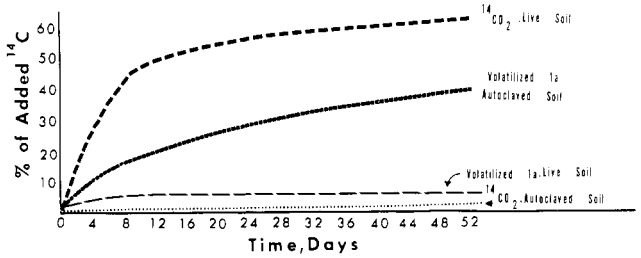

Figure 4. Degradation and volatilization of NDPA in live (nonsterile) and autoclaved Matapeake loam (room temperature).

significantly different (a subsequent experiment included NDEA (**2a**) whose rate of decomposition was also within experimental error of those of **1a**–**1c**), and, (3) that ^{14}C evolution does not depend on the location of the label in the NDPA. This last result is important with respect to the possible formation of degradation products that might retain the N–NO functionality. The experiments suggested that whatever the mechanism of soil degradation, there are no chain-shortening reactions that form stable products.

Tate and Alexander (1975, 1976) reported that they were unable to isolate microorganisms capable of metabolizing nitrosamines from soils or from sewage. We therefore conducted some experiments in which we attempted to determine whether the degradation we observed was in fact microbiological or simply chemical. Attempted sterilization of soil samples with sodium azide, chloroform, or mixtures of streptomycin and cycloheximide gave variable results. Incubation of **1a** in nonsterile and autoclaved soils produced the results illustrated in Figure 4. Portions of the aqueous sodium hydroxide trapping solutions were counted for ^{14}C as usual, and the NaOH solutions remaining after counting were then extracted with dichloromethane and recounted. We found that almost all of the ^{14}C from the sterile soils was extractable into the dichloromethane and was in fact (by TLC and autoradiography) unchanged **1a** that had volatilized from the soil. Initially, some NDPA was also trapped from the nonsterile soils, but after about the fourth day, all further ^{14}C trapped from this soil was $^{14}CO_2$. In contrast, unchanged NDPA continued to be trapped from the sterile soil throughout the experiment. A study of the volatilization of nitrosamines from soils will be published elsewhere (Oliver, 1979).

Unfortunately, autoclaving a soil alters its chemical and physical properties as well as its microbiological properties. Kaufman et al. (1968) demonstrated that amitrole was degraded in chemically sterilized soil but not in autoclaved soil; they concluded that the degradation was the result of an oxidative, free-radical chemical reaction and that autoclaving had drastically reduced the chemical reactivity of the soil. Our $^{14}CO_2$ production curves resemble those from Kaufman's study in the sense that induction periods were not observed prior to maximum activity. We therefore conducted an experiment with **2a** in which some of the soils were sterilized with ethylene oxide. The results, displayed in Figure 5, resemble those of the previous experiment; $^{14}CO_2$ was produced rapidly in the nonsterile soils, but only slowly in the sterilized soils. Volatilization of unreacted nitrosamine initially occurred from all soils, but, as was the case in the previous experiment, the process was significant only a few days in the nonsterile soil, whereas it continued much longer in the sterilized soil. That more NDEA than NDPA volatilized was observed is not surprising; the former is a more volatile compound, and also, the temperature was somewhat higher in the NDEA experiment. The significance of the limited amount

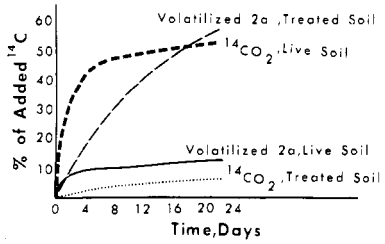

Figure 5. Degradation and volatilization of NDEA in live (nonsterile) and ethylene oxide treated Matapeake loam (30 °C).

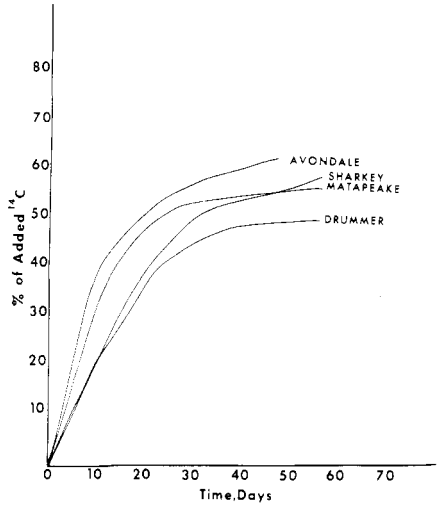

Figure 6. Dissipation of ^{14}C from four soils treated with NDPA (33 °C).

of $^{14}CO_2$ formed in the ethylene oxide treated soils is not known at this time; incomplete sterilization is a possibility, but we also cannot rule out the possibility that there is a nonmicrobiological reaction that is partly, but not entirely, responsible for the $^{14}CO_2$ production.

