The evaluation of 5-fluorouracil toxicity on
Corbicula striatella (Deshayes, 1830)

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Abstract
The calculation of toxicity evaluation LC10 and LC50 values for 24 and 96 hours. In present study, the toxicity of 5-Fluouracil on Corbicula striatella. It is allow that which is the increasing time of concentration, mortality rate will be increased. This investigation the accurate sources of their death is not known but it is proved that 5-Fluouracil brings some changes in physiochemical properties of water and it affects the metabolic as well as physiological rate leading to the death of animals. The toxicity study provides the level of safe concentration of toxicant.

Keywords: Corbicula striatella, 5-fluorouracil, toxicity

Introduction
In oceanic water comprises over 70% of the earth’s surface covered by water is most precious natural resource that exists on our planet. Without the seemingly invaluable compound compromise of hydrogen and oxygen, the life on earth would be non-existent, it essential for everything on our planet to grow proper. Subsequently, we are slowly but surely harming our planet to the point where organisms are dying at very alarming rate. In addition to innocent organism dying off, our drinking water has become greatly affected as is our ability to use water for recreational purposes. 5-Fluouracil is the anticancer drug and is used as multidrug administration in vitro in the human breast cancer cell line. This drug induced 60% cell death at 5 μg/ml dose. It is also sometimes used to induce apoptosis. In order to test the hypothesis that elemental diets protect the intestinal mucosa against 5-fluouracil toxicity, (Jane 1983) also, none of the elemental diets reduced the body weight losses observed after 5-fluouracil administration. Although the effects of the sodium salt of 5-fluouracil on body weight and food intakes were much less severe than those of the Tris salt. Results do not support the suggestion that elemental diets might reduce the intestinal toxicity of 5-fluorouracil.

Materials and Methods
The method described by Finney (1951), Medium sized bivalves, Corbicula striatella were collected from the Girna Dam near Chalisgaon city, M.S. India, latitude 200 28’ 58” N, longitude 740 43’ 13” E and brought to the laboratory. The room temperature was 23 °C – 26 °C and the pH of water was in the range of 7 – 8. Lethal toxicity tests were conducted over 96 hours. The experimental troughs containing 5 liters dechlorinated water were used to expose the animals. Stock solutions of 5-fluorouracil were prepared in double glass-distilled water and added to the test medium to get the desired concentration. Ten animals were exposed to 10 – 12 different concentrations of heavy metals. After every 12 hours, the water was changed by the fresh solution of the same concentration of 5-fluorouracil. Dead bivalves were counted individually. The resulting mortality was noted in the range of 10 to 90 % for each concentration for the duration of 24, and 96 hrs. Each experiment was repeated thrice till constant results were obtained.

Acute toxicity tests were carried out under static conditions up to 96 hours. The data collected was then analyzed statistically by means of the Probit method on transforming the toxicity curve (% mortality versus log of Conc.) into regression lines (Mortality in Probit / log Conc.) which allows the average medium lethal concentration of LC50 to be calculated for 24 and 96 hrs.

