KATHMANDU UNIVERSITY JOURNAL OF SCIENCE, ENGINEERING AND TECHNOLOGY
VOL. I, No. V, SEPTEMBER 2008, pp 12-16 .

TRICLOSAN AS A GENOTOXIC COMPOUND FOR *DROSOPHILA MELANOGASTER*

[1]Anitha D, [1]Shantha SL, [2]Krishnamurthy V*, [2]Vanishree R

[1]Post Graduate Studies, Department of Biotechnology (PG), PESIT Campus, 100 Ft. Ring Road, BSK III Stage, Bangalore-560 085 .

[2] PES Institute of Technology, 100 Ft. Ring Road, BSK III Stage, Bangalore-560 085

*Corresponding author: profvkm55@yahoo.com

Received 7 August; Revised 11 September

ABSTRACT

Triclosan [5, chloro-2-(2, 4-dichlorophenoxy) phenol] (CAS No. 3380-34-5) is extensively used as an antimicrobial agent in many pharmaceutical formulations. Understanding its genotoxic potential is the aim of this experimental study. Drosophila fulfils dual function in the field of genetic toxicology – firstly as an experimental organism for short term tests to identify carcinogens and secondly as a model for learning mechanistic pathways of mutagenesis induced by environmental toxicants. The mutagenesis in *Drosophila melanogaster* contributed by triclosan is the theme of this study. The Sex-linked recessive lethal (SLRL) test was conducted on stock of Drosophila melanogaster. Males of well-defined stock and appropriately marked balancer females of FM 7 stock with multiple inverted X-chromosomes were used for the experiment. The triclosan concentration was administered with exposure concentration ranging from 0.5 to 2 mg for adults. The results indicated significant reduction in the number of males in the F2 generation. The mortality of larval population was 100% suggesting the test required lowering of concentrations of triclosan that was administered particularly with respect to larvae.

INTRODUCTION

There has been a substantial proliferation of toxic chemicals produced and used. There is a scanty regard to the health effects caused by these on chronic usage and on human exposure. Triclosan is a potent wide spectrum antibacterial and antifungal agent. It is found in soaps, deodorants, toothpastes, mouth wash preparations. Triclosan has demonstrated immediate, persistent, broad-spectrum antimicrobial effectiveness and utility in clinical health care settings. A report highlights the utility and effectiveness of a 1% triclosan formulation for use in high-risk, high-frequency hand-washing (Rhonda, 2000; Adolfsson, 2002). Newer applications are its usage in increasing number of consumer products, such as kitchen utensils, toys, bedding, socks, and trash bags. It has been shown to be effective in reducing and controlling bacterial contamination on the hands, on body and on treated products.

The fruit-fly *D. melanogaster* provides a good experimental system to dissect embryonic development, cell biology, physiology. However, their use in unraveling the role of toxic chemicals in human disease is new. Studies exploring suitability of simple organisms to

be used as models to learn the mutagenic, teratogenic and carcinogenic potential of chemical substances are reported. The Comet assay showed that triclosan treatments led to a dose-dependent DNA damage of *C. ehrenbergii*; 0.25 mg/L caused significant genotoxic effects and higher concentrations irreversibly altered the DNA strands (Ciniglia, 2005). The evaluation of toxicity of pharmaceuticals and personal care products have become relevant giving valuable information for a risk assessment owing to aggressive marketing proliferation of chemical ingredients

MATERIALS AND METHODS

Stock maintenance

The baseline population of *D. melanogaster* maintained in constant light served as ancestral population. They were reared in the laboratory at constant temperature (24±1ºC), constant humidity (80 to 90%) and constant light (100 lux intensity) on a 21 day discrete generation cycle. The populations typically contained of ~1500 adults (number of males and females being approximately equal) in a Plexiglas cage ($25\times20\times15cm^3$). For starting a new generation the adult flies were given jaggery food supplemented with live yeast paste for 2 days. Subsequently were allowed to lay eggs on Petri plates. Approximately 60-80 eggs were collected into glass vials (9cm height×2.4 cm diameter) containing ~6ml banana food in which larvae developed into adults. Forty such vials were used for each population of LL and FM7. Adult flies emerging from these vials were transferred to Plexiglas cages on the 12^{th} day of egg collection, forming the breeding population for the next generation. For starting a new generation next set of eggs were collected after 21days from the previous egg collection date and adults were discarded, thus maintaining a 21 day discrete generation cycle.

