test was conducted in an open outdoor field.

This study has shown that fosamine ammonium is rapidly degraded in pasture species. Twelve months after treatment at 13.4 kg of a.i./ha, no (<0.05 ppm) fosamine ammonium or its main metabolite (CPA) could be found in either grass or clover. The small amount of residues which did remain (~0.42 ppm) appeared to be bound carboxyphosphonic acid moieties.

ACKNOWLEDGMENT

I thank Robert W. Reiser for analytical assistance, especially the GC/MS studies, Arthur G. Jelinek for synthesis of [$^{14}$C]fosamine ammonium, and Colin L. McIntosh for synthesis of the nonradioactive compounds fosamine ammonium, carbamoylphosphonic acid (diammonium salt), and carboxyphosphonic acid (trisodium salt).

**Registry No.** CPA, 6874-57-3; CPA·3Na, 63585-09-1; CPA·3NH$_3$, 83665-50-3; fosamine ammonium, 25954-13-6; carboxyphosphonic acid, 4428-95-9.

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# Diphenyl Ether Herbicides: Mutagenic Metabolites and Photoproducts of Nitrofen

William M. Draper* and John E. Casida

Nitrofen [2,4-dichloro-1-(4-nitrophenoxy)benzene] (1-$NO_2$; 1 = $Cl_2PhOPh$–) in thin films undergoes rapid photochemical reduction in near-UV light by the pathway 1-$NO_2$ → 1-NO → [1-NHOH] → 1-$NH_2$ and 1-N=N(O)-1. Rat liver enzyme preparations with NADPH reduce 1-$NO_2$ or 1-NO to 1-$NH_2$ under anaerobic conditions, confirming earlier in vivo studies of 1-$NO_2$ with rats and suggesting 1-NO and 1-NHOH as intermediary metabolites. This enzyme system in air oxidizes 1-$NH_2$ to 1-NO. Nitrofen's photoproducts and metabolites (1-NO, 1-NHOH, and 1-$NH_2$) are mutagens in the Ames *Salmonella typhimurium* (strain TA 100) assay with microsomal activation, i.e., 11–13 revertants/nmol in each case. Nitrofen is known to be a herbicide, teratogen, and carcinogen. The current study also identifies 1-$NO_2$ as a promutagen activated on nitro reduction. The 1-NO and 1-NHOH intermediary metabolites or their activated derivatives may be involved in the mutagenesis and perhaps in other aspects of the biological activity of nitrofen.

Diphenyl ethers (nitrofen or 1-$NO_2$ and related compounds) are one of the most important classes of herbicides, requiring light to disrupt membrane permeability, elicit biochemical changes, and kill plant cells (Matsunaka, 1969; Suzuki et al., 1981; Orr and Hess, 1981). The role of light in the herbicidal effect is not clearly defined (Matsunaka, 1969; Orr and Hess, 1981). 1-$NO_2$ is also a mutagen (Jeang and Li, 1980), carcinogen (Milman et al., 1978), and teratogen (Gray et al., 1982), properties not observed to date with other diphenyl ether herbicides. Definition of the mechanism of mutagenesis might contribute to an understanding of the carcinogenic action (Ames et al., 1975) and possibly other types of biological activity. As a first step in this direction the present study evaluates the possible activation of 1-$NO_2$ using photochemical and metabolic systems and the Ames *Salmonella typhimurium* mutagenesis assay. The compounds examined are indicated in Figure 1.

## MATERIALS AND METHODS

**Chromatography.** Thin-layer chromatography (TLC) utilized 0.25-mm silica gel 60 F-254 plates (E. Merck), 0.25-mm aluminum oxide F-254 Type T plates (E. Merck), or 0.25-mm RPSF fluorescent reverse phase plates (Analtech, Inc.) developed in mobile phases as specified in chromatography tanks without liners; 0.5-mm layers were used for preparative isolations. Sorbed compounds were eluted with diethyl ether (apolar bands) or methanol–diethyl ether (polar bands). Photoproducts were determined by gas–liquid chromatography (GLC) with a Varian 1400 instrument fitted with a hydrogen flame ionization detector (FID) and a glass column (1 m × 2 mm i.d.) packed with Chromosorb W (60–80 mesh) coated with 5% SE-30: nitrogen carrier gas flow rate, 35 mL/min; hydrogen gas flow rate, 20 mL/min; air flow rate, 200 mL/min; column temperature, 180 °C; inlet temperature, 230 °C; detector temperature, 300 °C. Metabolites were analyzed with a Hewlett-Packard 5830A instrument fitted with a nickel-63 electron capture (EC) detector and a glass column (1.5 m × 4 mm i.d.) packed with GHP (80–100 mesh) coated with 5% OV-101; the carrier gas was 5% methane–95% argon with a flow rate of 28 mL/min; the operating temperatures were inlet 230 °C, column 230 °C, and detector 250 °C.

