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The acute toxicity of phenol and unionized ammonia, separately and together, to the ephemeropteran *Baetis rhodani* (Pictet)

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Abstract

The acute toxicity of phenol and ammonia, singly and in combination, to larvae of the ephemeropteran, *Baetis rhodani*, was examined using a computerized continuous flow system in the laboratory. The 24 h LC_{50} values (with 95% confidence intervals) were calculated to be 29.9 (17.3–51.5) mg phenol litre^{-1} and 8.2 (2.0–33.0) mg un-ionized ammonia litre^{-1}. When phenol and ammonia were together in low concentration (<20 mg litre^{-1} and <3 mg litre^{-1} [un-ionized], respectively), they expressed their toxicity additively, but at higher concentrations they behaved in a greater-than-additive manner. When the 24 h LC_{50} values were used to predict the toxicity of a coking plant effluent, containing principally ammonia and phenol, it was found that the *B. rhodani* larvae died quicker than expected indicating the presence of other toxic chemicals. This exemplifies the value of using direct toxicity assessments to detect the presence of unknown toxicants.

Keywords: *Baetis rhodani*; Ephemeroptera; Macro-invertebrates; Phenol; Ammonia

1. Introduction

In the UK, river water quality is protected through the application of discharge consents (National Rivers Authority, 1994). Usually, maximum permitted concentrations are specified for each toxicant in the discharge. The data used to determine these concentrations are based principally on laboratory studies in which monospecific populations of a small range of species have been exposed to single toxicants under controlled environmental conditions. However, in reality, surface waters are frequently affected by a combination of toxicants operating in a variable environment. Often, the interaction between two or more toxicants is additive, but synergistic and antagonistic interactions are also known (Hellawell, 1986). Because studies on mixtures of poisons are rare, the occurrence of synergistic and antagonistic interactions may well be commoner than the literature indicates.

The objective of the investigation reported here was to determine the sensitivity of larvae of the ephemeropteran, *B. rhodani* (Pictet), to ammonia and phenol, separately and together. This was part of a broader study on the impact of a chemically complex effluent from a coking works on the macro-invertebrate fauna of the receiving stream (Khatami, 1996). The Cwm Coking Works at Llantwit Fardre, near Pontypridd, South Wales, was constructed in 1958 and currently produces some 350 000 tonnes of coke a year, primarily for use in the European metallurgical industry. The process results in a waste liquor which after treatment on site was, until 1994, discharged intermittently into the Nant Myddlyn, a third order channel (1:50 000 scale; Strahler, 1964). This liquor is now discharged into the sewerage system. The discharge into the Nant Myddlyn contained a complex mixture of chemicals; principally ammonia and phenol, as well as coal particles (Khatami, 1996).

Apart from its presence in the effluent from coking works, ammonia occurs widely as a pollutant, usually derived from sewage works' effluents, combined sewer overflows and farm-animal husbandry. Its toxicity to aquatic life is very variable and dependent on water chemistry conditions (Alabaster and Lloyd, 1980). Relatively little is known about its toxicity to macro-invertebrates and studies are needed to justify consents. Phenol is a frequent contaminant of freshwaters due to its presence in effluents from the resin, oil, chemical and coal industries. Knowledge about its toxicity to macro-invertebrates is limited.

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B. rhodani is widespread and often abundant in Britain (Elliott et al., 1988) and was found to be a numerically important component of the macro-invertebrate fauna throughout most of the Nant Myddlyn. However, it was absent from samples collected 0.6 km and 1.1 km downstream of the coking works' outfall, although present in samples collected upstream of the outfall and 2.4 km downstream of the outfall (Khatami, 1996). A provisional explanation for the absence of *B. rhodani* downstream of the coking works was that the effluent was toxic to it. Although some information about the acute toxicity of ammonia and phenol as single toxicants to this species is available (Williams et al., 1984, 1986; Green et al., 1985), nothing is known, as far as we are aware, about the toxicity of these chemicals in combination.

2. Materials and methods

2.1. The test animal

Larvae of *B. rhodani* (*ca.* 7 mm long) were collected on several occasions by kick-sampling an unpolluted reach of the Nant Myddlyn upstream (National Grid Reference ST068868) of the coking works. Conductivity, total hardness, pH and un-ionized ammonia content were measured on each occasion. The methods used are described later. The conductivity varied from 266 to 397 $\mu S\,cm^{-1}$, total hardness from 66.5 to 77.1 mg $CaCO_3$ $litre^{-1}$, pH from 7.06 to 7.35, and the un-ionized ammonia content was 0.005 mg $litre^{-1}$. Once collected, the larvae were transferred to a tank containing stream-water and transported to the laboratory in Cardiff where they were placed in a stock tank containing continuously aerated dechlorinated mains-water maintained at the experimental temperature (13°C). Acclimation prior to use in experiments was for at least 24 h, and during this period the larvae were fed on discs of conditioned (Bird and Kaushik, 1985) horse chestnut (*Aesculus hippocastanum* L.) leaves.

2.2. Single toxicant experiment

A computerized, continuous flow-through dosing system (Water Research Centre Continuous Flow Toxicity Test Rig, Medmenham, England), housed in a constant temperature room at 13°C, was used to maintain the concentration of these volatile toxicants. Approximately 96% of the test solution was replaced in 9 h.