Discussion
Experiments during recent years have shown that higher dose of anticancer drug compounds are injurious to molluscs. Further, in the extremely low concentration level of these
substances, the bivalves and other organisms in aquatic environment are exposed for as long as they persist in the medium. The evaluation of LC50 concentrations of anticancer drugs or toxicant is the first step before carrying further studies on physiological changes in animals. Anticancer drugs, 5-fluorouracil effective antitumour agents utilized in the treatment of a wide variety of malignancies. Nuclear transfer technology (Campbell, 1999; Prather et al., 1999; Gomez et al., 2003) [1, 9, 3], in studies of the cell cycle, the most frequently measured attribute is DNA content, whereby this marker indicates cell maturity in the cycle. The identification of a relatively highly proliferative tissue type from bivalves could be relevant across molluscan taxa and will contribute to the attempts being made at establishing cell culture lines from oysters or bivalves in order to delineate disease pathogenesis mechanisms. Moreover, the choice of an actively dividing tissue is useful in cytogenetics, for examining proliferation as a biomarker because of stressor impacts in feral oysters, and for assessing proliferative or non proliferative influences of chemical compounds. Different tissues of complex multicellular organisms are composed of cells having characteristic morphologies, with each tissue having its own growth properties (Tapon et al., 2001) [11]. The proliferative capability of cells derived from specific organs of bivalves has been studied, as has that of cells from digestive gland (Marigomez et al., 1999) [8] and gill cells from mussels (Gomez-Mendikutte et al., 2005; Sollid et al., 2005) [10]. The identification of a relatively highly proliferative tissue type from bivalves could be relevant across molluscan taxa and will contribute to the attempts being made at establishing cell culture lines from oysters in order to delineate disease pathogenesis mechanisms. Moreover, the choice of an actively dividing tissue is useful in cytogenetics, for examining proliferation as a biomarker because of stressor impacts in feral oysters, and for assessing proliferative or non proliferative influences of chemical compounds. The pyrimidine analog 5-fluorouracil is still one of the most useful antitumour agents for treatment of solid cancers (Longley et al. 2003) [9]. In spite of this, its mechanism of action is still not fully understood. Thymidylate synthase is thought to be one important target of 5-fluorouracil (Parker and Cheng 1990) [3]. The 5-Fluorouracil plays an important role in the systemic treatment of various solid tumors, such as gastrointestinal cancer, breast cancer, and head and neck cancer. Extensive knowledge has been acquired on the metabolic pathways and mechanism of action of 5-FU. (Pinedo and Peters, 1998) [8].

Observation tables
Calculation of LC10 and LC50 values
Calculation of LC10 and LC50 5-fluorouracil calculated from regression equation Y = 3.7184 and Y = 5.00 (values from Finney’s Table 1) were used to calculate LC10 and LC50 values in ppm of 5-fluorouracil respectively for 24 and 96 hours.

Calculation of percent mortality
Abbots formula (1925) was used for getting the exact mortality due to toxicant as below.

\[ \text{P} = \frac{\text{Om} - \text{Cm}}{100} \]

Where,
- \( \text{P} \) - Corrected mortality
- \( \text{Om} \) - Observed mortality
- \( \text{Cm} \) - Control mortality

(All percentages)

It was observed that there was no mortality in control group of bivalves. The mortality data obtained in experimental set of bivalves for each dose was calculated by Finney’s formula.

\[ r = \frac{\text{P}}{n} \times 100 \]

Where,
- \( r \) - Percentages mortality
- \( n \) - Number of animals exposed in batch.

Results
The mortality data thus obtained was put into empirical probit / log concentration to plot Probit regression lines. The 50% mortality and 10% mortality causing Corbicula striatella were 0.5-fluorouracil. Static acute toxicity test were carried out in the laboratory condition until 96 hours duration for 5-fluorouracil. Toxicity test were conducted for 24 and 96 hrs by the method described by Finney (1951). The regression equations were obtained for 5-fluorouracil. The result obtained after toxicity evaluation of 5-fluorouracil on Corbicula striatella are cited in tables 1. To 2.

The LC10 and LC50 values for 5-fluorouracil are 4.2257 ppm respectively. The LC10 values for 24 and 96 hrs are 3.193 and 31.39 ppm respectively. The LC50 values for 24 and 96 hrs exposures to 5-fluorouracil for 24 and 96 hrs are 10.58 and 37.16 ppm respectively.

Conclusion
5-Fluorouracil is the anticancer drug safe and minimum dose are useful to treatment of animals otherwise excess or high dose are harmful. 5-fluorouracil to inhibit the extra mass of malignancies such as dose depends upon the animal body structure. Safe dose to prevent the abnormal growth of cells or apoptosis. High dose are regulate toxicity of animal get increase mortality.

