Genotoxic evaluation of lambda-cyhalothrin in *Mystus gulio*

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Abstract: Genotoxic potential of pyrethroid pesticide lambda cyhalothrin (Karate) on *Mystus gulio* was studied using chromosomal aberration test in the gill cells. Fishes exposed in vivo to 6.4 ppb (10% 96hr LC50) concentration of compound and sacrificed at four different sampling times 24, 48, 72, and 96 hr against the parallel-untreated tap water control. The different types of aberrations observed were chromatid breaks, centromeric fusion, acentric fragments, chromosomal gaps, sticky plates, aneuploidy and ring chromosomes. Chromosomal aberrations were found to be significantly increased in number than in control, and are statistically significant at (p<0.05) level.

Key words: *Mystus gulio*, Lambda-cyhalothrin, Gill cells, Chromosomal aberrations, Genotoxicity.

Introduction

Pesticides are an important group of environmental pollutants and many of which are reported to be mutagenic (Sandhu et al., 1985, Waters et al., 1982). Fish are excellent material for the study of the mutagenic and/or carcinogenic potential of contaminants present in the water samples since they can metabolize, concentrate and store water borne pollutants (Al-Sabti, 1991).

Introduced commercially less than 20 years, synthetic pyrethroids now account for more than 30% of insecticide used worldwide in agricultural, domestic, veterinary applications (Eisler, 1992). These halogenated and lipophilic compounds are generally recognized as potent neurotoxins, characterized by high insecticidal properties and low mammalian toxicity. Synthetic pyrethroids are extremely toxic to fish and other aquatic organisms (Bradbury and Coats, 1989, Clark, 1995). According to Coats et al. (1989) they exhibit deleterious effects at sublethal levels.

Fish and other aquatic animals can potentially be used to assess the genotoxicity of xenobiotics using endpoints as chromosomal aberrations, sister chromatid exchange, and micronuclei (Hooffman, 1981; Alink et al., 1980; Al-Sabti, 1986).

The present study evaluates the genotoxic effect of Lambda cyhalothrin in the catfish, *Mystus gulio*.

Materials and Methods

The studied compound lambda-cyhalothrin (trade name ‘karate’) CAS chemical name: (R+5) - α - cyano - 3 - (phenoxyphenyl) methyl - (1S+1R) - Cis - 3- (z-2-chloro-3,3,3 – trifluoroprop-1-enyl)-2,2 - dimethyl - cyclopropene- carboxylate, CASRN 91465 - 06 - 06 was from Zeneca.

The choice of *Mystus gulio* was based on the fact that it is commonly available in most of the lakes in Tamil Nadu. Fishes weighing 9.36g ± 0.56 (mean ± SD) and length of 6-8cm were procured from Tamil Nadu fish seed farm, Pattabiram, Chennai. Prior the experiments, fish were acclimatized in an aquarium of 150 litre of well-aerated water. Fish were fed *ad libitum* with artificial feed.

The acute toxicity of Lambda cyhalothrin to *Mystus gulio* for 96 hr exposure was found to be 64 ppb. The fishes were maintained during the exposure period in aquaria, with fresh water containing 6.4 ppb of (10 % 96 hr LC50) Lambda cyhalothrin. Fishes were not fed for 24 hr before testing. The study was accompanied by using parallel tap water control. The chromosomal aberrations such as chromatid breaks, centromeric fusion, acentric fragments, chromosomal gaps, sticky plates, aneuploidy and ring chromosomes were examined at four sample times (24, 48, 72, 96 hr) for the treated animals and control.

Chromosomal preparations were made following standard method (Kligerman, 1982). The test fishes were injected with 0.02% Colchicine intra-peritoneally. The fishes were left undisturbed for 3-4 hr. After this time duration, the inner most gill arch was excised from the treated fish and was placed in 0.4% KCl (hypotonic solution) for 30-60 mts. Then fixed in Carnoy’s fixative at regular intervals of time, after which cell suspension was made using 50% acetic acid. Spreads were prepared on flame-dried slides by dropping method and were stained with 5% Giemsa for 30 min. The slides were then screened for metaphase plates.

Statistical analysis: The data collected were subjected to one way analysis of variance (ANOVA) and the mean were separated by using least significant different test, in order to detect the statistically significant differences in the chromatid break, centromeric fusion, acentric fragments, chromatid gap, sticky plate, ring chromosome at different periods of exposure.

Results

The diploid number of chromosomes in *Mystus gulio* was found to be 58 for both sexes. From every pooled preparation 100 metaphase plates were analysed for each time intervals. Aberrations observed were chromatid breaks, acentric fragments, centromeric fusions, aneuploidy, condensation, sticky plates and ring chromosomes. Frequencies of chromosomal aberration determined in different treatments are summarized in Table 1. The higher frequency of chromosomal
Fig. 1: Chromosomal aberrations in Mystus gulio exposed to the pyrethroid insecticide lambda-cyhalothrin. Giema-stained Metaphase plates, 1000x.
1. Normal metaphase plate
2. Condensed chromosome and Acentric fragments
3. Chromatid gap and Ring Chromosome
4. Breaks
5. Breaks and fragments
6. Centromeric fusion
7. Sticky plate
8. Ring chromosome and fragments
9. Aneuploidy.
al. repress the nucleolar activity. According to Ayla Celik et al. (1998), many chemicals produce similar aberrations though their mechanism may be different in different cases (Manna, 1990). Organophosphorus pesticides (Das and John, 1999). It seems for the control of malaria and other tropical diseases (Farmer et al., 2003), lambda cyhalothrin induces chromatid and isochromatid breaks (6.33) at 72 hr exposure. And followed by sticky plates and ring chromosomes. Similar to those observed by Ayla Celik, Birgili Mazanci, Yusuf Camalica, Aliaskin and Ulku Comelekoglu: Cytogenetic effect of lambda cyhalothrin on Wistar rat bone marrow. Mutat. Res., 539, 91-97 (2003).