In the first experiment, phenol was supplied from a 400 mg phenol $litre^{-1}$ stock solution to give nominal concentrations in the six tanks (each 15.5×25×15 cm and containing about 5 litres of solution) of 0 (control), 4, 10, 20, 30 and 40 mg phenol $litre^{-1}$. The dilutant was dechlorinated mains-water that had passed through carbon filters. In the second experiment, ammonia was added to the six tanks from a stock solution of 6000 mg NH_4Cl $litre^{-1}$ to give nominal concentrations of 0, 10, 30, 60, 100 and 125 mg $litre^{-1}$. Sodium hydroxide was added to maintain the pH at about 8.0 so that approximately 2% of the ammonia was un-ionized. The actual quantities of un-ionized ammonia present in each tank were calculated using the formula provided by Alabaster and Lloyd (1980) which takes account of the environmental pH and temperature. The un-ionized fraction was ascertained because previous studies on the toxicity of ammonia (Alabaster and Lloyd, 1980; Williams et al., 1986) had shown that it was the un-ionized molecule that determined the acute toxicity of ammonia to the fish and macro-invertebrates investigated.

In both experiments, the concentrations of toxicant used were based on information gained from the literature and took account of the concentrations known to have occurred in the Nant Myddlyn.

Ten larvae were assigned randomly to each tank and sufficient discs of conditioned horse chestnut leaves were added to provide food for the duration of the experiment. The larvae were observed regularly and death was recorded if an animal failed to respond to 15 s of mechanical stimulation. Dead animals were removed from the tanks. Computerized versions of the Litchfield (1949) time-response and the Litchfield and Wilcoxon (1949) concentration-response methods were used to analyze and statistically compare the mortality data. These versions enabled not only the LC_{50} values to be calculated, but also their confidence intervals. The latter are important when comparing LC_{50} values, although they are rarely provided in the toxicological literature.

Various water-quality variables were monitored regularly throughout the experiments. Temperature (±1°C), pH (±0.1), dissolved oxygen (±1% of the measured value) and electrical conductivity (±1% of the measured value) were determined using appropriate digital meters. Total hardness was determined by atomic absorption spectrophotometry after filtering the water sample through 0.45 μm porosity filter paper. Ammonia and phenol concentrations were measured using a WPA Hydrocheck HC 6000 Photometer (WPA Ltd, Linton, Cambridge, England). This colorimetric system used p-nitroaniline to measure phenol concentration at 445 nm and indophenol to measure ammonia concentration at 690 nm. The limits of detection were 0.2 mg phenol $litre^{-1}$ and 0.05 mg ammonia $litre^{-1}$.

2.3. Combined toxicant experiments

The first experiment was carried out as described already, except that the stock solution supplied comprised a mixture of phenol (400 mg $litre^{-1}$) and ammonia (6000 mg $litre^{-1}$), which, when diluted by the dosing

system, provided phenol–ammonia mixtures of increasing total toxicity in the five test tanks.

In the second experiment, *B. rhodani* larvae were exposed to effluent collected from the coking plant. This was obtained from the effluent pipe leading from the biological treatment plant. Except for the different stock solution, the experiment was carried out as before. Dilution of the stock solution was such that the six tanks contained effluent concentrations of 99, 90, 80, 70, 60 and 0% of the full strength effluent. The flow rate to each tank ensured 98% replacement of the effluent every 6 h.

3. Results

3.1. Single toxicant experiment

Water quality data, phenol and ammonia concentrations are displayed in Tables 1 and 2. The dissolved oxygen concentration was at least 85% of the air saturation value throughout. No mortality occurred amongst the control animals during the phenol study; a 10% control mortality was recorded during the ammonia study.

All the phenol concentrations used were toxic to *B. rhodani*; the lowest concentration (2.1 mg litre^{-1}) caused a 50% mortality within 11 days (Fig. 1). However, comparison of reaction time ratios (Litchfield, 1949) showed that there was a sharp and significant ($p=0.05$) increase in phenol toxicity once the concentration exceeded 17.6 mg litre^{-1}. There was no significant difference amongst the slope functions. The 24 h LC_{50} value (with 95% confidence interval) was calculated to be 29.9 (17.3–51.5) mg phenol litre^{-1}.

Un-ionized ammonia was also toxic to the *B. rhodani* larvae at the concentrations used in the experiment; the lowest concentration, 0.32 mg litre^{-1}, caused a 50% mortality in 36 h (Table 2). The LT_{50} values declined as the un-ionized ammonia concentration increased, but unlike phenol, there was no sharp increase in toxicity over the range of ammonia concentrations used (Fig. 2). There was no significant difference amongst the slope functions. The 24 h LC_{50} value (with 95% confidence interval) was calculated to be 8.2 (2.0–33.0) mg un-ionized ammonia litre^{-1}.