**Spectroscopy.** Chemical ionization mass spectra (CI-MS) were recorded with a Finnigan 3200 spectrometer interfaced with a System Industries 150 data system. Methane (0.8 torr) was used as the reagent gas and the ionization voltage was 70 eV. Samples were introduced with a solid probe or by GLC. Nuclear magnetic resonance ($^1$H NMR) spectra were recorded on a 250-MHz Fourier transform instrument; compounds were dissolved in $CDCl_3$ containing 0.2% $Me_4Si$ as the internal reference.

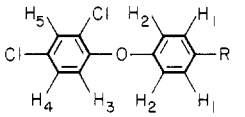
**Chemicals.** Table I gives the compounds considered and properties useful in their characterization as described below.

Pure 1-$NO_2$ (>99%) was obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC) or Chem Service (West Chester, PA) and technical

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

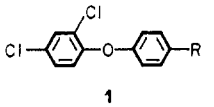
0021-8561/83/1431-0227$01.50/0 

Table I. Physical, Spectroscopic, Chromogenic, and Chromatographic Properties of Nitrofen and Its Derivatives



| property | 1-$NO_2$ | 1-NO | 1-NHOH | 1-$NH_2$ | 1-N=N(O)-1 | |
|---|---|---|---|---|---|---|
| mp, °C | 68-70 | | | 54-55 | 118-120 | |
| $^1$H NMR ($CDCl_3$ or acetone-$d_6$)[a] chemical shift ($\delta$) and coupling constants ($J$, Hz) | | | | | | |
| $H_1$ | 8.22 (d), $J$ = 9.3 | 7.92 (d), $J$ = 8.8 | 6.93 (d),[b] $J$ = 9.1 | 6.68 (d), $J$ = 8.8 | 8.36 (d),[c] $J$ = 9.2 | 8.32 (d),[d] $J$ = 9.2 |
| $H_2$ | 6.96 (d), $J$ = 9.2 | 7.03 (d), $J$ = 9.1 | 7.01 (d),[b] $J$ = 9.1 | 6.85 (d), $J$ = 8.8 | 7.17 (d),[c] $J$ = 9.2 | 7.12 (d),[d] $J$ = 9.2 |
| $H_3$ | 7.11 (d), $J$ = 8.7 | 7.13 (d), $J$ = 8.7 | 6.81 (d), $J$ = 8.8 | 6.75 (d), $J$ = 8.8 | 7.34 (d),[e] $J$ = 8.8 | 7.29 (d),[e] $J$ = 8.8 |
| $H_4$ | 7.33 (dd), $J$ = 8.7, 2.4 | 7.34 (dd), $J$ = 8.7, 2.4 | 7.14 (dd), $J$ = 8.8, 2.5 | 7.10 (dd), $J$ = 8.8, 2.5 | 7.51 (dd),[e] $J$ = 8.7, 2.6 | 7.48 (dd),[e] $J$ = 8.7, 2.6 |
| $H_5$ | 7.54 (d), $J$ = 2.4 | 7.54 (d), $J$ = 2.4 | 7.44 (d), $J$ = 2.5 | 7.42 (d), $J$ = 2.5 | 7.71 (d),[e] $J$ = 2.5 | 7.69 (d),[e] $J$ = 2.5 |
| NH | | | | 3.65 (s) | | |
| CI-MS base peak, $m/e$ | 284 (M + 1) | 268 (M + 1) | 268 + 254[f] | 254 (M + 1) | 519 (M + 1) | |
| color compound | none | blue-green | lt yellow | none | lt amber | |
| PCAF spray (TLC)[g] | none | violet | violet | gray[h] | none | |
| TLC, $R_f$ | | | | | | |
| silica gel[i] | 0.81 | 0.83 | 0.34 | 0.31 | 0.87 | |
| aluminum oxide[j] | 0.41 | 0.53 | | 0.06 | 0.43 | |
| reverse phase[k] | 0.30 | 0.22[l] | | 0.47 | 0.05 | |
| GLC, $t_R$[m] | 385, 371 | 197, 214 | | 274, 279 | | |

