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The toxicity of phenol to Phoxinus phoxinus, Gammarus duebeni, and Mesidotea entomon in brackish water[1]

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The toxicity of phenol to the minnow, *P. phoxinus* (L.), and to the crustaceans *M. entomon* (L.) and *G. duebeni* Lillj., all taken from the brackish water of the Baltic Sea, was determined at two temperatures using continuous flow bioassay. The lethal threshold concentrations (LTC) and the conventional 96-h LC50 values were estimated by the log-probit method. For *Mesidotea* the LTC of phenol is 85—100 ppm, for *Gammarus* 32—41 ppm and for *Phoxinus* 10 ppm. For the crustaceans the 96-h LC50 values were two to four times higher than the LTC, whereas for the minnow they were equivalent.

In tests on *Mesidotea* 4-chlorophenol was two to three times as toxic as phenol. A temperature rise from 5 °C to 10 °C had no effect on the toxicity of phenol at LTC. At higher concentrations a temperature increase accelerated the toxic effect of both phenol (*Gammarus*) and 4-chlorophenol (*Mesidotea*).

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1. Introduction

The toxicity of phenols to aquatic animals under laboratory conditions has been described in several papers. Most studies have been carried out on fish and in freshwater conditions (EIFAC 1973). Evaluation of the toxicity to brackish-water animals needs further study, because salinity has been said to be one of the physical factors affecting toxicity (Brown et al. 1967a).

Like many other foreign substances, phenols may accumulate in bottom sediments at concentrations up to tenfold (König 1968). Thus the effects of pollution may in many cases be reflected earlier in bottom-dwellers than in economically important pelagic species which swim actively and belong to higher trophic levels. Although many of the benthic invertebrates serve as important fish food, very little information is available on their ability to tolerate phenols.

This paper deals with the toxicity of phenols to *Mesidotea entomon* (L.), Isopoda), and to *Gammarus duebeni* Lillj. (Amphipoda), two euryhaline species which are typical representatives of the brackish-water fauna of the Baltic. The two species have a similar circumpolar distribution (Lockwood et al. 1976). The *Mesidotea* used in this work lives at depths of about 30 m, where water temperature and salinity are relatively constant throughout the year. The *Gammarus* species used lives in rock-pools in coastal areas, where it has adapted itself to very wide variations of water temperature, salinity and dissolved oxygen content. The minnow, *Phoxinus phoxinus* (L.), which is locally a brackish-water fish, was used as reference animal.

Usually the results of toxicity tests are presented as LC50, the concentration of the toxicant lethal to 50 per cent of the test animals after an arbitrary period of exposure, usually 24, 48 or 96 h. As the LC50 value depends greatly on the length of exposure, a test for determination of the lethal threshold concentration (LTC) with elimination of the effect of time is of the

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utmost importance and is to be recommended (Sprague 1969). The median lethal threshold concentration (= asymptotic LC50 = incipient lethal level = ultimate median tolerance limit) indicates the concentration of toxicant that is lethal to 50 % of the test animals which have been exposed for periods long enough to allow cessation of acute lethal action. From that point on, the toxicity curve becomes parallel to the time axis. Prolongation of the exposure time no longer increases mortality. In practice the median lethal threshold concentration means that in more dilute concentrations the test animal survives without acute damage. In this paper the LTC for the test animals is compared with the more routinely used 96-h LC50 values.

2. Materials and methods

Mesidotea were collected in June-July with a bottom trawl from a depth of about 30 m in the water area of Tvärminne Zoological Station (Storfjärden). *Gammarus* specimens were collected during July-August from rockpools (Långskär) and *Phoxinus* with dip nets from the surrounding near-shore waters of the Station.

For at least 3 weeks before the tests the animals were acclimated in 50- to 100-l glass aquaria with washed dune sand on the bottom. The brackish-water flow in each aquarium was about 1 l/min. and the temperature was kept at 10 °C. The LD cycle was 12:12 in all aquarium rooms. The aquaria had continuous aeration.

The *Phoxinus* were fed twice a week with rolled oats, dried *Daphnia* and pieces of fresh fish. The *Gammarus* were fed with rolled oats and dried aspen leaves, and the *Mesidotea* with pieces of fresh fish. During the acclimation period the mortality of the test animals was negligible. Feeding was stopped 2 days before tests were started.