3.2. Combined toxicant experiments

3.2.1. Phenol and ammonia

Water quality data, phenol and ammonia concentrations are shown in Table 3. Control mortality during this experiment was 10%. The concentrations of phenol and ammonia used in the combined toxicant experiment differed from those used in the single toxicant experiments. Therefore, a direct comparison of LT_{50} values between the single and combined toxicant experiments was not possible. Instead, we predicted the LT_{50} values for the combined toxicants from the sum of fractions of the 24 h LC_{50} values (Brown, 1968) for phenol and un-ionized ammonia, as derived from the single toxicant experiments (Table 4). A value of 1.0 for the sum of fractions indicates that 50% of the animals would be expected to die within 24 h if the toxicity of the combined toxicants is additive. If the value is less than 1.0, the LT_{50} would be expected to be greater than 24 h and if more than 1.0, the LT_{50} value would be less than 24 h. The predicted LT_{50} values for the combined toxicants, assuming a linear relationship between the potency of the combined toxicants and the LT_{50} value, are compared with the observed LT_{50} values in Table 5. The results indicate that at the lower concentrations of phenol and un-ionized ammonia, at least up to 2.73 mg

Table 1
Water chemistry (mean ± SE), median lethal times (LT_{50}) and slope functions (S) recorded for the phenol toxicity experiment

Parameter	Tank					
	Control	1	2	3	4	5
T (°C)	13.1 ± 0.1 ($n=12$)	13.1 ± 0.1 ($n=12$)	13.1 ± 0.1 ($n=12$)	13.1 ± 0.1 ($n=12$)	13.1 ± 0.1 ($n=12$)	13.1 ± 0.1 ($n=12$)
pH	7.85 ± 0.03 ($n=12$)	7.79 ± 0.04 ($n=12$)	7.74 ± 0.07 ($n=12$)	7.73 ± 0.07 ($n=11$)	7.86 ± 0.03 ($n=4$)	7.88 ± 0.02 ($n=4$)
Electrical conductivity (μS cm^{-1})	251 ± 3.8 ($n=12$)	249 ± 3.4 ($n=12$)	248 ± 3.3 ($n=12$)	247 ± 3.0 ($n=10$)	240 ± 1.8 ($n=4$)	240 ± 1.6 ($n=4$)
Hardness (mg litre^{-1} $CaCO_3$)	144.8 ± 18.6 ($n=12$)	140.9 ± 20.4 ($n=12$)	138.5 ± 17.0 ($n=12$)	137.5 ± 15.9 ($n=11$)	125.8 ± 11.4 ($n=4$)	120.2 ± 2.8 ($n=3$)
Phenol (mg litre^{-1})	Below detectable limits	2.1 ± 0.15 ($n=15$)	8.5 ± 0.22 ($n=14$)	17.6 ± 0.39 ($n=12$)	26.4 ± 0.87 ($n=4$)	36.3 ± 0.44 ($n=3$)
LT_{50} (hour)	—	250	132	110	21.5	12.6
(95% C.I.)		(136–461)	(61–288)	(67–180)	(11.9–38.6)	(6.6–24)
S	—	2.5	3.4	2.1	2.5	2.8
(95% C.I.)		(1.7–3.6)	(2.1–5.3)	(1.6–3.1)	(1.6–3.9)	(1.7–4.5)

n = number of samples.

NH_3 litre^{-1} and 17.6 mg phenol litre^{-1}, these toxicants conformed to the additive model and their combined toxicity could be predicted by summing fractions of the 24 h LC_{50} values. However, at higher concentrations, phenol and ammonia in combination behaved in a greater-than-additive, i.e. more toxic, manner.

3.2.2. Coking plant effluent

Water chemistry data, phenol and ammonia concentrations are displayed in Table 6. The conductivity of the water in this experiment was much higher than in the previous experiments, except for the control. The predicted LT_{50} values for the various dilutions of the coking

Table 2
Water chemistry (mean ± SE), median lethal times (LT_{50}) and slope functions (S) recorded for the ammonia toxicity experiment

Parameter	Tank					
	Control	1	2	3	4	5
T (°C)	13.1 ± 0.1 (n = 8)	13.1 ± 0.1 (n = 8)	13.1 ± 0.1 (n = 8)	13.1 ± 0.1 (n = 7)	13.1 ± 0.1 (n = 7)	13.1 ± 0.1 (n = 6)
pH	7.80 ± 0.05 (n = 9)	7.98 ± 0.10 (n = 9)	8.25 ± 0.07 (n = 8)	8.26 ± 0.04 (n = 7)	8.28 ± 0.03 (n = 7)	8.32 ± 0.01 (n = 6)
Electrical conductivity (μS cm^{-1})	296 ± 6.6 (n = 9)	354 ± 1.5 (n = 9)	516 ± 3.0 (n = 8)	760 ± 5.5 (n = 7)	1022 ± 6.2 (n = 7)	1235 ± 12.5 (n = 6)
Hardness (mg litre^{-1} $CaCO_3$)	153.4 ± 13.0 (n = 8)	152.7 ± 5.3 (n = 8)	156.3 ± 12.1 (n = 8)	157.7 ± 8.0 (n = 7)	156.8 ± 13.4 (n = 7)	144.2 ± 4.5 (n = 6)
Total ammonia (mg litre^{-1})	Below detectable limits	10.39 ± 0.8 (n = 8)	32.86 ± 1.9 (n = 8)	62.71 ± 3.5 (n = 7)	99.69 ± 3.9 (n = 7)	127.38 ± 7.3 (n = 5)
NH_3 (mg litre^{-1})	Below detectable limits	0.32 ± 0.07 (n = 8)	1.39 ± 0.1 (n = 8)	2.69 ± 0.2 (n = 7)	4.34 ± 0.38 (n = 7)	6 ± 0.37 (n = 5)
LT_{50} (hour)	—	36	30	26	18	13.5
(95% C.I.)		(27–49)	(18–51)	(15–44)	(10–32)	(5–34)
S	—	1.6	2.2	2.3	2.5	4.3
(95% C.I.)		(1.3–1.9)	(1.7–3)	(1.5–3.4)	(1.6–3.8)	(2.2–8.6)

n = number of samples.

