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Effect of pH on Acute Toxicity of Dehydroabietic Acid and Chlorinated Dehydroabietic Acid to Fish and *Daphnia*

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The toxicity of pulp and paper mill effluents and process compounds has generated a considerable effort to identify specific toxic compounds, their source in the mill and their effect on receiving stream animals. As of 1979, as many as 1668 studies were indexed by The Institute of Paper Chemistry (LOUDEN 1979). Recently, studies have determined that the significant and primary sources of toxicity to rainbow trout in Canadian softwood pulping effluents are resin acids (dehydroabietic, abietic, isopimaric, palustric, pimaric, sandaraco-pimaric, neoabietic), fatty acids (oleic, linoleic, linolenic and palmitoleic), and the chlorinated analogs of these compounds (LEACH & THAKORE 1975, 1977).

Some related work done by MCLEAY et al. (1979b) also suggests that the characteristics of the dilution water in the receiving stream may influence the toxicity of pulp and paper effluents (MCLEAY et al. 1979 a, b). One of the most important variables affecting the toxicity level was pH. McLeay found that when bleached kraft mill effluents were bioassayed at different pH values a range of responses was found. The effects of pH on toxicity of other chemicals such as zinc (MOUNT 1966) and dichlorophenol (HOLCOMBE et al. 1980) have been documented. In both of those cases toxicity decreases as pH increases. While it is common knowledge that test conditions in the laboratory influence the response to toxicants in a bioassay, it is important to remember that some of these conditions exist as variables in the natural environment and will affect impacts of waste discharges.

The work discussed in this report was conducted to determine the degree to which pH affects the toxicity of a common wood resin acid, dehydroabietic acid, and its chlorinated analogs, monochlorodehydroabietic acid and dichlorodehydroabietic acid (LEACH & THAKORE 1977, EASTY et al. 1978).

MATERIALS AND METHODS

Dehydroabietic acid (DHA) (Figure 1) is a relatively stable resin acid found in many coniferous resin by-products. For the bioassays, pure DHA was prepared by an alkaline hydrolysis of the dehydroabietonitrile to the sodium salt followed by

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conversion of the salt to the free acid (SANDERSON 1979). DHA was chlorinated according to the procedures of THAKORE et al. (1977).

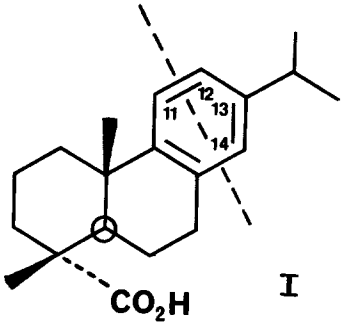

Figure 1. Dehydroabietic acid, $C_{20}H_{28}O_2$, m.w. 300.4

Toxicity response information was collected for fathead minnow (Pimephales promelas) and bluegill sunfish (Lepomis macrochirus) and for the crustacean Daphnia magna. Bluegill came from hatcheries in Wisconsin and Missouri. Fathead minnows were obtained from hatcheries and local bait distributors which provided wild-trapped local fish. At the end of the experimental period, fatheads were used that were reared in the laboratory. Daphnia magna were produced in the laboratory with a culture that originated from a single purchased individual and which has been maintained for more than 100 generations (estimated).

Dilution water was Appleton, Wisconsin city water which had been dechlorinated by activated carbon.

Fish bioassays were done using 96-h static exposures. Aeration was provided with compressed air when oxygen levels could not be maintained otherwise. Twenty-liter containers with 10 fish per container were used for bluegills and fatheads. Five or more serial dilutions plus a dilution water and solvent control were employed for each test. Fish were acclimated to laboratory water for at least two weeks prior to use and were not fed within 24 h of the start of a bioassay. To dissolve DHA, it was necessary to use ethanol and NaOH to form a soluble salt. When this was done, a solvent control was tested which consisted of ethanol and NaOH combined in a concentration equivalent to that found in the highest DHA concentration tested.

Stock solutions were tested by gas chromatography to check levels of test compounds present in the solutions.

Daphnia magna was assayed with an exposure time of 48 h using neonates less than 24 h old. A series of 8 dilutions, a dilution water control, and solvent control were used for each assay.

Twenty individuals were exposed to each concentration in groups of five in 125-mL widemouth glass jars containing 100 mL of solution. Adequate surface area was provided, and dissolved oxygen levels never declined below 2 ppm, which is quite adequate for Daphnia. No food was provided during the test, and neonates were not fed but were collected from a culture medium which included food. The jars were kept in a constant temperature room at 21°C. Survival and chemical parameters (temperature, pH, dissolved oxygen, and conductivity) were monitored at 24 and 48 h. Chemical parameters were monitored in a 5th jar of solution for each concentration at 24 h to avoid damaging the test organisms. At 48 h, or complete mortality, solutions containing Daphnia were monitored.

