Effect of temperature and salinity on the toxicity of chromium to three estuarine invertebrates (*Corophium volutator*, *Macoma balthica*, *Nereis diversicolor*)

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ABSTRACT: Acute toxicity of hexavalent chromium to 3 estuarine animals (*Corophium volutator*, *Macoma balthica*, *Nereis diversicolor*) has been studied at 3 temperatures (5, 10, 15 °C) and a range of salinities (5 to 40 ‰, in 5 ‰ increments), at time intervals of up to 384 h. Toxicity increased as temperature increased and as salinity decreased. From an analysis of variance, the significant factors and their interactions were included in a response surface model for *C. volutator* and *M. balthica* separately. The range of toxicity values for a single species under a variety of environmental conditions exceeded the previously reported range of toxicity values for several species maintained in constant conditions. It is emphasized that due account should therefore be made of environmental conditions when evaluating the toxicity of chromium, in the estuarine environment.

INTRODUCTION

The limited data available on the acute toxicity of chromium to vertebrates and invertebrates in both marine and fresh-water environments have been summarized by Taylor (1981) and Mance et al. (1984). However, data on the effects of variations in salinity and temperature, which are comparable to those encountered in estuaries, in relation to chromium toxicity are not available for animals. Where such studies have been conducted with other metals (Theede, 1980; Coglianese, 1982; Cotter et al., 1982; Denton and Burdon-Jones, 1982), they have generally shown that the acute toxicity of heavy metals increases as temperature increases and as salinity decreases. Frey et al. (1983) have reported that hexavalent chromium is very inhibitory to the growth of estuarine phytoplankton in fresh-water, but less inhibitory as salinity increases. Many industrial effluents containing chromium are discharged into estuaries, but predictions of their possible effects based on the results of marine or fresh-water studies, rather than estuarine studies, may be inappropriate. In the study reported here the effects of temperature and salinity on chromium toxicity were investigated using species representing taxonomic

diversity as well as ecological importance within the estuarine ecosystem. The following species were chosen: the amphipod crustacean *Corophium volutator* (Pallas); the bivalve mollusc *Macoma balthica* (L.) and the polychaete annelid *Nereis diversicolor* (O. F. Müller).

MATERIALS AND METHODS

Animals were collected throughout the year from the Tay estuary, Eastern Scotland, with *Macoma balthica* and *Corophium volutator* from Tayport, and *Nereis diversicolor* from Balmerino. Tayport and Balmerino experience a salinity range, at low tide, of 11 to 32 ‰ and 0.2 to 20.6 ‰ respectively, with both having an annual temperature range of 3 to 16 °C (Khayrallah and Jones, 1975). Experiments were conducted in a constant temperature room at 5, 10 or 15 °C (± 0.05 °C). A regime of 12 h light, 12 h darkness was maintained.

The acute toxicity of hexavalent chromium (as potassium dichromate) was determined under static conditions following SCA protocol (Anonymous, 1980). Stock solutions of analar grade $K_2Cr_2O_7$ were prepared in water of the appropriate salinity and test solutions obtained by dilution. Chromium levels were checked

colorimetrically using S-diphenylcarbazine and sulphuric acid (J. Riley, pers. comm.). Measured concentrations closely approximated to nominal values and there was no appreciable loss of metal from solution during experimentation.

Salinities of 5 to 30 ‰ were prepared by dilution of natural sea-water with deionized water. Salinities of 35 and 40 ‰ were prepared by the addition of Gerard's sea salt to natural sea-water. The sea-water was passed through polystyrene filters to remove suspended solids before storage in sea-water tanks.