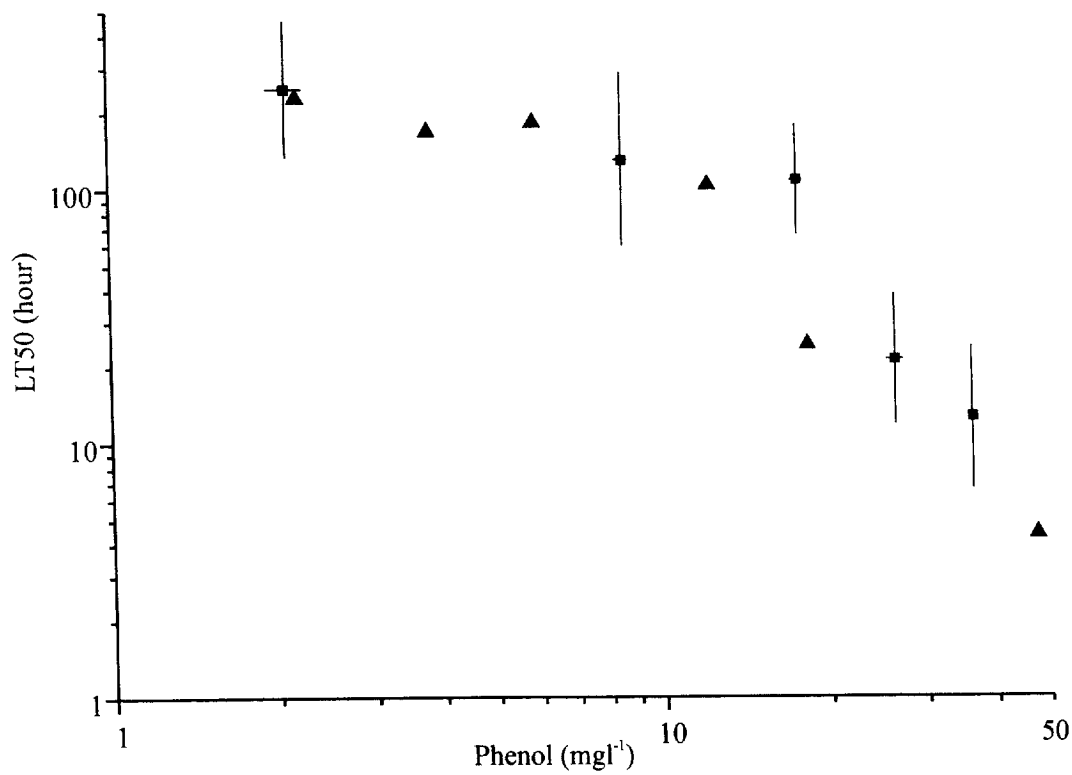

Fig. 1. Concentration-mortality curve for the toxicity of phenol to larvae of *Baetis rhodani* obtained by the present authors (■) compared with that obtained by Green et al. (1985; ▲). Vertical and horizontal error bars indicate 95% confidence intervals.

plant effluent, based on the summed fractions of the 24 h LC_{50} values for the phenol and un-ionized ammonia present in the effluent, are shown in Table 7 along with the observed LT_{50} values. The predicted LT_{50} values assume a linear relationship between the potency of the combined phenol and un-ionized ammonia concentrations

Table 3
Water chemistry (mean ± SE), median lethal times (LT_{50}) and slope functions (S) recorded for the experiment that used a combination of phenol and ammonia

Parameter	Tank					
	Control	1	2	3	4	5
T (°C)	13.1 ± 0.1 (n = 10)	13.1 ± 0.1 (n = 10)	13.1 ± 0.1 (n = 8)	13.1 ± 0.1 (n = 4)	13.1 ± 0.1 (n = 3)	13.1 ± 0.1 (n = 3)
pH	8.03 ± 0.03 (n = 11)	8.18 ± 0.03 (n = 11)	8.32 ± 0.06 (n = 11)	8.36 ± 0.1 (n = 6)	8.42 ± 0.18 (n = 3)	8.48 ± 0.2 (n = 3)
Electrical conductivity ($\mu S\,cm^{-1}$)	307 ± 5.9 (n = 11)	370 ± 3.6 (n = 11)	553 ± 2.8 (n = 11)	870 ± 8.7 (n = 6)	1211 ± 7.2 (n = 3)	1483 ± 9.0 (n = 3)
Hardness (mg litre^{-1} $CaCO_3$)	163.7 ± 8.2 (n = 10)	157.9 ± 6.1 (n = 10)	145.4 ± 7.9 (n = 8)	142 ± 6.4 (n = 4)	141.7 ± 15.1 (n = 3)	139.9 ± 8.9 (n = 3)
Phenol (mg litre^{-1})	Below detectable limits	1.9 ± 0.15 (n = 11)	8.5 ± 0.28 (n = 9)	17.6 ± 0.58 (n = 6)	26.2 ± 1.01 (n = 3)	37.4 ± 1.21 (n = 3)
Total ammonia (mg litre^{-1})	Below detectable limits	8.83 ± 1.20 (n = 11)	24.92 ± 2.04 (n = 9)	65.48 ± 5.63 (n = 6)	83.65 ± 3.68 (n = 3)	105.23 ± 8.31 (n = 3)
NH_3 (mg litre^{-1})	Below detectable limits	0.28 ± 0.49 (n = 11)	1.01 ± 0.12 (n = 9)	2.73 ± 0.28 (n = 6)	3.74 ± 0.37 (n = 3)	5.31 ± 0.69 (n = 3)
LT_{50} (hour)	—	213	63	24	2.9	1.6
(95% C.I.)		(159–286)	(46–86)	(8–74)	(1.8–4.7)	(0.9–3.6)
S	—	1.6	1.6	6.1	2.1	2.4
(95% C.I.)		(1.3–1.8)	(1.3–2.0)	(2.6–14.1)	(1.5–3.0)	(1.6–2.6)