[a] $CDCl_3$ except for 1-N=N(O)-1 in acetone-$d_6$. [b] Arbitrary assignments for $H_1$ and $H_2$ signals. [c] =N(O)—aryl protons. [d] =N—aryl protons. [e] Arbitrary assignments for dichlorophenoxy substitutents. [f] Composite of 1-NO and 1-$NH_2$. 1-$NHOSi(CH_3)_3$ analyzed by GLC-CI-MS gave $m/e$ (rel intensity) 342 (M + 1, 14), 341 ($M^+$, 14), 326 (M − 15, 9), and 306 (M − 35, 5). [g] Pentacyanoammineferroate spray: 0.5% in methanol-water (3:1 v/v). [h] Gray on silica gel; blue-green on aluminum oxide; purple on reverse-phase plates; emerald green in solution. [i] Carbon tetrachloride-diethyl ether (3:1 v/v). [j] Hexane-diethyl ether (9:1 v/v). [k] Methanol-water (3:1 v/v). [l] Tailing resulted in poor resolution of 1-$NO_2$ and 1-NO. [m] Values for the 5% SE-30 column operated at 180 °C followed by those for the 5% OV-101 column at 230 °C; this is the standard elution sequence for a series of nitro, nitroso, and amino compounds analyzed by GLC (Gorrod, 1968).



1

1—$NO_2$ nitrofen (R = $NO_2$)
1—NO, 1—NHOH, 1—$NH_2$ and 1—N=N(O)—1 designate nitroso, hydroxylamino, amino and azoxy derivatives of nitrofen

**Figure** 1. Nitrofen and derivatives examined.

nitrofen (TOK, 95.7%) was from Rohm and Haas Co. (Spring House, PA).

Reduction of 1-$NO_2$ to 1-$NH_2$ with iron under acidic conditions proceeds via 1-NO and 1-NHOH, but these intermediates are obtained in higher yield by peracid oxidation of 1-$NH_2$ to 1-NO and mild reduction of 1-$NO_2$ to 1-NHOH. Product isolation by preparative TLC utilized solvent systems given in Table I.

1-$NH_2$, previously synthesized by Nakagawa and Crosby (1974a), was prepared by refluxing a mixture of 1-$NO_2$ (460 mg), powdered iron (270 mg), and concentrated HCl (0.3 mL) in ethanol (15 mL) for 2 h; additional iron (ca. 200 mg) was added during the reaction (Haworth and Barker, 1939). The mixture was filtered, combined with water (40 mL), and neutralized. 1-$NH_2$ was recovered on extraction with diethyl ether (3 × 40 mL), drying ($MgSO_4$), and silica gel TLC (35% yield). 1-$NH_2$ sorbed on silica gel autoxidized slowly, yielding brown products.

1-NO was obtained by treatment of 1-$NH_2$ (50 mg) with 2 equiv of *m*-chloroperoxybenzoic acid MCPBA, 69 mg) in methylene chloride (10 mL), immediately yielding a brilliant green color, and holding for 30 h at 0 °C (Okazaki et al., 1969). The methylene chloride was extracted with 5% aqueous $Na_2CO_3$ (2 × 15 mL) and dried ($Na_2SO_4$), and 1-NO was recovered by aluminum oxide TLC (20% yield). 1-NO was also isolated in lower yield from the iron/HCl reduction mixture of 1-$NO_2$ by chromatography on silica gel followed by aluminum oxide. The blue-green color of 1-NO ($\lambda_{max}$ = 758 nm, $\epsilon$ = 24.9 L $mol^{-1}$ $cm^{-1}$) is a typical n → $\pi^*$ transition of monomeric aryl nitroso compounds with characteristic intensity and wavelength (Boyer, 1969).

