TOXICITY, UPTAKE AND SURVEY STUDIES OF
BORON IN THE MARINE ENVIRONMENT

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Abstract—Little is known about the toxic and bioaccumulative dangers to aquatic life posed by borate
discharge recently initiated by coastal British Columbia groundwood pulp mills. Bioassays with sodium
metaborate and underyearling coho salmon (Onchorhynchus kisutch) in fresh water yielded a 283-h
LC50 of 113 μg ml⁻¹ (104, 123 = 95% confidence limits). Toxicity to underyearling coho in sea water
appeared considerably greater with a 283 h LC50 of 12.2 μg ml⁻¹ (10.89, 14.56 = 95% confidence limits).
The disparity between fresh and saltwater boron toxicity to coho is not understood at this time.
In salmonids, boron enters the tissues slowly, necessitating prolonged bioassay tests. Sockeye salmon
(O. nerka) and juvenile oysters (Crassostrea gigas) exposed to sublethal doses of boron take up boron
roughly in relation to its availability. Oysters show no bioaccumulative potential or prolonged retention
of boron following cessation of dosage. Field surveys conducted before and after industrial borate
emission confirm the lack of evidence for tissue bioaccumulation. Results of a survey of boron levels
in receiving waters are reported. No hazard to salmonids of oysters at the present level of industrial
discharge of boron (< 1 μg B ml⁻¹) is apparent from this work.

INTRODUCTION

In the pulp and paper industry, a conventional
method for the brightening of groundwood (mechani-
cal) pulp has been by zinc dithionite (ZnS₂O₄; tri-
vially named zinc hydrosulfité). Because of the con-
cern that high levels of zinc in pulp mill effluents
may be resulting in the accumulation of the metal
in shellfish, a survey of waters and shellfish was un-
under taken in 1971. The results of the survey clearly
indicated that zinc concentrations in the vicinity of
one mechanical pulping mill were abnormally high.
Even in areas quite remote from the mill, where zinc
levels in the sea water were apparently normal, oys-
ters still exceeded the maximum permissible level of
100 mg g⁻¹ (marketed weight).

With this in mind, representatives of the pulp and
paper industry in British Columbia indicated in
March 1973 that they would be switching to a new
brightening compound, sodium dithionite (Na₂S₂O₄)
to overcome the zinc contamination problem. How-
ever, in doing so, they would be using the Borol pro-
cess (equation 1) to produce sodium hydrosulfité from
sodium borohydride (NaBH₄) with sodium metabor-
ate (NaBO₂) as a by-product released to the sewer.
NaBH₄ + 8NaOH + 8SO₂ =
4Na₂S₂O₄ + NaBO₂ + 6H₂O (1)

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The chemistry of boron in sea water has been well
studied (Byrne and Kester, 1974; Noakes and Hood,
1961) and the importance of the borate system to the
overall buffering capacity of the oceans is established.
Boron is a major constituent in sea water and is a
conservative element (concentration varies directly
with salinity). More recent work by Uppström (1974)
has established the mean boron-chlorinity ratio in
Pacific Ocean waters to be 0.232 ± 0.005 B (mg kg⁻¹)
Cl⁻(‰)⁻¹. In oceanic coastal waters, away from the
immediate influence of fresh water, the boron content
should bear a linear relationship to chlorinity.
Little is known about the chemical transformations
that boron from industrial sources might undergo
upon introduction into saline waters. It can be
assumed, however, from our knowledge of aqueous
boron chemistry, that metaborate ion will undergo
rapid hydrolysis to the borate anion (equation 2).

BO₂⁻ + H₂O = B(OH)₄⁻ (2)

B(OH)₄⁻ + H₂O ^ H₃BO₃ + 2H₂O (3)

Since boric acid (H₃BO₃) is weakly dissociated, it will
be the predominant boron species found in sea water
(equation 3). Byrne and Kester (1974) have shown
that the acid accounts for 76% of the total boron
species. The borate anion accounts for ~13%, while
the remainder is made up of neutral and positively-
charged borate complexes of sodium, magnesium and
calcium. Little or no contribution can be expected
from organically-bound boron (Noakes and Hood, 1961; Williams and Strack, 1966).