RESULTS AND DISCUSSION

Dehydroabietic Acid Toxicity. Results for the effects of DHA on Daphnia, fathead minnows, and bluegill sunfish are summarized in Table 1. Because of the unavailability of test animals during the study period, there are fewer data available for fish than for Daphnia. However, this and other work (ZANELLA et al. 1982) have shown that Daphnia 48-h LC_{50}'s are comparable to fathead minnow 96-h LC_{50}'s.

Table 1. Summary of Bioassay Results for Dehydroabietic Acid. Mean and Standard Deviation for LC_{50} in mg/L.

pH	6.5	7.0	7.5	8.0	8.5	9.0	10.0
Daphnia magna	2.47	6.35 (4.7)	10.8 (1.7)	21.7 (7.9)	--	38.3 (8.7)	76.9 (20)
Fathead minnow	1.5 (0.07)	3.2		9.9 (7.2)		45.5 (25)	
Bluegill	4.6[a]	6.4 (4.1)	3.8 (0.10)				

[a]Single assay, not a mean.

From these data, the effect of pH on the toxicity of DHA is clearly evident. Mean values for toxicity to Daphnia at pH 9 are six times greater than at pH 7 and 15 times greater than at pH 6.5. A similar relationship is seen for fish. The data plotted in Figures 1 and 2 show the curve is roughly sigmoid in shape. Because of pH effects and possible differences in Daphnia culture conditions, the standard deviation for LC_{50}'s increased with increasing pH. A wide range of responses was observed.

The curves in Figures 2 and 3 show an inverse relationship to the solubility curve for DHA included in Figure 4. This curve (Figure 3) was calculated on the basis of pKa = 5.7 (NYREN & BACK

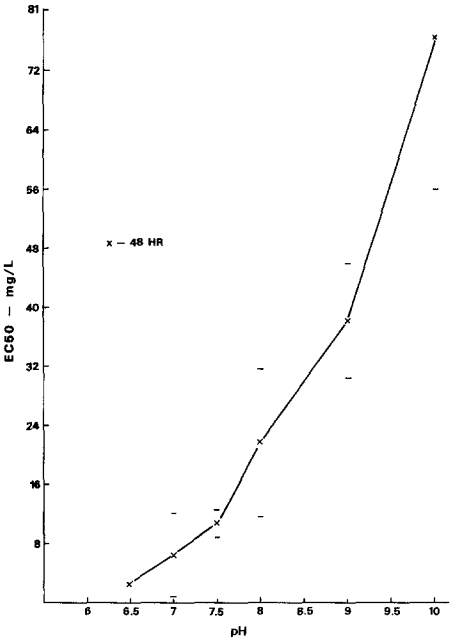

Figure 2. Toxicity curve for Daphnia magna exposed to DHA at different pH values.

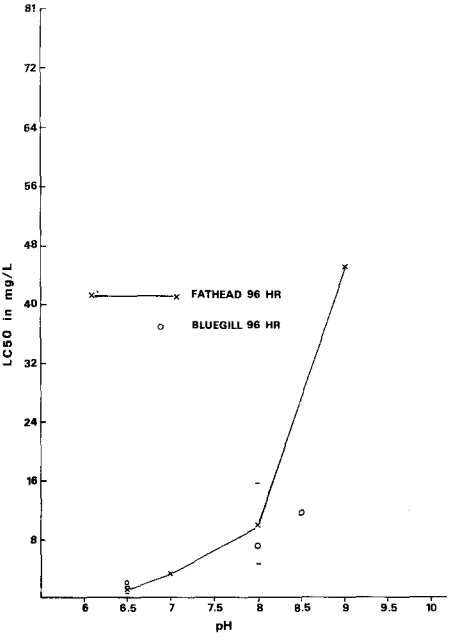

Figure 3. Toxicity curves for fathead minnow and bluegill sunfish exposed to DHA at different pH values.

1958). It can be seen that the greater the percent of free DHA, the greater is the corresponding acute toxicity. At high pH values, the DHA becomes increasingly dissociated and decreasingly toxic. The range of pH at which this effect can be measured is, of course, limited by the upper and lower limits where stress caused by pH alone begins to have an effect on the test organism survival. Nonetheless, it appears that even slight changes in pH have a marked effect on the acute toxicity of DHA.

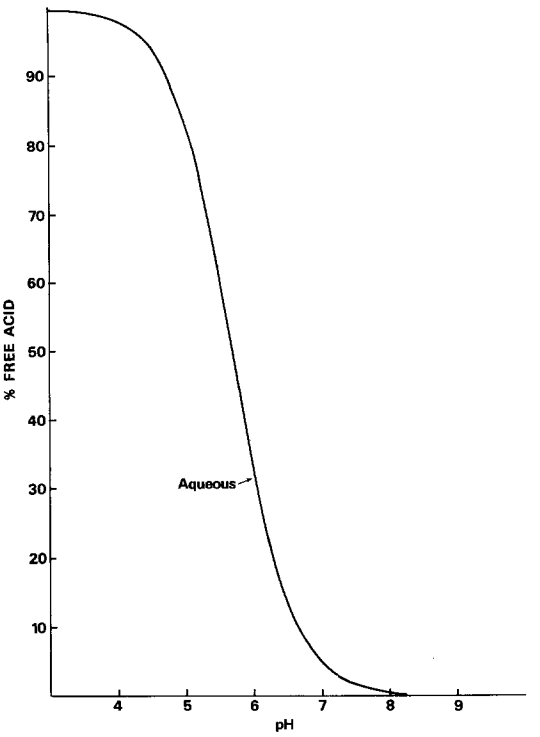

Figure 4. Dissociation curve for dehydroabietic acid in water.