So that 1-NHOH could be obtained, 1-$NO_2$ (0.5 g) was added to ethanol (25 mL) containing demineralized water (0.3 mL), $NH_4Cl$ (0.3 g), and zinc powder (0.5 g). After being stirred 1 h under argon at 25 °C, the mixture was filtered and the product isolated by silica gel TLC. 1-NHOH was recovered (30% yield) by elution with ether and concentration under dry nitrogen; it was stored under argon at 0 °C. For maximization of yields product isolation required reduced temperature (5 °C, including chromatography). 1-NHOH was degraded upon addition of water to the reaction mixture (Gilman and Kirby, 1926) or exposure to air while sorbed on silica gel. Chromatography of 1-NHOH on aluminum oxide was not possible due to instability and in aqueous base a red-brown polymer formed quickly. Attempted reduction of 1-$NO_2$ with ammonium sulfide (Lotlikar et al., 1965) resulted in cleavage of the ether linkage. The trimethylsilyl ether [1-$NHOSi(CH_3)_3$] was formed by reaction of 1-NHOH with bis(trimethylsilyl)acetamide in dry acetonitrile (Beckett and Achari, 1977).

1-N=N(O)-1 was obtained as amber, glassy cyrstals in near quantitative yield on holding the filtrate from the 1-NHOH reaction mixture under argon at 5 °C for 2 weeks.

The pentacyanoammineferroate (PCAF) spray reagent (Feigl, 1975) gave characteristic colors and high sensitivity for 1-NO, 1-NHOH and 1-$NH_2$ but not for 1-$NO_2$ or 1-N=N(O)-1 (Table I). The Liebermann spot test (red solution with phenol and $H_2SO_4$, changing to blue-green with base) is considerably more specific for nitroso com-

Table II. Metabolism of Nitrofen and Its Reduced Derivatives on Incubation with Rat Liver S9 Fraction in Air and Argon in the Presence and Absence of NADPH

| | | substrate recovery, %[a] | | metabolite yield, % | | |
|---|---|---|---|---|---|---|
| substrate | atmosphere | -NADPH | +NADPH | compd | -NADPH | +NADPH |
| 1-$NO_2$ | argon | 74 | 28 | 1-$NH_2$ | <0.1 | 38 |
| 1-NO | argon | 2 | 0[b] | 1-$NH_2$ | <0.1 | 22 |
| 1-NO | air | 1 | 0 | | | |
| 1-$NH_2$ | air | 54 | 33 | 1-NO | <0.1 | 1.3 |

[a] Relative to buffer control. [b] Control with NADPH but no S9 gave 72% conversion of 1-NO, probably to 1-NHOH (Sternson, 1975).

pounds (Feigl, 1975). As a reducing agent, 1-NHOH was the only compound giving colored derivatives with ammoniacal $AgNO_3$ (Tollen's reagent, immediate black) and alkaline 2,3,5-triphenyl-2*H*-tetrazolium chloride (brilliant red). 1-NHOH also gave the only positive test with 4-(*p*-nitrobenzyl)pyridine (red-brown color on heating and then blue upon addition of base); this reagent detects alkylating agents (Hammock et al., 1974).

CI-MS revealed quasimolecular ions (M + 1) for 1-$NO_2$, 1-NO, 1-$NH_2$, 1-N=N(O)-1 and 1-NHOSi$(CH_3)_3$ (Table I). Solid probe, CI-MS of 1-NHOH indicated disproportionation as a spectrum appropriate for a composite of 1-$NH_2$ and 1-NO was observed in spite of a source temperature of <40 °C. $^1H$ NMR showed two separate ring sytems and five types of nonequivlent protons for each derivative (Table I). An AX system was apparent for protons of the N-substituted ring in 1-$NO_2$ and 1-NO; the three-proton dichlorophenyl ring exhibited *o*, *m*, and *o*/*m* coupling. In 1-N=N(O)-1 examined in acetone-$d_6$ the resonances were fully resolved for all protons in both rings (Table I).