Experiments for *Corophium volutator* and *Nereis diversicolor* each had a $3 \times 7 \times 8$ factorial design with temperatures of 5, 10, 15 °C, chromium concentrations of 2, 4, 8, 16, 32, 64 and 128 ppm (+ controls) and salinities of 5, 10, 15, 20, 25, 30, 35 and 40 ‰. The experiment for *Macoma balthica* was a $3 \times 7 \times 6$ factorial design with the same temperatures and chromium concentrations as above and salinities of 15 to 40 ‰ in 5 ‰ increments. *M. balthica* is unable to survive for periods of longer than 24 h in salinities below 15 ‰. Animals were fully acclimated to each salinity and temperature for 5 d before use. Temperature acclimation was accomplished by conducting

experiments at the appropriate season (e.g. 5 °C experiments in winter). Oxygen concentration, pH, temperature, salinity, and chromium concentrations were monitored regularly. Twenty *M. balthica* and *C. volutator*, and 10 *N. diversicolor* were used for each temperature, salinity and chromium combination. Sterile sand was provided as substrate in all test vessels and no food was provided throughout the experiment. Vessels were examined, dead animals removed and test solutions changed daily for 384 h. Death was noted as gaping in *M. balthica*, and lack of muscular or pleopod activity in *N. diversicolor* and *C. volutator*.

At each time interval the cumulative % mortality was calculated following the method of Lloyd (1979). This value (expressed as probits) was plotted as a function of time (expressed logarithmically) directly onto logarithmic-probability graph paper for each of the concentrations of chromium used. A straight line was fitted by eye to each set of data, giving greater weight to those values between 25 and 75 % response. The time for 50 % mortality, the median period of survival (LT_{50}), was then read from the graph. Concentration response curves were plotted on double log paper using the LT_{50} values calculated for each

Table 1. *Corophium volutator*. Median periods of survival in hours (LT_{50}) derived graphically at 5, 10, 15 °C, 5 to 40 ‰ S, and chromium concentrations of 2 to 128 ppm

	5 °C	5	10	15	20	25	30	35	40 ‰ S
Chromium conc.	2	125	245	350	370	> 384	> 384	> 384	265
	4	88	190	270	320	> 384	> 384	> 384	135
	8	64	105	220	210	300	370	220	240
	16	39	54	105	120	170	210	310	220
	32	15	16.5	40	58	110	135	115	120
	64	5.8	14.5	22	26	50	80	85	56
	128	3.2	9.5	13.5	14	29	48	31	41
	10 °C	5	10	15	20	25	30	35	40 ‰ S
Chromium conc.	2	110	220	220	> 384	> 384	320	> 384	> 384
	4	68	130	200	220	360	230	> 384	> 384
	8	29	56	120	190	330	180	210	330
	16	15	37	100	125	155	128	170	150
	32	9	22	52	70	88	96	115	90
	64	7.5	14	22	46	56	54	85	72
	128	*1	6.5	12.5	20	18.5	36	29	34
	15 °C	5	10	15	20	25	30	35	40 ‰ S
Chromium conc.	2	44	54	115	195	140	230	145	100
	4	33	58	105	133	100	180	130	72
	8	24	37	90	76	80	155	105	56
	16	11	18.5	64	56	60	60	60	50
	32	6.6	12.5	21	30	32	48	28	25
	64	*1	6	13.5	13.5	23	27	20	19.0
	128	*1	*1	6.2	9	12	12.5	13	12.5

* Values estimated, as data was unsuitable for graphical estimation of LT_{50}

chromium concentration at each temperature and salinity combination, and a straight line fitted through the data. The median lethal concentration (LC_{50}) for 24, 48, 96, 192 and 384 h was read from each graph.

STATISTICAL ANALYSIS

Three-factor analysis of variance of untransformed LT_{50} values was partitioned into linear and quadratic forms for the main factors (temperature, concentration and salinity) and their second order interactions. Since there were no replicates, the third order interaction was taken as the error term (Davies, 1979). *Corophium volutator* and *Macoma balthica* data were analysed separately (see Tables 3 and 6). Terms significant at $p < 0.01$ were included in a response surface model for each species (*Nereis diversicolor* data could not be analysed in a similar way because the data set for this species was incomplete). Coefficients for the terms were found by multiple regression and the resulting equations used to draw isopleths of LT_{50} . (N.B. T =

temperature °C, C = concentration chromium ppm, S = salinity ‰ NaCl).