The present result obtained from in vivo exposure of Mystus gulio to lambda cyhalothrin showed the genotoxic effects such as chromatid breaks, acentric fragments, centromeric fusions, aneuploidy, condensation, sticky plates and ring chromosomes. Similar to those observed by Channa punctatus treated with Dichlorvos (Rishi and Grewal, 1995), Oreochromis mossambicus treated with pyrethroid fenvalerate (Arokia Rita and Selvanayagam, 1998), Heteropeustes fossilis exposed to pentachlorophenol (Niamat Ali and Waseem Ahmad, 1998), and Etropus suratensis exposed to organophosphorus pesticides (Das and John, 1999). It seems that many chemicals produce similar aberrations though their mechanism may be different in different cases (Manna, 1990).

The genetic toxicity and mutagenecity studies concerning the synthetic pyrethroids produced controversial results depending on the assay used. Lambda cyhalothrin produced negative results in all Ames assay using five different test strains, with or without metabolic activation (Royal Society of Chemistry 1991, USEPA, 1995). Genotoxic evaluation of lambda cyhalothrin on fish was performed by Campana et al. (1999), who reported an increase in the micronuclear frequency in erythrocytes of Cheirodon interruptus exposed to different doses of this insecticide. Cavas and Ergene-Gozukara (2003) have reported that lambda cyhalothrin has the ability to repress the nucleolar activity. According to Ayla Celik et al. (2003), lambda cyhalothrin induces chromatid and isochromatid types of gaps and breaks, double minute, exchanges, dicentric chromosomes and fragments on Wistar rat bone marrow cells.

The observed results clearly indicate that lambda cyhalothrin brings chromosomal aberrations even when the fishes are not injected with the pesticide. In the conclusion, the results indicate the frequency of chromosomal aberrations in the fish serves as a tool to assess genotoxic pollutants.

### References


Farmer, D., J.R. Hill and S.J. Maund: A comparison at the fate and effects at two pyrethroid insecticides, Lambda cyhalothrin and Lambda cyhalothrin. Occasionally used for the control of malaria and other tropical diseases (Farmer et al., 1995). Lambda cyhalothrin is a newer pyrethroid, first marketed in 1985. Apart from agricultural uses, it has public and animal health applications, and was first insecticides to be passed by the WHO Pesticide Evaluation Scheme (WHO-PS) and animal health applications, and was first insecticides to be marketed in 1985. Apart from agricultural uses, it has public and animal health applications, and was first insecticides to be passed by the WHO Pesticide Evaluation Scheme (WHO-PS). Lambda cyhalothrin results depending on the assay used. Lambda cyhalothrin brings chromosomal aberrations even when the fishes are not injected with the pesticide. In the conclusion, the results indicate the frequency of chromosomal aberrations in the fish serves as a tool to assess genotoxic pollutants.

### Table – 1: Frequency of chromosomal aberrations induced by Lambda-cyhalothrin in gill cells of Mystus gulio (Ham).

<table>
<thead>
<tr>
<th>Treatment (hr)</th>
<th>Chromatid break</th>
<th>Centromeric fusion</th>
<th>Acentric fragments</th>
<th>Chromatid gap</th>
<th>Sticky plate</th>
<th>Aneuploidy</th>
<th>Ring chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>4.6±0.88</td>
<td>0.3±0.00</td>
<td>5.3±0.66</td>
<td>±0.00</td>
<td>1.66±0.33</td>
<td>0±0.00</td>
<td>0.66±0.33</td>
</tr>
<tr>
<td>48</td>
<td>5.66±0.88</td>
<td>0.33±0.33</td>
<td>7.66±1.45</td>
<td>0.66±0.33</td>
<td>3.33±0.88</td>
<td>0±0.00</td>
<td>1.33±0.88</td>
</tr>
<tr>
<td>72</td>
<td>6.33±1.20</td>
<td>3.66±0.88</td>
<td>12.3±0.88</td>
<td>5±1.15</td>
<td>2.33±0.88</td>
<td>3.33±0.88</td>
<td>2.66±1.20</td>
</tr>
<tr>
<td>96</td>
<td>5.66±1.20</td>
<td>4.33±0.88</td>
<td>7±1.15</td>
<td>4.33±0.88</td>
<td>5.33±1.45</td>
<td>2.33±1.45</td>
<td>2.66±0.66</td>
</tr>
<tr>
<td>Control</td>
<td>1±0.57</td>
<td>0.33±0.33</td>
<td>0±0.00</td>
<td>0.66±0.33</td>
<td>0±0.00</td>
<td>0.00±0.00</td>
<td>0.33±0.33</td>
</tr>
<tr>
<td>LSD</td>
<td>3.43</td>
<td>2.105</td>
<td>3.52</td>
<td>2.105</td>
<td>3.39</td>
<td>2.76</td>
<td>2.67</td>
</tr>
</tbody>
</table>

p<0.05


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