Bull. Environ. Contam. Toxicol. (1988) 41:108–113

Environmental Contamination and Toxicology

Toxicity of Sodium Dichloro S-Triazinetrione to *Drosophila melanogaster*

S. Shayela, H. Hathi, and C. V. Rao

St. Xavier's College, Zoology Department, Bombay 400 001, India

The compound sodium dichloro S-triazinetrione or cyanuric acid has been used as a water purifier in swimming pools and the drinking water tanks (Veger 1976). Cyanuric acid is known to be toxic to some fresh water molluscs, such as Anodonta, Vivipara and Planorbis (Protean and Labat 1980). It is easily biodegradable into carbon dioxide and ammonia in nature by microorganisms, provided there is no other source of nitrogen for these organisms for their metabolic requirements (Zeyer et al. 1980; Zeyer et al. 1981), which is a rare possibility. Subcutaneous application of cyanuric acid once a week to rats and mice and five times weekly during feeding produced low blastogenic activity with tumors appearing in 30% of the cases with an average latent period of 1-5 years (Pliss and Zabezhinski 1970). In mice spontaneous motor activity was temporarily inhibited when 500 and 100 mg of Na-dichloro isocyanurate was administered and in rats oral dose of 62.5 mg/kg showed piloerection, diarrhea and writhing, decrease in heart rate and blood pressure, transient respiratory disorder, which was exhibited in rabbits when the dose of 20 mg/kg and above was administered (Kaneto and Takahashi 1984). Brewer et al(1979) found that triazines and their derivatives were effective in inhibition of photosynthetic electron transport chain.

Therefore, it was thought to assess the toxic effects of Na-dichloro S-triazinetrione on certain metabolic enzymes of Drosophila melanogaster.

MATERIALS AND METHODS

Drosophila melanogaster flies obtained from Tata Institute of Fundamental Research(Bombay) were

Send reprint request to Dr.C.V.Rao at the above address.

divided into four groups in which each group had three subgroups having 1 gm of flies in each vial. The group I flies were maintained at 26°C in vials containing 5% sucrose moistened cotton swab stuck to the bottom of the vial. The experimental groups were maintained in similar manner but the sucrose solution contained respective concentration of Na-dichloro S-triazinetrione. The concentration of the compound used for treating the flies was 0.0083 mg/mL (this concentration has been recommended for human consumption in drinking water), 0.1 mg/mL and 2.5 mg/mL respectively for groups II, III and IV flies. The flies were maintained in the vials for 48 h treatment at 26°C.

After 48 h the flies were etherised and 10% homogenate in 0.6% ice cold saline was prepared. The homogenates were cold centrifuged at 1000 g for 20 min and the clear supernatants were used for estimation of enzyme activities. Total protein was estimated by the method of Lowry et al(1951). The enzyme activities were estimated by following methods:

1. Succinate dehysrogenase (SDH) (Nachlas et al. 1960).
2. Lactate dehydrogenase (LDH) (Bergmeyer and Bernt 1965).
3. Acid and alkaline phosphatase (ACP and ALP) (Andersch and Szcypinski 1947).
4. Alanine and aspartate aminotransferase (GPT and GOT) (Reitman and Frankel 1957).
5. Isocitrate dehydrogenase (IDH) (Bell and Baron 1960).
6. Glutamate dehydrogenase (GDH) (John 1965).
7. γ-glutamyltranspeptidase (γGTP) (Szasz 1969).

The standard deviation was calculated by using student 't' test at 95% confidence limit.

RESULTS AND DISCUSSION

There was appreciable change in the activities of enzymes in treated flies, which is tabulated in Table 1.

The succinate dehydrogenase activity was remarkably decreased in group II($P<0.02$), group III ($P<0.05$) and group IV ($P<0.1$) flies. It indicates that the compound has inhibitory action on this enzyme. The inhibitory activity was more prominent in group II (lowest concentration) flies than in higher

Table 1. Enzyme activities in control and Na-dichloro S-triazinetrione treated Drosophila melanogaster. Mean ± S.D .

