# Acute Toxicity of Ammonia-Base Neutral Sulfite Pulp Mill Waste Liquor to Rainbow Trout

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Western North Carolina is noted for its fine trout fishing. In recent years fishing has declined in some areas. Industrial pollution is the cause in some cases. A paper and pulp mill in Jackson County discharged, until its recent closing, untreated ammonia-base neutral sulfite waste liquor into Scott's Creek which flows into the Tuckaseegee River. There is good trout fishing above the creek's entrance into the river but downstream from it trout fishing is poor. The purpose of this study was to determine by laboratory static bioassay the LC50 of this effluent on fingerling rainbow trout (Salmo gairdneri Richardson).

## Materials and Methods

Each bioassay was conducted according to standard methods (APHA, 1965; SPRAGUE, 1969). All trout were obtained from the Waynesville Hatchery of the N.C. Wildlife Resources Commission. They were acclimated to laboratory conditions for seven days in a 160-liter tank having a recirculating water filtration system. After acclimation, ten fish were measured individually and placed into separate 40-liter aquaria. Assignment of a specific concentration of toxicant to each aquarium was accomplished using random number tables. This minimized possible bias due to the fact that either the weaker fish or perhaps the larger fish were caught initially and put into the first aquaria filled. Each aquarium contained premeasured amounts of diluent; dechlorinated, aerated Western Carolina University tap water with a mean pH of 7.7 and methyl orange alkalinity of 38.0 mg/l. The amount of diluent and the number of aquaria depended upon the concentration of neutral sulfite waste liquor (NSWL) desired. For each bioassay, fresh untreated NSWL was collected from the pulp mill effluent pipeline, analyzed and used in bioassays. Using standard procedures (APHA, 1965; HOWELL, 1962) undiluted NSWL was analyzed for ammonia, sulfur dioxide, pH, transmittance, specific gravity, BOD, and filterable solids. Lignin was determined gravimetrically after CaOH precipitation as calcium lignin sulfonate (PEARL, 1967). Temperature, dissolved oxygen, pH, and ammonia were measured daily at the same time of day in both test and control aquaria. An attempt was made to keep dissolved oxygen and temperature levels within the ranges specified by APHA (1965). Fish were allowed one hour to recover before NSWL was added to the diluent. A total volume of 30-liters of test solution was used in each aquarium, and 30-liters of water only in the control. All aquaria received aeration at



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rates made equal as possible. Percent survival to 96 hours was plotted against NSWL concentration. The LC50 was determined on semi-log paper by straight-line graphical interpolation and 95% confidence interval was computed (APHA, 1965).

## Results

Composition of the concentrated NSWL used to make all test solutions and trout length is summarized in Table 1. As these bioassays were conducted over several months, the fish were progressively larger. Regression analysis of the average fish length in each bioassay and the LC50 gave correlation coefficient (r) of 0.49; thus variation in length and LC50 were not highly correlated.

Fish survival data in four 96 hour bioassays are presented in Table 2. Excellent survival rates were obtained in the controls. Almost all of the mortality in the test aquaria occured in the first 2-3 hours. Subsequent mortality was rare. The LC50s obtained were 0.72%, 0.41%, 0.66%, and 1.5% respectively. A mean LC50 of 0.82% and 95% confidence interval of 0.08 - 1.56% was obtained.

Daily analysis of control and test aquaria solutions showed that dissolved oxygen, with few exceptions, did not fall below 5 mg/l and tended to be lower in the higher concentrations of NSWL. This was probably due to the oxygen lowering capacity of NSWL. Ammonia concentration was highest on the first day, generally fell on the second and third day, and increased on the fourth. The range of ammonia concentration in test aquaria was 0.5 - 9.0 mg/l. The pH in all test aquaria was generally lower than 8.5 except in the highest NSWL concentrations where it was 9.0. This parameter was relatively stable in each aquarium throughout the 96 hours. Temperature averaged 16.0 $\pm$ 2 degrees centrigrade in the four bioassays. However, temperature variation in each bioassay rarely exceeded one degree. Dissolved oxygen, ammonia, and pH stayed within acceptable levels in control aquaria.

Regression analysis indicated high correlation between 5 of 8 measured NSWL constituents and the LC50. Lignin content correlated most highly with LC50 with r = 0.96, followed by specific gravity (r = 0.90), sulfur dioxide (r = 0.81), ammonia (r = 0.78), and BOD (r = 0.77). Transmittance, pH, and filterable solids did not appear correlated with LC50 with correlation coefficients being 0.59, 0.32, and 0.28 respectively.

## Discussion

Toxicity similiar to that observed in this study has been reported by other workers. ZIEBELL et al. (1970) noted fish mortalities in field studies when sulfite waste liquor was 8,870 mg/l (0.89%). HOLLAND et al. (1964) reported total mortal-

TABLE 1. Analysis of Neutral Sulfite Waste Liquor Used to Make Test Solutions, and Ranges and Means of rainbow trout lengths

| Bioassay | pH | $SO_2$ mg/l | $NH_3$ mg/l | S.G.[a] | T.[b] % | F.S.[c] g/l | Lignin[d] g/l | B.O.D.[e] | Length (mm) Range | Length (mm) Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10.2 | 210 | 894 | 0.99 | 14 | 0.94 | -- | 1092 | 105-133 | 118.5 |
| 2 | 10.2 | 500 | 1883 | 0.98 | 50 | 0.25 | 1.16 | 2213 | 85-165 | 145.0 |
| 3 | 10.0 | 300 | 1983 | 1.00 | 36 | 0.08 | 1.20 | 821 | 132-180 | 152.8 |
| 4 | 9.6 | 150 | 664 | 0.90 | 70 | 0.07 | 0.39 | 523 | 130-190 | 162.0 |
| Means | 10.0 | 290 | 1356 | 0.97 | 42.5 | 0.34 | 0.92 | 1162 | | |