Corophium volutator:

$$LT_{50} = 172.4 - 25.5 T - 7.02 C + 13.24 S + 0.687 T^2 + 0.0477 C^2 - 0.2846 S^2 + 0.4067 TC + 1.469 TS - 0.1623 CS - 0.00221 TC^2 - 0.0867 T^2S + 0.00213 CS^2 + 0.00000565 C^2S^2$$

$$R^2 = 82.3 \%$$

Macoma balthica:

$$LT_{50} = 237.7 - 14.45 T - 1.77 C + 8.56 S - 0.02 SC + 0.00349 T^2S + 0.000475 C^2S - 0.00000992 C^2S^2$$

$$R^2 = 84.8 \%$$

RESULTS

Corophium volutator

LT_{50} values are shown in Table 1. The combined effect of temperature and salinity on median period of survival (LT_{50}) at 2 chromium levels (16 and 64 ppm) is demonstrated in Fig. 1a, b. The susceptibility of

Fig. 1. *Corophium volutator*. Effect of temperature and salinity on median periods of survival (h) at chromium concentrations of (a) 16 ppm, (b) 64 ppm

Corophium volutator to chromium depends on both factors, greatest tolerance occurring at low temperature and high salinity, greatest sensitivity occurring at high temperature and low salinity. Comparison of Fig. 1a with 1b shows the higher LT_{50} values recorded for the lower chromium concentration, though the overall pattern of response was similar at both levels.

LC_{50} values for *Corophium volutator* (Table 2) reveal an ameliorating effect of high salinity and low temperature; 192 h LC_{50} values are much lower than 96 h values.

Analysis of variance (Table 3) indicates that both linear and quadratic effects of temperature (T), concentration (C) and salinity (S) significantly affect LT_{50} values. The following interactive terms were all significant ($p < 0.01$), $T \times C$, $T \times C^2$, $T \times S$, $T^2 \times S$, $C \times S$, $C \times S^2$, $C^2 \times S^2$. These 2-way significant interactions indicate that the effect of one variable on LT_{50} is dependent on exposure levels of the other variable.

Fig. 2 shows the surface response isopleths representing the effect of concentration of chromium and salinity on LT_{50} values for *Corophium volutator* at 5 °C. The shape of the contours reveals an interactive effect

between chromium concentration and salinity on LT_{50} at low salinities and low chromium levels. As the chromium concentration increases, the effect of salinity is diminished, until at 32 ppm, at salinities above 15 ‰, there is virtually no change in LT_{50} up to 40‰. At chromium levels below 8 ppm the shape of the contours indicates that salinity exerts a greater effect than chromium concentration on LT_{50} at salinities below 15 ‰. As chromium concentration increases, the interactive effect between salinity and chromium diminishes with chromium exerting an increasingly strong influence over a greater part of the salinity range. The shape of the contours was similar for 10 and 15 °C.

The effect of temperature on the interactive effect between chromium and salinity may be clarified by examining the range of conditions over which a median survival period of 150 h occurs. At 5 °C, 50 % of *Corophium volutator* survived for 150 h or more over a salinity range of 6 to 40 ‰ and 2 to 32 ppm chromium, at 10 °C the range is reduced to 11 to 40 ‰ S and 2 to 32 ppm chromium, at 15 °C the range was further reduced to 24 to 31 ‰S and 2 to 3 ppm chromium.

Table 2. *Corophium volutator*. Median lethal concentrations (LC_{50}) of hexavalent chromium (ppm) at 5, 10, 15 °C, at 5 to 40 ‰ S

Time (h)	5 °C							
	5	10	15	20	25	30	35	40 ‰ S
12	34	80	×	×	×	×	×	×
24	18	38	78	90	190	×	×	×
48	9.5	16	34	50	80	120	85	100
96	5	8	15	17	36	38	40	36
192	•	3	7	7.5	15	19	20	12
384	•	•	3	3.2	•	•	•	•
Time (h)	10 °C							
	5	10	15	20	25	30	35	40 ‰ S
12	27	65	×	×	×	×	×	×
24	12	29	64	110	×	×	×	×
48	5.4	13	27	48	60	100	90	90
96	2.3	5.8	120	20	25	25	36	38
192	•	2.3	5.2	8.5	11	6.2	14	15
384	•	•	•	•	4	•	•	•
Time (h)	15 °C							
	5	10	15	20	25	30	35	40 ‰ S
12	17	32	180	90	×	×	×	×
24	6	12	30	38	65	75	52	44
48	2	4.2	11	15	17	26	17	9.5
96	•	•	4.4	6	4.7	9.5	5.8	2.2
192	•	•	•	2.5	•	3.6	2	•
384	•	•	•	•	•	•	•	•

