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Daphnia magna as an Indicator of the Acute Toxicity of Waste Waters

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The water flea, Daphnia magna Straus (Cladocera, Crustacea) is the most commonly used zooplankton species in toxicological tests. The use of D. magna began with the works of Einar Naumann who in 1933 and 1934 published a series of 17 papers on the utilization of D. magna as test animal (ref. Anderson 1980). After that the Daphnia toxicity tests have been applied for a wide range of purposes including mostly acute lethality assays for pure materials and for complex industrial discharges.

At present, the use of D. magna especially in the toxicology has achieved a level of standardization (DIN 1982; ISO 1982; BS 1983; NF 1983) as an acute test. As the data obtained often suggest that Daphnia species are among the most sensitive aquatic organisms tested (Buikema et al. 1980), the results have been used to set water quality criteria (EPA 1973). Recommendations have also been given by e.g. OECD (1981) concerning long-term ($\geq$14 d) reproduction bioassays conducted on Daphnia. Long-term chronic tests with pure materials have been used to estimate maximum allowable toxicant concentrations, MATCs (Winner and Farrel 1976).

In this paper the proposed Finnish standard (SFS 1984) for both chemical and waste water testing is described and results obtained from different industrial waste waters by the test method concerned are presented.

MATERIAL AND METHODS

In the proposed Finnish standard (SFS 1984) it has not been decided to standardize the method by which Daphnia magna are bred. In this experiment the stock culture on water fleas was fed ad libitum with mixed population of algae, dominated by unicellular Chlorophyta species.

Prior to test, adult *Daphnia*, sorted by size are isolated in separate beakers and young water fleas less than 24 hours old are used in the tests the following day. Five individuals are placed in each test tube in at least 10 ml of test solution. Three replicates are used in each concentration. The stock solutions are made according to the recommendation of ISO-standard (ISO 1982) but the final solution is diluted to one fifth with distilled or deionized water, having a maximum conductivity of 1.0 mS/m. The dilution water thus prepared has a Ca:Mg ratio 4:1 and a hardness of 50 mg $CaCO_3$ which is in the same order of magnitude as the measured median in the Finnish recipient waters (Laaksonen 1970). The pH of the dilution water and the samples investigated also is adjusted to pH 7.0 $\pm$ 0.1 with dilute NaOH or HCl. When necessary, the dilution water is aerated until the oxygen concentration achieves the air saturation value. During the test, the test vessels are kept at 20 $\pm$ 2 °C in the same illumination where the breeding was done (L:D $\geq$ 16 h: $\leq$ 8 h).

For the calculation of 24h EC 50 (effective concentration 50 %) the number of mobile *Daphnia* is counted in each container. Those which are not able to move in the 15 s following a gentle agitation of the test vessel are considered to be immobilized. The estimation method of EC 50 has not been standardized. The method of Litchfield and Wilcoxon (1949), probit analyses (Finney 1971), ISO-standard method (ISO 1982) and the power curve method (Nikunen 1983) are mentioned as possible alternatives.

As in the other standards, $K_2Cr_2O_7$ is mentioned as a possible reference compound. The toxicity of it is bigger (24h EC 50 0.1 - 0.2 mg/l) (SFS 1984) than with the ISO-standard method (24h EC 50 0.9 - 2.0 mg/l) (ISO 1984) mainly because of the lower hardness of the dilution water used in the Finnish standard proposal.

In this study the number of mobile *Daphnia* was calculated three times during the 24 hours' test period. Based on those observations, the median survival time (MST) in each concentration was calculated by linear regression. The EC 50 values were estimated from at least four concentration/MST pairs with power curve $y = ax^b$, where y = concentration and x = MST. In this calculation method no preliminary tests are usually needed, which is particularly advantageous in waste water testing due to the minimization of the storage time of the sample.

When necessary, the waste water samples are filtered (e.g. Whatman GF/C) in order to avoid the influence of suspended solids on the swimming movements of water fleas which might have some impact on the results obtained.

The toxicity emission rate (TER) and toxicity emission factor (TEF) were calculated as follows (Wong et al. 1978):

$$TER = \frac{100}{EC\ 50\ (\%)} \times Q\ (1000\ m^3/d)$$

$$TEF = \frac{100}{EC\ 50\ (\%)} \times \frac{Q}{P}\ (m^3/d)$$

where Q = effluent volume per unit of time and P = the corresponding production per unit of time.

The waste water samples taken for the experiments were mainly chosen by the local water authorities. The majority of tested effluents were taken from the pulp and paper industry which is the most important polluter of the Finnish waters. The other waste waters chosen for the experiments came primarily from various plants of chemical industry. All the investigated samples were stored frozen (-20 °C) till the beginning of the bioassays.

