Toxic Interaction of Mixtures of Two Coal Conversion Effluent Components (Resorcinol and 6-Methylquinoline) to *Daphnia magna**

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The anticipated development of a large-scale coal conversion industry within the next two decades will result in the potential production of substantial quantities of contaminated process water. Aqueous effluents may contain many organic compounds found in wastewater from other industrial pyrolysis processes, such as coking operations. Major classes of contaminants will include phenols, which have been detected at levels of 6000 mg/ℓ in a coal liquefaction wastewater (WENDER 1975), and arylamines, which were present at a total concentration of 310 mg/ℓ in the same sample. As many as 35 individual compounds of each class have been detected in coking effluents (BARKER and HOLLINGWORTH 1959; HUGHES 1962; BARK et al. 1972); a corresponding complexity undoubtedly will be present in coal liquefaction effluents.

Although biological wastewater treatment is able to remove greater than 99.9 percent of total phenols (KOSTENBADER and FLECKSTEINER 1969), degradation of dihydric phenols is far less complete than is removal of monohydric phenols (ASHMORE et al. 1967). Moreover, arylamines may pass through biological treatment processes substantially unchanged (COOPER and CATCHPOLE 1973). Both dihydric phenols and arylamines may therefore be released through coal liquefaction effluents into natural waters at mg/ℓ levels. Although acute toxicity thresholds to aquatic organisms of many individual dihydric phenols and arylamines have been tabulated (McKEE and WOLF 1963), potential toxic interactions between compounds of the two classes are not known. We have, therefore, investigated acute toxicities of mixtures of resorcinol and 6-methylquinoline to Daphnia magna (a representative zooplankter) to assist in assessing the potential effects of coal conversion effluents on aquatic biota.

MATERIALS AND METHODS

Daphnia magna were maintained in laboratory cultures; two-to four-day old animals were isolated and utilized in the test. No reproduction occurred during the test period. Ten Daphnia were incubated in 250 ml of each toxicant test solution in 400 ml beakers covered with watch glasses. Temperature was maintained at 20 ± 0.5°C in a growth chamber under a 12-hour light/dark fluorescent lighting regime. Ten drops of homogenized "Trout Chow" (Ralston Purina Co.) were added initially as food.

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Toxicant test solutions were composed of resorcinol and 6-methylquinoline, both individually and in mixtures. Reagent-grade dry chemicals (Aldrich, Chicago) were dissolved in membrane-filtered spring water to obtain stock solutions. Preliminary 48-hour LC_{50} values obtained with adult Daphnia were 2.0 and 11 mg/ℓ for resorcinol and 6-methylquinoline, respectively. Stock solutions of each compound were prepared in spring water at concentrations 10 times the preliminary 48-hour LC_{50} values (i.e., resorcinol:20 mg/ℓ and 6-methylquinoline:110 mg/ℓ). Three stock solutions of mixtures were prepared by combining aliquots of the single-component stock solutions in 1:3, 1:1, and 3:1 proportions (v/v). Stock solution compositions are listed in Table 1.

TABLE 1

Compositions of Stock Solutions

Stock Solution	Proportion of Stock Solutions (v/v; Resorcinol: 6-Methylquinoline)	Concentrations of Components, mg/ℓ	
		Resorcinol	6-Methylquinoline
A	1:0	20.0	0
B	0.75:0.25	15.0	27.5
C	0.5:0.5	10.0	55.0
D	0.25:0.75	5.0	82.5
E	0:1	0	110.0

Individual test solutions were formulated by dilution of appropriate aliquots of the five stock solutions with filtered spring water to final volumes of 250 ml. In each test solution the sum of the concentrations of resorcinol and 6-methylquinoline was equal to 0.06, 0.08, 0.10, 0.12, 0.14 or 0.16 times the sum of the stock solution concentrations; the range was selected to bracket 48-hour LC_{50} values predicted from initial toxicity data. Control beakers of spring water without added toxicants were included. The pH remained 7.5 both after addition of toxicants and through the course of the experiment. Test solution compositions are presented in Table 2. The entire test matrix was run in duplicate; data for 70 individual test solution beakers were therefore obtained. Values for 48-hour LC_{50} toxicities and 95 percent confidence intervals, expressed as fractional concentrations of the stock solutions, were obtained by computerized PROBIT analytical procedures. Concentrations of resorcinol and 6-methylquinoline in each 48-hour LC_{50} mixture were then calculated from compositions of the stock solutions (Table 1).

