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Acute Lethal Toxicity of Some Reference Chemicals to Freshwater Fishes of Scandinavia

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Relevance of the choice of a test organism intended to be representative for a given environment seems to be under continual debate in aquatic ecotoxicology. For instance, it is commonly argued that acute toxicity tests with rainbow trout (Salmo gairdneri Richardson), the species most often recommended as a standard cold water teleost, were not representative for Nordic countries because the species is an alien in local faunas. A comparative study with several freshwater species was therefore initiated to clarify the validity of this assumption. As a first approximation, standard LC 50 assays were conducted. The species used were chosen only on the basis of their local availability, i.e., they randomly represented the fish fauna of Nordic inland waters. Furthermore, inter-species variation of toxicity response was compared with certain other, quantitatively more important, intra-species sources of variability affecting the toxicity of chemicals.

Use of reference toxicants has been recommended as a means of standardizing bioassays (Davis and Hoos 1975; OECD 1981; ISO 1982; Nordforsk 1982). Davis and Hoos (1975) used dehydroabietic acid (DHAA) and pentachlorophenol (PCP) as reference toxicants when assessing inter-laboratory variation of acute lethality to salmonid fishes, including rainbow trout. These compounds, characteristic of effluents from the pulp and paper industry (Holmbom 1980; Voss et al. 1980), were selected also for the present study.

The toxicity of organic acids such a phenols and resin acids, as well as that of pulpmill effluents, strongly depends on water pH (Dave 1978, 1984; McLeay et al. 1979; Saarikoski and Viluksela 1981). Because of the possibility that species differences could exist in this respect, effects of water acidity on toxicity of these types of substances to a randomly selected local species was investigated. Finally, as an example of the biological source of assay variability, the effect of yolk absorption was studied with a subsequent crisis period due to moderate starvation under laboratory conditions.

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MATERIALS AND METHODS

The fishes studied, all native species in Scandinavia, are listed in Table 1. Whitefish and pike were newly hatched young, which were fed ad libitum with mixed zooplankton species. Lake trout young had almost completely absorbed their yolk. Other fishes were seine-netted from local waters, transported to the laboratory, and acclimatized to the dilution water as described in Table 1.

Static acute lethality tests (LC 50) were conducted in all glass 40 L aquaria according to OECD (1981) guidelines. Compared with the OECD standard, however, the reconstituted dilution water (pH 7.0) contained reduced hardness components (Ca/Mg ratio 4:3 with their total conc. 0.5 mmol/L), i.e. more characteristic of most Nordic inland waters. Concentrations of sodium and potassium were 160 µmol/L and 16 µmol/L, respectively. Lethality tests for chemicals were conducted 1-3 times for each fish species. During the tests the fish loading density in aquaria varied from 0.05 g/L (whitefish) to 0.5 g/L (Crucian carp) of water.

Stock solutions of DHAA (> 98 % purity) and chlorophenolics (> 99 %) were prepared in sodium hydroxide solution (pH ca.11). The pulp bleachery effluent (PBE) was a one-week composite sample from a Finnish kraft pulp mill and contained the same chlorophenolic compounds as reported before (Holmbom 1980; Voss et al. 1980). The test solutions, 5-6 concentrations per chemical, were prepared in well-aerated dilution water a few hours before transfer of animals. Fish were monitored twice a day and dead specimens were removed. Concentrations of toxicants in water, used for calculation of LC 50 values (48 or 96 h) according to probit analysis (SAS 1982), were nominal values. Random check-out determinations, conducted by capillary GC after methylation or silylation of chemicals, revealed that 85 % or more of the calculated level was present in water at the end of tests. No confidence limits of LC 50 are given for tests where only one partial mortality between 0 and 100 % was observed.

RESULTS AND DISCUSSION

The LC 50 values, with their 95 % confidence intervals, of DHAA and PCP for five and six fish species are given in Tables 2 and 3, respectively. Inter-species coefficients of variation ($CV = SD \times 100/\bar{x}$) of LC 50 values for DHAA and PCP were 37 % and 23 %, respectively. Inter-species variability was, in fact, smaller or of the same order as the inter-laboratory variability reported for rainbow trout (CV of DHAA and PCP being 31 % and 36 %, respectively; Davis and Hoos 1975). Therefore, when general ecotoxicological screening and classification of chemicals are in question, it is not very important what particular species of fishes available will be selected for determination of acute toxicity. Rainbow trout, in this sense, is as valid a choice as any other. In particular, its sensitivity towards DHAA and PCP is similar to other teleost fishes (Tables 2 and 3; Davis and Hoos 1975; Johnson and Finley 1980).

Table 1. Water temperatures, seasons and sizes of the fishes used in acute toxicity tests. The source of animals and their acclimatization time in the laboratory are also presented.