[a]specific gravity; [b]transmittance at 565 nm; [c]filterable solids; [d]calcium lignin sulfonate; [e] biochemical oxygen demand

TABLE 2 Percent Survival to 96 Hours and the Computed LC 50

| | | | Percent Neutral Sulfite Waste Liquor | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bioassay | LC50 | Control | 0.10 | 0.18 | 0.32 | 0.56 | 0.75 | 1.0 | 1.8 | 3.2 | 5.6 | 10.0 |
| 1 | .72% | 100 | 100 | 100 | 40 | 90 | -- | 0 | -- | -- | -- | -- |
| 2 | .41% | 90 | 90 | 80 | 90 | 0 | -- | 0 | -- | -- | -- | -- |
| 3 | .66% | 90 | -- | -- | 100 | 70 | -- | 0 | 0 | 0 | -- | 0 |
| 4 | 1.50% | 100 | -- | -- | 80 | 100 | 90 | 100 | 30 | -- | 0 | -- |

-- Concentration not used

ity in young chum salmon Oncorhynchus keta Walbaum after six days exposure to 3,290 mg/l (0.32%) ammonia-bass neutral sulfite waste liquor. In our experiments most mortality occured within the first few hours of each bioassay. Additional deaths were few. Perhaps, as DAVIS (1973) suggested this is because the apparent toxicity of the pollutant in static bioassays decreases with time. Another possibility is that the fish became acclimated to the stress.

Fresh NSWL was collected for use in each bioassay. The concentration of components varied between collections. Slight changes in pulping process, sources of supplies, and different rates of drainage from the plant floor and log yard (part of the total effluent), could account for this variability. Correspondingly, a different LC50 was obtained by bioassay using different effluent samples. This suggested a causal relationship between changing LC50 and varying NSWL component concentration. High correlation coefficients for lignin, pH, sulfur dioxide, ammonia, BOD, and LC50 suggested that these components were at least partly responsible for the toxicity of NSWL. Specific gravity also correlated highly. It was probably not in itself toxic but was due to the high concentration of dissolved materials.

The toxicity of ammonia and sulfur dioxide to fish is well known (ELLIS, 1940; McKEE and WOLF, 1963). Little is known about the toxicity of lignin (WILBER, 1969). The unavoidable variation in trout length used in each bioassay was not highly correlated with LC50. Therefore the differing LC50 was attributed to the varying concentration of the constituents of NSWL used in each bioassay.

The poor trout fishing noted in the polluted portion of the Tuckaseegee River might be attributed to the toxicity of NSWL. This could be due to several reasons. The rainbow trout population could have been reduced by the lethality of the pollutant, the reproductive success of the species could have been limited, or the fish food organisms could have been affected. RIDENHOUR and WEST (1973) demonstrated that the latter did occur. Any one or a combination of these situations could explain the poor trout fishing observed in the areas below the mill's outfall into the river. Environmental factors such as temperature and dissolved oxygen may have acted synergistically with the pollutant adding to the variability of the toxic action. The mill cited in this study closed December 31, 1974 due to the cost of needed pollution abatement programs, and uneconomic operation. The recovery of the river will provide an interesting continuing study.

## Summary

The acute toxicity to rainbow trout of untreated ammonia-base neutral sulfite pulp mill waste liquor was demonstrated in these experimants. A mean LC50 of 0.82% and 95% confidence interval of 0.08 - 1.56% was obtained in four static bioassays.

Lignin, sulfur dioxide, and ammonia were implicated as toxic components. The latter two were probably the most toxic.

## References


AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). Standard Methods for the Examination of Water and Wastewater. 12th ed. New York: American Public Health Association (1965).

DAVIS, JOHN C. J. Fish. Res. Bd. Can. 30: 369-377 (1973).

ELLIS, M. M. Pollution of the Coeur de'Alene River and Adjacent Waters by Mine Wastes. In McKee, Jack Edward and Harold W. Wolf. Water Quality Criteria. The Resources Agency of California, State Water Resources Control Board. Pub. No. 3-A (1940).

HOLLAND, G. A., J. E. LASATER, E. D. NEUMAN, AND W. E. ELDRIDGE. Toxic Effects of Organic and Inorganic Pollutants on Young Salmon and Trout. State of Washington Department of Fisheries. Res. Bull. No. 5 (1964).

HOWELL, N. Titrimetric Determination of Combined Ammonia in Ammonium Salts, p. 745. In Standard Methods of Chemical Analysis. D. Van Nostrand Co., New York. (1962).

McKEE, JACK EDWARD AND HAROLD W. WOLF. Water Quality Criteria. The Resources Agency of California, State Water Resources Control Board. Pub. No. 3-A (1963).

PEARL, IRWIN A. The Chemistry of Lignin. New York. Marcel Dekker. (1967).

RIDENHOUR, GEORGE S., JR. AND JERRY L. WEST. ASB Bull 20, No. 2:78.

SPRAGUE, J. B. Wat. Res. 3: 793-821 (1969).

WILBER, CHARLES G. The Biological Aspects of Water Pollution. Springfield, Ill. Charles C. Thomas. (1969).

ZIEBELL, CHARLES D., ROLAND E. PINE, ALVIN D. MILLS, AND RICHARD K. CUNNINGHAM. J. Wat. Pollut. Control Fed. 42(2, part 1): 229-236 (1970).