The Sex-linked recessive lethal (SLRL) test using *D. melanogaster* detects the occurrence of mutations, both point mutations and small deletions, in the germ line of the insect. This test is a forward mutation assay capable of screening for mutations at about 800 loci on the X-chromosome; this represents about 80% of all X-chromosomal loci. The X-chromosome represents approximately one fifth of the entire haploid genome (Donner, 1983), Mutations in the X-chromosome of *D. melanogaster* are phenotypically expressed in males carrying the mutant gene. When the mutation is lethal in the hemizygous condition, its presence is inferred from the absence of one class of male offspring out of the two that are normally produced by a heterozygous female. The SLRL test takes advantage of these facts by means of specially marked and arranged chromosomes.

Males of a well-defined LL stock (Sheeba, 1999) and appropriately marked balancer females of FM 7 stock with multiple inverted X-chromosomes were used (Lirim, 1990). Stock cages were yeasted for 2 days and on the third day cut plates of banana jaggery food were put on a petri plate and were placed inside the LL stock cage. After 2 hours (i.e. after enough eggs were laid on cut plates) the cut plates were removed and put inside a labeled zip-lock cover to avoid contamination. Preliminary assessment was carried out with 280 vials containing 6 ml of banana jaggery food. Eggs were collected from cut plates, placed in LL stock cage and approximately 40-50 eggs were placed in each of these vials. These vials were monitored for any eclosion from the sixth day after egg collection. The possibility of mating was prevented by separating the eclosing flies at an interval of two hours. Carbon dioxide was used for separation of male and female flies.

Virgin males which were collected were placed in food vials containing 6 ml banana jaggery food. Initial assessment of exposure to triclosan commenced once half of the population emerged from each vial.

Virgin males were starved for 6 hours by transferring them into empty vials. The starved virgin males were later transferred to vials which had a thin layer of tissue paper impregnated with triclosan dissolved in ethanol and 5% sucrose solution. The concentration steps of triclosan procured from M/s. Sigma Chemicals, U.S.A. used was 5µg, 25µg, 50 µg, 100 µg, 200 µg, 500 µg, 1 mg, 2.5 mg, 5 mg, 25mg and 50mg taken in separate vials for the purpose of 6 hour exposure to the flies. As a control, vial containing only 5% sucrose solution was used. Four replicate vials were used for each concentration step of test substance. The number of virgin males in each test vial was 30

The steps of concentration range of triclosan, and the number of replicates were decided on an arbitrary basis since it was a preliminary trial. The data obtained from this preliminary assessment therefore formed the baseline concentration for SLRL.

A total number of 11 different concentration steps of triclosan compound administered in quadruplicate vials. Each vial comprised a total of 30 flies. The Table 1 presents concentrations determined after observing the rate of mortality of flies. It was observed that all the 30 flies survived up to the exposure dose of 0.5 mg, indicating that 0.5 mg as sub-lethal dose. On an average, 15 flies survived in vials of 2.5 and 14 flies survived in vials of 1 mg. The vials in which more than 2.5 mg concentration was administered no fly survived inferring that the lethal value stands above 2.5mg. It is relevant to indicate here that, the period of exposure was 6 hours in all the concentration steps and fly counts. Since all the flies died above the level of 2.5 mg, it was decided to consider 6 hrs. as the cut off time for fixing lethal concentration.

Preliminary assessment of lethal concentration exposure of triclosan showed a sublethal value below 0.5 mg, LD50 value of 2.5 mg and the lethal value however remained above 2.5 mg.

