

POECILIID RESEARCH

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Investigation on acute toxicity of lindane in guppies, *Poecilia reticulata* Peters, 1859

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Abstract. The objective of this paper was to present a correlation concerning the acute toxicity of lindane and the color markers in guppies; also, the main fish measurements were investigated. In this view, a number of 36 healthy Red Blond guppy fish (*Poecilia reticulata*) were selected for the experiments and randomly separated into four experimental groups, as follows: group I – tap water, group II – 0.007 ppm lindane, group III – 0.08 ppm lindane, group IV – 0.16 ppm lindane. Our results supported our hypothesis that the increasing of lindane concentration used in experiment was responsible for the predicted effect of decreasing of red pigmentation and fish dimensions. According to such results as perturbation of red pigment expression in the skin, lindane should be considered endocrine disrupting pesticide as long as red pteridine synthesis is related to the level of testosterone. The fish measurements and color analysis in Red Blond variety of guppy has the potential to become the key test to identify the concentration of lindane in aquatic ecosystems. These results are useful in mathematical models to mark the toxicity risk prediction on guppies.

Key words: guppy, *Poecilia reticulata*, red, orange area, lindane, HCH toxicity.

Introduction. Many pesticides inhibit specific metabolic processes in animal organisms and therefore they are included in the group of endocrine disrupting substances (Baatrup & Junge 2001; Fleseriu 2010; Georgescu et al 2005).

Endocrine disruptors are exogenous substances that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (Crisp et al 1998). They are sometimes also referred to as hormonally active agents (Krimsky 2001), endocrine disrupting chemicals (Diamanti-Kandarakis 2009), or endocrine disrupting compounds (Petrescu-Mag et al 2010).

Lindane (γ -HCH) is an organochlorine pesticide that has been used for long time as an agricultural insecticide. Lindane (Figure 1) is a neurotoxin that interferes with GABA neurotransmitter function by interacting with the GABA_A receptor-chloride channel complex at the picrotoxin binding site.

It is not yet very clear whether lindane is an endocrine disruptor or not, but it affects liver, kidneys, nervous system, and it is considered carcinogenic (Reuber 1979). However, even classified as moderately hazardous by World Health Organization, in 2009 the production of lindane for agricultural use was banned under the Stockholm Convention on persistent organic pollutants. A specific exemption to that ban allows it to continue to be used as a pharmaceutical product for the treatment of scabies and lice.

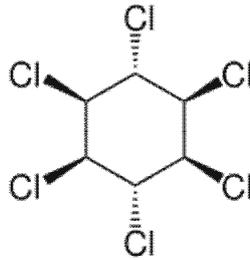


Figure 1. Lindane (gamma-hexachlorocyclohexane)(wikipedia.org).

In the last decades, there is considerable interest in computer-assisted image analysis of biological images for the assessment of endocrine disrupting amplitude (Baatrup & Junge 2001), specific adaptations of the living organisms, or pathologic biomedical issues (Tălu 2012ab). Image textures are defined as complex visual patterns composed of entities having characteristic brightness, colors, slopes, sizes etc. Thus, texture can be regarded as a similarity grouping in an image (Rosenfeld 1982; Materka & Strzelecki 1998).

Purpose. The aim of this paper is to study the acute toxicity of lindane, a toxic pesticide and potential toxic pollutant contaminating aquatic ecosystems, in guppy (*Poecilia reticulata*). We also intend here to open a new sub-field of research, based on a possible correlation of color markers or size markers in guppies with the pesticide concentration (with lindane in this particular case).

Materials and Method. A number of 36 healthy Red Blond guppy fish (*P. reticulata*) were selected for the experiments (Figure 2). The young fish had on average the age of ten days and were randomly separated into four experimental groups (nine fish per tank of 10L volume each), as follows: group I – tap water, group II – 0.007 ppm lindane, group III – 0.08 ppm lindane, group IV – 0.16 ppm lindane. These concentrations reflect: II - the maximum concentration in surface waters of Cluj county (data not shown here), III – 2x maximum admissible concentration, IV – 4x maximum admissible concentration. They were kept under experimental conditions for five months in the same water (the aquatic environment was permanently recycled). Water parameters: guppies were kept in aquaria at $25 \pm 1^\circ\text{C}$ under a 14/10 h light/dark cycle. They were fed with a commercial diet, with 48% protein, three times a day ad libitum. Water quality parameters (O_2 , pH, ammonia, nitrite and nitrate) were monitored every 10 days during the experiment.

