N-Nitrosamine Formation from Atrazine

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The reaction of organic nitrogen compounds to form N-nitroso derivatives is attracting widespread interest, most of which has been stimulated by evidence that N-nitroso derivatives as a general class of compounds are carcinogenic. Although a great deal of this interest has centered around N-nitrosodimethylamine, there has been increasing concern with the formation of N-nitroso derivatives from man-made chemicals that receive widespread agricultural and industrial use. This, along with the recent finding of atrazine and other triazine herbicides in both New Orleans (U.S. EPA, 1974) and Midwest (JUNK, 1975) drinking water, prompts a preliminary report of our studies of N-nitrosation of the triazine herbicide, atrazine.

N-Nitrosamine formation with several amines has been demonstrated under a variety of environmental and biological reaction conditions. AYANABA et al. (1974) have demonstrated dimethylnitrosamine formation in soils treated with high concentrations of the fungicide thiram and nitrate. MIRVISH (1971) has investigated N-nitroso derivative formation from substituted ureas and urethanes under reaction conditions similar to those in the human digestive system. ELESPURU and LIJINSKY (1973) reported N-nitroso derivative formation for several agricultural chemicals, including the widely used carbamate insecticide carbaryl.

Atrazine (1) is the most widely used pesticide in the United States (U.S. EPA, 1972). It is moderately persistent and in many cases is applied with nitrogen fertilizers, giving rise to chemical reaction conditions that might favor N-nitrosamine formation. Atrazine has a low basicity (pK_a 1.68) (WEBER, 1970) and therefore the chemical environment of the stomach (WALTERS et al., 1972) may offer a suitable site for N-nitrosamine formation from atrazine ingested through drinking water.

Under our synthetic reaction conditions (Figure 1) the N-nitrosation of atrazine was anticipated to give rise to three products. We have isolated and

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characterized the 2-chloro-4-(N-nitroso-N-ethylamino)-6-isopropylamino-s-triazine (2) (NNA) and assigned its structure on the basis of infrared, nuclear magnetic resonance, ultraviolet, and mass spectral data. The other possible isomer, 2-chloro-4-ethylamino-6-(N-nitroso-N-isopropylamino)-s-triazine (3), and the dinitroso derivative, 2-chloro-4-(N-nitroso-N-ethylamino)-6-(N-nitroso-N-isopropylamino)-s-triazine (4), which may be formed in the presence of excess nitrite, were not isolated. Our findings are consistent with previously reported data in the patent literature for nitrosation of related substituted diamino-s-triazines (BASF, 1964; GOLD and ROSSLER, 1965).

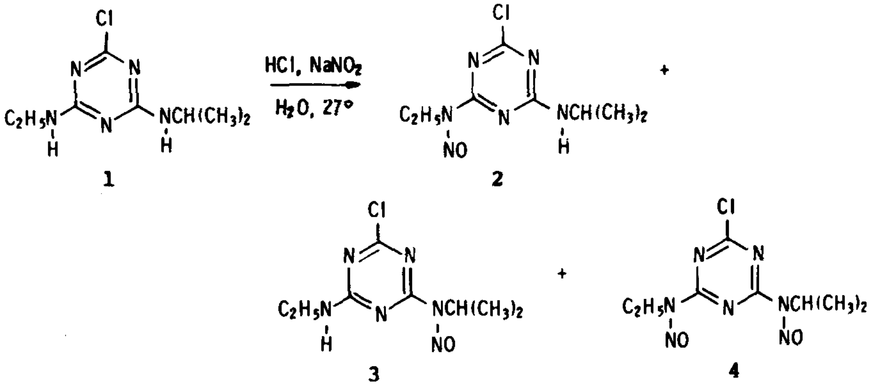

Figure 1. Formation of N-nitrosoatrazine in water.