RESULTS AND DISCUSSION

Mixtures of resorcinol and 6-methylquinoline were less toxic than either pure compound tested alone (Table 3). Concentrations of resorcinol and 6-methylquinoline in each LC_{50} mixture were cal-

TABLE 2

Preparation of Test Solutions from Stock Solution Mixtures

Volume of stock solution added, ml[1]	Fractional concentration of stock solution	Concentrations of resorcinol (R) and 6-methylquinoline (MQ) in diluted stock solution mixture, mg/ℓ									
		A[2]		B		C		D		E	
		R	MQ	R	MQ	R	MQ	R	MQ	R	MQ
0	0	0	0	0	0	0	0	0	0	0	0
15	0.06	1.2	0	0.9	1.65	0.6	3.3	0.3	4.95	0	6.6
20	0.08	1.6	0	1.2	2.2	0.8	4.4	0.4	6.6	0	8.8
25	0.10	2.0	0	1.5	2.75	1.0	5.5	0.5	7.75	0	11.0
30	0.12	2.4	0	1.8	3.3	1.2	6.6	0.6	9.9	0	13.2
35	0.14	2.8	0	2.1	3.85	1.4	7.7	0.7	11.55	0	15.4
40	0.16	3.2	0	2.4	4.4	1.6	8.8	0.8	13.2	0	17.6

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[1] Diluted to 250 ml with filtered spring water.

[2] Stock solution series identification letter.

TABLE 3

Toxicities of Mixtures of Resorcinol and 6-Methylquinoline to *Daphnia magna*

Stock Solution Series	48-hr LC_{50}, fraction of stock solution (95% C.I. in parentheses)	Standard Error of 48-hr LC_{50}	Concentrations of Resorcinol (R) and 6-Methylquinoline (MQ) at 48-hour LC_{50} (95 percent Confidence Intervals in parentheses)			
			mg/ℓ		Toxic Unit	
			R	MQ	R	MQ
A	0.064 *(0.025-0.081)*	0.0096	1.28 *(0.50-1.62)*	0	1.00[1] *(0.39-1.26)*	0
B	0.119 *(0.102-0.146)*	0.0095	1.79 *(1.53-2.19)*	3.28 *(2.80-4.00)*	1.40 *(1.20-1.71)*	0.26 *(0.22-0.32)*
C	0.142 *(0.128-0.169)*	0.0091	1.42 *(1.28-1.69)*	7.8 *(7.0-9.3)*	1.11 *(1.00-1.31)*	0.63 *(0.56-0.75)*
D	0.127 *(0.116-0.140)*	0.0056	0.63 *(0.58-0.70)*	10.5 *(9.6-11.5)*	0.49 *(0.45-0.55)*	0.85 *(0.77-0.93)*
E	0.113 *(0.103-0.123)*	0.0047	0	12.4 *(11.3-13.5)*	0	1.00[1] *(0.90-1.08)*

[1] By definition.

culated in terms of Toxic Units of each (after SPRAGUE and RAMSAY 1965). One Toxic Unit (T.U.) is defined to be the 48-hour LC_{50} of either compound tested individually. The total toxicant concentration in each mixture is thus the sum of the number of Toxic Units each of resorcinol and 6-methylquinoline present in the mixture. Mortality among controls was minimal (two percent) and thus was not included in data analysis.

The data are presented graphically in Figure 1 according to SPRAGUE (1970) by plotting concentrations (in T.U.) of each toxic component along the x- and y-axes. Lines and areas of interaction are labelled according to both SPRAGUE and WARREN (1971). SPRAGUE has adapted the nomenclature of GADDUM (1948) to aquatic toxicological situations. An additive interaction occurs when the effect of a combination of toxicants is identical to that produced by an equivalent concentration of either toxicant alone. Thus, if 1.0 Toxic Units (T.U.) of A in the absence of B kills 50 percent of test organisms within 48 hours, and 1.0 T.U. of B in the absence of A produces the same effect, then an additive interaction would occur if 50 percent were killed by a mixture containing 0.5 T.U. each of A and B. Definition of non-additive interactions, based upon the earlier works of SPRAGUE and GADDUM, have been presented unambiguously by WARREN (1971). No interaction occurs when the effect observed may be explained as due entirely to the major toxic component: a mixture of 1.0 T.U. of A and 0.5 T.U. of B would thus produce the same effect as 1.0 T.U. of A alone.