There are limited data available on the boron content of marine organisms or on the physiological effects of this element. Recently Yamamoto et al. (1973) reported that boron in marine zooplankton of various species ranged from 18 to 216 ng g⁻¹ (dry matter). Seaweeds averaged 106 ng g⁻¹. Mann (1973) reported that borates have a comparatively low toxicity to fish. Similar low toxicity to phytoplankton has been noted by Antia and Cheng (1975).

Because of this paucity of information, a study was undertaken as part of a joint Department of Environment project (DOE, 1973) to determine: (a) boron levels in waters and selected marine organisms adjacent to pulp mills where dihydroxides are used for bleaching; (b) the acute toxicity of boron to salmon; and (c) the uptake of boron by coho (Onchorhynchus kisatch) and sockeye (O. nerka) salmon and the Pacific Oyster, Crassostrea gigas. The results of the field surveys and laboratory experiments are described below.

METHODS

Toxicity bioassays with salmon

Underyearling and alevisn coho salmon (Onchorhynchus kisatch) were used for toxicity tests in 301. all-glass aquaria. Solutions were changed every 24 h by transferring the fish to a new tank. For freshwater bioassays, aerated well water at 11.0 ± 0.5 °C with a hardness of 47° as CaCO₃ was used. Seawater bioassays utilized aerated sea water at 8.0 ± 0.5 °C with a salinity of 28 ± 1°. Fish loading density in all bioassays was 0.4-0.7 g 1⁻¹, and test fish were not fed 24 h prior to the test or during the test. Coho alevisn used for freshwater bioassays ranged in weight from 0.19 to 0.7 g, while coho underyearlings for salt water bioassays weighed 1.5-3.8 g. These latter fish were acclimated to sea water during three weeks of gradual increase in salinity. Use of larger fish for seawater tests was necessary, as more mature fish are required for seawater acclimation. For freshwater bioassays using alevisn, 20 fish were used per test tank. For saltwater bioassays, the larger size of test fish and need to stay within fish solution loading guidelines allowed the use of only 6 fish per test tank. During bioassays, the criterion for death was cessation of all movement, including respiratory movement. Dissolved oxygen, pH and temperature were monitored in all tanks throughout the test.

The desired boron concentration was obtained by dissolving analytical grade sodium metaborate (Na₂B₄O₇·5H₂O). Solutions were adjusted to the pH of diluent water by addition of H₂SO₄ or NaOH prior to introduction of the fish. Tests were conducted in this manner for a period of 12 days. Results were expressed as μg B 1⁻¹. The concentration lethal to 50%, of the test organisms (Sprague, 1969) complete with 95% confidence limits (Litchfield and Wilcoxon, 1949).

Boron uptake studies

Boron uptake was studied in young Pacific oysters, Crassostrea gigas, (4.0-6.2 g wet tissue wt) by exposing the animals to elevated background boron levels. In one experiment, 30 oysters were placed in each of 5 identical 80-l. fiberglass tanks containing 60 l. of test solution. A continuous flow of fresh sea water of 500 ml min⁻¹ supplied each tank, together with a continuous flow of 2.0 ml min⁻¹ of concentrated borate solution from a Mar-
All reagents were of analytical grade and used without further purification. Boron standards were prepared from anhydrous boric acid.

