Acute Toxicity of Lignosulphonates on Rainbow Trout (*Salmo gairdneri*)[1]

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Waste products from pulp mills, especially liquors from the sulphite pulping process, have attracted attention as major industrial pollution factors in salmon rivers and their estuaries.

Sulphite spent liquor (SSL) contains a relatively high concentration of organic material, a major part of which consists of various hexoses, pentoses, and lignosulphonates. The lignosulphonate content of the liquor ranges from 5 to 6% (TØTTERMAN 1958). Considerable work has been carried out on the effects of SSL on aquatic organisms (WILLIAMS et al. 1953; ALDERDICE and BRETT, 1957; SPRAGUE and MCLEESE, 1968 a, b; WILSON, 1972), but, up to the present, no adequate information on the dose response of fish population to lignosulphonates seems available. The aim of the present work was to determine the acute toxicity of lignosulphonates to rainbow trout.

MATERIALS AND METHODS

The high purity sodium lignin sulphonate powder used in this study was kindly supplied by A/S Alwatech, Norway and had the following characteristics:

Lignosulphonates:		Sulphur:	
high molecular fraction	39.0 %	total	6.67%
low molecular fraction	61.0 %	SO_2, titrated	0.75%
Ash, as sulphate	18.7 %	organic fixed SO_2, soluble	1.18%
Total N, as NH_3	0.32%	Reducing substances, as glucose	0.14%
Solubility in water	99.9 %		

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The rainbow trout used in the toxicity tests were clinically normal fish obtained from the Fish Breeding Experimental Station, Sunndalsøra, and ranging in size from 20.5 - 26.0 g (mean 23.6 g). The fish were environmentally settled for eight days prior to the addition of the lignosulphonates. Poly-ethylene-lined tanks containing 300 liters of test solution served as containers. Artificial aeration was necessary since natural reaeration was insufficient to maintain a suitable dissolved oxygen concentration in the test water. Prior experiments showed that artificial aeration did not increase volatilization of lignosulphonates from the test water. The toxicity tests were conducted under static conditions with aeration. Fresh solutions of lignosulphonates in water were prepared every second morning. The fish density never exceeded 1.0 g of fish per liter of water. Groups of ten healthy fish were each incubated in one of eleven different concentrations of lignosulphonates ranging from 0.625 - 20.000 g/l. At least two, but usually three replicates were run for each concentration. Control groups of ten rainbow trout each, kept under comparable conditions were included. The fish were fed a dry trout feed twice a day during the test period.

The water temperatures, dissolved oxygen, N_2 saturation, conductivity and pH were recorded daily, whereas the NH_3 concentration, total hardness and C.O.D were determined in each tank once a week. The samples were brought to room temperature before analysis, and the measurements were made in accordance with Standard Methods (AMERICAN PUBLIC HEALTH ASSOCIATION 1971). The NH_3 concentrations were calculated as described by EMERSON et al. (1975).

The 48 - hr LC_{50} values were calculated from mortality during the first 2 days of the 28 - day exposures as outlined by the EUROPEAN INLAND FISHERIES ADVISORY COMMISSION (1975), and LITCHFIELD and WILCOXON (1949). The toxicity response curve was calculated and drawn as described by WILSON (1974).

The water temperature during the entire experiment was 9.0° C $\pm$ 0.5°C; and dissolved oxygen was maintained between 80 and 95% air saturation. The average NH_3 concentration was 0.005 ppm with a range from 0.001 to 0.009 ppm. Mean values of the remaining parameters, tested at different concentrations of lignosulphonates are listed in Table 1.

TABLE 1

Mean values of various test parameters at different concentrations of lignosulphonates.

Lignosulphonates (ppm)	pH	Conductivity (microhm/cm)	Total hardness (mg CaO/l)	C.O.D (mg O/l)	N_2 (%)
Control	6.44	15	5	8	99.1
625	6.35	66	37	840	98.3
1,250	6.78	114	69	1,680	97.6
1,875	6.84	138	100	2,320	102.5
2,500	6.91	190	135	3,240	102.7
3,750	7.16	265	188	4,940	101.5
5,000	7.24	340	256	6,600	100.0
7,500	7.31	485	370	9,840	102.3
10,000	7.46	625	501	12,960	99.3
12,500	7.48	785	670	16,200	98.6
15,000	7.51	910	828	19,600	100.8
20,000	7.54	1,015	945	25,000	101.1

RESULTS AND DISCUSSION

The information listed in Table 1 show that the test conditions were satisfactory and did not influence the registered toxicity of the lignosulphonates.