The experiments were performed in continuous-flow aquaria (size 39 × 24 × 12 cm, water volume 8.5 l). The experimental arrangement was similar in principle to that described by Connor & Wilson (1972). Each set of experiments consisted of one control aquarium and five test aquaria. The water temperature was regulated to within ± 0.7 °C. Natural brackish water was used (mean salinity 6 ‰). The phenols (Merck pa.) were added from stock solutions through the water inlets with a peristaltic pump (Desaga). The concentrations desired in different test aquaria were obtained by minor adjustments of pump speed and water inflow. The concentration of both phenol and 4-chlorophenol in each test aquarium was checked daily by the spectrophotometric method described by Ochynski (1960).

The water flow in each aquarium was about 100 ml/min. This means a 90 % water replacement in 3 h. Dissolved oxygen was determined by the Winkler method. During the tests the oxygen concentration in the aquaria was 8.4 ± 0.3 mg O_2/l. The pH was 7.7 ± 0.1, and additions of phenol did not change the pH of the water. At this pH phenol is in non-ionized form (Wuhrmann & Woker 1950) and penetrates biological membranes more easily than in its ionized form.

Because no data were available on the phenol tolerance of the animals used, preliminary tests were carried out with a smaller number of test animals (cf. Doudoroff et al. 1951). The final experiment consisted of 5—6 different phenol concentrations, which were selected for further probit analysis in such a way that successive concentrations increased by a given multiple. This made the final calculations (with simple units) much easier (Ph. NORD. IV, 1961).

All three species were tested at a water temperature of 5 °C (±0.6). The effect of temperature was also studied by testing *Gammarus* at 16 ± 0.6 °C, and *Mesidotea* at 10 ± 0.7 °C.

Because sensitivity to pollutants depends partly on body size (Luk'yanenko & Flerov 1966), test animals of equal size were selected in order to obtain comparable results. Moreover, moulting in Crustacea has been reported to affect their susceptibility to toxic substances (Emery 1970; Swedmark et al. 1971). Therefore recently moulted animals with soft exoskeletons were not used.

The lengths and weights ($\bar{x}$ ± SD) of the test animals were as follows:

	cm	g	n
Phoxinus	5.5±0.4	1.6±0.4	40
Gammarus	1.8±0.2	0.08±0.03	222
Mesidotea	3.8±0.4	0.9±0.3	427

The number of animals per batch was 6—8 for *Phoxinus*, 10—20 for *Gammarus* and 15—20 for *Mesidotea*. The test animals were placed at random in the six aquaria 2 days before the toxicity test was started. The animals were not fed during the test.

The animals were considered dead when there were no respiratory or other movements and no response to mechanical stimuli. Dead specimens were removed from the aquaria, measured and weighed. Mortality in control aquaria occurred only sporadically and was always less than 10 % in all experiments.

The median survival times for the animals at each phenol concentration were first estimated. To show the general pattern of response, toxicity curves were constructed by plotting the survival times against the respective phenol concentrations on logarithmic paper. The tests were continued until the toxicity curve became parallel to the time axis, indicating the LTC. A more accurate value for the LC50 was calculated for the longest exposure time from the original data by the log-probit method (Ph. NORD. IV, 1961). Likewise, the LC50 values for 96 h were calculated.

3. Results

Figs. 1 and 4 show the toxicity curves obtained (survival time against concentration). Figs. 2, 3, 5 and 6 show mortality as probits.

For *Phoxinus* the median lethal threshold concentration was reached after exposure for only 96 h (Fig. 1); the 96-h value is the same as the asymptotic LC50, 9.5 ppm phenol (Fig. 2). However, 96 h was not a sufficient time for evaluating the acute toxicity to the invertebrates