Enzymes	Control group I	0.0083 mg/mL treated, group II	0.1 mg/mL treated group III	2.5 mg/mL treated group IV
GPT[1]	803.05 ± 567.87	920.80 ± 83.26[b]	991.73 ± 133.41[c]	984.43 ± 128.25[c]
GOT[2]	574.37 ± 406.14	469.79 ± 73.94[b]	425.33 ± 105.38[c]	371.04 ± 143.77[d]
ALP[3]	11.10 ± 7.84	11.18 ± 0.05	7.18 ± 2.77[d]	9.04 ± 1.45[c]
ACP[3]	23.75 ± 16.79	27.96 ± 2.97[d]	6.78 ± 11.99[b]	7.23 ± 11.68[b]
LDH[4]	28.30 ± 20.01	34.11 ± 4.10[d]	38.18 ± 6.98[c]	40.72 ± 8.78[b]
SDH[4]	9.22 ± 6.52	1.41 ± 5.52[a]	2.12 ± 5.02[b]	3.13 ± 4.30[c]
GDH[5]	28.92 ± 2.44	57.94 ± 20.52[c]	55.74 ± 18.96[c]	44.34 ± 10.90[e]
IDH[5]	57.02 ± 40.31	57.60 ± 0.41	75.11 ± 12.79[b]	51.13 ± 4.16[c]
γGTP[6]	0.22 ± 0.16	0.30 ± 0.05[c]	0.34 ± 0.07[a]	0.309± 0.05[e]

[a]P<0.02, [b]P<0.05, [c]P<0.1, [d]P<0.2, [e]P<0.01

[1]GPT= Pyruvate μmol*/30 min/mg protein, [2]GOT= Oxaloacetate μmol/60 min/mg protein

[3]ALP and ACP= ηmol** of p-nitrophenol/30 min/mg protein, [4]LDH and SDH= μg*** formazan/ 15 min/mg protein, [5]GDH and IDH= μg oxoglutarate/60 min/mg protein,

[6]γGTP= μg p-nitroanilide/min/mg protein. *micromol, **nanomol, ***microgram.

concentration treated flies (group III and IV), which is probably due to inhibition of the co-enzyme or the enzyme itself.

The activity of lactate dehydrogenase increased appreciably in group II ($P<0.2$), group III ($P< 0.1$) and group IV ($P<0.05$) flies respectively as compared to controls. The increase in activity was in accordance with increase in concentration of the compound. The significant increase in activity of LDH in flies treated with higher concentration of compound is indicative of shifting of respiratory pathway towards anaerobic side of metabolism.

The GOT activity was significantly decreased in experimental flies (group II $P<0.05$, group III $P<0.1$ and group IV $P<0.2$) as compared to controls. The decrease in activity of GOT in lower concentration treated flies was highly significant ($P<0.05$) than the higher concentration treated flies. This indicates inhibitory action of the compound on GOT, which might have led to accumulation of ketoglutarate. On the other hand the activity of GPT remarkably increased in experimental groups (group II $P<0.05$, group III and IV $P<0.1$) than the controls. This correlates well with the fact that, the decrease in GOT activity in experimental flies leading to increase in ketoglutarate which in turn is getting converted to pyruvate by GPT, hence the increase in GPT activity in experimental groups.

The activity of glutamate dehydrogenase was found to be significantly increased in group II and III ($P<0.1$) flies as compared to group IV ($P<0.01$) and controls. The activity of GDH gradually decreased as the concentration of the compound increased, but the values were still higher than the control group. The increase in activity of GDH might have led to increase in production of ketoglutarate, which in turn has increased the activity of GPT to convert ketoglutarate to pyruvate. Indeed, this assumption holds good because, in the present experiment the experimental groups did show increase in GPT activity.

Moreover, IDH showed appreciable ($P<0.05$) increase in activity only in group III flies as compared to group II, IV and control flies. It is apparent from the data that, the compound at highest and lowest concentration has inhibitory action on IDH or its co-enzyme. It is rather difficult to explain

the mechanism involved in this inhibitory action. But one can assume that, the compound at higher concentration probably down regulates the enzyme (IDH) activators and at lower concentration it might be acting as irreversible inhibitor.

The activity of enzyme such as acid phosphatase has been significantly (group III and IV $P<0.05$) inhibited at higher concentration. Similarly the alkaline phosphatase activity was also found to be appreciably decreased in group IV ($P<0.1$) flies. The decrease in activity of ACP and ALP is indicative of inhibition of lysosomal activity by the compound.

The enzyme γ-glutamyltranspeptidase showed remarkable (group II $P<0.1$, group III $P<0.02$ and group IV $P<0.01$) increase in activity of tissue level stimulus for increase in transpeptidase reaction and hence may increase intermediary metabolism.

From this data it is apparent that the compound Na-dichloro S-triazinetrione sufficiently interacts with cellular metabolism and biochemical processes, which may cause harmful effects in long course of time if it is consumed with water, since it is used for water purification.

Acknowledgments

We are grateful to the Department of Zoology, St. Xavier's college for providing us all the facilities and to the Principal of the college for providing us sufficient grant to conduct this project.

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Received May 8, 1987; accepted December 28, 1987.