

Preliminary studies on the toxicity and mutagenicity of 1-amino-2-naphthol-4-sulphonic acid in *Drosophila melanogaster*

M. V. GAYATHRI and N. B. KRISHNAMURTHY

Department of Post-Graduate Studies and Research in Zoology, University of Mysore, Manasagangotri, Mysore 570 006

MS received 27 August 1979; revised 4 February 1980

Abstract. The toxicity and mutagenicity of 1-amino-2-naphtho-4- sulphonic acid were analysed in *Drosophila melanogaster*. Rate of development and viability were the two parameters employed to study the toxicity. The frequency of dominant lethals was scored to evaluate the mutagenic effect of the chemical on male and female germ cells. Concentrations of 250 mg and above/100 ml wheat cream agar medium were found to be significantly toxic. Significant number of dominant lethals was induced even by a concentration as low as 50 mg/100 ml medium. Male germ cells were more sensitive than female germ cells.

Keywords. 1-Amino-2-naphtho-4-sulphonic acid; *Drosophila melanogaster*; rate of development; viability; dominant lethals.

Introduction

Sulphonic acids are used in organic synthesis and in the manufacture of dyes and synthetic drugs (Hackh, 1930). Dye intermediates are prominent among the sulphonates commonly found in the market and they include many sulphonic acids. Many of the benzene sulphonic acids are used in the manufacture of synthetic detergents (Richardson, 1957). With such a wide use in industry, it is of importance to analyse the effect of sulphonic acids on biological systems to ensure the safety of occupational exposure of human beings. Several esters of sulphonic acids possess insecticidal, acaricidal, ovicidal, fungicidal and herbicidal properties (Konecny and Demecko, 1973). The fungicidal effects of organic salts of aryl sulphonic acids have also been studied by El-Nawawy *et al.* (1973). Some aromatic sulphonic acids are also known to possess antiviral activity (Akerfeldt *et al.*, 1971). Present investigations report the preliminary studies on the toxic and mutagenic effects of a dye-stuff intermediate 1-amino-2-naphthol-4-sulphonic acid on *Drosophila melanogaster*.

Materials and methods

Toxicity studies

For toxicity studies, 1-amino-2-naphthol-4-sulphonic acid in concentrations of 250, 300, 350 and 400 mg was dissolved in 2 ml alcohol and was thoroughly mixed in 100 ml wheat cream agar medium and these media were poured into vials (3" × 1"). Normal (control I) and 2 ml alcohol supplemented media (control II) served as controls. *D. melanogaster* Oregon K eggs collected by Delcour's technique (1969) were transferred into these vials (25 eggs/vial) so that throughout the development, the larvae were exposed to treated and control foods. Twenty replicates were

maintained for each of the controls and treated media. The census of the emerged flies along with their sexes was taken every day from first to the last day of emergence. From this data, the pattern of emergence as well as the mean development time of the whole group and of the two sexes in each of the chemical concentrations and controls were calculated.

Induction of dominant lethals

Tests for the induction of dominant lethals in male and female germ cells after larval feeding of 1-amino-2-naphthol-4-sulphonic acid were carried out according to the methods of Sankaranarayanan (1967). Since all the concentrations upto 250 mg were found to be lethal, sub-lethal concentrations of 200, 100 and 50 mg were employed to screen for mutagenicity. All the experiments were carried out at a constant temperature of $24 \pm 1^\circ\text{C}$.

Results

Pattern of emergence

Pattern of emergence of flies in controls and the treated series is shown in figure 1. It is clear from the figure that, in the treated series there is a developmental delay. In

