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Toxicity and Teratogenicity of Aromatic Amines to *Xenopus laevis*

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Coal accounts for approximately 75% of the fossil fuel reserve of the United States. To be of greater industrial and commercial value, coal can be refined or converted into a gas or liquid fuel. With any coal conversion technology that reaches commercial operation, there are several sources of potential environmental hazards. One of the most serious of these is the aqueous effluents (FORNEY et al. 1974). Chemical analyses of possible effluents from pilot coal conversion facilities reveal that they contain five major classes of organic compounds: (1) phenols, (2) monoaromatic hydrocarbons, (3) polyaromatic hydrocarbons, (4) arylamines, and (5) sulfur-containing compounds. The efficiency with which these organics can be removed from aqueous process streams depends on several parameters including their molecular size and their degree of alkyl and heteroatom substitution (HERBES et al. 1976); i.e., heavily methyl-substituted, N-containing multi-ring compounds are difficult to remove by current depuration technology and thus pass readily through treatment processes (COOPER and CATCHPOLE 1973, HERBES et al. 1976).

Quinoline and its methyl-substituted derivatives are examples of such compounds and are present in significant quantities in waters from coal conversion processes (FORNEY et al. 1974, PETERSEN 1975, FRUCHTER et al. 1977). It is important to know the effects of these compounds on the aquatic biota. It is the purpose of this report to examine the toxicity and teratogenicity of several pure aromatic amines known to be present in coal conversion process waters on the early embryonic stages of, South African clawed frog, Xenopus laevis.

MATERIALS AND METHODS

Embryos of Xenopus laevis were obtained from paired adults injected with human chorionic gonadotropin following the procedures of BROWNE and DUMONT (1978).

For acute static testing, replicates of 25 embryos each were tested by exposing early cleavage to mid-blastula embryos at 20°C in finger bowls containing 250 ml of test solution. Stock solutions of aniline, pyridine, quinoline, 2-methylquinoline and 2,6-dimethylquinoline were prepared biweekly by dissolving the arylamines in deionized water. Embryos reared in water without aromatic amines served as controls. Observations were made at representative time points; and the number of deaths and abnormal

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embryos, as well as their state of pigmentation, motility, and stage of development, were recorded. The abnormalities were so severe that normal development into free swimming larvae was impossible. For statistical analyses, abnormal embryos were counted as dead. The concentrations of the quinolines causing 50% mortality (LC50) at 96-hr and 95% confidence intervals were obtained by PROBIT analyses. The mortality rates of embryos exposed to various concentrations of quinolines were examined by linear regression (SPRAGUE 1969).

RESULTS

Since preliminary experiments using aniline, pyridine, and quinoline at concentrations of 10 and 50 mg/liter showed quinoline to be the most toxic and teratogenic of the three aromatic amines (Table 1), the effects of quinoline and two of its methyl derivatives were studied more closely. Quinoline and 2-methylquinoline in concentrations <10 mg/liter are neither toxic nor teratogenic to early Xenopus embryos (Tables 1,2). Embryos exposed to 2,6-dimethylquinoline in concentrations as low as 5 mg/liter, however, showed high rates of mortality and abnormal development (Table 2). Both quinoline and 2-methylquinoline, in concentrations ranging from 10 to 100 mg/liter, are parallel with respect to their toxicity and teratogenicity (Table 2). The concentration range over which mortality and abnormality rates for 2,6-dimethylquinoline are linear is narrower; from less than 25 mg/liter to less than 5 mg/liter (Table 2). Early embryos (Stages 8–28) are most susceptible to the toxic and teratogenic potential of the quinolines tested. However, changes during the first 24 hr of exposure (early embryonic stages) are often difficult to discern.

TABLE 1

Lethality and Teratogenicity of Aniline, Pyridine, and Quinoline

Amine	Concentration (mg/liter)	Day							
		1		2		3		4	
		A/S[a]	%	A/S	%	A/S	%	A/S	%
Control		0/50	0	0/50	0	0/50	0	0/50	0
Aniline	10	0/50	0	0/47	0	4/36	11	4/36	11
	50	1/50	2	3/48	6	3/48	6	3/48	6
Pyridine	10	3/50	6	3/49	6	3/49	6	3/49	6
	50	2/50	4	16/48	33	16/48	33	16/48	33
Quinoline	10	0/48	0	0/48	0	0/48	0	0/48	0
	50	16/46	35	43/43	100	—	—	—	—