RESULTS

Toxicity tests with fish

Boron toxicity to coho alevins in fresh water (from sodium metaborate) was evident for nearly twelve days, after which time there was an indication that the toxicity curve became asymptotic to the time axis (Fig. 2). The 283 h LC50 for freshwater coho alevins was 113 μg ml⁻¹ with 95% confidence limits of 104 and 123 μg ml⁻¹ boron. Boron toxicity to coho un­deryearlings in sea water was considerably greater. The 283 h LC50 for these fish was 12.2 g ml⁻¹ boron with 95% confidence limits of 10.89 and 14.56 μg ml⁻¹ boron.

Boron uptake studies

Tissue analysis was performed on some of the freshwater coho that died during bioassay in the higher boron concentrations. One gram of wet tissue from the homogenate of several fish exposed to a given concentration gave the following results, indicating a dose-related uptake of boron:

<table>
<thead>
<tr>
<th>Sample</th>
<th>μg B ml⁻¹ in water</th>
<th>μg B g⁻¹ in tissue</th>
<th>Median survival time of groups (h)</th>
<th>No. in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(a)</td>
<td>656</td>
<td>354</td>
<td>33.4</td>
<td>20</td>
</tr>
<tr>
<td>1(b)</td>
<td>656</td>
<td>391</td>
<td>33.4</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>328</td>
<td>224</td>
<td>84.0</td>
<td>20</td>
</tr>
</tbody>
</table>

Tissue boron levels in juvenile sockeye (32.2–100.6 g) removed from the aquarium stock tank and not exposed to elevated boron levels are shown in Table 1. Results from individual fish from the same stock exposed for three weeks to 10 μg ml⁻¹ boron in the second experiment are shown in Table 2. Values for control fish are similar for those of stock fish in Table 1, while elevated boron levels in all bor­on-exposed fish are evident, particularly in bone and kidney tissue. Tissue boron levels were not vastly different from water boron levels, suggesting no evidence for active bioaccumulation of boron in cockeye tis­sues.

Results of the boron uptake study with Pacific oys­ters are shown in Fig. 3. It is apparent that tissue boron concentrations approximated the levels in the water within 36 days exposure. Following cessation of dosage, tissue boron levels returned to background levels (3.67–4.01 μg g⁻¹ wet weight, x = 3.84) by the 71st day of the study, illustrating a fairly rapid clearance and no evidence of long-term boron retention. Boron uptake in oysters was slow as the eight day sampling failed to show increased tissue boron levels, even in the group exposed to 10 μg B ml⁻¹. Unfortunately, no results were obtained from the 16-day sampling owing to technical problems.

Field surveys

A total of 71 seawater samples was collected from the four areas shown in Fig. 1. Boron values obtained using the curcumin colorimetric method averaged 3.53 μg ml⁻¹ (s = 26.1%,.) at the surface and
3.86 µg ml⁻¹ (± 28.8%) at 5 m. Concentrations ranged from 0.22 µg ml⁻¹ in Alberni Harbour (Station C14) to 4.68 µg ml⁻¹ in Discovery Passage (Station B13). A linear relationship, B = 0.249 Cl⁻ 0.088, (r = 0.99), was obtained by linear regression analysis of 18 data points chosen at random. The slope of the line (or boron/chlorinity ratio) is 0.249 B(µg ml⁻¹) Cl⁻ 0.088, a value in harmony with those of world surface waters. This is somewhat higher than the value of 0.232 reported by Uppström (1974) for deep Pacific Ocean waters, but very near those values obtained by a less precise method by Igelrud et al. (1938) for waters from Hecate Strait, British Columbia and Portland Canal, Alaska. These

![Graph showing results of acute toxicity bioassays with boron on young coho salmon. Arrows indicate cessation of acute mortality.](image)

**Fig. 2.** Results of acute toxicity bioassays with boron on young coho salmon. Arrows indicate that observations continued up to 23 days for fresh water coho, illustrating cessation of acute mortality.

Table 1. Boron levels in sockeye salmon tissues in normal seawater prior to the boron uptake experiment.