**Photochemical Reactions.** Thin films of 1-$NO_2$ (120 $\mu g/cm^2$), 1-NO (34 $\mu g/cm^2$), and 1-$NH_2$ (90 $\mu g/cm^2$) in glass Petri dishes with borosilicate glass covers were irradiated in a Rayonet photoreactor (The Southern New England Ultraviolet Co., Middletown, CT) under RPR 3500 lamps (360-nm maximum energy output) or RPR 3000 lamps (300 nm). Photolysis of 1-$NO_2$ was also examined in acetonitrile and 2-propanol solutions (2 mg/mL) irradiated at 360 nm. The photolysates were analyzed by FID-GLC and TLC.

**Metabolism.** 1-$NO_2$, 1-NO, and 1-$NH_2$ (0.1 $\mu$mol each) were individually incubated with S9 preparation (~10 mg of protein, 0.25 mL) from Aroclor-1254-induced rats (Ames et al., 1975) in pH 7.4 0.05 M phosphate buffer (2.5 mL) for 1 h at 37 °C. Incubations were carried out with 0 or 10 $\mu$mol of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and in air or argon. Incubated mixtures were extracted with diethyl ether (3 volumes) and the organosoluble fraction was analyzed by EC-GLC. Controls consisting of unfortified microsomes and substrate in buffer were analyzed as well.

**Mutagenesis Assays.** The *S. typhimurium*/microsome assay (Ames et al., 1975) was used with the TA 100 tester strain (sensitive to "base-pair substitution" mutagens) or, in limited comparative studies, with the TA 98 strain (sensitive to "frame-shift" mutagens) to determine the mutagenicity of pure compounds and chemical mixtures. Mutagenic activity was assayed by incorporating the test substance in dimethyl sulfoxide ($Me_2SO$) in the top agar at levels up to 1.0 mg/plate; 1-$NO_2$ was also tested under preincubation conditions (Nagao et al., 1977). Microsomal activation studies used the normal S9 preparation from Aroclor-1254-pretreated rats (Ames et al., 1975). In tests for photochemical activation, the photolysate was dissolved in $Me_2SO$ and the solution was stored in a sterile glass vial at −5 °C until assay. The background

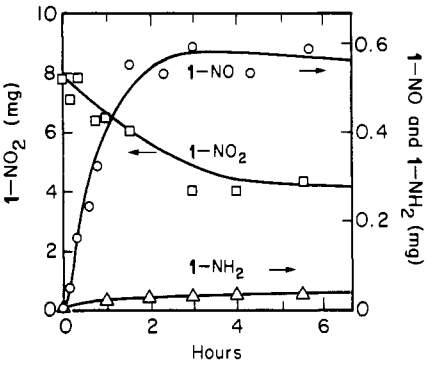



**Figure 2.** Photoreduction of nitrofen irradiated as a thin film at 360 nm.

reversion to prototrophy for histidine (120–150 revertants for TA 100 and 15–20 revertants for TA 98) was subtracted.

## RESULTS

**Photodecomposition and Metabolism of 1-$NO_2$ and Derivatives.** *Photodecomposition.* 1-$NO_2$ in thin films irradiated at 360 nm photodecomposed rapidly, forming 1-NO and 1-$NH_2$ (Figure 2) along with brown polymeric material and other photoproducts. 1-NO was detected by TLC, the PCAF spray, and FID-GLC after 15 min of irradiation and reached a yield of 20% of the reacted 1-$NO_2$ after 2 h (Figure 2). 1-$NH_2$ was a minor component in thin film irradiations (Figure 2). The composition of the photoproducts early in the photodecomposition of 1-$NO_2$ (thin film, 360 nm, <6 h) reflected their photostabilities (half-lives were 1.5 h for 1-$NO_2$, 5.5 h for 1-NO, and 1.0 h for 1-$NH_2$). Analyses of photoproduct mixtures established the photochemical conversions

$$\text{1-NO}_2 \rightarrow \text{1-NO} + \text{1-NH}_2 + \text{1-N=N(O)-1}$$

$$\text{1-NO} \rightarrow \text{1-NH}_2 + \text{1-NO}_2$$

$$\text{1-NH}_2 \rightarrow \text{1-NO}_2$$

Photodecomposition rates for each compound decreased with time, possibly due in part to light attenuation by the red-brown polymeric photoproducts which formed most rapidly in 1-$NH_2$ thin films.