[a] Abnormals/survivors

The 96-hr LC50 for quinoline and its 2-methyl and 2,6-dimethyl derivatives was calculated from a least-squares linear regression of the

TABLE 2

Lethality and Teratogenicity of Quinoline, 2-Methylquinoline and 2, 6-Dimethylquinoline

Amine	Concentration (mg/liter)	Day								
		1		2		3		4		4
		A/S[a]	%	A/S	%	A/S	%	A/S	%	Dead + Abnormal (%)
QUINOLINE	0	0/100	0	0/96	0	2/96	2	3/96	3	7
	10	0/96	0	1/95	1	3/95	3	3/95	3	8
	25	3/93	3	20/91	22	30/86	35	37/86	43	51
	50	6/93	6	39/87	44	49/83	59	56/83	67	73
	75	6/96	6	46/86	53	63/86	72	68/86	79	82
	100	12/86	14	46/63	73	60/62	97	62/62	100	100
	125	14/77	18	14/14	100	0/0		0/0		0
2-METHYLQUINOLINE	0	0/100	0	0/98	0	1/98	1	1/97	1	4
	10	1/97	1	2/97	2	6/97	6	6/97	6	9
	25	1/96	1	3/96	3	38/96	40	38/96	40	42
	50	0/100	0	15/92	16	57/86	66	57/86	66	71
	75	0/98	0	17/89	19	53/85	62	64/75	85	89
	100	9/88	10	38/71	54	70/71	99	71/71	100	100
	125	11/71	15	15/15	100	0/0		0/0		0
2, 6-DIMETHYLQUINOLINE	0	0/99	0	1/97	1	3/97	3	3/97	3	6
	5	0/95	0	14/92	15	26/91	29	36/91	40	45
	10	0/81	0	12/78	15	31/77	40	36/76	47	60
	15	1/42	2	13/32	40	23/31	74	25/31	81	94
	25	0/4	0	0/0		0/0		0/0		0

[a] Abnormals/survivors

PROBIT of survival versus log concentration (Table 3). Quinoline and 2-methylquinoline have identical toxicities, but 2,6-dimethylquinoline is four times more toxic.

TABLE 3

96-hr LC50 values for Xenopus embryos exposed to quinolines

Quinoline derivative	LC50 (mg/liter)	Regression equation	Correlation coefficient
Quinoline	26.3 (22.1–31.3)	Y= -3.72X + 10.28	-.917
2-Methylquinoline	26.4 (22.4–31.1)	Y=-3.86X + 10.48	-.935
2,6-Dimethylquinoline	6.5 (5.6–7.5)	Y=-4.65X + 8.78	-.947

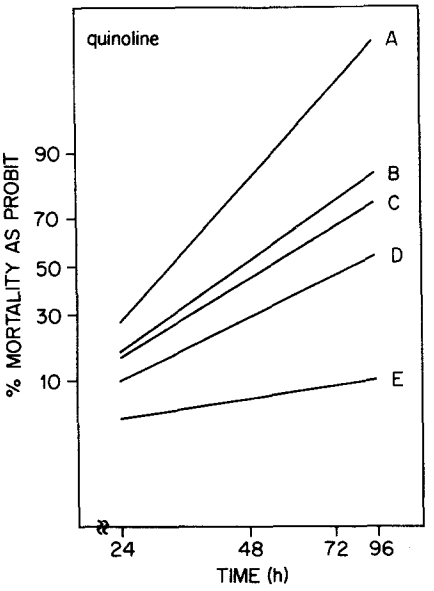
To examine the possibility that more than a single mode of action is involved in the toxicity of the quinolines, the percentage of mortality for several concentrations of each derivative was plotted against the log of exposure time (Figs. 1–3). The significant changes in the slope suggest different modes of action (SPRAGUE 1969).

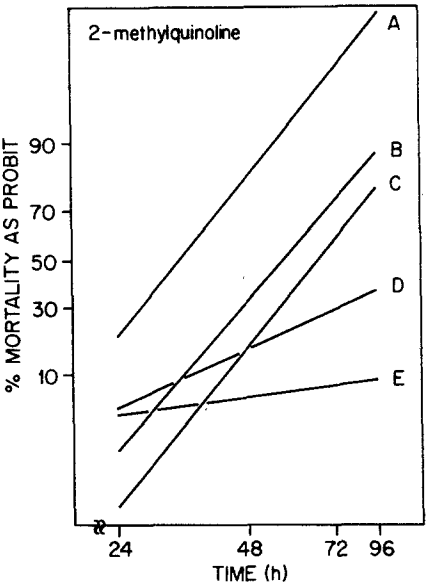
All quinolines tested reduce the motility and decrease the pigmentation of the embryos by the time of hatching (Table 4). At the termination of the exposures the size of embryos exposed to quinolines is smaller than controls. These reductions occur at much lower concentrations in embryos exposed to 2,6-dimethylquinoline.

