Bull. Environm. Contam. Toxicol. 23,814-819 (1979)

Toxicity and Toxic Interaction of Aniline and Pyridine[1]

T. Wayne Schultz and Terry C. Allison

Department of Biology, Pan American University, Edinburg, Texas 78539 (TWS and TCA), and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830 (TWS)

Chemical characterization of organic contaminants of coal conversion processes such as gasification or liquefaction has revealed them to be a potential source of environmental hazards (FORNEY et al. 1974, FRUCHTER et al. 1977, HO et al. 1976, PETERSEN 1975). The majority of these compounds may be divided into four major classes: (1) phenols, (2) aromatic hydrocarbons, (3) aromatic amines, and (4) sulfur-containing compounds. HERBES et al. (1976) note that little is known about nitrogen-substituted aromatics. The data available, however, suggest that they are toxic (DUMONT et al. 1979, SCHULTZ et al. 1978b) and, even more importantly, are resistant to structural degradation (COOPER and CATCHPOLE 1973). Examination of their water solubility reveals that although they are not as soluble as low-molecular-weight phenols, arylamines are more soluble than their nonsubstituted or sulfur-containing analogs. Thus, with existing technologies there is a real possibility that nitrogen-containing aromatic compounds may be discharged from coal conversion facilities as aqueous effluent in large enough concentration to have a pernicious effect on aquatic biota. Acute toxicity thresholds of several nitrogen-containing aromatic compounds have been tabulated for aquatic organisms (VERSCHEUREN 1977), but potential toxic interactions between these compounds have not been investigated.

Sprague (1970) has pointed out that pollutants have been shown to impair reproduction and that tests for this damage should be regarded as among the most important in determining "safe" concentrations of pollutants. He further stated that "with invertebrates and algae, as much as with fish, tests of sublethal effects such as growth and development are more meaningful than tests of acute toxicity," and "reproduction seems to be one of the most sensitive of chronic or sublethal responses which is clearly meaningful in nature" (SPRAGUE 1970, 1971).

The advantages of using Tetrahymena pyriformis as a test organism have been noted (CARTER and CAMERON 1973, SCHULTZ et al. 1978a). This ciliate is a relatively large, mobile cell which is easy and inexpensive to maintain in the laboratory. Tetrahymena has a brief, well-characterized life cycle, and its biochemistry, physiology, and subcellular structure have been extensively reviewed (ELLIOTT 1973, HILL 1972, NILSSEN 1976). This report examines the acute static toxicities of aniline and pyridine and the toxic interaction of the two compounds to population growth and density of the ciliate Tetrahymena pyriformis.

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[1]This study was supported in part by Pan American University Faculty Grant #1538 to TWS and by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation

0007-4861/79/0023-0814 $01.20

MATERIALS AND METHODS

General

Tetrahymena pyriformis, strain GL-C (obtained from Dr. J. R. Kennedy, Department of Zoology, University of Tennessee, Knoxville), was reared axenically at 28°C in a 2% proteose peptone medium. Test cultures were in 250-ml Erlenmeyer flasks each containing 50 ml of medium and inoculated with 0.2 ml of log-phase culture.

Stock solutions of aniline and pyridine (Aldrich Chemical Co., Milwaukee, Wisconsin) were prepared by dissolving the aromatic amines in absolute ethanol.

Population Growth and Density Experiments

The effects of replicates of a graded concentration series of pure aniline and pyridine on population growth and near-stationary-phase density of *Tetrahymena* were recorded from a Bausch & Lomb Spectronic 20 spectrophotometer at 540 nm. Cultures without arylamines served as controls. The concentration response was examined by plotting the absorbance, Y, versus the concentration, X. The best line was fitted by the least-squares method of linear regression and the 72-hr IGC 50, the concentration which inhibits growth by 50% after 72 hr, and 95% confidence intervals were calculated.

Toxic Interaction Experiments

The effects of mixtures of aniline and pyridine on the reproduction of *Tetrahymena* were also studied. A preliminary stock solution of each compound was prepared at a concentration approximately 500X the calculated 72-hr IGC 50 value (Table 1). From these preliminary stock

TABLE 1

Composition of Stock Solutions

Solution	Proportion of aniline:pyridine (v/v)	Concentration of components (ppm)	
		Aniline	Pyridine
Mixture I	0:1.00	0	575,000
Mixture II	0.25:0.75	18,750	431,250
Mixture III	0.50:0.50	37,500	287,500
Mixture IV	0.75:0.25	56,250	143,750
Mixture V	1.00:0	75,000	0

solutions three mixture stock solutions with ratios of 1:3, 1:1 and 3:1 (v/v) were prepared by combining aliquots of the pyridine and aniline solutions, respectively. Compositions of these mixture stock solutions are presented in Table 1. Final test solutions were prepared by adding 0.05, 0.10, or 0.15 ml of one of the three mixture stock solutions to 250-ml Erlenmeyer flasks containing 50 ml of medium each. Thus the final test concentration range bracketed the calculated IGC 50 values of the two toxicants.