The results of another early experiment are shown in Figure 6. The experiment was intended to compare the rates of $^{14}CO_2$ production from **1a** in four soils but was also conducted before we became aware of the extent to which volatilization initially competed with degradation. Furthermore, the soils were collected from various parts of the country and probably were not handled or stored uniformly, so we could not conclude that any one of the soils was inherently more active in degrading nitrosamines than the others. For example, the Avondale loam, from which the most total ^{14}C was trapped, was subsequently found to be the soil from which NDPA volatilized most rapidly (Oliver, 1979). We mention this experiment only to indicate that soil degradation of NDPA seems to be a general process and not one limited to a particular soil.

At the end of the experiment in which the control soils had been autoclaved (3 months), one of the duplicate samples of each soil was steam distilled, and the other was extracted with methanol. The two methods gave comparable results; 1.4–3% of the added radioactivity was recovered from the nonsterile soils, and 6.5–7.4% was recovered from the autoclaved soils. The extracts were carefully concentrated and analyzed by TLC and autoradiography. In each case a single spot with R_f equal to that of NDPA was observed. Thus at least two processes, volatilization and microbiological degradation, appear to compete for the nitrosamines in the soil. Note that the volatilization process appears to terminate before the soil has been depleted of nitrosamines; perhaps a reversible (because nitrosamine can be recovered by extraction or steam distillation) binding occurs that inhibits movement

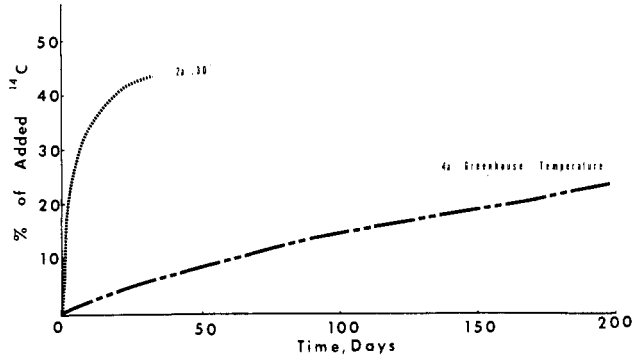

Figure 7. $^{14}CO_2$ production from two nitrosamines in Matapeake loam.

of the compounds to the soil surface. We have not examined leaching, but that process has been addressed by Saunders et al. (1979). These experiments collectively indicate that the actual lifetimes of nitrosamines in soil depend on soil type, temperature and probably other variables. In most cases the first half-life appears to have elapsed within 3 weeks.

In another experiment, soybeans were grown to maturity in Matapeake loam treated with 100 ppb **1b** or **4a** in 6-in. pots maintained in an outdoor soil bed. After 4 months, a portion of the **1b**-treated soil was extracted with methanol, the extract was concentrated and then partitioned between dichloromethane and water. The dichloromethane solution, after washing with aqueous HCl and with aqueous NaOH, contained 1.6% of the radioactivity initially incorporated in the soil. TLC and autoradiography revealed a single compound whose R_f corresponded to that of NDPA; however, the intensity of the spot was low, and we have not absolutely confirmed the presence of NDPA or the absence of other materials. However, several degradation products of NDPA that might be anticipated, e.g., carboxylic acids and amines, are eliminated because they would have been removed by the HCl and NaOH washes. If the recovered material was, in fact, NDPA, the result is in good agreement with our earlier results; if NDPA decomposition in soil follows first-order kinetics, and its half-life is 20 days, 6 half-lives would have elapsed in 4 months, and 1.5% of the original material should have remained.

The most common types of nitrosamines thus far found in herbicides have been the low-molecular-weight nitrosodialkylamines just discussed. Our major interest has been in those nitrosamines associated with the dinitroaniline herbicides. Most of the latter are dialkylamine derivatives, and the nitrosamine impurities, if any, have corresponded to the dialkylamino group of the dinitroaniline. At least two of the dinitroaniline herbicides, however, are secondary amines; thus the herbicides themselves are capable of forming *N*-nitroso derivatives. Nitrosobutralin and its stability in soil have already been mentioned, and we have also accumulated data that show that nitrosopendimethalin 4, a contaminant of pendimethalin (Bontoyan et al., 1979) is also quite stable in soil.