Fish species	Temp. °C	Season (month)	Mean weight g	Source N lat., E long.	Accl.time in laboratory d
Alburnus alburnus (L.) Bleak	11 - 12	Oct. - Dec.	0.90	Lake Pyhäselkä 62° 30´, 29° 30´	30 - 70
Rutilus rutilus (L.) Roach	11 - 12	Sept. - Oct.	1.26	Pond Öllölä 62° 20´, 30° 05´	10 - 20
Esox lucius L. Northern pike	11 - 12	May	0.011	Lake Pyhäselkä 62° 30´, 29° 30´	2 (posthatch)
Carassius carassius (L.) Crucian carp	12	August	2.20	Pond Hermanni 62° 40´, 29° 35´	15 - 20
Salmo trutta m.lacustris (L.) Lake trout	12 - 13	June	0.082	Lake Inari * 69° 00´, 28° 00´	4 - 6 (posthatch)
Coregonus muksun (Pallas) Whitefish	11 - 12	May	0.004	Taivalkoski * 65° 30´, 28° 00´	2 - 4 (posthatch)

* Fertilized and cultured in Taivalkoski Fish Culture Research Station, NE Finland.

Table 2. Acute toxicity (mg/L) of dehydroabietic acid to five species of freshwater teleost fishes.

Species	Test time, h	LC 50	(95 % confidence limits)
Bleak	96	1.85	(1.81 - 1.90)
Pike	96	0.70	(0.66 - 0.75)
Crucian carp	96	1.69	
Lake trout	48	1.20	(0.50 - 1.52)
Whitefish	96 *	1.01	(0.87 - 1.39)

* Tested 2 d posthatch

By contrast, it must be born in mind that much wider variation, occasionally up to a factor 10 000, may exist between species and taxa more distantly related than those studied here (Dave 1978; Sloof and Canton 1983; Chapman 1983). On the scale of acute toxicity, however, fishes are in general among the most sensitive organisms. Despite this relative sensitivity of teleosts, it is ecotoxicologically important that a variety of taxa are used for acute toxicity screening of chemicals and effluents, instead of being too much concerned about the tight local relevance of a species chosen from a fairly narrow taxonomic group. On the other hand, when chronic and subchronic toxicity is determined, there may be good grounds to place the locally important species first. Accordingly, in biomonitoring programs of ecosystems, locally relevant indicator species certainly are a rational choice in laboratory verifications of ecosystem responses.

Table 3. Acute toxicity (µg/L) of pentachlorophenol to six species of freshwater teleost fishes.

Species	Test time, h	LC 50	(95 % confidence limits)
Bleak	48	78	(74.6 - 84.6)
	96	66	(46.4 - 77.8)
Roach	96	38	(35.5 - 40.7)
Pike	96	45	
Crucian carp	48	87	(81.9 - 92.2)
Lake trout	96	54	(52.2 - 56.5)
Whitefish	48 *	65	(58.3 - 77.3)
	96 *	43	(40.0 - 45.2)
	48 **	22	(14.5 - 25.2)

* Tested 4 d posthatch; yolk still present
** Tested 14 d posthatch; fish, although fed ad libitum with zooplankton items, suffered undernutrition (empty intestines, slow growth)

It is well known that both biological and environmental factors may greatly affect the outcome of acute toxicity tests (Blank et al. 1978). Accordingly, the developmental/nutritional status of young

Table 4. Effect of water pH on acute toxicity (96 h LC 50) of tetrachloroguaiacol (CG-4) and pulp bleachery effluent (PBE) to bleak, Alburnus alburnus.

Water pH	CG-4 µg/L	PBE vol. %
7.0	110 (96.0 - 121.1) *	8.2 (6.60 - 9.09)
6.0	67 (57.3 - 76.9)	7.7 (7.13 - 11.10)
5.0	56 (49.6 - 62.4)	3.9 (3.30 - 4.42)

* 95 % confidence limits

whitefish (Table 3) and water pH (Table 4) were shown to cause more variability among the LC 50 values than that observed among the six fish species studied. The effects of pH on acute toxicity of tetrachloroguaiacol and pulp bleachery effluent, containing a variety of chlorinated phenolics, to bleak were essentially similar to other related weak organic acids as well as bleached kraft pulp mill effluent reported before (McLeay et al. 1979; Saarikoski and Viluksela 1981; Dave 1984). Therefore, this response was basically not dependent on the species chosen for the study, but merely on a common mechanism affecting all freshwater teleosts fairly indiscriminately.

In conclusion, the present results with freshwater teleosts indicate that fairly free choice can be made in selection of test species within narrow taxonomic groups concerning general screening of the acute toxicity of chemicals as well as their basic toxicity mechanisms.

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