Analysis for NNA by glc was unsuccessful since the compound decomposed during chromatography under all glc conditions tried. With sample sizes of 2 µg two peaks appeared in the gas chromatogram; the first had a retention time identical to that of atrazine. The second was believed to be NNA, thus indicating partial decomposition. With smaller sample sizes (2 ng) a single peak with the same retention time as atrazine appeared. Therefore, a liquid chromatographic (lc) method (strong cation exchange column - 5% acetonitrile - water mobile phase) was developed by which we could directly analyze for NNA, atrazine, and NNA photoproducts down to the one ppm level in water.

The rate of NNA formation was studied as a function of pH between pH 1 and 5. With initial concentrations of 2.5 x 10^{-2}M nitrite and 1.0 x 10^{-4}M atrazine (27°), maximum rate of formation occurred at pH 1.8. The reaction rate was also found to increase with the square of the nitrite concentration between 0.01M and 0.1M nitrite. This is in keeping with the accepted third-order-overall reaction mechanism (RIDD, 1961).

To make our data easier to interpret we have expressed them in terms of half-lives of nitrosamine formation. Concentrations of pesticide residues in water and foodstuffs are generally quite low. However, the concentration of nitrite added to foodstuffs is likely to be orders of magnitude larger and under these conditions pseudo-first-order reaction kinetics would prevail. At a given pH under these conditions the half-life for nitrosamine formation is independent of amine concentration. Table 1 gives a crude comparison of calculated pseudo-first-order-rate constants and half-lives for N-nitrosamine formation for some pesticides based on literature data. At pH 2.0, (1) reacts approximately two orders of magnitude faster than dimethylamine and about three orders of magnitude faster than carbaryl.

TABLE 1

Calculated Rates of N-nitrosamine Formation for Some Pesticides and Pesticide Derivatives at pH 2 and 0.01 M Nitrite at 25°.

Pesticide	k_1 (hr^{-1})[d]	$t_{1/2}$ (hr)[e]
Dimethylamine[a]	1.72×10^{-4}	4,000
Methylurea[b]	4.08	0.17
N-Methylurethane[b]	1.34×10^{-1}	5.2
Carbaryl[c]	3.33×10^{-5}	21,000
Atrazine	4.1×10^{-2}	17

[a] MIRVISH (1970). [b] MIRVISH (1971). [c] Estimated from data in ELESPURU and LIJINSKY (1973), assuming N_2O_3 as the nitrosating reagent at this high nitrite concentration. [d] Pseudo-first-order rate constants under pH buffered conditions and 0.01 M nitrite. [e] Half-lives calculated from pseudo-first-order rate constants.

NNA was very stable in water at pH values greater than 4 (25°C). No change in NNA concentration was detected over a three-week period either in distilled waters buffered at pH 5.5 and pH 8.0 or in a natural water sample collected from a local river (pH 7.1). At pH values less than 4, NNA slowly decomposed to atrazine. For example, at pH 3.0, NNA was 50% decomposed after 114 hours.

In contrast to its hydrolytic stability in solution, NNA was rapidly decomposed by light. The photoreactivity of NNA was somewhat surprising, because simple N-nitrosodialkylamines are not photoreactive in neutral water or organic solvents (BURGESS and LAVANISH, 1964; CHOW, 1973); the latter compounds are photoreactive only in strongly acidic media.

Major products derived from photolysis of NNA in water were 4-amino-2-chloro-6-isopropylamino-s-triazine (5) (desethylatrazine) and atrazine (Figure 2). In chloroform and ether solvents, atrazine was the major product. The photoproducts were isolated by preparative lc and identified by comparison of their mass spectra with those of authentic samples. Interestingly (5) was also found in New Orleans drinking water samples (U.S. EPA, 1974). However, this product can also form via microbial or free-radical oxidation of atrazine (KAUFMAN and KEARNEY, 1970; PLIMMER *et al.*, 1971).

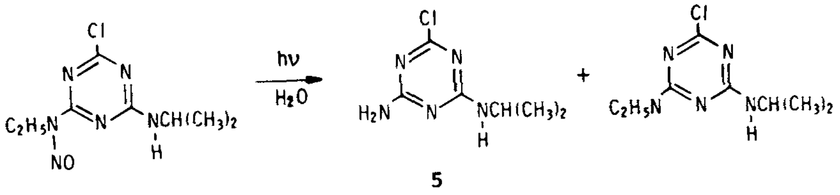

Figure 2. Photodegradation products of N-nitrosoatrazine in water.