The 48- hr LC_{50} value for lignosulphonates for rainbow trout was calculated to be 7,300 ppm (confidence limits at P = 0.05, 5,700 - 9,340 ppm). During the entire 28 - day exposures, 100% survival was recorded at concentrations of lignosulphonates as high as 1,875 ppm (the concentration at which the mortality ceased to be affected by further exposure). These fish showed no visible response to the presence of the toxicant. On exposure to 2,500 ppm the period of time to reach 50% mortality was 275 hours (Fig. 1).

Before dying, the fish kept at 2,500 ppm lignosulphonates, or higher concentrations, showed very definite signs of illness. The first effect observed was a loss of reaction to sudden movements, and affected fish swam close to the water surface. At this stage rapid and irregular breathing occurred. Death was preceded by loss of coordination, and the fish kept lying on their sides for hours before finally dying.

The discharge of SSL is known to affect the natural pH, BOD, COD, and the transparency of the water recipient (BAGGE 1969). These effects reflect the

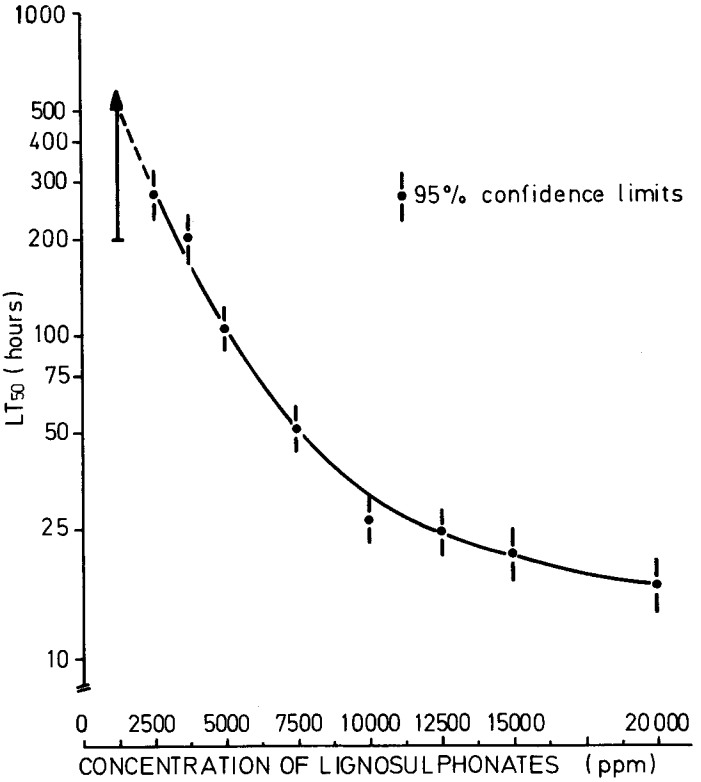

Figure 1. Toxicity response curve for rainbow trout exposed to different concentrations of lignosulphonates for 28 days. LT_{50} (the time in hours to cause 50% mortality) and the concentration of the toxicant are drawn in semilogaritmic scales.
The vertical arrow indicates the median lethal threshold concentration (the concentration at which toxicity ceases to be affected by further exposure).

essential characteristics of the liquor; it contains a substantial amount of organic material, which causes turbidity in its native state and oxygen depletion during its degradation, and it contains a quantity of acidic hydrogen ions. The toxicity is due, therefore, to the interaction of effluent toxicity, lowered pH and lowered oxygen availability (ALDERDICE and BRETT 1957). In fresh water tests with spent sulphite liquor, GRANDE (1964) found that about 600 ppm for 9 days was completely lethal to brown trout (Salmo trutta). The 96-hour LC_{50} of SSL on Atlantic salmon (Salmo salar) varied from 2,000 to 2,400 ppm (WILSON 1972).

The results of the present investigation indicate that the lignosulphonates may be an important factor in the effluent toxicity of SSL responsible for fish

mortality, and that further research on the effects of lignosulphonates on aquatic organisms is needed to elucidate the reaction mechanisms of importance in this connection.

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