Chlorpyrifos-Induced hsp70 Expression and Effect on Reproductive Performance in Transgenic Drosophila melanogaster (hsp70-lacZ) Bg

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Abstract. Expression of hsp70 in the third-instar larval tissues of transgenic Drosophila melanogaster (hsp70-lacZ) following dietary exposure to organophosphate insecticide chlorpyrifos for various time intervals was investigated. Effect of the chemical on different developmental stages of the fly was also evaluated by looking at survivorship, hatchability, emergence, fecundity, fertility, and reproductive performance. Our results showed that the toxicant evokes profound cytotoxic effect as evidenced by dark blue staining in salivary gland, proventriculus, brain ganglia, and midgut in the lowest concentration of the chemical following 24 and 48 h of exposure. On the other hand, a significant increase (61%) in hsp70 expression in the above larval tissues was observed in the next higher concentration of the toxicant after 6 h exposure when compared with that of the lowest dose. A further increase in exposure time caused 100% larval mortality. Similarly, larvae exposed to higher concentrations of the toxicant, exhibited 100% mortality within 1 h of treatment. The insecticide caused a delay in emergence and a severe reduction in survivorship of the flies in a dose-dependent manner with 100% mortality within a day of exposure in the highest dosed group. A delay in emergence by 3 days was evident even in the lowest concentration of the chemical. A drastic effect of the chemical on hatchability was found in the highest dosed group with 100% embryonic mortality at post-16 stage of the embryo. Reproductive performance was significantly affected even in the lowest dosed group. The present study suggests that certain larval tissues of Drosophila, a nontarget organism, are vulnerable to chlorpyrifos as evidenced by hsp70 expression. Further, the adverse effect of the toxicant is reflected on various stages of development of the fly including reproductive performance.

The extensive use of pesticides has been of great concern due to their adverse effects on health of humans and wildlife. Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a broad-spectrum organophosphate insecticide used widely in India in fruit orchards and also has application in commercial pest control (U.S. Public Health Service 1997). Its exposure is reported to affect respiratory system, cardiovascular system, and nervous system in addition to causing skin and eye irritation in humans (Gallo and Lawryk 1991). Thrasher et al. (1993) reported that certain components of human immune system may be affected by chlorpyrifos. In rodent and insect cell lines, chlorpyrifos has been shown to cause genotoxic effects (Amer and Fahmy 1982; Sobti et al. 1982; Patnaik and Tripathy 1992). In mice, chlorpyrifos exposure has been shown to increase the incidence of erythroblast chromosomal aberration (Amer and Fahmy 1982). Deacon et al. (1980) have shown that chlorpyrifos-exposed mouse litters had increased skeletal abnormalities. Genotoxicity in both somatic and germ line cells as well as X-chromosome loss in Drosophila by this insecticide has been reported (Woodruf et al. 1983; Patnaik and Tripathy 1992).

In a nontarget toxicity study, chlorpyrifos (as Dursban) applied to kill mosquitoes was shown to cause marked mortality of young mallard ducks and certain invertebrates (Hurhbert et al. 1970). Miyazaki and Hodgson (1972) reported 100% mortality in 8-day cockerels exposed to chlorpyrifos for 24 h. In another study, the pesticide was shown to be highly toxic to fourth-instar Chironomus tentans—a nontarget organism (Pape-Lindstrom and Lydy 1997).

Experimental evidence in past years has confirmed that stress proteins play an active role in cellular defence (Welch 1993). The stress proteins are believed to buffer cells from harm under stressful conditions by maintaining the proteins in the correct conformation, by aiding the proteins to be properly translocated to different organelles in the cell, and by guiding the newly formed as well as denatured proteins to attain their proper conformation, thus protecting the cells from further damage (Craig et al. 1983, 1994). Among all the stress protein families, the ubiquitous hsp70 is one of the most highly conserved and also one of the most commonly expressed stress protein family (Lindquist and Craig 1988; Nover 1991). In response to damaging stimuli, the synthesis of Hsp70 increases, providing defense to the cell against proteotoxicity (Hightower 1991).

Drosophila was chosen as a test system because of its well-dissected-out genetics and developmental biology; moreover, its genome can easily be manipulated by P-element
mediated germ line transformation, and the expression of a given gene can be studied by reporter gene assay.

Therefore, the present study was conducted to examine the cytotoxic potential of chlorpyrifos by hsp70 expression qualitatively (in situ) and quantitatively in transgenic Drosophila melanogaster. The study was further extended to investigate whether the pesticide has any effect on the life cycle of Drosophila by looking into survivorship, hatchability, emergence, fecundity, fertility, and reproductive performance of the exposed flies.