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### Table 1: Calculation of Regression equation for LC$_{10}$ and LC$_{50}$ values of Corbicula striatella exposed to 5-fluouracil for 24 hrs.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. of 5-FU (PPM)</th>
<th>Log of Conc. to base 10 'x'</th>
<th>No. of bivalves exposed 'n'</th>
<th>Mortality for 24hrs. 'r'</th>
<th>% mortality P=$(_{100r}/n$</th>
<th>Empirical probit 'X'</th>
<th>Expected probit 'Y'</th>
<th>Weighing coefficient 'w'</th>
<th>Weight W= nw</th>
<th>Working probit 'Y'</th>
<th>Wx</th>
<th>Wy</th>
<th>Wx$^2$</th>
<th>Wy$^2$</th>
<th>Wxy</th>
<th>Improved expected probit 'Y'</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>2.5</td>
<td>0.3979</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>3.7184</td>
<td>3.25</td>
<td>0.17994</td>
<td>1.7994</td>
<td>4.012</td>
<td>0.7159</td>
<td>7.2191</td>
<td>0.2848</td>
<td>28.9630</td>
<td>2.8721</td>
<td>3.45</td>
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<td>II</td>
<td>5.0</td>
<td>0.6989</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>4.1584</td>
<td>4.15</td>
<td>0.47144</td>
<td>4.7144</td>
<td>4.160</td>
<td>3.2948</td>
<td>19.6119</td>
<td>2.3027</td>
<td>81.5855</td>
<td>13.7063</td>
<td>4.19</td>
</tr>
<tr>
<td>III</td>
<td>7.5</td>
<td>0.8790</td>
<td>10</td>
<td>3</td>
<td>30</td>
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<td>0.60052</td>
<td>6.0052</td>
<td>4.479</td>
<td>5.2545</td>
<td>26.8972</td>
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<td>1.0000</td>
<td>10</td>
<td>4</td>
<td>40</td>
<td>4.7467</td>
<td>5.02</td>
<td>0.63662</td>
<td>6.3662</td>
<td>4.749</td>
<td>6.3642</td>
<td>30.233</td>
<td>6.3662</td>
<td>30.233</td>
<td>4.93</td>
<td></td>
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<td>V</td>
<td>12.5</td>
<td>1.0969</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>5.0000</td>
<td>5.22</td>
<td>0.62742</td>
<td>6.2742</td>
<td>4.977</td>
<td>6.8821</td>
<td>31.321</td>
<td>7.5489</td>
<td>156.6664</td>
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<tr>
<td>VI</td>
<td>15.0</td>
<td>1.1760</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>5.5244</td>
<td>5.52</td>
<td>0.58099</td>
<td>5.8099</td>
<td>5.524</td>
<td>6.8324</td>
<td>32.0938</td>
<td>8.0349</td>
<td>177.2861</td>
<td>37.7421</td>
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<tr>
<td>VII</td>
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<td>1.2430</td>
<td>10</td>
<td>8</td>
<td>80</td>
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<td>0.53159</td>
<td>5.3159</td>
<td>5.834</td>
<td>6.6076</td>
<td>31.0129</td>
<td>8.2132</td>
<td>180.9292</td>
<td>38.5487</td>
<td>5.53</td>
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</tbody>
</table>

\[
l_c = \frac{\sum Wx}{\sum W} = 35.9535 = 0.9908\]

\[
l_{c_{50}} i.e. X = \frac{5+(b(x^- - y^-))}{b}  \]

\[
l_{c_{50}} = \frac{5+(2.4622 \times 0.9908) - 4.9171}{2.4622} = 5.5424 \times 0.9908 - 4.9171 \]

\[
Y = y^- + b(X - x^-) = 4.9171 + 2.4622X - 2.4622X \times 0.9908 = 2.4622X - 2.4775
\]