At the end of the experiment, fish were photographed from the left and right sides, as presented in Figs 2-5. The best left and right flank photos were selected for digital examination and statistics. The average value of left view and right view for each fish was calculated for statistical analysis.

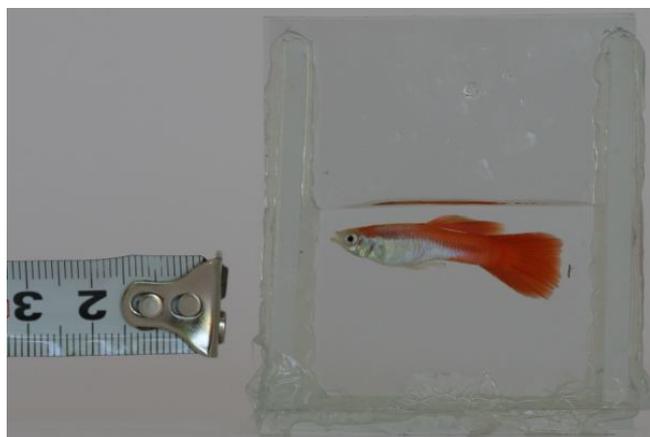


Figure 2. Red Blond variety of guppy fish in tap water (left flank view).



Figure 3. Red Blond variety of guppy fish in water containing 0.007 ppm lindane (left flank view).



Figure 4. Red Blond variety of guppy fish in water containing 0.08 ppm lindane (left flank view).

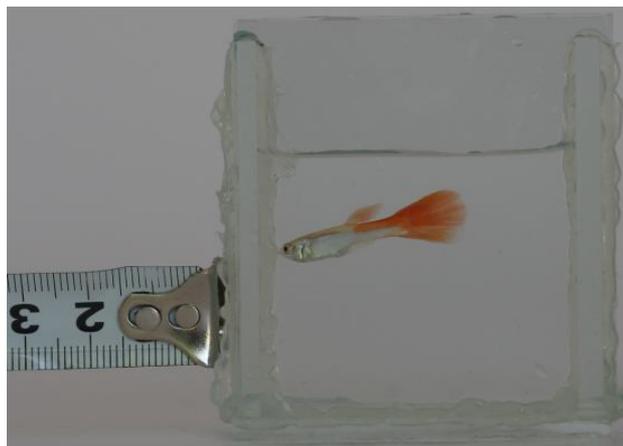


Figure 5. Red Blond variety of guppy fish in water containing 0.16 ppm lindane (left flank view).

A set of 72 (36 left flank views + 36 right flank views) fish images (Figure 2), corresponding to normal and contaminated guppy groups, was analyzed. An algorithm for fish measurements (for TL, SL, BD, ED; see Table 1 and Figure 6) was made and applied using the Image J software software package (Wayne Rasband, National Institutes of Health, in Bethesda, Maryland, USA) (<http://imagej.nih.gov/ij/>). After adjustment of the color digital images, the images were analyzed following a standardized protocol. The

digital images were processed in .jpg format. The red areas were extracted using the morphologic operations from the original images with the structuring elements.

Lastly, all the raw data were exported and analyzed in Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) and GraphPad InStat software program (<http://www.graphpad.com/instat/instat.htm>).