The concentration response for each mixture was studied on least-squares linear regression plot of absorbance, Y, versus concentration, X, with the 72-hr IGC 50 value for each mixture being calculated in milliliters. Initially the IGC 50 values were converted to parts per million (ppm) of each toxicant in the mixture by use of Table 1 and the dilution factor of 50 to account for the medium. The 72-hr IGC 50 values in ppm were then converted to "toxic units" (SPRAGUE and RAMSAY 1965), with a toxic unit in this study being defined as the 72-hr IGC 50 value for the pure compounds. Toxic interaction was examined graphically following the procedure of HERBES and BEAUCHAMP (1977).

RESULTS AND DISCUSSION

Preliminary experiments revealed that ethanol at a concentration of $\leq 0.5\%$ does not alter Tetrahymena population growth. In addition, population densities of culture exposed to aniline or pyridine administered in an ethanol solution were not significantly different from those grown in the presence of aniline or pyridine added without prior ethanol dilution. Thus, there is no apparent toxic effect of or toxic interaction between ethanol and aniline nor ethanol and pyridine over the concentration range used in these studies.

The effects of aniline and pyridine on population growth and maximum cell density of Tetrahymena are inversely related to concentration over the ranges tested. Aniline inhibits population growth at a concentration approximately an order of magnitude less than pyridine; the concentrations required to completely inhibit growth are less than 250 and 2500 ppm, respectively. Addition of pyridine to the medium to form a final concentration of 2500 ppm increases the pH approximately 0.15 units. A similar addition of aniline, however, has no effect on pH. Since the pH of 72-hr control cultures is acidic compared with sterile medium (6.3 ± 0.2 vs. 7.0 ± 0.1), but well within the pH growth range reported by PRESCOTT (1958), this pH change is not the factor which limits population growth. The phenomenon of maximum population density being inversely related to concentration has been observed for Tetrahymena grown in the presence of compounds having different modes of action (SCHULTZ et al. 1978a). Thus, this inverse relationship is a general trend and not specific for a given class of compounds or a given mode of action. The linear regression equations, correlation coefficients, and calculated 72-hr IGC 50 values with their 95% confidence interval are presented for aniline and pyridine in Table 2.

TABLE 2

Toxicities of Mixtures of Aniline (A) and Pyridine (P) to Tetrahymena

Mixture stock solution	Linear regression equation	Correlation coefficient	72-hr IGC 50 value[a] (ml)	Concentration of A and P at 72-hr IGC 50[a]			
				ppm		Toxic units	
				A	P	A	P
I	$Y = 0.712 - 0.001X$	-0.948	—	—	1193.70 (559.63–1824.71)	0	1.00 (0.47–1.53)
II	$Y = 1.039 - 5.792X$	-0.984	0.1129 (0.0854–0.1493)	127.01 (96.08–167.96)	324.59 (245.53–429.24)	0.82 (0.62–1.09)	0.27 (0.21–0.36)
III	$Y = 1.183 - 6.549X$	-0.951	0.1218 (0.0974–0.1522)	91.35 (73.05–114.15)	700.35 (560.05–875.15)	0.59 (0.47–0.74)	0.59 (0.47–0.73)
IV	$Y = 1.110 - 6.337X$	-0.964	0.1144 (0.0892–0.1467)	42.90 (31.08–55.01)	986.70 (715.01–1265.25)	0.28 (0.20–0.36)	0.83 (0.60–1.06)
V	$Y = 1.133 - 0.005X$	-0.969	—	154.27 (123.37–192.90)	— —	1.00 (0.80–1.25)	0

[a]95% confidence intervals in parentheses.

Mixtures of the two nitrogen-containing monoaromatic compounds are less toxic than either aromatic amine tested separately (Table 2). These data, when presented graphically following the example of HERBES and BEAUCHAMP (1977), reveal a symmetrical toxic interaction curve which lies above the diagonal "additive interaction" line (Fig. 1). The shape