The disappearance quantum yield (CALVERT and PITTS, 1966) for NNA in air-saturated water was 0.30 at both 313 nm and 366 nm. Both the quantum yield and electronic absorption spectrum [vis max 427 nm (ε 61.8); 407 nm (ε 92.7)] were pH-independent in the pH 5.5 to 8.0 range. Using the spectral and quantum yield data, we calculated that, near the surface of a water body, the half-life for photodecomposition of NNA under sunlight is less than ten minutes throughout the year in the United States (ZEPP and CLINE). Experiments under sunlight in both distilled water and water from a local river confirmed the calculated half-life. Photodecomposition of NNA under ordinary fluorescent lighting in the laboratory is sufficiently rapid that it, as well as the afore-mentioned analytical problem, may have prevented detection of NNA in environmental samples.

These results indicate that sunlight photolysis is likely to prevent any build-up of NNA in the aquatic environment. However, the most likely location for environmental formation of NNA is in the soil where no light is present. Recent studies by JUNK (1975) have demonstrated that triazines are wide-spread in groundwater drinking supplies in the Midwest, presumably as a result of leaching from the soil. Since NNA is more water-soluble (227 ppm at 25°C) than atrazine, it is likely that it would also be leached into ground waters if it forms in the soil.

For lack of a sensitive method of analysis we have not carried out any studies at pesticide concentrations anticipated under environmental conditions. It is questionable if present methods of pesticide residue analysis based on glc techniques would detect N-nitrosamine derivatives of pesticides.

CONCLUSIONS

Previous studies have focused on N-nitroso derivatives of relatively non-persistent pesticides or pesticides that form unstable N-nitroso derivatives. We feel that more persistent pesticides or their degradative products capable of forming N-nitroso derivatives should be investigated. Also pathways of degradation, both chemical and biological, should be elucidated.

LITERATURE CITED

AYANABA, A., W. VESTRAETE, and M. ALEXANDER: Soil Sci. Soc. Amer. Proc. 37, 565 (1973).

BASF: Ger. Patent 1, 184, 148 (1964); Chem. Abstr. 62, 12384B (1965).

BURGESS, E. M., and J. M. LAVANISH: Tetrahedron Lett. 1221 (1964).

CALVERT, J. G., and J. N. PITTS: Photochemistry. New York: John Wiley and Sons, Inc. 1966. p. 587.

CHOW, Y. L.: Accounts Chem. Res. 6, 354 (1973).

ELESPURU, R. K., and W. LIJINSKY: Fd. Cosmet. Toxicol. 11, 807 (1973).

GOLD, D., and S. ROSSLER: Belg. Patent 663,328 (1965); Chem. Abstr. 65, 2280c (1966).

JUNK, G. A.: Personal Communication (1975).

KAUFMAN, D. D., and P. C. KEARNEY: Residue Reviews 32, 235 (1970).

MIRVISH, S. S.: J. Nat. Cancer Inst. 44, 633 (1970).

MIRVISH, S. S.: J. Nat. Cancer Inst. 46, 1183 (1971).

PLIMMER, J., P. C. KEARNEY, and U. KLINGEBIEL: J. Agr. Food Chem. 19, 572 (1971).

RIDD, J. H.: Quart. Rev. (London) 15, 418 (1961).

U.S. ENVIRONMENTAL PROTECTION AGENCY: The Pollution Potential in Pesticide Manufacturing. EPA#TS-00-72-04 (1972).

U.S. ENVIRONMENTAL PROTECTION AGENCY: Draft Analytical Report, New Orleans Area Water Supply Study, Region VI (Nov. 1974).

WALTERS, C. L., M. C. ARCHER, and S. R. TANNENBAUM: Science 179, 96 (1972).

WEBER, J. B.: Residue Reviews 32, 93 (1970).

ZEPP, R. G., and D. M. CLINE: Paper in Preparation.