n = number of samples.

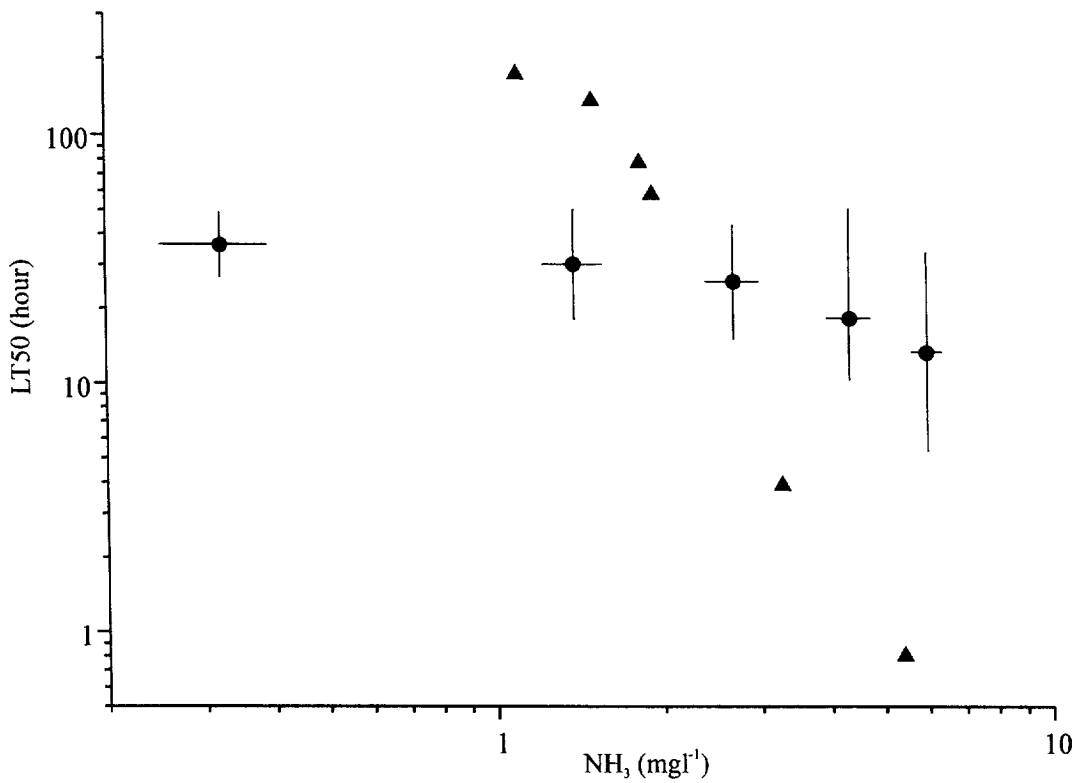

Fig. 2. Concentration mortality curves for the toxicity of un-ionized ammonia to larvae of *Baetis rhodani* obtained by the present authors (●) and by Williams et al. (1986; ▲). Vertical and horizontal error bars indicate 95% confidence intervals.

and the LT_{50} values. The *B. rhodani* larvae died much faster than predicted on the basis of the phenol and un-ionized ammonia components of the effluent alone. No mortality was observed amongst the control animals.

4. Discussion

There are few studies with which the present investigation can be compared. In particular, the need for further data on the toxicity of ammonia to aquatic invertebrates has been emphasized by Adams and Bealing (1994).

Green et al. (1985) studied the toxicity of phenol to several aquatic macro-invertebrate species including *B. rhodani*. They used a flow-through system, as in this study, but their experiments were carried out at a lower temperature (11°C) and in softer water (99.5 mg $CaCO_3$ $litre^{-1}$). Also, their *B. rhodani* larvae were obtained from a different river and were larger (8–10 mm; pers. comm). Despite these differences, the results obtained by Green et al. (1985) for *B. rhodani* are very similar to

Table 4
The concentrations of phenol and un-ionized ammonia used in the combined toxicant experiment, and expressed as fractions of the 24 h LC_{50} values obtained for phenol and un-ionized ammonia, and the sum of these fractions for each mixture

Combined toxicant	Concentration ($mg\ litre^{-1}$)					24 h LC_{50} of single test ($mg\ litre^{-1}$)	Toxic unit of 24 h LC_{50}				
	Tank						Tank				
	1	2	3	4	5		1	2	3	4	5
NH_3	0.28	1.01	2.73	3.74	5.31	8.2	0.03	0.12	0.33	0.46	0.65
Phenol	1.90	8.50	17.60	26.20	37.40	29.9	0.06	0.28	0.59	0.88	1.25
Sum toxic unit							0.09	0.40	0.92	1.34	1.90

Table 5
Predicted and observed median lethal times (LT_{50} values) for phenol and un-ionized ammonia in combination

	Tank				
	1	2	3	4	5
Sum of toxic units	0.09	0.40	0.92	1.34	1.90
Predicted LT_{50} based on toxic unit value (hour)	267	60.0	26.0	17.9	12.6
Observed LT_{50} (hour)	213	63	24	2.9	1.6
(95% C.I)	(159–286)	(46–86)	(8–74)	(1.8–4.7)	(0.9–3.6)

The predicted values assume that, when in combination, these toxicants express their toxicity in an additive manner, and also that a linear relationship exists between the potency of the combined toxicants and the LT_{50} value.