1-$NO_2$ underwent no detectable (TLC) degradation after 52-h irradiation at 360 nm in acetonitrile, but in 2-propanol it rapidly yielded 1-$NH_2$ (CI-MS, M + 1 = 254) and other products and the parent compound was completely destroyed within 48 h.

*Metabolism.* The rat liver S9 fraction efficiently reduced 1-$NO_2$ and 1-NO to 1-$NH_2$ when incubated with NADPH under an argon atmosphere (Table II). These conversions were not detected in the absence of NADPH or under aerobic conditions. Oxidation of 1-$NH_2$ to 1-NO was evident under aerobic conditions with cofactor (+NADPH) (Table II). 1-NHOH could not be detected by using the GLC assay employed.

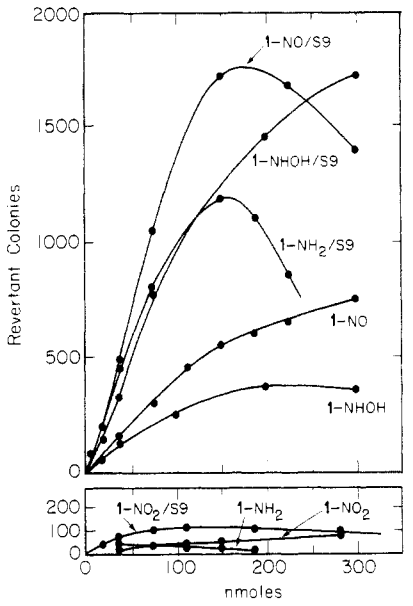



**Figure 3.** Metabolic activation of the mutagenic activity of nitrofen and some of its derivatives assayed with *S. typhimurium* strain TA 100 in the presence and absence of the liver S9 mix. Mutagenic activity values in revertants/nmol were as follows: 1-NO, 3.7 (–S9) and 12 (+S9); 1-NHOH, 2.7 (–S9) and 11 (+S9); 1-$NH_2$ 13 (+S9). 1-$NO_2$ (±S9) and 1-$NH_2$ (–S9) were not active as mutagens. 1-N=N(O)-1 (±S9) was not mutagenic at levels up to 4000 nmol/assay plate. A positive mutagenic test requires a net reversion rate more than twice that of the solvent control (Brusick, 1980). 1-$NH_2$ and 1-NHOH were bactericidal at 200 and 400 nmol/assay plate, respectively.

**Mutagenic Activity of 1-$NO_2$ and Derivatives.** *Direct Assay.* The *S. typhimurium* strain TA 100 assay detected 1-NO and 1-NHOH as mutagens but not 1-$NO_2$ and 1-$NH_2$ (Figure 3). 1-N=N(O)-1 was also inactive by direct assay.

*Metabolic Activation Assay.* The S9 mix converted 1-$NH_2$ to a mutagen and increased the mutagenic activity of 1-NO and 1-NHOH so that with activation all three compounds were of similar potency (Figure 3). 1-$NO_2$ and 1-N=N(O)-1 were not active even with the S9 mix. Preincubation of 1-$NO_2$ with the bacteria also failed to detect this compound as a mutagen, even with S9. Supplementation of the S9 assay system with acetyl coenzyme A and ATP (2.5 and 7 mM, respectively, in the standard top agar; Seiler, 1979) did not increase the mutagenic activity of 1-$NO_2$ 1-NO, 1-NHOH, and 1-$NH_2$. The mutagenic activity of 1-NHOH was considerably less with the TA 98 strain of *S. typhimurium*, i.e., 0.7 revertant/nmol (–S9) and 2 revertants/nmol (+S9).

*Photochemical Activation Assay.* 1-$NO_2$ undergoes photochemical activation and the photolysate can then be further activated with the S9 mix (Figure 4). The degree of photochemical activation of mutagenic activity (–S9) was dependent on the extent of photolysis, i.e., 4 revertants/μg after 2 h (360 nm) or 8 h (300 nm) with diminished activity after 16 h (360 nm). TLC fractionation enhanced the mutagenic activity (–S9) of the photolysate by removing a bactericidal component; maximum mutagenic activity (–S9) then appeared after 2.5–4 h and persisted for up to 24 h of irradiation (360 nm).