<table>
<thead>
<tr>
<th>Fish Identification</th>
<th>Gross Weight (g)</th>
<th>Fork Length (cm)</th>
<th>Gill</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100.6</td>
<td>20.0</td>
<td>0.7</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>B</td>
<td>82.2</td>
<td>18.7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>C</td>
<td>48.6</td>
<td>15.0</td>
<td>0.6</td>
<td>0.9</td>
<td>0.7</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>D</td>
<td>32.2</td>
<td>13.5</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Mean</td>
<td>65.9</td>
<td>16.8</td>
<td>0.6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Individual fish are designated as A, B, C or D.

Table 2. Boron levels in individual sockeye (A, B, C) in control seawater and two boron exposure tanks containing 10 µg ml⁻¹ B above background.

<table>
<thead>
<tr>
<th>Fish Identification</th>
<th>Gross Weight (g)</th>
<th>Fork Length (cm)</th>
<th>Micrograms Boron Per Gram Tissue (Wet Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Tank, A</td>
<td>43.02</td>
<td>16.5</td>
<td>1.3, 1.0</td>
</tr>
<tr>
<td>(no Boron)</td>
<td>48.49</td>
<td>17.3</td>
<td>1.0, 0.9, 0.7</td>
</tr>
<tr>
<td></td>
<td>50.86</td>
<td>17.5</td>
<td>0.3, 0.3, 0.3</td>
</tr>
<tr>
<td>Tank One, A</td>
<td>40.84</td>
<td>16.5</td>
<td>4.6, 4.8, 5.8</td>
</tr>
<tr>
<td>(after 3 weeks at 10 µg/ml B)</td>
<td>44.76</td>
<td>16.3</td>
<td>6.7, 6.5, 6.8</td>
</tr>
<tr>
<td>C</td>
<td>65.90</td>
<td>18.0</td>
<td>3.5, 3.3</td>
</tr>
<tr>
<td>Tank Two, A</td>
<td>49.40</td>
<td>17.2</td>
<td>3.0, 3.5</td>
</tr>
<tr>
<td>(after 3 weeks at 10 µg/ml B)</td>
<td>49.69</td>
<td>17.4</td>
<td>10.1, 8.8, 11.9</td>
</tr>
</tbody>
</table>

Values are tissue levels of boron following three weeks exposure under the experimental conditions. Values are means obtained from triplicate analysis of each sample.
higher values probably reflect the influence of freshwater runoff.

Two series of oyster tissue surveys were carried out. The initial and more extensive sampling series was made prior to conversion to the Borol process by any of the mills. Concentrations of boron found in oysters from these surveys are given in Table 3 for stations near mills at Powell River and Crofton, B.C. before and after conversion to the new process. The data in Table 3 indicate that there is not a significant difference between the May 1973 (before) and the November 1973 and June 1974 (after) conditions; although there is a slightly higher mean for the latter Crofton data.

A field survey was conducted by the Vancouver Fish Inspection Laboratory to provide a frame of reference for background boron concentrations in various marine shellfish found along the British Columbia coast. These data are given in Table 4.

Results of the bioassay tests with coho salmon indicate that boron is moderately toxic to these fish upon prolonged exposure. Toxicity tests of at least 12 days duration are required. Admittedly, the bioassay data are weakened by the long length of the test and the fact that fish were not fed. These factors could increase the apparent toxic effect. Fish that died in the bioassays had high internal boron concentrations, suggesting that some internal mode of toxic action may be involved. Seawater acclimated coho yearlings were considerably more sensitive to boron than freshwater coho alevins. Differences in fish age, test temperature and available boron levels (due to the background levels of boron in sea water) may account in part for the difference in apparent toxicity in fresh and salt water. It seems unlikely that these results can be explained in terms of salinity stress

Table 3. Boron levels in tissues of Pacific oysters obtained during field sampling