Materials and Methods

Transgenic D. melanogaster lines that express bacterial β-galactosidase as a response to stress (Lis et al. 1983) were used during the study. In the said strain of flies, the transformation vector is inserted within a P-element, the lines contain wild-type hsp70 sequence up to the lacZ fusion point. The flies and larvae were cultured on standard Drosophila food containing agar, corn meal, brown sugar, and yeast at 24°C ± 1°C. Additional yeast suspensions were provided for healthy growth.

Treatment Schedule

Technical-grade chlorpyrifos (97.1% purity), obtained from DE-NO-CIL Crop Protection Ltd. was used for the study. Four different concentrations of the pesticide (3,000 ppm corresponding to the recommended concentration for agricultural use, 30 ppm [1/100 of recommended agricultural concentration], 0.5 ppm corresponding to the permissive maximum residue level (MRL) on fruits and vegetables [PEA 1992] and 0.005 ppm [1/100 of MRL concentration]) dissolved in dimethyl sulfoxide (DMSO) and finally mixed with food were fed to larvae/adults. Two sets of control (set I and II) were used. In set I control, normal food was fed, while in set II control, DMSO-mixed food was fed to the larvae/adults.

Hatchability of Eggs

Effect of chlorpyrifos on the hatchability of eggs was examined by transferring freshly laid eggs, following synchronous egg laying for 0.5 h on normal food to the control and different concentrations of chlorpyrifos-contaminated food (500 eggs/group). The number of first-instar larvae hatched from control and treated food was recorded in each group (Marchal-Segault et al. 1985).

Survivorship of Adult Flies

Effect of chlorpyrifos on adult longevity was studied after transferring the fertile flies of same age (± 0.5 h) grown on normal food to the side wall of the vials (50 fertile flies/vial and 10 vials/group) containing food supplemented with or without different concentrations of the pesticide. Flies were transferred to fresh vials every alternate day and the number of dead flies per day were scored until the death of the last fly (Marchal-Segault et al. 1985).

Emergence of Flies

First-instar larvae hatching from the eggs following synchronous egg laying for 0.5 h were transferred to control and different concentrations of pesticide-contaminated food throughout their development (50 larvae/vial and 10 vials/group). The number of emerging flies was recorded daily until all the flies emerged. From the data obtained, the pattern of emergence of the flies in different groups was evaluated according to the method of Gayathri and Krishnamurthy (1981).

Fecundity, Fertility, and Reproductive Performance of Flies

Virgin male and female flies emerging from the control and treated food were separated and pair-mated in vials having normal Drosophila food. Ten pairs of flies were included per group. Flies were transferred to fresh vials everyday for the next 10 days, and the number of eggs laid during this period were scored. From these data, total fecundity and mean daily egg production were calculated. The number of flies emerging from these vials were counted and the percentage of the flies emerging from the number of eggs laid presented a measure of the fertility. For reproductive performance, total number of flies produced by each pair for 10 days was counted, and, from the same, the mean number of flies per pair for a period of 10 days was calculated (Gayathri and Krishnamurthy 1981).

Stress Gene Assay

To examine the effect of chlorpyrifos on cytotoxicity, third-instar larvae (100 larvae per group) were allowed to feed on chlorpyrifos-free or -contaminated food for various time intervals (2, 4, 6, 12, 24, and 48 h). Following feeding, the larvae were removed from the food, washed thoroughly with Poels’ modified salt solution (Lakhotia and Mukherjee 1980) to get rid of the adhering food particles, and subsequently used for hsp70 expression studies by quantitative (spectrophotometric) and qualitative (histochemical staining) stress gene assays as stated below:

Soluble O-Nitrophenyl-β-D-Galactopyranoside (ONPG) Assay (Quantitative). A modified version of the method described by Stringham and Candido (1994) was followed for quantitative assay. Briefly, after washing, the larvae were taken in a microcentrifuge tube (20 larvae/tube, 5 replicates/group), permeabilized for 10 min by acetone, and incubated overnight at 37°C in 600 μl of ONPG staining buffer. Following incubation, the reaction was stopped by adding 300 μl of 1 M Na2CO3. The extent of reaction was quantified by measuring the absorbance at 420 nm on Cintra 20 GBC UV spectrophotometer.

In Situ Histochemical β-Galactosidase Activity (Qualitative). In brief, the larval tissues were explanted in Poels’ modified salt solution and after brief fixing in 2.5% glutaraldehyde and postwashing in 50 mM phosphate buffered saline (pH 8.0), staining was performed as described earlier (O’Kane and Gehring 1987; Kar Chowdhuri et al. 1999).

Temperature Shock Treatment

For both qualitative and quantitative assays, healthy third-instar larvae were placed on a petri dish lined with moist filter paper and given temperature shock at 37°C ± 1°C for 1 h as described previously (Lakhotia and Singh 1989).