Table 6
Water chemistry (mean ± SE, $n = 5$), median lethal times (LT_{50}), and slope functions (S) recorded for the experiment that used dilutions of the coking plant effluent

Parameter	Control	Effluent concentration (%)				
		99	90	80	70	60
T (°C)	16.96 ± 0.4	16.96 ± 0.4	16.96 ± 0.4	16.96 ± 0.4	16.96 ± 0.4	16.96 ± 0.4
pH	8.06 ± 0.05	6.41 ± 0.42	6.83 ± 0.31	6.93 ± 0.29	6.99 ± 0.30	7.32 ± 0.21
Electrical conductivity ($\mu S\ cm^{-1}$)	390 ± 0.01	7500 ± 0.08	7270 ± 0.14	6590 ± 0.11	5870 ± 0.12	4920 ± 0.12
Total ammonia ($mg\ litre^{-1}$)	Below detectable limits	0.258 ± 0.03	0.228 ± 0.03	0.201 ± 0.31	0.178 ± 0.02	0.162 ± 0.02
NH_3 ($mg\ litre^{-1}$)	Below detectable limits	0.0010 ± 0.0007	0.0013 ± 0.0008	0.0014 ± 0.0009	0.0015 ± 0.001	0.0018 ± 0.001
Phenol ($mg\ litre^{-1}$)	Below detectable limits	4.619 ± 0.08	4.265 ± 0.11	3.800 ± 0.13	3.338 ± 0.10	2.280 ± 0.05
LT_{50} (hour)	—	21.3	21.9	28.6	29.2	> 42
(95% C.I.)		(19.7–23)	(18.4–26.0)	(9.1–89.6)	(20.3–41.8)	
S	—	1.1	1.3	6.2	1.7	—
(95% C.I.)		(1.0–1.2)	(1.1–1.5)	(2.6–14.7)	(1.3–2.1)	

ours (Fig. 1). This helps confirm the validity of their results and hence their conclusion that *B. rhodani* has a greater sensitivity to phenol than most aquatic species, including fish species, that have been investigated (Green et al., 1985). However, the 24 h LC_{50} value of 19 mg phenol litre^{-1} obtained by Green et al. (1985) indicates a greater sensitivity to phenol than is shown by our results (24 h LC_{50} = 29.9 mg litre^{-1}). While this may be due partly to differences between the experimental conditions provided, particularly temperature (e.g. Green et al., 1988, reported increased toxicity of phenol to the isopod *Asellus aquaticus* at lower temperatures), we suspect that the difference largely reflects experimental variation (Fig. 1); the 95% confidence interval calculated for the 24 h LC_{50} value in the present study (17.3–51.5) overlaps the 24 h LC_{50} value obtained by Green et al. (1985). Unfortunately, these authors did not provide a confidence interval for their value.

The sensitivity of *B. rhodani* to ammonia has been studied previously by Williams et al. (1986) who also used a flow-through system. However, they used a lower temperature (11.5°C) and softer water (97.6–105.8 mg $CaCO_3$ litre^{-1}) than we did. Their results differ markedly from ours (Fig. 2); the *B. rhodani* larvae used in their experiment were less sensitive than ours at the lower concentrations of un-ionized ammonia (< 2 mg NH_3 litre^{-1}), but more sensitive at higher concentrations. The environmental differences between the

Table 7
Predicted and observed median lethal times (LT_{50}) for dilutions of the coking plant effluent

	Dilution of coking plant effluent (%)				
	99	90	80	70	60
Sum of fractions of the 24 h LC_{50} values	0.15	0.14	0.13	0.11	0.076
Predicted LT_{50} value (hour)	160	171	184	218	316
Observed LT_{50} value (hour)	21.3	21.9	28.6	29.2	> 42
(95% C.I)	(19.7–23.0)	(18.4–26.0)	(9.1–89.6)	(20.3–41.8)	—

The predicted values are based on the sum of fractions of the 24 h LC_{50} values for phenol and un-ionized ammonia. The actual concentrations are given in Table 6.