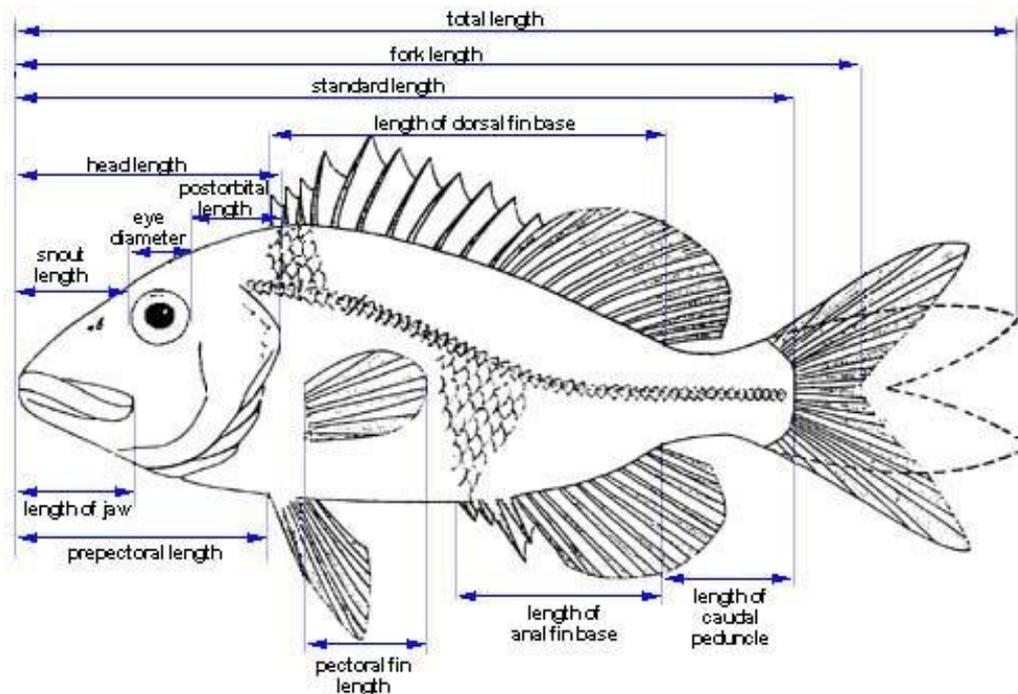


Figure 6. Standard measurements of bony fish (source: www.flmnh.ufl.edu).

Results and Discussion. Morphometrics is generally used in ichthyology and taxonomy to extract different information and by combining this information we can describe the fish shape, age, health, or size more accurately. Such analyses are also helpful for the characterization of some fish populations and show on what extent one population is related to another (Nowak et al 2009).

In this particular case, morphometrics and meristic analysis had in view to make possible the comparison between the four experimental groups as regards corporal development and red pteridine expression. Both body size and red pteridine expression are related to testosterone levels in males, where testosterone is a key regulator (Rodd & Sokolowski 1995; Rodd et al 2002; Jayasooriya et al 2002). Moreover, acute toxicity of lindane may cause delay of corporal development or sexual maturity in guppies (see similar research in Lozinsky 2011).

The results of analysis obtained for the mentioned images are given in Table 1. It can be seen that the fish red color palette decrease with the increasing of the lindane concentration, reflecting its toxicity in a specific concentration.

The Kolmogorov-Smirnov test for testing the normality of the distributions of fish dimensions was performed. It has turned out that the fish dimensions obtained from the image measurements described above followed normal distributions.

One-way analysis of variance (ANOVA) was used to compare dimensions among the normal and contaminated groups as the variable category. Values with $p < 0.05$ were regarded statistically significant.

Conclusions. Our results support our hypothesis that the increasing of lindane concentration used in experiment was responsible for the predicted effect of decreasing of red pigmentation and fish dimensions. According to such results as inhibition of red pigment expression, lindane should be considered endocrine disrupting pesticide as long as red pteridine synthesis is related to the level of testosterone.

Table 1

Morphometric and meristic analysis of the four experimental groups

Average concentration of lindane	Total length TL [mm]	Standard length SL [mm]	Body depth BD [mm]	Eye diameter ED [mm]	Body red area/ Body total area
Tap water; Average \pm standard deviation	37.950 \pm 1.372	27.865 \pm 1.550	7.022 \pm 0.371	1.915 \pm 0.077	0.7177 \pm 0.027
Water containing 0.007 ppm lindane; Average \pm standard deviation	36.649 \pm 2.529	26.733 \pm 2.506	6.673 \pm 0.774	1.864 \pm 0.145	0.7040 \pm 0.027
Water containing 0.08 ppm lindane; Average \pm standard deviation	36.239 \pm 1.586	25.137 \pm 1.075	6.420 \pm 0.342	1.833 \pm 0.051	0.6717 \pm 0.034
Water containing 0.16 ppm lindane; Average \pm standard deviation	31.706 \pm 2.096	23.040 \pm 1.958	5.370 \pm 0.548	1.829 \pm 0.076	0.6144 \pm 0.043

The fish measurements and color analysis in Red Blond variety of guppy has the potential to become the key test to identify the concentration of lindane in aquatic ecosystems. The guppies also were found to be highly adapted to polluted waters and effective in cleaning microorganisms and organic matters. Computer-assisted image analysis of biological images can provide detailed information on guppies' morphometric analysis more sensitive than a visual examination. Further work with toxicity testing methods directly on guppies will be very necessary in assessing possible ecological risk assessment of pesticides and other toxicants. Our measurement methods can easily be adapted to other fishes with suitable adjustments.

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