× Less than 50 % mortality at time measured – • 50 % mortality at this exposure time would fall outside range of concentrations tested

Table 3. *Corophium volutator*. Analysis of variance of effects of 8 chromium concentrations, 8 salinities and 3 temperatures on median periods of survival. DF: degrees of freedom; MS: mean sum of squares; F: ratio of treatment mean square to error mean square; Lin: linear; Quad: quadratic

Source of variation		DF	MS	F	
Temp	Lin	1	272800	127.2	***
	Quad	1	37280	17.4	***
Conc	Lin	1	716800	334.2	***
	Quad	1	294500	137.3	***
Sal	Lin	1	251500	117.2	***
	Quad	1	79420	37.0	***
Temp · Conc	Lin · Lin	1	80240	37.4	***
	Quad · Lin	1	11350	5.29	*
	Lin · Quad	1	28100	13.10	***
	Quad · Quad	1	4962	2.31	NS
Temp · Sal	Lin · Lin	1	25980	12.1	***
	Quad · Lin	1	23030	10.7	***
	Lin · Quad	1	7613	3.5	NS
	Quad · Quad	1	3612	1.68	NS
Conc · Sal	Lin · Lin	1	46890	21.86	***
	Quad · Lin	1	7332	3.42	NS
	Lin · Quad	1	32160	15.0	***
	Quad · Quad	1	16780	7.8	**
Temp · Conc · Sal (Error)		149	2145		
Total		167			

*** Significant at $P = < 0.001$ NS: not significant
 ** Significant at $P = < 0.01$
 * Significant at $P = < 0.1$

Fig. 2. *Corophium volutator*. Response surface showing combined effect of chromium concentration and salinity on median period of survival (h) at 5°C

Fig. 3a and b show response surface isopleths representing the effect of temperature and salinity on LT_{50} values at chromium levels of 2 and 16 ppm, respectively. At the lowest salinity of 5‰ an increase in temperature from 5 to 15°C results in a 7-fold decrease in LT_{50} . As salinity is increased to 20‰, the temperature effect on LT_{50} is diminished. The ameliorating effect of salinity lessens at salinities of about 30‰, and at salinities of 40‰ interacts more strongly with temperature.

Response surface contours are similar in shape at concentrations of 2, 4, 8 and 16 ppm, with salinity exerting an increasingly strong effect at salinities below 20‰ over the whole temperature range. At 32 ppm chromium there is virtually no temperature effect at salinities below 20‰, at salinities above 20‰ and temperature above 10°C there is no temperature effect. At 64 and 128 ppm the prediction of the model is weak.

The effect of chromium concentration on the interactive effect of temperature and salinity is demonstrated by examining the range of conditions over which an LT_{50} of 150 h occurs, at 2 ppm 50% of

Fig. 3. *Corophium volutator*. Response surface showing combined effect of temperature and salinity on median period of survival (h) at chromium concentrations of (a) 2 ppm, (b) 16 ppm

Corophium volutator would survive over a range of 6 to 40 ‰ S from 5 to 15 °C, at 4 ppm the range is 7 to 40 ‰ at 5 to 14 °C, at 32 ppm the range is further reduced to 26 to 40 ‰ at 6 to 10 °C. The range narrows gradually up to 16 ppm with a dramatic contraction at concentrations of 32 ppm and above.

Fig. 4a and b are response surface isopleths showing the combined effects of chromium concentration and temperature on LT_{50} values for *Corophium volutator* at salinities of 10 and 35 ‰. Maximum LT_{50} values occur at combinations of low temperature and low chromium concentrations at all salinity levels. At low salinity, 10 ‰, temperature does not influence LT_{50} at

chromium levels above 16 ppm, however temperature and chromium concentration interact at levels below 16 ppm. As salinity is increased concentration interacts to a greater degree with temperature to affect LT_{50} .