The principal mutagen (–S9) in the photolysates cochromatographed with 1-$NO_2$ on TLC with silica gel (carbon tetrachloride–diethyl ether, 3:1, or benzene) or with a reverse-phase system (aqueous methanol), but they separated on aluminum oxide (hexane–diethyl ether, 9:1). Desorption of the silica gel TLC band containing 1-$NO_2$

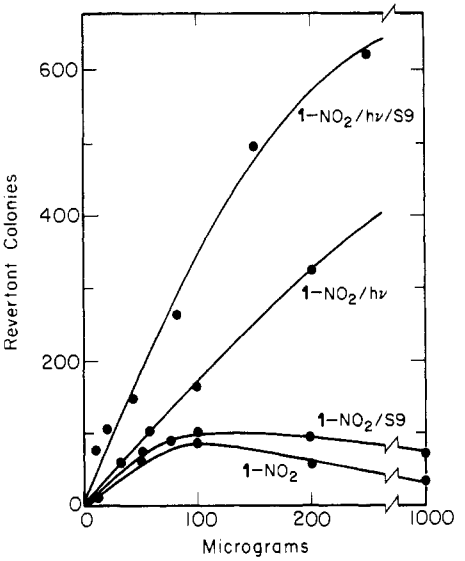



**Figure 4.** Photochemical activation of the mutagenic activity of nitrofen irradiated as thin films at 360 nm for 2 h and assayed with *S. typhimurium* strain TA 100 in the presence and absence of the liver S9 mix. These photolysates were bactericidal at 500 μg/assay plate or higher.

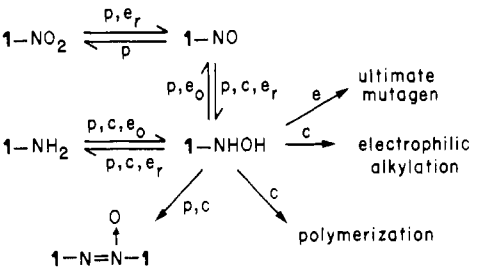



**Figure 5.** Photochemical, metabolic, and chemical interconversions of nitrofen and some of its metabolites and photoproducts. Abbreviations: p = photochemical; c = spontaneous chemical reaction (autoxidation, disproportionation); e = enzymatic; $e_r$ = enzymatic reduction; $e_o$ = enzymatic oxidation.

and the mutagenic photoproduct yielded a green oil whose mutagenic component was identified as 1-NO based on GLC, TLC and chromogenic reactions, and comparative bioassays.

The bactericidal component of the photolysate isolated by TLC (silica gel; carbon tetrachloride–diethyl ether, 3:1) was identified as 1-$NH_2$ by GLC, TLC, and bioassay for inhibition of the bacterial "lawn". 1-$NH_2$ was also identified by TLC and GLC–CI-MS in photolysates of 1-$NO_2$ in 2-propanol, a treatment yielding primarily bactericidal but not mutagenic (–S9) products. The bactericidal activity of 1-$NH_2$ in the standard TA 100 assay was greater than that of 4-nitrophenol and 2,4-dichlorophenol (potential 1-$NO_2$ photoproducts) and of 4-chlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol; none of these phenols showed mutagenic activity (–S9).

## DISCUSSION

1-$NO_2$ undergoes photochemical and metabolic reduction to 1-$NH_2$ via 1-NO and the highly unstable 1-NHOH (Figure 5). The hydroxylamine reacts in several ways, i.e., electrophilic alkylation, polymerization to unidentified products, disproportionation to 1-NO and 1-$NH_2$, and coupling to 1-N=N(O)-1 (the azoxy dimer may ultimately result from reaction of 1-NO with 1-NHOH; Pak and Testa, 1981). The trifluoroacetoxy derivative, isolable for other hydroxylamines (Beckett and Achari, 1977), may be considerably more electrophilic than 1-NHOH itself since

attempted preparation resulted in rapid breakdown to a variety of products.