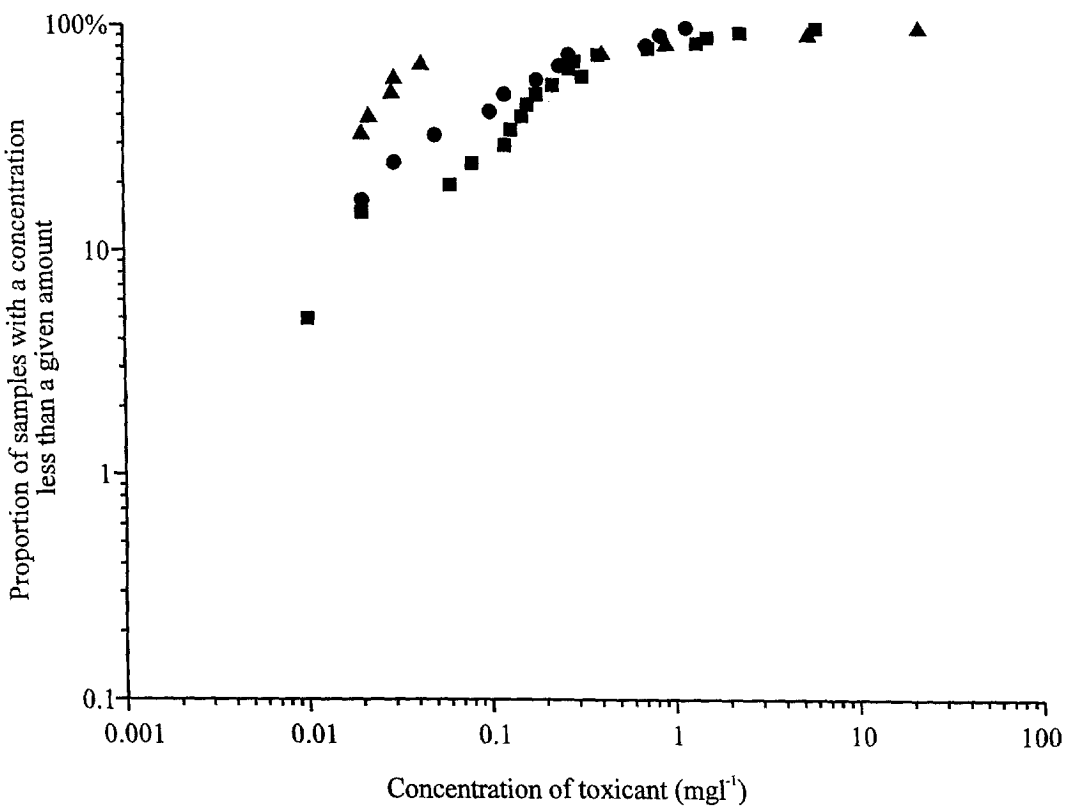

Fig. 3. Concentrations of phenol (▲) and un-ionized ammonia (■) 0.6 km downstream of the coking plant outfall, and the concentration of un-ionized ammonia (●) 1.1 km downstream of the outfall in 1993. These data were provided by the National Rivers Authority.

experiments carried out by Williams et al. (1986) and ourselves do not appear to account for the different results obtained. The 24 h LC_{50} value of 2.3 mg NH_3 litre^{-1} (no confidence interval provided) obtained by Williams et al. (1986) is less than the 8.2 mg NH_3 litre^{-1} calculated by us, although it lies just within the confidence interval (2.0–33.0). These values show *B. rhodani* to be one of the less sensitive macro-invertebrate species to un-ionized ammonia amongst those studied (Williams et al., 1986; Maltby, 1995) and they are well in excess of the 24 h LC_{50} values that have been obtained for various fish species, and of the water quality standard (95 percentile; 0.025 mg un-ionized NH_3 litre^{-1}) set to protect freshwater fisheries in the European Union (Alabaster and Lloyd, 1980; Lloyd, 1992; Adams and Bealing, 1994).

No study has been carried out previously on the toxicity of mixtures of phenol and ammonia on *B. rhodani*; in fact, few such studies exist for any species (Alabaster et al., 1994). Our study indicated that these toxicants were additive at lower concentrations (less than about 3 mg un-ionized NH_3 litre^{-1} and 20 mg phenol litre^{-1}) but greater than additive at higher concentrations. Herbert (1962) examined the toxicity of mixtures of phenol and ammonia to rainbow trout. He observed a greater than additive relationship over the range of concentrations used (almost entirely within our lower concentration as defined earlier) so that the fish died faster than predicted. However, he considered the deviation from additive to be small enough for the toxicity of a mixture of phenol and ammonia to be acceptably predictable on the basis of the additive model although a physiological mechanism that would account for an additive interaction between these toxicants remains elusive (Alabaster et al., 1994). The differences between our observations and those of Herbert (1962) probably reflect the different physiologies of the species involved, but differences between the experimental conditions provided may be important. Further work is needed.

One of the reasons for investigating the toxicity of ammonia and phenol was to establish whether the absence of *B. rhodani* from sites immediately downstream of the coking works was caused by the coking works' effluent. It is apparent from our results that, during 1993, when the longitudinal distribution of *B. rhodani* in the Nant Myddlyn was surveyed (Khatami, 1996), the concentrations of phenol and, in particular, un-ionized ammonia at the sites downstream of the coking works from which *B. rhodani* was absent were sufficiently high when considered individually (Fig. 3) or in combination to be acutely toxic to this species. These two chemicals were the major components of the coking works' effluent, but direct toxicity assessment using dilutions of the effluent (Table 7) showed that it was about 7.5 times more toxic to *B. rhodani* than expected from the concentrations of ammonia and phenol alone. Other chemicals toxic to *B. rhodani* must also have been present. This exemplifies the difficulties in setting consent conditions for such complex discharges and supports the use of direct toxicity assessment as part of the strategy for protecting surface water (Hunt et al., 1993).