Macoma balthica

Median periods of survival for *Macoma balthica* are shown in Table 4. The combined effect of temperature and salinity on LT_{50} at a low (16 ppm) and a high (64 ppm) chromium level is demonstrated in Fig. 5a and b. Greatest sensitivity to chromium occurs at low

Table 4. *Macoma balthica*. Median periods of survival (h) (LT₅₀) derived graphically at 5, 10, 15°C, 5 to 40‰ S, and chromium concentrations of 2 to 512 ppm

		LT ₅₀ h					
5 °C		15	20	25	30	35	40 ‰ S
Chromium conc.	2	> 384	> 384	> 384	> 384	> 384	> 384
	4	> 384	> 384	> 384	> 384	> 384	> 384
	8	> 384	> 384	> 384	> 384	> 384	> 384
	16	280	> 384	> 384	> 384	> 384	> 384
	32	190	280	> 384	> 384	> 384	> 384
	64	140	185	240	270	320	340
	128	130	125	175	195	230	280
	256	85	110	120	140	180	210
	512	NT	NT	NT	98	90	170
10 °C		15	20	25	30	35	40 ‰ S
Chromium conc.	2	> 384	> 384	> 384	> 384	> 384	> 384
	4	310	> 384	> 384	> 384	> 384	> 384
	8	250	> 384	> 384	> 384	> 384	> 384
	16	190	300	360	360	> 384	> 384
	32	145	210	260	310	310	> 384
	64	92	160	170	200	360	> 384
	128	68	68	110	145	145	155
15 °C		15	20	25	30	35	40 ‰ S
Chromium conc.	2	380	> 384	> 384	> 384	> 384	> 384
	4	200	> 384	> 384	> 384	> 384	> 384
	8	160	250	> 384	> 384	> 384	> 384
	16	125	195	210	250	320	> 384
	32	98	*70	135	175	185	> 384
	64	*70	*70	105	130	130	> 384
	128	42	58	64	78	78	90

NT: Not tested; * Values estimated, as data was unsuitable for graphical estimation of LT₅₀

NT: Not tested; * Values estimated, as data was unsuitable for graphical estimation of LT₅₀

salinity and at high temperature. At the highest salinities a temperature effect was not apparent. Table 5 lists LC₅₀ values for *M. balthica*, 192 h LC₅₀ values are lower than 96 h values.

Table 6 shows the analysis of variance for LT₅₀ data for *Macoma balthica*. The response surface model is simpler than that derived for *Corophium volutator* as few of the interactive terms were significant. The

linear effects of temperature, concentration and salinity were all highly significant. The following interactive terms were all significant, T×S₂, C×S, C₂×S, C²×S². None of the possible interactions between temperature and concentration was significant and only 1 of the possible 4 interactions between T×S was significant, namely linear temperature × quadratic salinity.

Table 5. *Macoma balthica*. Median lethal concentrations (LC₅₀) of hexavalent chromium (ppm) at 5, 10, 15°C, and 15 to 40‰ S

Time (h)	5°C						10°C				15°C				
	15	20	25	30	35	40	15	20	25	30	15	20	25	30	35‰ S
96	190	220	×	600	640	×	70	120	160	320	29	46	64	98	110
192	36	62	105	135	180	340	20	36	50	68	65	16	19	26	34
384	*	17	*	*	52	52	*	*	*	*	*	*	*	*	*

× Less than 50 % mortality at time measured

* 50 % mortality at this exposure time would fall outside range of concentrations tested

Fig. 5. *Macoma balthica*. Effect of temperature and salinity on median period of survival (h) at chromium concentrations of (a) 16 ppm, (b) 64 ppm

Fig. 6 illustrates response surface isopleths of the effects of chromium and salinity on LT_{50} at 10 °C. The shape of the contours shows the interactive effect; LT_{50} values decrease with decreasing salinity and increasing chromium concentration, maximum values occurring at high salinity and low concentrations. Unlike *Corophium volutator*, interaction occurs over the whole concentration range, although at salinities above 35 ‰ and concentrations above 64 ppm, chromium concentration exerts a stronger effect. Increasing temperature decreases the range over which a certain survival time would be expected, although the pattern of response was the same at all 3 temperatures.

Although analysis of variance indicated a significant interaction between linear temperature and quadratic salinity effects the value of the regression coefficient for this term was small ($b = 0.00349$), so the effect of this interaction on LT_{50} is small, as indicated by response surface isopleths shown in Fig. 7. Increasing temperature and decreasing salinity acted additively to reduce LT_{50} .