<table>
<thead>
<tr>
<th>Sampling Locations</th>
<th>Station</th>
<th>B (μg/g)a</th>
<th>Sampling Date</th>
<th>May 1973b</th>
<th>Nov. 1973c</th>
<th>June 1973c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crofton, B.C.</td>
<td>D-10</td>
<td>2.6</td>
<td></td>
<td>3.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>D-11</td>
<td>3.2</td>
<td></td>
<td>4.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>D-15</td>
<td>2.8</td>
<td></td>
<td>2.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>D-16</td>
<td>3.8</td>
<td></td>
<td>4.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>D-17</td>
<td>3.6</td>
<td></td>
<td>4.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.2</td>
<td></td>
<td>4.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Powell River, B.C.</td>
<td>A-14</td>
<td>3.9</td>
<td></td>
<td>-</td>
<td>4.1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>A-17</td>
<td>3.1</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>A-19</td>
<td>3.4</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>A-20</td>
<td>3.8</td>
<td></td>
<td>5.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>A-25</td>
<td>3.6</td>
<td></td>
<td>-</td>
<td>3.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.6</td>
<td></td>
<td>-</td>
<td>3.8</td>
<td>-</td>
</tr>
</tbody>
</table>

a Calculated on wet-weight basis. Data represent means of five replicate analyses.
b Samples taken prior to introduction of Borol processes.
c Samples taken subsequent to introduction of Borol process.
Table 4. Summary of boron levels found in various marine shellfish from various locations in British Columbia

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Samples</th>
<th>µg/B·g wet weight</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter Clam (Meretrix litorea)</td>
<td>62</td>
<td>2.4-5.3</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Native Little Neck Clam (Protothaca staminea)</td>
<td>19</td>
<td>0.9-3.4</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Manila Little Neck Clam (Venerupis Japonica)</td>
<td>17</td>
<td>1.5-3.8</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Native and Manila Little Neck Clam</td>
<td>8</td>
<td>1.4-2.8</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Native &amp; Japanese Oyster (Ostrea lurida &amp; Ostrea gigas)</td>
<td>6</td>
<td>3.1-4.0</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Razor Clam (Ensis pustulatus)</td>
<td>9</td>
<td>1.7-3.7</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Sea Mussel (Mytilus edulis)</td>
<td>7</td>
<td>3.7-5.5</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Bay Mussel (Mytilus eilatensis)</td>
<td>2</td>
<td>2.0-4.9</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Cockle (Ctenoidopoma mutaellis)</td>
<td>2</td>
<td>1.6-2.4</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Mya Clam (Mya arenaria)</td>
<td>1</td>
<td>-</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Horse Clam (Goniobranchus asper)</td>
<td>1</td>
<td>-</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Octopus (Polyplotus bimaculatus)</td>
<td>1</td>
<td>-</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Dungeness Crab (Cancer magister)</td>
<td>7</td>
<td>0.9-3.3</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

*These samples were received "mixed".

Boron emissions from the groundwood pulping process in B.C. coastal mills are reported to be below 1 µg B ml⁻¹ in discharged effluent. The results obtained here indicate no obvious hazard at this concentration to salmon or oysters. Boron levels acutely toxic to salmon are at least an order of magnitude higher than the reported discharge concentration. Furthermore, at concentrations close to the incipient LC₅₀, approximately twelve days exposure are required to cause death. No evidence for bioaccumulation of boron in either fish or oysters was found.

The results of the oyster field surveys also suggest that no significant accumulation in tissue was occurring at that time. A slightly higher mean tissue concentration for the November sampling probably reflects the higher salinities existing during a period of lower freshwater runoff. This also reflects our experimental observation that oysters adjust to changes in available boron concentration (Fig. 3).

The survey data shown in Table 4 indicate that, in all species tested, levels of boron approximating that in the water are attained in the tissues. There is no evidence to suggest that bioaccumulation of boron does occur. These data are also of interest in that they represent the first comprehensive survey of boron in shellfish to be reported in the literature.

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Studies of boron