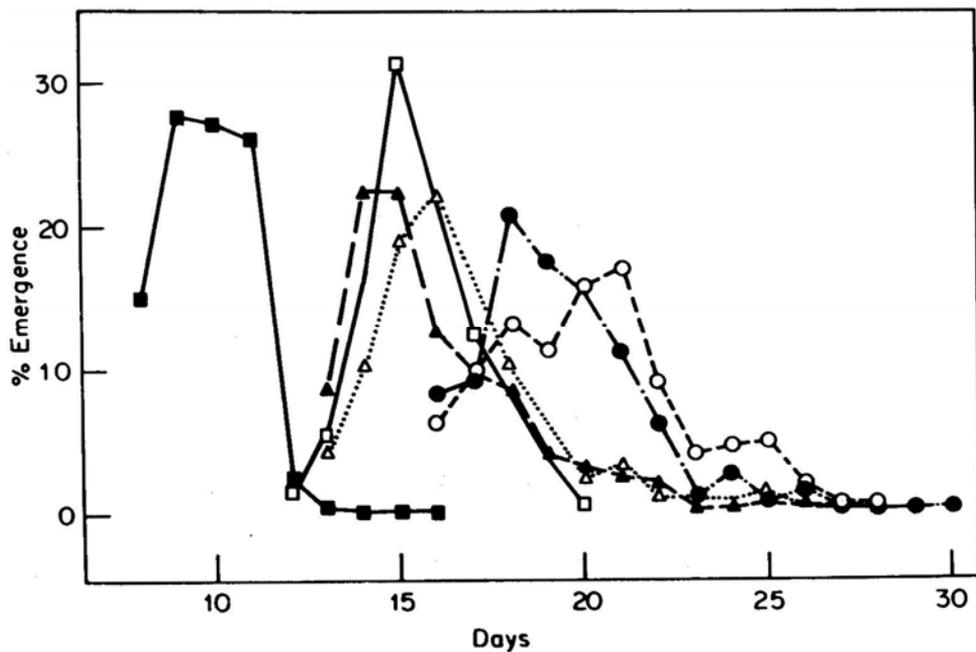


Figure 1. Pattern of emergence of *D. melanogaster* flies in controls and in different concentrations of 1-amino-2-naphthol-4-sulphonic acid.

■—■ control; □—□ alcohol control; Δ-----Δ 250 mg; ▲---▲ 300 mg; O---O 350 mg; ●---● 400 mg.

the control I, emergence started on the 9th day and ended on the 17th day. In control II eclosion which started on the 12th day terminated by the 20th day; whereas in 250 mg concentration, emergence commenced on the 13th day extending upto the 27th day. In the highest concentration of 400 mg tested, emergence started on the 16th day and continued upto the 30th day.

Rate of development

Mean development time for different groups and for the two sexes in each group are presented in table 1. Mean development time for the control I is 9.83 ± 0.03 days and in the control II it is 15.54 ± 0.06 days. In the lowest concentration of 250 mg tested, it is 16.77 ± 0.13 days, while in 400 mg concentration it is 19.14 ± 0.21 days.

Table.1 Mean development time of *D. melanogaster*.

Treatment	Mean development time		
	For group	For males	For females
Control	9.83 ± 0.03	10.02 ± 0.06	9.73 ± 0.06
Alcohol (2 ml)	$15.54 \pm 0.06^*$	15.44 ± 0.09	15.46 ± 0.15
1-Amino-2-naphthol-4-sulphonic acid			
250 mg	$16.77 \pm 0.13^*$	16.89 ± 0.12	16.94 ± 0.21
300 mg	$16.08 \pm 0.14^*$	16.56 ± 0.08	16.21 ± 0.19
350 mg	$20.18 \pm 0.15^*$	20.34 ± 0.15	20.19 ± 0.24
400 mg	$19.14 \pm 0.21^*$	20.29 ± 0.32	19.81 ± 0.07

* Control vs treatment significant at 5% level.

Viability

Table 2 gives the viability as affected by the chemical. In control I and II lethality is 7.2% and 8.4% respectively, whereas in different concentrations of the chemical, there is a dose-dependent increase in lethality. Thus in 250 mg it is 28.2%, in 300 mg it is 30.0% whereas in 350 and 400 mg it is 39.4% and 50.2% respectively. The number of males and females emerged in each group is also given in table 2.

Many brown and shrunken larvae were found on the sides of the vials with chemical supplemented media. Developmental defects in the form of upheld wings were noticed at all concentrations of the chemical tested (0.5%).

Table 2. Viability of *D. melanogaster*

Treatment	No. of adults emerged out of 500 eggs		Mean No. of offsprings/vial	Lethality (%)	Corrected lethality (%)
	Males	Females			
Control	227	237	23.2 ± 0.22	7.2	—
Alcohol (2 ml)	215	247	22.9 ± 0.22	8.4	1.29
1-Amino-2-naphthol-4-sulphonic acid					
250 mg	180	179	17.95 ± 0.22	28.2*	22.63
300 mg	165	185	17.50 ± 0.22	30.0	24.57
350 mg	172**	131**	15.50 ± 0.22	39.4*	34.7
400 mg	141**	108**	12.45 ± 0.23	50.2*	46.34

* Control vs treatment, significant by analysis variance ($P < 0.05$).