Nereis diversicolor

LT_{50} values for *Nereis diversicolor* at all combinations of temperature, chromium concentration and salinity are shown in Table 7. LC_{50} values for *N. diversicolor* are listed in Table 8. Detailed analysis of interactions between variables was not possible due to an incomplete data set. This arose as *N. diversicolor* could survive for periods up to 140 h under certain conditions and 24 h later all animals were dead, thus preventing graphical estimation of LT_{50} . LT_{50} decreased with increasing chromium concentration at all salinity and temperature combinations. The combined effect of temperature and salinity on LT_{50} of *N. diversicolor* at 2 chromium levels is demonstrated in Fig. 8a and b.

As with *Corophium volutator* and *Macoma balthica* maximum tolerance to chromium occurs at high salinity and low temperature. Salinity has a slight effect on LT_{50} , this is least obvious at the highest temperature. Temperature has a greater effect on LT_{50} , greater changes occurring between 5 and 10 °C than between 10 and 15 °C.

Table 6. *Macoma balthica*. Analysis of variance of effects of 8 chromium concentrations, 6 salinities and 3 temperatures on median periods of survival. DF: degrees of freedom; SS: sum of squares; MS: mean sum of squares; F: ratio of treatment mean square to error mean square; Lin: linear; Quad: quadratic

Source of variation		DF	MS	F
Temp	Lin	1	188300	***
	Quad	1	2862	NS
Conc	Lin	1	342100	***
	Quad	1	8614	*
Sal	Lin	1	285800	***
	Quad	1	8229	NS
Temp · Conc	Lin · Lin	1	498	NS
	Quad · Lin	1	2719	NS
	Lin · Quad	1	318.6	NS
	Quad · Quad	1	1099	NS
Temp · Sal	Lin · Lin	1	2981	NS
	Quad · Lin	1	828.8	NS
	Lin · Quad	1	18300	***
	Quad · Quad	1	2083	NS
Conc · Sal	Lin · Lin	1	8988	**
	Quad · Lin	1	198250	***
	Lin · Quad	1	1757	NS
	Quad · Quad	1	9671	**
Temp · Conc · Sal (Error)		53	1248	
Total		71		

*** Significant at $P = < 0.001$ NS: not significant
 ** Significant at $P = < 0.01$
 * Significant at $P = < 0.1$

Fig. 6. *Macoma balthica*. Response surface showing combined effect of chromium concentration and salinity on median period of survival (h) at 10 °C

Fig. 7. *Macoma balthica*. Response surface showing combined effect of temperature and salinity on median period of survival (h) at a chromium concentration of 16 ppm

Table 7. *Nereis diversicolor*. Median periods of survival (h) (LT₅₀) derived graphically at 5, 10, 15 °C, 5 to 40 ‰ S, and at chromium concentrations of 0.5 to 64 ppm

	LT ₅₀ h								
	5 °C	5	10	15	20	25	30	35	40 ‰ S
Chromium conc.	0.5	> 384	> 384	> 384	> 384	> 384	> 384	> 384	> 384
	1	> 384	> 384	> 384	375	> 384	> 384	> 384	> 384
	2	> 384	> 384	360	330	> 384	> 384	> 384	> 384
	4	360	320	305	275	300	340	> 384	> 384
	8	330	300	255	240	250	280	300	> 384
	16	210	220	215	205	220	210	230	250
	32	120	155	170	170	180	190	190	155
	64	62	95	120	125	160	150	125	140
	LT ₅₀ h								
	10 °C	5	10	15	20	25	30	35	40 ‰ S
Chromium conc.	0.5	> 384	390	> 384	> 384	> 384	> 384	> 384	> 384
	1	280	320	310	300	> 384	230	> 384	> 384
	2	190	240	240	*	260	270	310	> 384
	4	160	155	*	210	*	*	250	300
	8	130	132	*	*	*	*	190	210
	16	105	94	105	180	*	*	145	175
	32	85	*	95	8	115	105	105	110
	64	56	60	76	62	84	80	96	60
	LT ₅₀ h								
	15 °C	5	10	15	20	25	30	35	40 ‰ S
Chromium conc.	0.5	> 384	> 384	300	330	370	220	320	> 384
	1	180	190	200	*	240	190	210	360
	2	150	150	170	200	190	120	140	290
	4	120	*	100	130	105	90	120	115
	8	100	98	90	*	78	*	110	95
	16	78	80	85	85	*	*	100	76
	32	*	70	74	*	*	*	72	72
	64	*	52	*	62	68	*	*	*