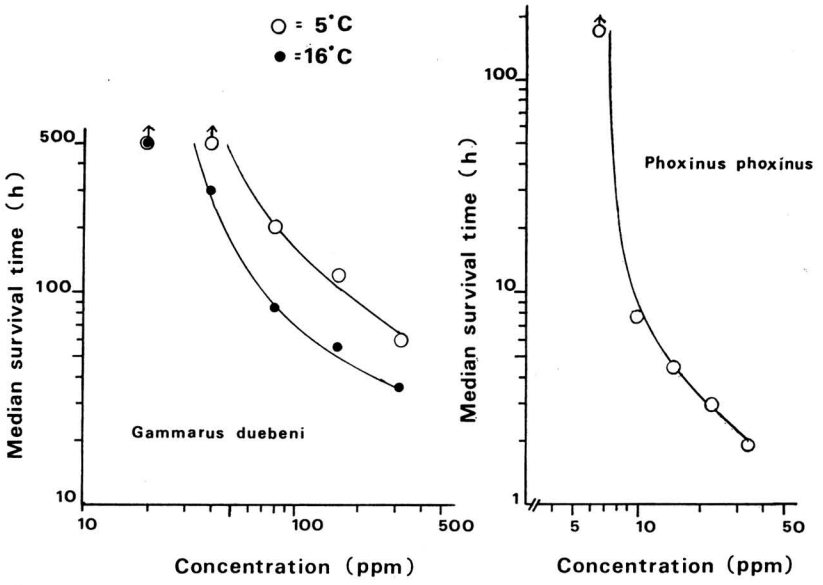

Fig. 1. Toxicity curves (survival time against concentration) for *G. duebeni* and *P. phoxinus*. Symbols with an arrow indicate that median mortality was not obtained.

tested. Because the toxicity curve had not become asymptotic by 96 h, the test was prolonged.

For *Gammarus* the curve did not show a clearly visible threshold even at 3 weeks (Fig. 1), the longest exposure time used in this study. The results were calculated for a period of that length for practical reasons (Fig. 3). An increase in temperature clearly shortened the survival times of *Gammarus* at high concentrations (Fig. 1), but had no effect on the threshold concentration (Table 1). The values for 96 h are 3- to 4-fold as compared with those obtained for the threshold concentration in 3-week exposures.

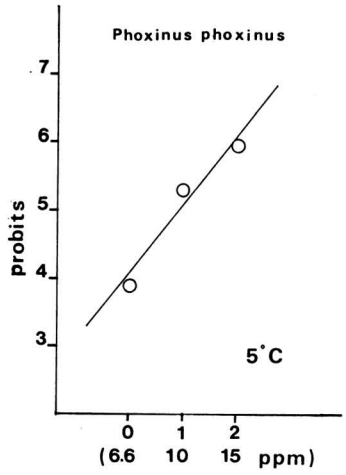

Fig. 2. Mortality (as probits) of *P. phoxinus* as a function of phenol concentration after 96-h exposure. The regression line was calculated on arbitrarily chosen units; the actual concentrations are indicated in parentheses. Probit 5 = 50 % mortality.

Mesidotea reached the threshold in phenol after 2 weeks' exposure (Fig. 4), and these values were found to be only about half the corresponding 96-h LC50 values (Fig. 5 A and B). No temperature effect could be demonstrated for phenol toxicity in *Mesidotea*. With 4-chlorophenol, in contrast, an increase in temperature from 5 °C to 10 °C accelerated toxicity (Fig. 4), and the threshold value was reached in half the time, but even with this phenol temperature had no effect on the median lethal threshold concentration (Fig. 6). The toxicity of 4-chlorophenol was about three-fold that of phenol. If the only test used is the 96-h routine test, temperature is seen to have a marked effect on the toxicity of 4-chlorophenol (Table 1): the two values obtained differed significantly.

Gravid females were found to be exceptionally sensitive; they died more quickly, and abortions were often observed.

In *Phoxinus* and *Gammarus* signs of phenol exposure were evident even at low concentrations: *Phoxinus* was restless, showed increased

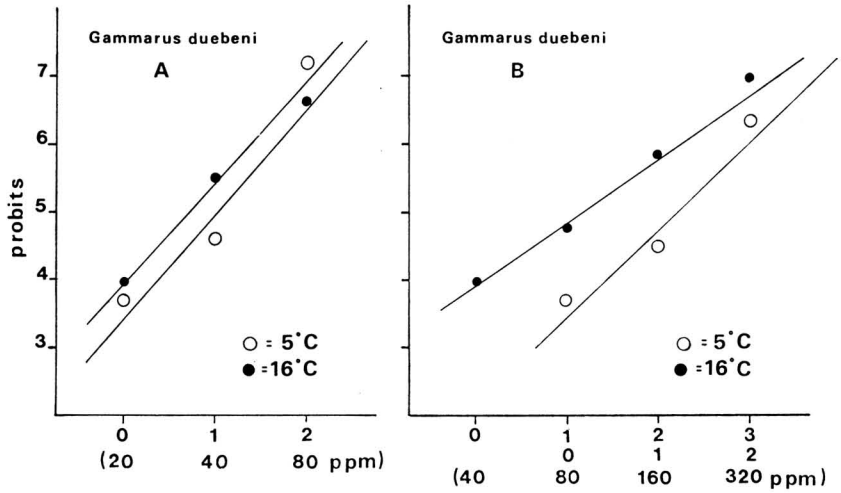

Fig. 3. Mortality (as probits) of *G. duebeni* as a function of phenol concentration. A = 3-week exposure, B = 96-h exposure. For further explanation, see Fig. 2.