RESULTS AND DISCUSSION

The acute toxicity (24h EC 50) and the toxicity emission values (TER and TEF) of different pulp and paper process streams are expressed in Table 1. When no acute lethal toxicity was detected (EC 50 > 100 %) the toxicity emissions are expressed as zero even though the non-detectability of toxicity can also be due to the high dilution caused by large waste water volume.

The debarking waste waters gave the highest acute toxicity for Daphnia. Their 24h EC 50 after filtration were 0.63 and 3.4 percent. In addition, it has earlier been noted that the toxicity of debarking effluents is many times higher for rainbow trout (Salmo gairdneri Richardson)(Nikunen 1983) and that they can be responsible for the major part of the toxicity emission of the whole mill (Holmbom and Lehtinen 1980; Nikunen 1983). In this study the toxicity emissions were not especially large due to the small waste water volumes.

The bleaching effluent samples from two relatively old whole bleaching plants were also quite toxic (7.8 and 18 %). The difference between the toxicity emissions of the two bleaching plants were striking and apparently mainly due to the process technical differences. The greater emission values were obtained from a conventional whole bleaching plant and the smaller ones were obtained from a one-stage hypochlorite bleaching. In the case of bleaching effluents it must be borne in mind that their ecological impacts are mainly caused by the sublethal effects of bioaccumulative chlorinated compounds which cannot be estimated by bioassays measuring acute lethal toxicity.

Table 1. Acute toxicities and toxicity emissions of neutralized pulp and paper waste waters.

Waste water (n)	24h EC 50 (%)	TER (1000m³/d)	TEF (m³/t)
	$\bar{x}$ (Range)	$\bar{x}$ (Range)	$\bar{x}$ (Range)
Log basin (2)	63(51-74)	-	-
Debarking (2)	2.2(0.63-3.4)	150(103-206)	-
Evaporation (1)	94	6.4	24
Bleaching (2)	13(7.8-18)	190(11-360)	980(61-1900)
Sulphite, total (2)	35(25-45)	160(110-210)	905(880-930)
Kraft, total, old (7)	23(8.7->100)	570(0-2100)	740(0-2000)
Kraft, total, new (8)	74(17->100)	150(0-790)	92(0-220)
Building board (1)	49	1.4	8.0
Fluiting board, semi-chemical pulp (1)	24	43	81

The kraft pulp mills were roughly divided into two groups according to the age of the mill. If the main part of the factory was built or modernized during the last 20 years it was classified in the group of new mills. The results obtained gave some support to the idea that the toxic emissions of modern mills are in general smaller than the corresponding values of older mills. However, the differences between various kraft mills was marked. In three cases studied the acute toxicity of effluents treated in aerated lagoons was observed to be non-detectable which indicates that a considerable reduction in the quantities of toxic substances may occur in the biological purification. Also a mechanical purification of one kraft pulp mill effluent studied led to a toxicity reduction of 94 percent.

The EC 50 value and toxicity emissions of whole mill, debarking and bleaching were all in the same order of magnitude as reported in an earlier comparable study (Nikunen 1983). The reduction of acute lethal toxicity of waste waters to a non-detectable level in an aerated lagoon has also been noted earlier with water fleas (Nikunen 1983). However, the detoxication percents were in this study bigger than those revealed by bioassays conducted on rainbow trout (Salmo gairdneri) (Walden and Howard 1974; Holmbom and Lehtinen 1980; Miettinen et al. 1982).

In the other 30 industrial waste water samples tested the largest acute toxicity was detected in effluents discharged from plants producing chlorine (24h EC 50/ TER: 0.39/11.5), glue (4.7/0.07), viscose fibers (0.17/12,000), stainless steel products (24/2.6), dairy products (20/47) and pesticides and special chemicals (26/0.98).

Toxic, biologically purified waste water was also released from wool staining plant (24h EC 50/TER: 46/0.18 and 22/1.4) in which some process streams were extremely toxic to Daphnia (24h EC 50's 0.12 - 26 %). The toxicity the of whole mill effluent was at the same level as that of U.S. textile mills on average (Walsh et al. 1980). All the other biologically purified waste waters from different plants of chemical industry and municipal waste waters were not acutely toxic to Daphnia. A chemical precipitation with $CaCO_3$ reduced the toxicity of an effluent from chlori-alkali plant with 99 percent (from 0.39 to 50 %).

One sample of the four landfill leachates investigated proved to be moderately toxic (24h EC 50 46 %). In a study by Nuorteva et al. (1983) the acute toxicity of 17 other Finnish landfills was studied with a comparable Daphnia magna method and in 12 cases the samples proved to be acutely toxic (24h EC 50 ranged 11 - 100 %). All these results give evidence that the Finnish landfill leachates are probably somewhat less toxic than Canadian ones from which Cameron and Koch (1980) have obtained results with rainbow trout.