* Insufficient data to derive LT₅₀ graphically

Table 8. *Nereis diversicolor*. Median lethal concentration (LC₅₀) of hexavalent chromium (ppm) at 5, 10, 15 °C, and 5 to 40 ‰ S

Time (h)	5 °C								10 °C								15 °C							
	5	10	15	20	25	30	35	40	5	10	15	20	25	30	35	40	5	10	15	20	25	30	35	40 ‰ S
96	56	80	×	×	×	×	×	×	19	22	27	65	55	34	52	40	7.5	9.5	8.5	12	12	7.5	22	16
192	15	18	19	17	26	26	27	26	2.9	3.5	4	5.4	5.8	4	8	12	*	1	1.5	2	2	1.5	3.3	5.2
384	3.8	4.4	*	*	*	*	*	*	*	.7	*	*	*	*	*	*	*	*	*	*	*	*	*	*

× Less than 50 % mortality at time measured
 * 50 % mortality at this exposure time would fall outside range of concentrations tested

DISCUSSION

The results have clearly documented significant effects of temperature, salinity and chromium concentration on the acute toxicity of chromium to *Corophium volutator* and *Macoma balthica*. In general, toxicity increased with increased temperature and increased

chromium concentration and decreased with an increase in salinity. However, in *C. volutator* there was a decrease in toxicity with an increase in salinity over the range 5 to 30 ‰, with evidence of further salinity increase up to 40 ‰ causing increased toxicity.

Temperature, salinity and chromium concentration interacted in a complex way to affect median survival

Fig. 8. *Nereis diversicolor*. Effect of temperature and salinity on median period of survival (h) at chromium concentrations of (a) 16 ppm, (b) 64 ppm

times of *Corophium volutator*. The combined interactions of chromium concentration $\times$ salinity, temperature $\times$ salinity and chromium concentration $\times$ temperature all significantly decreased LT_{50} values. However the effect of chromium on *C. volutator* at concentrations above 64 ppm is so great that these interactive effects are only significant at lower concentrations.

In contrast, the interactive effect of these variables has less influence on the median survival times of *Macoma balthica*. Only the interactive effect of salinity and increasing chromium concentration significantly decreased LT_{50} .

The percentage variance accounted for by the multiple regression analysis was 82.3 % for *Corophium volutator* and 84.8 % for *Macoma balthica*, illustrating that the experimental models provide a reasonable fit for the test results and that they each represent a good predictive model of the effect of salinity, temperature and chromium concentration on median survival periods.

In *Nereis diversicolor*, an increase in toxicity occurred with increased temperature, however the effect of salinity was less marked than in the other two species.

All experiments documented a progressive increase in toxicity values (as LC_{50}) with time. The 'log concentration-log response' curves for all species revealed a straight line relation. Lloyd (1979) and Franklin (1980)

state that a linear concentration/response relation indicates that the test organism has no method of detoxification for this pollutant, and that there is no lethal threshold concentration (or asymptote), so that prolonged exposure to concentrations below that of the 96 h LC_{50} will be lethal. In the present study this effect of time was clearly seen in *Corophium volutator* with progressive increase in toxicity with time, at up to 384 h. The other species under most conditions showed a similar response. The effect of chromium on these 3 species may be compared with their known ecological and physiological tolerances. All 3 species are well adapted to life in estuaries, and show considerable euryhalinity. *C. volutator* and *Nereis diversicolor* are classified as 'true estuarine species', whereas *Macoma balthica* is classified as 'euryhaline marine species' (McLusky, 1971). This distinction is made on the basis of their physiology and ecology. *M. balthica*, although it occurs widely in estuaries, exhibits no osmoregulation and its tissue remains iso-osmotic with the medium in all salinities; consequently it is unable to live in estuaries below 10 to 12 ‰. It can tolerate lower salinities for brief periods by closing its shells, but cannot endure prolonged periods of low salinities. This fact was also seen in the present study, as control animals died at 5 and 10 ‰, and consequently no toxicity studies were possible at these salinities. An

increase in salinity above 15 ‰ caused a progressive decrease in toxicity, up to 35 to 40 ‰, a response pattern which may be typical of a euryhaline marine animal.