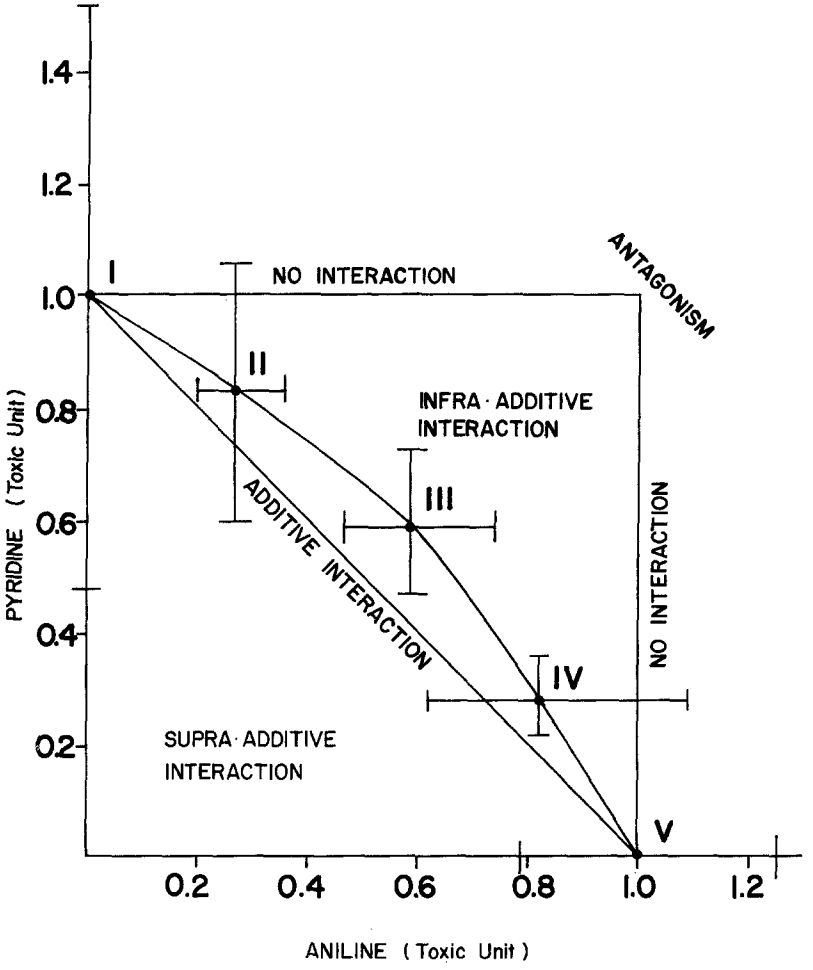

Figure 1. Toxic interaction of aniline and pyridine.

and position of the curve suggest that even though there is better than a 7-fold difference in the 72-hr IGC 50 values for aniline and pyridine (Table 2), mixtures in which either one is the major toxic component (points II and IV, respectively) are still slightly infra-additive (Fig. 1).

However, interpretation of these data must be tempered by the fact that the 95% confidence interval for aniline in point II and pyridine in point IV cross not only the "additive interaction" line but also the appropriate "no interaction" line.

The present study indicates that simple addition of fractional toxicities of organic compounds even from the same general class may produce considerable error. This lessens the value of simple addition as a productive tool in ascertaining total toxicity of mixtures of even similar organic compounds. Furthermore, it demonstrates Tetrahymena as an excellent short-term environmental screening system which allows sublethal population effects to be monitored easily and rapidly.

REFERENCES

CARTER, J. W., and I. L. CAMERON: Water Res. 7, 951 (1973).

COOPER, R. L., and J. R. CATCHPOLE: Water Res. 7, 1137 (1973).

DUMONT, J. N., T. W. SCHULTZ, and R. JONES: Bull. Environ. Contam. Toxicol. 22, 159 (1979).

ELLIOTT, A. M.: Biology of Tetrahymena. Stroudsburg, Pennsylvania: Dowden Hutchinson & Ross Inc. 1973.

FORNEY, A. J., W. P. HAYNES, S. J. GASIOR, G. E. JOHNSON, and J. P. STRAKEY, Jr.: U.S. Bur. Mines Tech. Prog. Rep. 76 (1974).

FRUCHTER, J. S., M. R. PETERSEN, J. C. HAUL, and P. W. RYAN: In: Fossil Energy Update, ERDA-9500022 (1977).

HERBES, S. E., and J. J. BEAUCHAMP: Bull. Environ. Contam. Toxicol. 17, 25 (1977).

HERBES, S. E., G. R. SOUTHWORTH, and C. W. GEHRS: Proc. 10th Annu. Conf. on Trace Substances in Environmental Health, University of Missouri, Columbia (1976).

HILL, D. L.: The Biochemistry and Physiology of Tetrahymena. New York: Academic Press, 1972.

HO, C. H., B. R. CLARK, and M. R. GUERIN: J. Environ. Health Sci. A 11, 481 (1976).

NILSSEN, J. R.: C. R. Trav. Lab. Carlsberg 40, 215 (1976).

PETERSEN, M. R.: Prog. Rep. Battelle Pacific Northwest Lab., BNWL-B-470 (1975).

PRESCOTT, D. M.: Physiol. Zool. 31, 111 (1958).

SCHULTZ, T. W., J. N. DUMONT, and L. M. KYTE: J. Protozool. 25, 502 (1978a).

SCHULTZ, T. W., L. M. KYTE, and J. N. DUMONT: Arch. Environ. Contam. Toxicol., 7, 457 (1978b).

SPRAGUE, J. B.: Water Res. 4, 3 (1970).

SPRAGUE, J. B.: Water Res. 5, 245 (1971).

SPRAGUE, J. B., and A. B. RAMSAY: J. Fish Res. Board Can. 22, 425 (1965).

VERSCHEUREN, K.: Handbook of Environmental Data on Organic Chemicals. New York: Van Nostrand Reinholt Co. 1977.