A sample of **4a** was incubated in Matapeake loam under the same general conditions as were the nitrosamines already discussed. Figure 7 illustrates the rate of $^{14}CO_2$ production from **4a** and includes a similar reaction of **2a** for comparison. The incubations of **4a** were followed 200 days, and less than 25% of the added ^{14}C was evolved at $^{14}CO_2$. Samples were extracted periodically, and after 49 days, 73% of the added radioactivity could be extracted from the soil with organic solvents. As judged by TLC and autoradiography, almost all of the ^{14}C was still present as

4a. Even after 200 days, ca. 50% of the added radioactivity could be extracted, and unchanged 4a still accounted for most of the ^{14}C. We observed little or no pendimethalin as a degradation product. This constrasted with our earlier observations of nitrosoatrazine where denitrosation to atrazine was always a major degradation pathway (Kearney et al., 1977).

A portion of the 4a-treated (100 ppb) soil in which soybeans were grown was also extracted after 4 months, and 58% of the ^{14}C was recovered. As was the case in the biometer flask experiments, unchanged 4a seemed to account for the majority of the ^{14}C as judged by TLC and autoradiography.

In addition to the experiments just described, a considerable number of additional $^{14}CO_2$ evolution experiments haave been conducted with NDPA-^{14}C, and the results, except when influenced slightly by temperature, soil type, etc., have been essentially identical with those described. Some observations from some of the additional experiments are: exposure of biometer flasks to light did not influence the rate of $^{14}CO_2$ production from either 1a or 4a [most incubations were performed in the dark because of the known photolability of nitrosamines (Chow, 1973)]. Addition of 1 ppm trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) to the soil had no effect on the rate of $^{14}CO_2$ production from NDPA-^{14}C. The rate of $^{14}CO_2$ production did not vary with NDPA-^{14}C concentration over the range of 5 ppb to 10 ppm.

In summary, nitrosamines are degraded to CO_2 in nonsterile, but not in sterile soils. The low-molecular-weight nitrosodialkylamines seems to have half-lives of about 3 weeks, and under these experimental conditions, significant losses of nitrosamines can result from volatilization during the first few days. The *N*-nitroso derivative of pendimethalin is more stable in soil and appears to be able to persist at least several months.

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Total Carbonyls and Phenols in Experimental Burley and Bright Tobacco

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Soluble carbonyls and phenols were determined in several experimental tobacco materials that were produced by practices that altered concentrations of these health- and quality-related components. An improved spectrophotometric method was used for measuring total carbonyls as quinoidal anions of their 2,4-dinitrophenylhydrazones. Phenolic estimations were based on the extent of hydrogen bonding of phenols to insoluble polyvinylpyrrolidone. Carbonyl and phenolic levels decreased in bright tobacco that was grown at higher-than-normal plant population density or reconstituted into sheets after flue curing. Carbonyl concentrations reduced in air-cured burley leaves that were harvested from successively higher leaf positions on the stalk, whereas phenolics increased with ascending stalk position. Phenolics were reduced in tobacco that was treated for removal of soluble protein.

Cured tobacco contains many compounds with carbonyl and phenolic functional groups that contribute to the organoleptic and biological properties of the leaf and smoke. Phenolic compounds are considered important to leaf quality and usability (Tso, 1969). Volatile and semivolatile carbonyl-containing compounds influence the flavor and aroma of tobacco smoke (Weybrew and Stevens, 1962; Demole and Berthet, 1972; Kimland et al., 1972; Demole and Demole, 1975; Demole and Enggist, 1975; Davis et al., 1976; Dickerson et al., 1976; Lloyd et al., 1976). Health-related effects in the respiratory systems of mammals have also been attributed to specific carbonyl compounds in smoke (Kensler and Battista, 1963; Fenner and Braven, 1968; Schoental and Gibbard, 1972; Sabine et al., 1973; Sprince et al., 1975).

Recent statistical evidence based on correlations of the chemical composition of experimental cigarettes and the biological activity of their derived smoke suggested that high levels of soluble phenols in leaf are undesirable [DHEW Publication No. (NIH) 76-1111, 1976; DHEW

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