** Sex ratio significant at 5% level.

Dominant lethals

Table 3 shows the data on the frequency of dominant lethals in controls and in the treated flees. In control I the percentage of dominant lethals is 3.69%, in control II the percentage of dominant lethals in males is 3.62% and in the females it is 3.75%. While in the males treated with 50 mg of the chemical, it is 12.55% and in the females 8.13%. In the highest concentration (200 mg) of the chemical screened for dominant lethals, it is 20.21% for the treated males and 14.68% for the treated females.

Table 3. Frequency of dominant lethals

Treatment	No. of eggs counted	No. unhatched	% Dominant lethals
Control	3445	127	3.686
Alcohol (2 ml)			
Treated males	2811	102	3.628
Treated females	3410	128	3.754
1-Amino-2-naphthol-4-sulphonic acid			
50 mg			
Treated males	2916	366	12.55*
Treated females	2880	234	8.13*
100 mg			
Treated males	3222	638	19.80*
Treated females	2660	340	12.78*
200 mg			
Treated males	3998	808	20.21*
Treated females	3976	584	14.69*

* $P < 0.01$

Discussion

Survival value and rate of development represent two parameters for evaluating the toxicity (Luning, 1966). Bonnier (1960) has demonstrated that change in the rate of development is due to the compound effects of the genotype and environment. Taking these points into consideration, in the present experiment, the space, the amount of food, the temperature and the number of eggs per vial are identical to that in the controls, and therefore the difference in the rate of development and viability must be due to the chemical and the differences in these parameters are due to the different concentrations used. A significant lengthening of development time is evident in all the concentrations of the chemical tested ($P < 0.05$, table 1). But, the delay in development cannot be entirely attributed to the effect of 1-amino-2-naphthol-4-sulphonic acid, because a significant delay is noticed in the alcohol-supplemented control (control II) too. Compared to control II, a significant delay in development is noticed in 350 and 400 mg supplemented diets ($P < 0.05$). However, no significant variation in development time has been observed between the two sexes in each treatment (table. 1).

Data in table 2 indicate that 1-amino-2-naphthol-4-sulphonic acid reduces viability in *D. melanogaster*. Analysis of variance has shown that the chemical has significant effects on viability at all the concentrations tested ($P < 0.05$). Table 2 clearly indicates that there is a linear relationship between the degrees of lethality and the concentration of the chemical employed. Developmental defects were also encountered in the form of upheld wings in the chemical-treated flies.

So, from the foregoing discussion it is seen that 1-amino-2-naphthol-4-sulphonic acid is toxic to *Drosophila*. Many derivatives of aromatic sulphonic acids are also shown to have low or moderate toxicity in mice (Kurnatowska and Kurnatowski, 1972). Some of the compounds when injected subcutaneously caused simple fatty degeneration of liver cells, slight proliferation of the white pulp in the spleen and necrosis of a few epithelial cells of the neural tubules. But, the experiments of Gaunt *et al.* (1974) have shown that the food colouring agent Sunset Yellow FCF (disodium salt of 1-R-sulpho-phenyl azo-2-naphthol-6-sulphonic acid) does not exert any long term toxic effect on mice. No evidence for teratogenic or carcinogenic effects of the above chemical was noted by them.

χ^2 homogeneity test for the viability of males and females in each treatment has shown that, at concentrations of 350 and 400 mg, the sex ratio is significantly altered ($P < 0.05$, table 2), The viability of females is more affected than those of males thus indicating that the females are more susceptible to the toxic effects of the chemical than males. An alteration in sex ratio due to the effect of chemical in *Drosophila* was reported with Ceresan (Rajasekarasetty *et al.*, 1979; Gayathri and Krishnamurthy, 1979).