Corophium volutator and *Nereis diversicolor* are both regarded as 'true estuarine' species. Both are able to osmoregulate when kept in low salinities (McLusky, 1968; Oglesby, 1969). *C. volutator* can tolerate salinities from 2 to 50 ‰, but only grows and reproduces between 5 and 15 ‰. Salinity experiments reveal a preference for the 10 to 30 ‰ range, with the 'optimal' salinity at 20 ‰ (McLusky, 1970). The present study has shown a decrease in toxicity with increased salinity up to 30 ‰, with evidence of increased toxicity beyond that point.

Nereis diversicolor is the most typical annelid of British estuaries, living down to salinities of 1 ‰. Above about 20 ‰ it is replaced by *N. virens*, *N. pelagica* or *Nephtys hombergi* (McLusky, 1971). In the laboratory it is well able to live in salinities above 20 ‰, so its relative scarcity from higher salinities may be attributable to ecological competition rather than to physiological intolerance. It can osmoregulate well and, like *Corophium volutator*, maintains its blood hyper-osmotic to the medium in low salinities. Whereas chromium toxicity in *Macoma balthica* and *C. volutator* was markedly affected by salinity, this effect is not obvious in *N. diversicolor*. There is however still an effect, and the salinity at which minimal toxicity occurs (20 to 30 ‰) is, like *C. volutator*, higher than the normal ecological salinity of the animal.

The toxicity effects observed in the present study may be compared with the only other studies known of the effect of chromium on estuarine invertebrates. Frank and Robertson (1979) reported on the effect of chromium on juvenile blue crabs *Callinectes sapidus* at 3 salinities, and one temperature and, as in the present study, found that toxicity decreased with increasing salinity (48 h LC₅₀s: 34 ppm chromium at 1 ‰ to 98 ppm chromium at 35 ‰). Eisler and Hennekey (1977) compared the toxicity of several heavy metals to many species of estuarine invertebrates, at one temperature and salinity, and found the rank order of toxicity to be: $Hg^{2+} \gg Cd^{2+} > Zn^{2+} > Cr^{6+} \gg Ni^{2+}$, with annelida more sensitive to chromium than other phyla, which is in agreement with the studies of Oshida (1976, 1977), and Oshida et al. (1981) on the sublethal effects of chromium. Thus in both the literature and the present study, the taxonomic ranking of toxicity is generally annelida > crustacean > mollusc. In the present study, however, the order was *Corophium*, *Nereis*, *Macoma* under some conditions of temperature and salinity, and *Nereis*, *Corophium*, *Macoma* under other conditions. The bivalve mollusc *Macoma balthica* was always the most tolerant species.

The range of toxicity values for a single estuarine species exposed to different salinity and temperature combinations in the present study (*Nereis diversicolor* 0.7 to 80 ppm; *Corophium volutator* 3 to 120 ppm; *Macoma balthica* 6 to 640 ppm, all as the LC₅₀ over periods of 12 to 384 h), exceeds the total range of toxicity values reported in literature for all species in marine and fresh-water conditions (2 to 100 ppm) as reported by Taylor (1981) and Mance et al. (1984). The fact that the range of toxicity values for a single species under different environmental conditions can exceed the reported range for several species emphasizes that single values for the toxicity of chromium, or other metals, should be treated with extreme caution. It should be emphasized that these values are lethal toxicity values, and should not be confused with sublethal values, for example a value of 1.26 ppm maximum proposed by Oshida et al. (1981), which are based on the effects of chromium on the reproduction of annelids.

When an effluent containing chromium is being licensed, the present results emphasize the need for the controlling authority to determine the acute toxicity under conditions that approximate to the field discharge conditions, rather than relying on values from literature which are invariably derived under different environmental conditions. Similarly the results emphasize the need to consider the range of environmental conditions at the point of discharge, and to base toxicity studies on the 'worst' combination of environmental conditions that can possibly occur.

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