Procedure and Exposure level for SLRL test: Each concentration step for which the concentration and the time of exposure were fixed based on the preliminary assessment. However, the SLRL test was performed on duplicate vials with all the concentration steps. LL males (three days old) were starved for 6 hours and then were transferred to vials containing ethanolic triclosan test substance and 5% sucrose solution. Subsequent to exposure the flies were transferred to food vials for restoration up to a period of 24 hours.

The concentration of triclosan taken for SLRL test was 0.5, 1.0 and 2.0 mg. After restoration period, these males were placed in fresh food vials containing virgin females from FM 7 stock and 6 ml banana jaggery food. Each such vial contained a single virgin LL treated male and 3 virgin females of FM 7 stock. Every day the females in these test vials were replaced with fresh 3 virgin females to cover the entire germ cell cycle. Such an exercise was done for 2 days.

Heterozygous F1 females from the above crosses were allowed to mate individually (i.e. one female per vial) with their brothers. In the F2 generation, each vial was scored for the absence of males.

RESULTS AND DISCUSSION

Table 1. The population means of males of F2 generation and test of hypothesis

Triclosan Exposure concentration (mg)	N	Population mean ± (S.D.)	$t_{calculated}$	$t_{tabulated}$
0.5	17	0.60 ± 0.13	4.66 ± 0.13	1.60 ± 0.13
1.0	20	0.63 ± 0.05	14.82 ± 0.05	1.60 ± 0.05
2.0	10	0.57 ± 0.09	4.80 ± 0.09	1.7 ± 0.09
Control (0.0 mg)	5	0.44 ± 0.07	- 3.18 ± 0.07	1.80 ± 0.07

Data from the assay SLRL were subjected to statistical two-sample‘t’ test. Since sample size was small, it was appropriate to estimate population standard deviation. The ratio of males to females and mean was calculated and t test was applied considering control as reference value. The result indicate that at all the concentrations of administered triclosan concentrations (0.5, 1, 2 mg) there was a decrease in the male population. However, variations in the levels of significance were observed. An interesting observation was that at an exposure concentration of 1mg the degree of significance was considerable.

Triclosan may not be panacea and very safe chemical to be considered at all concentrations for its usage. This study casts a doubt about its genotoxic potential. A report relating to the study of triclosan effects on the early life and reproduction of Oryzias latipes in an aquatic toxicological experiment is reported (Ishibhishi, 2004). This experimental study points out that it possesses genotoxic potential. Well-designed and in-depth experimental studies could throw more light to the triclosan’s genotoxicity.

REFERENCES

1. Rhonda D. Jones, Hanuman B. Jampani , Jerry L. Newman, Andrew S. Lee 2000. Triclosan: A review of effectiveness and safety in health care settings, American Journal of Infection Control, 28(2), 184-196.

2. Adolfsson-Erici M, Pettersson M, Parkkonen J and Sturve J 2002. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. Chemosphere 46, 1485–1489.

3. Ciniglia C, Cascone C, Giudice RL, Pinto G, Pollio A, 2005. Application of methods for assessing the geno- and cytotoxicity of Triclosan to C. ehrenbergii. J Hazard Mater. 122(3), 227-32.

4. Donner, M, Hytonen, S., Sorsa, M., 1983. Application of sex-linked recessive lethal test in Drosophila melanogaster for monitoring the work environment of a rubber factory. Heriditas, 99:7-10.

5. Sheeba, V., Sharma, V.K., Chandrashekarn, M.K., Joshi, A., 1999. Perisitence of eclosion rhythm in Drosophila melanogaster after 600 generations in an aperiodic environment Naturwissens chafter, 86 (9), 448-9.

6. Lirim Shemshedini, Thomas G Wilson, 1990. Resistance to juvenile hormone an insect growth regulator in Drosophila is associated with an altered cytosolid juvenile hormone binding protein. Proceedings of the National Academy of Sciences, U.S. A., 87, 2072-76.

7. Ishibashi H, Matsumura N, Hirano M, Matsuoka M, 2004. Effects of Triclosan on the early life stages and reproduction of medake Oryzias latipes and induction of hepatic vitellogenin. Aquatic Toxicology 67, 167-179.