Third instar larvae are the most sensitive to the chemical treatment, since they crawl to the sides of the vials, become shrunken and brown, fail to pupate and die. Studies with the colour additive amaranth (a trisodium salt of 1-(4-sulpho-1-naphthyl-azo)-2-naphthol-3, 6-disulphonic acid) is embryotoxic as well foetolethal even in low concentrations (of. Arnald *et al.* 1976). Another naphthalene derivative 2, 4-dichloro-1-naphthol is found to affect both rate of development and viability in *D. melanogaster* (Krishnamurthy and Vijayan, 1979). Further, based on the findings of Martin and Grossmann (1972a, b) that rufianic acid (1, 4-dioxyanthraquinone-sulphonic acid) has an inhibitory effect on enzyme systems in *Rhizoctonia solani*, a similar enzyme inhibition may probably be operative in causing toxicity in the system in the present study. It can also be recalled that many of the polycyclic hydrocarbons are known to intercalate into DNA and are said to be converted into epoxides by microsomal enzyme systems (Ames *et al.*, 1972).

A dominant lethal mutation by definition is the one which kills the organism when present in a single dose (Auerbach, 1962). Table 3 reveals that, 1-amino-2-naphtho-4-sulphonic acid is significantly mutagenic in male and female germ cells at all concentrations tested ($P < 0.01$). But, a higher frequency is noticed in the male germ cells than the female germ cells at all concentrations tested. The experiments of Arnald *et al.* (1976) indicate that the colour additive amaranth has no significant effect on the frequency of dominant lethal mutations in male mice. Mono-, di-, and trisulphonic acids are also known to produce a reversible inhibition of sulphate equilibrium exchange in human red cells (Zaki *et al.*, 1975). The findings of Misra *et al.* (1975) indicate a neuroexcitatory and neurotoxic effects of sulphonic acids. In the light of all this, the effects of sulphonic acids on biological systems need careful monitoring.

Acknowledgements

The authors are grateful to Prof. M. R. Rajasekarasetty for his constructive criticism and constant encouragement. One of the authors (MVG) is grateful to University Grants Commission, New Delhi for financial assistance.

References

- Akerfeldt, S., Gertrud, W. and Torsten, J. (1971) *J. Med. Chem.*, **14**, 596.
- Ames, B. N., Sun, and Grover, P. L. (1972) *Science*, **176**, 47.
- Arnald, D. W. Kennedy, C. L. Jr., Keplinger, M. L. and Calendra, J. C. (1976) *Food Cosmet. Toxicol.*, **14**, 163.
- Auerbach, C. (1962) *Mutation—An introduction to research in mutagenesis, Part I. Methods*, (Edinburgh, London: Oliver and Boyd) p. 35.
- Bonnier, G. (1960) *Genetics*, **46**, 86.
- Delcour, J. (1969) *Dros. Info. Serv.*, **44**, 133.
- El-Nawawy, A. S., Mansour, S. A., Ali Tag El din, Khadous, E. A. and Khalifa, M. A. S. (1973) *Meded Fax Landbouwwet Rijksuniv Gent*, **38**, 1607.
- Gaunt, I. F., Grasso, M. P. and Kirs, I. S. (1974) *Food Cosmet. Toxicol.*, **12**, 1.
- Gayathri, M. V. and Krishnamurthy, N. B. (1979) *Indian J. Exp. Biol.*, **17**, 433.
- Hackh, I. W. D. (1930) *A chemical dictionary* (London: J and A Churchill) p. 703.
- Konecny, V. and Demecko, J. (1973) *Chem. Zvesti.*, **27**, 497.
- Krishnamurthy, N. B. and Vijayan, V. A. (1979) *Entomon*, **4**, 105.
- Kurnatowska, A. and Kurnatowski, A. (1972) *Patol. Pol.*, **23**, 203.
- Luning, K. G. (1966) *Nature (London)*, **209**, 84.
- Martin, J. and Grossmann, F. (1972a) *Phytopathol. Z.*, **75**, 38.
- Martin, J. and Grossmann, F. (1972b) *Phytopathol. Z.*, **75**, 97.
- Misra, C. H. and John, W. O. (1975) *Brain Res.*, **97**, 117.
- Rajasekarasetty, M. R., Gayathri, M. V. and Krishnamurthy, N. B. (1979) *Indian J. Hered.*, **11**, 90.
- Richardson, A. S. (1957) in *The encyclopedia of chemistry*, eds. G. L. Clark and G. G. Hawley (New York: Reinold Pub. Corporation) p. 903.
- Sankaranarayanan, K. (1967) *Mutat. Res.*, **4**, 641.
- Zaki, L., Fassold, H., Shumann, B. and Passow, H. (1975) *J. Cell. Physiol.*, **86**, 471.