Comparisons of the stages of development of embryos in different concentrations of quinolines reveal that in all concentrations the rate of development is significantly slowed, especially over the first 48 hr of exposure. The reduced development rate is concentration dependent for all three compounds, but again 2,6-dimethylquinoline is effective at much lower concentrations. By 96 hr organisms exposed to the lower concentrations of quinoline and 2-methylquinoline are approaching developmental stages equal to that of controls (Stage 45, NIEUWKOOP and FARBER 1956).

DISCUSSION

An interesting observation made in the course of this study is the sharp increase in the toxicity of 2,6-dimethylquinoline compared with quinoline, the parent compound, and 2-methylquinoline. In a model protozoan system, Tetrahymena pyriformis, a series of six quinolines were tested in which toxicity increased with methyl substitution (SCHULTZ et al.





Figures 1–3. Comparisons of time mortality for Xenopus embryos exposed to quinolines. Slopes of mortality rates are: Quinoline (A) (100 mg/liter = 5.62; (B) 75 mg/liter = 3.73; (C) 50 mg/liter = 2.93; (D) 25 mg/liter = 2.22; (E) 10 mg/liter = - 0.62). 2-Methylquinoline (A) (100 mg/liter = 6.30; (B) 75 mg/liter = 5.43; (C) 50 mg/liter = 6.09; (D) 25 mg/liter = 2.40; (E) 10 mg/liter = 0.68. 2, 6-Dimethylquinoline (A) (25 mg/liter = 1.92; (B)15 mg/liter = 2.40; (C) 10 mg/liter = 1.95 (D) 5 mg/liter = 2.54).

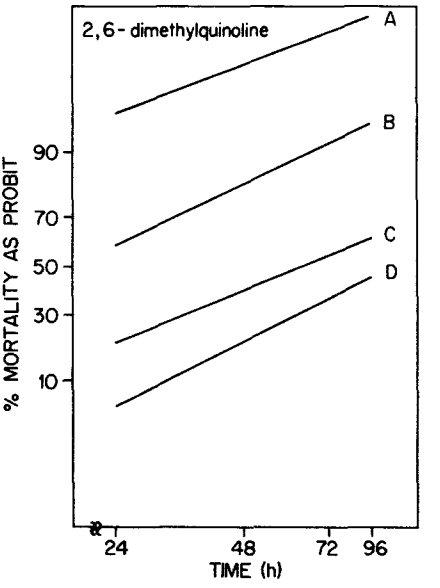


TABLE 4

Effects of Quinolines on the Motility and Pigmentation of Xenopus Embryos

Derivative	Control	Concentration (mg/liter)							
		5	10	15	25	50	75	100	125
Quinoline									
Motility	++++		+++		++	+	+/-	-	-
Pigmentation	++++		+++		+++	++	+	+/-	+/-
2-Methylquinoline									
Motility	++++		++		+	+/-	+/-	-	-
Pigmentation	++++		++		++	+/-	+/-	-	-
2,6-Dimethylquinoline									
Motility	++++	++	+/-	+/-	-	-			
Pigmentation	++++	++	+/-	+/-	-	-			

1978). This increased toxicity, coupled with the decrease in solubility and degradability associated with increased alkylation (HERBES et al. 1976), suggests that derivatives with two or more alkyl groups present a greater potential environmental hazard than unsubstituted or monosubstituted compounds. The fourfold increase in toxicity observed with 2,6-dimethylquinoline may be due to its ability to become concentrated in the hydrophobic compartments of the cell. Both hydrophobicity and partitioning in octanol–water is increased by additions in alkyl substitution (LEO et al. 1971). Correlations between partition coefficients and their bioconcentration (NEELY et al. 1974) or their toxicity (KOPPERMAN et al. 1974, SCHULTZ et al. 1978) have been demonstrated.