respiratory movements and jerky swimming movements, and had convulsions. At higher concentrations the fish — still swimming — lost their balance. After death the posture was bent and the opercula and mouth were wide open. *Gammarus* showed similar jerky swimming movements; at higher concentrations these soon ceased and the animals had convulsions. These symptoms were followed by complete immobilization and death. At low concentrations swimming activity was increased. In *Mesidotea* no such symptoms could be observed. Immediately before death the isopods just lay still on the bottom of the aquarium, often being clumped together. In dilute solutions the animals were clearly more aggressive than in control conditions.

4. Discussion

The finding that for *Phoxinus* the 96-h LC50 is equal to the 7-day asymptotic value agrees well with the general assumption that, for fish, a 96-h routine test is long enough to give satisfactory information on toxicity. For rainbow trout a 24-h exposure is sufficient (Brown et al. 1967a) and for common bluegill, 48 h (Trama 1955). Ruesinck and Smith (1975), with fathead minnow, found that at 25 °C the 96-h LC50 and the threshold concentration were almost identical, but that at 15 °C the reaction was retarded, the 96-h LC50 value being about 1.5-fold the threshold concentration.

The deleterious phenol concentrations for fishes presented in the literature vary widely, from 0.08 to 1900 ppm, thus reflecting differences in the way of expressing toxicity, in the conditions of exposure, and particularly in the duration of the tests. Figures for median lethal con-

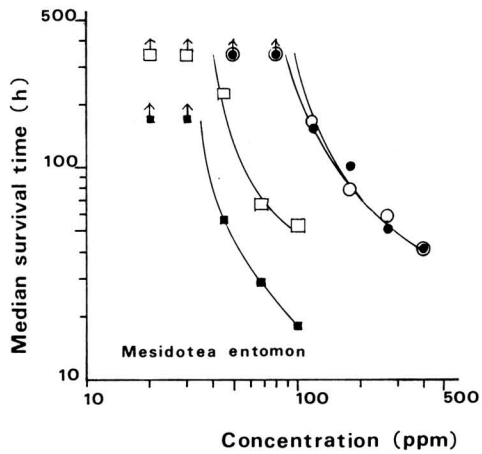

Fig. 4. Toxicity curves for *M. entomon*: circles = phenol, squares = 4-chlorophenol. Open symbols = 5 °C, solid symbols = 10 °C. Symbols with an arrow indicate that median mortality was not obtained.

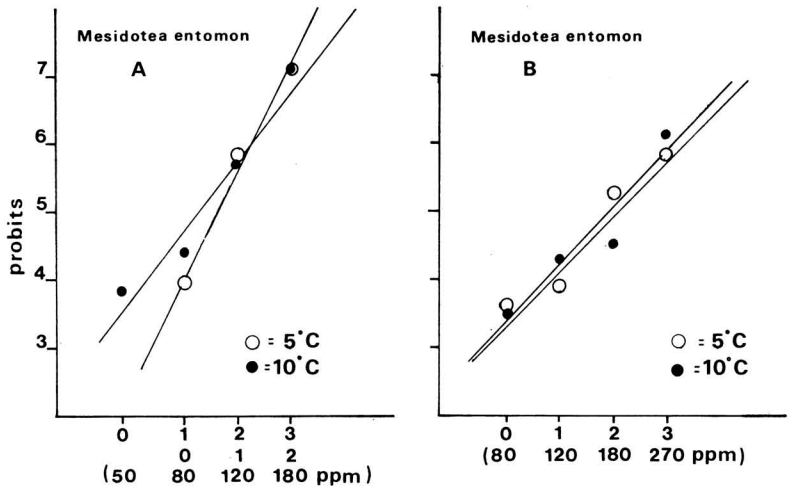

Fig. 5. Mortality (as probits) of *M. entomon* as a function of phenol concentration. A = 2-week exposure, B = 96-h exposure. For further explanations, see Fig. 2.