### Table 2: Calculation of Regression equation for LC$_{10}$ and LC$_{50}$ values of Corbicula striatella exposed to 5-fluouracil for 96 hrs.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. of 5-FU mg/lit</th>
<th>Log of Conc. to base 10 'x'</th>
<th>No. of animal exposed 'n'</th>
<th>Mortality 96 hours 'r'</th>
<th>Percentage mortality P=$(_{100r}/n$)</th>
<th>Empirical probit</th>
<th>Expe-ceted probit 'Y'</th>
<th>Weighing coefficient 'w'</th>
<th>weight W= nw</th>
<th>work-ing probit 'Y'</th>
<th>Wx</th>
<th>Wy</th>
<th>Wx$^2$</th>
<th>Wy$^2$</th>
<th>Wxy</th>
<th>Improved expected probit 'Y'</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>2</td>
<td>3.5</td>
<td>10</td>
<td>2</td>
<td>20%</td>
<td>4.7467</td>
<td>4.80</td>
<td>0.62742</td>
<td>6.2742</td>
<td>4.747</td>
<td>9.764573</td>
<td>29.783627</td>
<td>15.196</td>
<td>46.352</td>
<td>141.382</td>
<td>4.757</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>3.9</td>
<td>10</td>
<td>4</td>
<td>40%</td>
<td>5.2553</td>
<td>5.45</td>
<td>0.60052</td>
<td>6.0052</td>
<td>5.250</td>
<td>9.554273</td>
<td>31.5273</td>
<td>15.200</td>
<td>50.159</td>
<td>165.518</td>
<td>5.365</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>4.1</td>
<td>10</td>
<td>8</td>
<td>80%</td>
<td>5.8416</td>
<td>5.84</td>
<td>0.50260</td>
<td>5.0260</td>
<td>5.841</td>
<td>8.105430</td>
<td>29.356866</td>
<td>13.0715</td>
<td>47.3438</td>
<td>171.473</td>
<td>5.745</td>
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<tr>
<td>V</td>
<td>5</td>
<td>4.4</td>
<td>10</td>
<td>9</td>
<td>90%</td>
<td>6.2816</td>
<td>6.29</td>
<td>0.37031</td>
<td>3.7031</td>
<td>6.278</td>
<td>6.085674</td>
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<td>10.000</td>
<td>38.205</td>
<td>145.951</td>
<td>6.283</td>
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<td>VI</td>
<td>6</td>
<td>4.7</td>
<td>10</td>
<td>10</td>
<td>100%</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>SW = 25.7229</td>
<td>SWx = 40.66873</td>
<td>SWy = 133.52776</td>
<td>SWx$^2$ = 64.340</td>
<td>SWxy = 211.84255</td>
<td>SWy$^2$ = 705.911</td>
</tr>
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\[
S_W = \frac{\sum W}{n = 35.9535} = \frac{\sum Wx}{n} = 37.3483 = 23.5483 = 181.0272
\]

\[
S_{Wy} = \frac{\sum W}{n = 35.9535} = \frac{\sum Wy}{n} = 177.2861 = 177.2861 = 177.2861
\]

\[
S_{Wxy} = \frac{\sum W}{n = 35.9535} = \frac{\sum Wxy}{n} = 181.0272 = 181.0272 = 181.0272
\]
\[ Y = y + b \left( x - x_0 \right) \]

\[ SW_x = 40.66873 \]
\[ SW_y = 133.52776 \]
\[ SW_{xy} = x \cdot SW_y \]

\[ SW_y = 25.7229 \]
\[ SW_{xy} = x \cdot SW_y \]
\[ b = \frac{SW_{xy} - x \cdot SW_y}{SW_x^2 - x \cdot SW} \]
\[ LC_{50} = \frac{211.84255 - 1.5810321 \cdot 133.52776}{64.340287 - 1.5810321 \cdot 40.66873} = \frac{211.84255 - 211.11167}{-64.29856} = 0.0417194 \]

References