Chlorinated Dehydroabietic Acid. Bioassays were also run for monochlorodehydroabietic acid (MCDHA) and dichlorodehydroabietic acid (DCDHA) in pure form. These compounds may occur in effluents from mills which bleach with chlorine (LEACH & THAKORE 1977, EASTY et al. 1978). Bioassays were run at several pH values for Daphnia and fish, and the results are summarized in Table 2. Curves similar to those for DHA are presented in Figure 5.

For all test organisms, the chlorinated resin acid was more acutely toxic than the nonchlorinated acid. As the degree of chlorination increased, so also did the toxicity. At pH 7, the acute toxicity of DHA to Daphnia was 6.3 mg/L, that of MCDHA was 2.2 mg/L, and that of DCDHA was 1.75 mg/L. Figure 4 shows that this relationship was consistent at all pH values tested. It was also evident that the chlorinated resin acid was less affected by

increasing pH than was the nonchlorinated form. The acute toxicity of both MCDHA and DCDHA decreased much more slowly at higher pH values than DHA. At pH 9 the LC_{50} of DHA to Daphnia was 38.3 mg/L, whereas for MCDHA it was 12.1 mg/L and for DCDHA it was 11.2 mg/L. The dissociation constants for MCDHA and DCDHA are not available; these chlorinated acids may be more insoluble at higher pH and therefore more toxic.

Table 2. Summary of Bioassay Results for Monochlorodehydroabietic Acid (MCDHA) and Dichlorodehydroabietic Acid (DCDHA). Results are Means and Standard Deviations (in Parentheses) in mg/L.

pH	6.5	7.0	8.0	9.0
MCDHA				
Daphnia magna	0.87	2.2	4.1	12.1
	(0.05)	(0.1)	(0.7)	(2.2)
Fathead minnow		1.35 (0.0)		
Bluegill		0.48[a]		
DCDHA				
Daphnia magna	0.66 (0.01)	1.75 (0.2)	2.8 (0.2)	11.25 (0.4)
Fathead minnow		0.67[a]		
Bluegill		0.29[a]		

[a]Single assay, not a mean.

Fathead minnows and bluegills were tested only at pH 7 for MCDHA and DCDHA. The MCDHA 96-h LC_{50} for fatheads was 1.35 mg/L. It was 0.67 mg/L for DCDHA. Bluegills were slightly more sensitive to both chlorinated acids, with 96-h LC_{50}'s of 0.48 mg/L for MCDHA and 0.29 mg/L for DCDHA.

The acute toxicity of dehydroabietic acid is increased by chlorination. However, it is decreased by increases in pH. This response corresponds to changes in the solubility of DHA at different pH values. Chlorinated DHA does not decrease in toxicity with increasing pH to the same degree as nonchlorinated DHA, but it does decrease.

These responses indicate that a prediction of the possible impact of key indicator or "priority" compounds must take into

account the characteristics of the receiving stream. If the acute toxicity measured in an effluent is due to resin acids such as DHA, the effluent may have an enhanced or decreased effect on resident aquatic organisms in the receiving stream, depending on the pH of that stream.

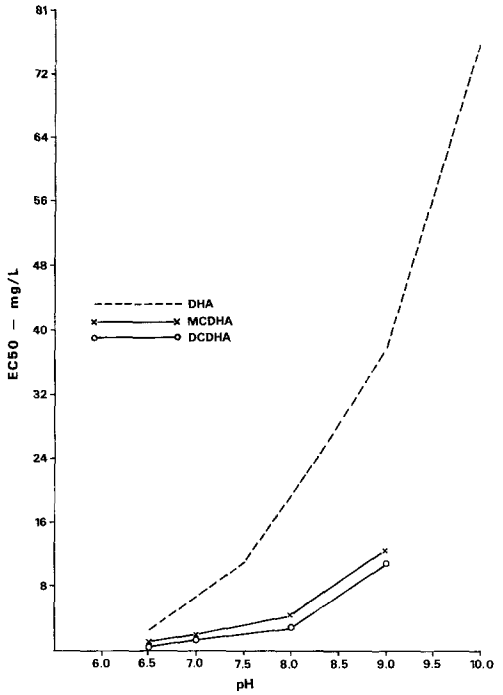

Figure 5. Toxicity curves for Daphnia exposed to chlorinated DHA at different pH values.

The implications of this work are twofold:

1. Control and environmental protection measures must take pH into account.

2. Much more needs to be learned about real and potential impacts of specific pollutants on aquatic ecosystems before reasonable control strategies can be formulated.

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