In most studies of the impact of a discharge on the fauna of a river, the assumption is made that, in the absence of the discharge, the faunal community downstream of the outfall would have had the same structure as that upstream. Therefore, any observed differences are considered to be the consequence of the discharge. There is rarely an opportunity to test this assumption which may be wrong. It is known that small-scale variation is manifest in rivers and that, even where replicate channels have been manipulated to make them similar, their faunal communities have displayed differences (Armitage, 1995). During the present investigation, the coking works' effluent was, in August 1994, permanently diverted into the sewerage system for further treatment at a sewage works. This has provided us with the opportunity to monitor the response of *B. rhodani* (and other macro-invertebrates) in the Nant Myddlyn to the cessation of the coking works' effluent. On three occasions in 1993, *B. rhodani* was not found in samples collected 0.6 km and 1.1 km downstream of the coking works' outfall, although it was present upstream of the outfall and in samples collected 2.4 km downstream. In October 1994, one *B. rhodani* larva was found at the 1.1 km site, and by October 1995, the abundance of *B. rhodani* at the 1.1 km site (but not at the 0.6 km site) was the same as that upstream of the coking plant. The assumption that the 1.1 km site would have supported a similar population density of *B. rhodani* to the upstream site appears to have been valid in this case, but monitoring is continuing.

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References

Adams, N., Bealing, D., 1994. Organic pollution: biochemical oxygen demand and ammonia. In: Calow, P. (Ed.). Handbook of Ecotoxicology, Vol. 2. Blackwell Scientific Publications, Oxford, pp. 264–285.

Alabaster, J.S., Lloyd, R., 1980. Water Quality Criteria for Freshwater Fish. Butterworth, London.

Alabaster, J.S., Calamari, D., Dethlefsen, V., Konemann, H., Lloyd, R., Solbé, J.F., 1994. Mixtures of toxicants. In: Howells, G. (Ed.). Water Quality for Freshwater Fish. Gordon and Breach Science Publishers, Switzerland, pp. 145–205.

Armitage, P.D., 1995. Faunal community change in response to flow manipulation. In: Harper, D.M., Ferguson, A.J.D. (Eds.). The Ecological Basis for River Management. J. Wiley and Sons, Chichester, pp. 59–78.

Bird, G.A., Kaushik, N.K., 1985. Processing of elm and maple leaf discs by collectors and shredders in laboratory feeding studies. Hydrobiologia 126, 109–120.

Brown, V.M., 1968. The calculation of the acute toxicity of mixtures of poisons to rainbow trout. Water Research 2, 723–733.

Elliott, J.M., Humpesch, U.H., Macan, T.T., 1988. Larvae of the British Ephemeroptera: a key with ecological notes. Scientific Publications of the Freshwater Biological Association 49, 1–145.

Green, D.W.J., Williams, K.A., Pascoe, D., 1985. Studies on the acute toxicity of pollutants to freshwater macroinvertebrates. 2. Phenol. Archiv fur Hydrobiologie 103, 175–182.

Green, D.W.J., Williams, K.A., Hughes, D.R.L., Shaik, G.A.R., Pascoe, D., 1988. Toxicity of phenol to *Asellus aquaticus* (L.)—effects of temperature and episodic exposure. Water Research 22, 225–231.

Hellawell, J.M., 1986. Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier Applied Science Publishers, London.

Herbert, D.W.M., 1962. The toxicity to rainbow trout of spent still liquors from the distillation of coal. Annals of Applied Biology 50, 755–777.

Hunt, D.T.E., Johnson, I., Milne, R., 1993. The control and monitoring of discharges by biological techniques. In: Currie J.C., Pepper, A.T. (Eds.). Water and the Environment. Ellis Horwood, Chichester, pp. 139–151.

Khatami, S.H., 1996. An ecotoxicological study of the impact of a coking plant effluent on a riverine fauna. Ph.D. thesis. University of Wales, Cardiff, UK.

Litchfield, J.T., 1949. A method for rapid graphic solution of time-per cent effect curves. Journal of Pharmacology and Experimental Therapeutics 97, 399–408.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. Journal of Pharmacology and Experimental Therapeutics 96, 99–113.

Lloyd, R., 1992. Pollution and Freshwater Fish. Fishing News Books, Oxford.

Maltby, L., 1995. Sensitivity of the crustaceans *Gammarus pulex* (L.) and *Asellus aquaticus* (L.) to short-term exposure to hypoxia and unionized ammonia: observations and possible mechanisms. Water Research 29, 781–787.

National Rivers Authority, 1994. Discharge consents and compliance: the NRA's approach to control of discharges to water. NRA Water Quality Series No 17. HMSO, London.

Strahler, A.N., 1964. Quantitative geomorphology of drainage basins and channel networks. In: Chow, V.T. (Ed.). Handbook of Applied Hydrology, Section 4–11. McGraw-Hill, New York.

Williams, K.A., Green, D.W.J., Pascoe, D., 1984. Toxicity testing with freshwater macroinvertebrates: methods and application in environmental management. In: Pascoe, D., Edwards, R.W. (Eds.). Freshwater Biological Monitoring: Advances in Water Pollution Control. Pergamon Press, Oxford, pp. 81–91.

Williams, K.A., Green, D.W.J., Pascoe, D., 1986. Studies on the acute toxicity of pollutants to freshwater macro-invertebrates. Archiv fur Hydrobiologie 106, 61–70.