The changes in the slope of the mortality curves (calculated by summing the number of dead and abnormal embryos) for quinoline and 2-methylquinoline (see Figs. 1,2) suggest that the mode of action of these two compounds changes as their concentration is increased. (SPRAGUE 1969). A comparison of abnormal/survivor data (Table 2) reveals that while the number of abnormalities remains relatively constant at the higher concentrations, the total number of animals surviving decreases. Midrange concentrations (50–75 mg/liter), where mortality is less, apparently permit a greater expression of their teratogenic potential. The data are further complicated in the case of 2-methylquinoline by the unusually low mortality of animals after 24-hr exposure to concentrations of 50 and 75 mg/liter (Fig. 2). It must be noted, however, that PROBIT transformation of percentage of mortality exaggerates this aspect of the data. In fact, the difference in 24-hr mortality for concentrations from 10 to 75 mg/liter is less than 5%.

The fact that the greatest increase in mortality is observed during 24 to 72-hr exposure must be tempered with the knowledge that mortality is difficult to ascertain at very early stages of embryonic development. Other toxicity and teratogenicity studies on amphibian embryos show this

developmental time to be most sensitive to environmental influences (BIRGE et al. 1973, DIAL 1976).

Initially, embryos exposed to toxicants lag behind in development. By 96 hr, however, many embryos, especially those exposed to lower concentrations, begin to reach stages comparable to the controls. Of course, developmental rate is not linear, i.e., it is quite rapid during gastrulation and neurulation and becomes slower thereafter. The experimental animals are always smaller than controls at 96 hr. The reduction in size, as well as motility and pigmentation of exposed animals, will obviously limit the animals' ability to survive in a natural, competitive ecosystem.

Although with Xenopus quinoline is more toxic and teratogenic than either aniline or pyridine, this is not so with other aquatic test species—some are more susceptible to aniline poisoning while others are most sensitive to pyridine (McKEE and WOLF 1963, VERSCHUEREN 1977). This is not surprising in light of the wide differences in the testing methodologies, especially the wide variation in the physiology and biochemistry of the test organisms used.

In summary, quinoline is more toxic to Xenopus embryos than either aniline or pyridine. The toxicity of quinoline and its 2-methyl derivative is equal, but the toxicity of the 2,6-dimethyl derivative is four times greater. The mortality rates of Xenopus embryos exposed to various concentrations of quinoline and 2-methylquinoline suggest two modes of action for these compounds. The curves for 2,6-dimethylquinoline suggest a single mode of action. In general, size, motility, pigmentation, and rate of development are inversely related to concentration.

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REFERENCES

BIRGE, W.J., J.J. JUST, A. WESTERMAN, and A.D. ROSE: Univ. Ky. Water Resour. Inst. Res. Rep. 71 (1973).

BROWNE, C.L., and J.N. DUMONT: J. Toxicol. Environ. Health. (in press).

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

DIAL, N.A.: Teratology 13, 327 (1976).

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

FRUCHTER, J.S., J.C. LAUL, M.R. PETERSEN, and P.W. RYAN: Fossil Energy Update. BNWL-CONF-770301. 1977.

HERBES, S.E., G.R. SOUTHWORTH, and C.W. GEHRS: Proc. 10th Annu. Conf. Trace Substances in Environmental Health. Columbia: Univ. Mo. 1976.
KOPPERMAN, H.L., R.M. CARLSON, and R. CAPLE: Chem-Biol. Interact. 9, 245 (1974).
LEO, A., C. HANSCH, and D. ELKINS: Chem. Rev. 71, 525 (1971).
McKEE, J.E., and H.W. WOLF: Water Quality Criteria. 2 ed. Sacramento: Calif., State Water Resour Control Board Publ. 1963.
NEELY, W.B., D.R. BRANSON, and G.E. BLAU: Environ. Sci. Technal. 8, 1113 (1974).
NIEUWKOOP, P.D., and J. FARBER: Normal table of Xenopus laevis (Daudin). Amsterdam: North Holland Publishing Co. 1956.
PETERSEN, M.R.: Prog. Rep. Battelle Pacific Northwest Lab. BNWL-B-470 (1975).
SCHULTZ, T.W., L.M. KYTE, and J.N. DUMONT: Arch. Environ. Contam. Toxicol. (in press).
SPRAGUE, J.B.: Water Res. 3, 793 (1969).
VERSCHUEREN, K.: Handbook of Environmental Data on Organic Chemical. New York: Van Nostrand Reinhold Co. 1977.