1-$NO_2$ photochemistry differs markedly in thin films, water, aqueous suspension, and solution in organic solvents. 1-NO is a major photoproduct in thin films; an analogous photochemical conversion to a nitroso derivative was noted previously with 3-COOMe-4-PhOPh-2,4,6-$Cl_3$ in concentrated aqueous suspensions (Ruzo et al., 1980). 1-$NH_2$ was only a minor product in thin films, probably due to both its lability and competing reactions of its precursor, 1-NHOH. Characteristics of the organic solvent affect the photochemistry as well; efficient hydrogen donors (isopropanol) enhance photoreduction whereas poor hydrogen donors (acetonitrile) greatly diminish the photochemical reactivity. Increasing concentrations of methanol enhance the rate of photochemical nitro reduction of 1-$NO_2$ in water (Nakagawa and Crosby, 1974a). In aqueous solutions, 2,4-dichlorophenol and 4-nitrophenol, from photonucleophilic displacement reactions (Nakagawa and Crosby, 1974b), predominate and lesser levels of 1-$NH_2$ are found; polymeric materials also appear on aqueous photolysis of 1-$NO_2$ and 1-$NH_2$. In concentrated aqueous suspensions 1-$NH_2$ is the major photoproduct and only traces of the phenolic derivatives are detected (Ruzo et al., 1980).

Metabolic reduction of 1-$NO_2$ is the principal pathway with S9 preparations in vitro under anaerobic conditions. Under aerobic conditions with NADPH, microsomal oxidation converts 1-$NH_2$ to 1-NO; the yield of the nitroso derivative in these studies may have been lowered considerably by its chemical reduction with NADPH (Sternson, 1975). The relevance of these findings to in vivo mammalian metabolism is established to studies showing that 1-$NH_2$ is a urinary metabolite in rats dosed with 1-$NO_2$ (Costlow and Manson, 1982).

1-$NO_2$ is a promutagen undergoing activation on photolysis and metabolism or both. The intermediary metabolites 1-NO, 1-NHOH, and 1-$NH_2$ are each increased in mutagenic potency by the S9 mix. Considerable variation (~2-fold) was encountered in the 1-NO/S9 assay; more consistent activities were found for 1-NO (-S9) and 1-NHOH, 1-$NH_2$, and 2-aminofluorene (each +S9). This variation (1-NO/S9) may be due to differing levels of liver microsomal nitro reductase activity which is sensitive to deactivation by oxygen (Rosenkranz and Speck, 1975). The identity of the ultimate mutagen derived from 1-$NO_2$ and its transformation products is unknown. The available data do not rule out the possibility that the highly reactive 1-NHOH is in fact the ultimate mutagen somehow stabilized by the S9 mix. 1-NO may undergo chemical reduction to 1-NHOH on reaction with NADPH (Sternson, 1975), a component of the S9 mix. Alternatively, the bacteria as well as the S9 mix may be capable of metabolizing 1-NO to the active mutagen via 1-NHOH. Other hydroxylamines (or hydroxamic acids) including [(*N*-hydroxyacetyl)amino]fluorene exhibit increased potency in the bacterial mutagen assay with the S9 mix. Metabolic activation of 1-NHOH by formation of the acetoxy ester [1-$NHOC(O)CH_3$] was not indicated by preliminary studies as increases in mutagenic potency were not associated with acetyl-CoA or ATP (Seiler, 1979). Alternative metabolic activation mechanisms for consideration are esterification with phosphate, sulfate, or glucuronic acid (Miller and Miller, 1969).

Technical nitrofen contained less than 0.06 wt% of 1-$NH_2$ or 1-NO (the limit of detection of the FID-GLC determination); the material was dark red-brown, suggesting that the labile reduction products of 1-$NO_2$ had polymerized. The mutagenic photoproducts are probably intermediates in the environmental degradation of nitrofen, leading to polymeric substances, phenols, and possibly 1-N=N(O)-1.

1-NO, 1-NHOH, and 1-$NH_2$, as mutagens, provide alternatives to 2,7-dichlorodibenzodioxin or other trace contaminants in accounting for the toxicity and teratogenicity of technical-grade 1-$NO_2$ (Woolson et al., 1972; Kimbrough et al., 1974; Gray et al., 1982). The mutagenic metabolites are also worthy of consideration as contributors to this herbicide's carcinogenicity (Milman et al., 1978).

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**Registry No.** 1-$NO_2$, 1836-75-5; 1-NO, 73143-91-6; 1-NHOH, 76532-45-1; 1-$NH_2$, 14861-17-7; 1-N=N(O)-1, 84254-28-4.

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