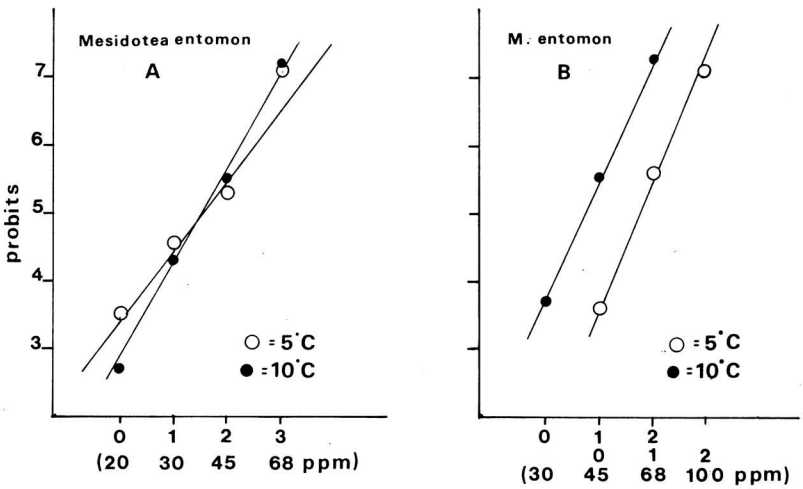

Fig. 6. Mortality (as probits) of *M. entomon* as a function of 4-chlorophenol concentration. A = 2-week exposure at 5 °C and 1-week exposure at 10 °C, B = 96-h exposure. For further explanation, see Fig. 2.

centrations during periods of 6—96 h cover a range of 9—25 ppm for adult fishes EIFAC 1973). The differences between species become still smaller if attention is confined to results for longer test periods. Although perch, pike and rainbow trout react to phenol more quickly than rudd and eel, the 7-day (asymptotic) LC50 is of the same magnitude, 8—11 ppm, for all these five species (EIFAC 1973). The value obtained for *Phoxinus* in this study, 9.5 ppm, falls well within these limits.

This study shows that the invertebrates tested are more resistant to phenol than are fish. *Gammarus* is about 4 times as resistant as *Phoxinus*, and *Mesidotea* about twice as resistant as *Gammarus*.

It is noteworthy that 96 h was insufficient for evaluation of the toxicity of phenol to the invertebrates. This agrees with the finding of Swedmark et al. (1971), who worked with surface-active agents, and Ahsanullah (1976), who studied the toxicity of cadmium.

A sudden temperature increase has been reported to shorten the survival time at high phenol concentrations in short-term tests with fish (Gersdorff 1943, Bucksteeg et al. 1955, Brown et al. 1967b). It has been suggested that, as a 10 °C rise in temperature approximately

Table 1. Lethal concentrations of phenol and 4-chlorophenol in test animals; the 95 % confidence limits, LC5 and LC95 and the slopes of regression (probit) lines.

		Time (h)	t °C	LC5 (ppm)	LC50 (ppm)	95 % confidence limits	LC95 (ppm)	Slope
Phenol	*Phoxinus phoxinus*	96	5	5.1	9.5	6.9 – 13.2	17.9	1.051
	Gammarus duebeni	96	16	27.0	89.5	63.9 – 125.2	296.2	0.950
		96	5	75.8	183.2	145.7 – 230.3	442.6	1.289
		504	16	14.3	32.5	26.0 – 40.6	73.9	1.382
		504	5	20.0	41.0	33.2 – 50.7	84.5	1.572
	Mesidotea entomon	96	10	82.7	186.2	149.2 – 232.3	419.2	0.819
		96	5	78.7	176.8	145.8 – 214.3	397.4	0.821
		336	10	45.4	85.8	71.3 – 103.2	162.0	1.046
		336	5	67.8	100.5	86.9 – 116.3	149.1	1.687
4-chloro-phenol	*Mesidotea entomon*	96	10	27.5	40.3	36.0 – 45.0	58.9	1.782
		96	5	42.2	59.7	52.7 – 67.6	84.4	1.825
		168	10	23.0	37.5	32.9 – 42.7	61.0	1.366
		336	5	18.9	36.8	29.7 – 45.5	71.6	0.997