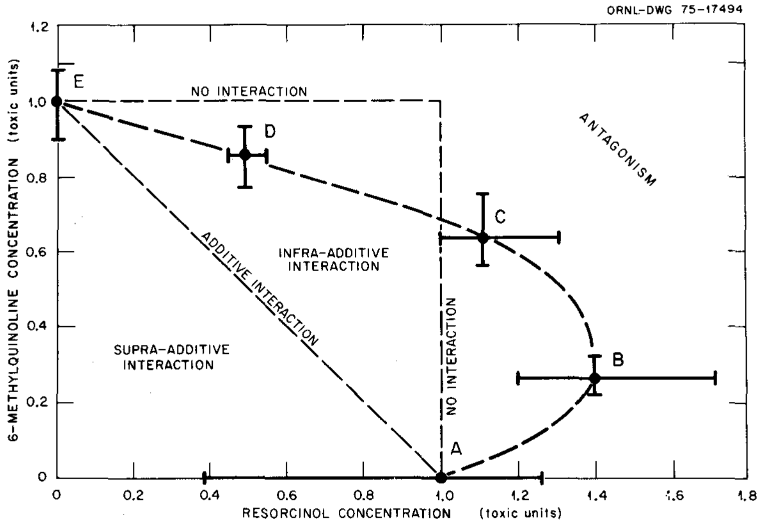

Figure 1. 48-hour LC_{50} values of resorcinol:6-methylquinoline mixtures to Daphnia magna. Vertical and horizontal bars indicate 95 percent confidence intervals. Interaction terms after SPRAGUE (1970) and WARREN (1971).

Antagonism occurs when the response is less than predicted on the basis of no interaction, while an infra-additive interaction is intermediate between non-interactive and strictly additive cases. A supra-additive interaction (potentiation in GADDUM's terminology) occurs when a response is more than additive (e.g., if a mixture of 0.3 T.U. each of A and B produced the same effect as 1.0 T.U. of either alone). Use of the term synergism was deliberately rejected by WARREN because it has been used ambiguously in the past; the term has therefore been avoided in the present study as well.

From examination of Figure 1, deviation of the data points from an additive interaction is quite apparent: none of the three "mixture" points (B, C, and D) or associated 95 percent confidence intervals lie on any diagonal line (representing additive interaction) drawn between the confidence intervals of the two pure compounds (A and E).

Whether or not the combined toxicity is non-interactive is less apparent. As plotted in Figure 1 the data suggest that antagonism occurs when resorcinol is the major toxic component, while an infra-additive interaction occurs when 6-methylquinoline is the major toxic component. F-statistics were calculated to determine whether the estimated resorcinol concentrations of points A, B and C differ significantly, and also whether the estimated 6-methylquinoline concentrations of points D and E differ significantly. The difference between the resorcinol concentrations of points A and B also was compared. Numerators of the F-statistics were estimates of variation between the concentrations of interest; denominators were pooled estimates of the variance within each estimated concentration. When only two estimated concentrations were compared the F-statistic was equivalent to the two-sample t test. Results of the calculations are summarized in Table 4. From examination of the results, departures of the estimated concentrations from the "no interaction" line appear to be real. However, further experimentation is required to determine whether the pattern of toxic interaction is characteristic of organic bases and phenols in general.

TABLE 4

Comparison of Differences Between Major-Component Concentrations of 48-Hour LC_{50} Values

Data points compared	Major component	Level of significance*	
		10%	5%
A, B, C	Resorcinol	yes	no
A, B	Resorcinol	yes	yes
D, E	6-Methylquinoline	yes	yes

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*No differences were significant at the one percent level.

The present results contrast with most earlier studies of toxic mixtures, which have generally demonstrated additive interactions between such aqueous pollutants as phenol mixtures (EUROPEAN INLAND FISHERIES ADVISORY COMMISSION 1973), metals (LLOYD 1961), ammonia and phenol (HERBERT 1962), and mixtures of ammonia, phenol, zinc, copper, and cyanide (LLOYD and JORDAN 1963). BROWN et al. (1969) found no interaction to occur between phenol, ammonia, and zinc when the latter toxicant comprised greater than 74 percent of the total predicted toxicity. Additive, infra-additive, and non-interactive toxicities to Daphnia, of pairs and trios of salts, were observed by FREEMAN and FOWLER (1953). These earlier investigations, however, examined effects of inorganic compound mixtures or highly-similar organic compounds; the present study, in contrast, has dealt with two dissimilar organic contaminants. The present work thus indicates that addition of fractional toxicities may produce substantial errors in prediction of toxicity of mixtures of non-homologous organic components.

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