The tests conducted on Daphnia magna indicated that the proposed Finnish standard (SFS 1984) is a fairly rapid and easy method for measuring the acute lethal toxicity of waste water samples. Especially it is recommended as a preliminary simple screening test method before any more complex sublethal bioassys are applied.

The toxicity emissions calculated show clearly that, due to the great differences in the waste water flows between plants the toxicity emission rates (TER) have ecologically much greater importance than mere EC 50 values.

The differences between various pulp mills was marked and when the productions were taken into account (TEF) the distinction became even more striking. The process technical reasons for the disparity concerned should be carefully studied in the future.

Pulp and paper mills had clearly the biggest toxicity emission rates of the samples investigated. Just one other plant, producing viscose fibers, had a TER value which was in the same order of magnitude as the median values of waste waters released from different pulp producing plants. The results obtained thus lend support to the thought that the pulp and paper industry is the most important polluter of the Finnish recipient waters also as far as the discharge of acutely lethal compounds is concerned.

REFERENCES

Anderson BG (1980) Aquatic invertabrates in tolerance investigations from Aristotle to Naumann. In: Buikema ALJr, Cairns JJr (eds) Aquatic invertabrete bioassays. ASTM STP 715 pp 3-35

BS (British Standard)(1983) British Standard 6068 Water Quality Part 5 Methods for biological testing. Section 5.1 Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera Crustacea)

Buikema AL, Geiger JG, Lee DR (1980) Daphnia toxicity tests. In: Buikema ALJr, Cairns JJr (eds) Aquatic invertabrete bioassays. ASTM STP 715 pp 48-69

Cameron RD, Koch FA (1980) Toxicity of landfill leachates. J Wat Pol Contr Fed 52:760-769

DIN (Deutsche Normen)(1982) Deutsche Einhetsverfahren zur Wasser-, Abwasser- und Schlamundersuchung. Testverfahren mit Wasserorganismen (Grouppe L). Bestimmung der Wirkung von Wasserinhaltstoffen auf Kleinkrebse (Daphnien- Kurtzeitstest) (L11) DIN 38 412

EPA (Environmental Protection Agency) (1973) Water quality criteria 1972. National Academy of Sciences, USEPA Research Series, Washington D.C

Finney DJ (1971) Probit analyses. Cambridge University press, 668 p

Holmbom B, Lehtinen K-J (1980) Acute toxicity to fish of kraft pulp mill waste waters. Paperi ja Puu Papper o Trä 63:673-684

ISO International Standard Organization)((1982) Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea). International Standard 6341 E
Laaksonen R (1970) Water quality in the water systems, a study based on observations carried out by the water pollution control authority 1962 - 1968. Doctoral Thesis, Univ. Helsinki, 132 p
Litchfield JF, Wilcoxon F (1949) A simplified method of evaluation dose-effect experiments. J Pharma exp Ther 96:99-113
Miettinen V, Lönn B-E, Oikari A (1982) Effects of biological treatment on the toxicity for fish of combined debarking and kraft pulp bleaching effluent. Paperi ja Puu - Papper o Trä 64:251-254
NF (Norme Francaise)(1983) Essai des eaux. Détermination de l'inhibition de la mobilité de Daphnia magna. Norme Francaise T 90-301
Nikunen E (1983) The acute toxicity of some Finnish pulp and paper mill effluents and comparison of three measurement methods. Paperi ja Puu - Papper o Trä 65: 726-730
Nuorteva P, Assmuth T, Cajander V-R, Heikkilä E, Kaarakka V, Lodenius M, Nikunen E, Tulisalo E, Virolainen E, Yrjänä E-R (1983) The acute toxicity of Finnish landfill leachates as measured with Daphnia magna test. In Finnish with English summary, Ympäristö ja Terveys 19:224-232.
OECD (Organization for Economical Cooperation and Development) (1981) Daphnia sp, 14-day reproduction test (including an acute immobilisation test). Guidelines for testing of chemicals, 202
SFS (Finnish Standards Association) (1984) Determination of the acute to the acute toxicity with water flea, Daphnia magna Straus. Proposed Finnish standard, in Finnish
Walden CC, Howard TE (1974) Effluent toxicity removal on the west coast. Pulp Paper Mag Can 75:T370-T374
Walsh GE, Bahner HL, Horning WB (1980) Toxicity of textile mill effluents to freshwater and estuarine algae, crustaceans and fishes. Environm Pollut (Ser A) 21:169-179
Walsh GE, Bahner HL, Horning WB (1980) Toxicity of textile mill effluents to freshwater and estuarine algae, crustaceans and fishes. Environm Pollut (Ser A) 21:169-179
Winner RW, Farrel MP (1976) Acute and chronic toxicity of copper to four species of Daphnia. J Fish Res Board Can 33:1685-1691
Wong A, LeBorhis M, Wostradowski R, Prahacs S (1978) Toxicity, BOD and color of effluents from novel bleaching processes. Pulp Paper Can 79:T234-T241

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