doubles the metabolic rate of poikilothermic aquatic animals, it simultaneously reduces the survival time in toxicity tests by half (Skidmore 1974, Cairns et al. 1975). In the present study the survival time of *Gammarus* at high phenol concentrations shortened in this manner when the temperature was raised from 5 to 16 °C. In *Mesidotea* an increase of as little as 5 °C had the same effect, but only when 4-chlorophenol was used. It should be noted, however, that temperature affects only the lower portion of the toxicity response curve, the threshold varying but slightly (Fig. 4). The negligible effect of temperature on the LTC presumably reflects the similarity of the temperatures used to those which the species encounter in their natural habitats. Data presented by Thurberg et al. (1973) and Jones (1975) suggest that the toxicity of pollutants is greater when abiotic factors such as salinity and temperature approach the extremes of the animals' tolerance. This illustrates the additive effects of pollutants and physiological stress.

Much less information is available on invertebrates than on fish. Portman (1972), studying the effect of phenol on *Cardium edule* and *Crangon crangon*, reported 48-h LC50 values of 330 and 10—30 ppm, respectively. For *Daphnia magna* the toxicity limit is 94 ppm when measured with the 16-h test (Liebmann 1960), but the threshold concentration is only 7 ppm (EIFAC 1973). Alekseev & Antipin (1976) have reported that at 20 °C the 48-h LC50 values for freshwater crustaceans are 14—132 ppm. Thus the crustaceans *Mesidotea entomon* and *Gammarus duebeni* seem both to be relatively resistant to phenol, particularly as the toxicity of phenol has been reported to increase with salinity (Brown et al. 1967a). The considerable differences in tolerance between species of Crustacea may be related to differences in general activity. The more active species in general appear to be more susceptible to pollutants than the more sluggish ones (Skidmore 1974). *Mesidotea* is a rather slow-moving benthic species.

In this study *Mesidotea* was found to be more resistant than *Gammarus duebeni*, although *G. duebeni* is a very inactive swimmer as compared with other *Gammarus* species (Rygg 1972).

The difference in resistance to phenol intoxication between *Mesidotea* and *Gammarus* may also be due to differences in surface permeability. The cuticle of *G. duebeni* is likely to be more permeable than that of Mesidotea (Lockwood et al. 1976).

The resistance of animals varies considerably during their life-cycle, the early phases usually being the most sensitive. Among adult crustaceans gravid females have been shown to be particularly sensitive (EIFAC 1973). Evidently the normal development of eggs and embryos is disturbed at even lower concentrations than those inducing the death of the female. The juvenile forms of crustaceans have been shown to be more susceptible to phenol and cresol than the adults (Dowden & Bennet 1965, Emery

1970). Experiments on the toxicity of surface-active agents to crustaceans have shown that adults, although very resistant in the intermoult stages, show a 2- to 10-fold increase in sensitivity during moults, being then about as sensitive as fish (Swedmark et al. 1971).

Addition of chlorine to phenolic waste may produce highly toxic chlorinated phenols. The more chlorinated the phenol, the more toxic it is to fish and to *D. magna* (Liebmann 1960, Kopperman et al. 1974). *Mesidotea*, too, was considerably more sensitive to 4-chlorophenol than to pure phenol.

The behaviour of *Phoxinus* when exposed to high phenol concentrations resembled that described in various other fish species (Jones 1951, Bucksteeg et al. 1955, Trama 1955, Wuhrmann & Woker 1959, Kristoffersson et al. 1973). Few reports have dealt with the effect of phenol on invertebrates. The behaviour of *Gammarus* induced by exposure to high phenol concentrations is similar to that described for water insects (Beer 1958/59, Butorin & Kamshilov 1973). The symptoms suggest that phenol exerts its primary effect on the central nervous system (Jones 1951).

The increased swimming activity observed in dilute concentrations has sometimes been interpreted as an escape or avoidance reaction, but the data available are contradictory (cf. Portmann 1973, EIFAC 1973).

The threshold for acute toxicity is not to be taken as a 'safe' concentration, but merely as a convenient and reproducible reference point, being the concentration that on long exposure kills the 'average animal' of the species tested. In tests of this type, records should not be restricted to mortality and survival times. Although great importance attaches to the increased mortality, the other harmful effects of the toxic substance should not be underestimated. Such effects as abortion and impairment of ionic regulation in *Mesidotea* (unpublished results) and abnormal behaviour in *Phoxinus* and *Gammarus* may be considered highly deleterious to the species concerned.

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