



Jelena ŽIVANOV-ČURILS¹
Jasmina TOMIN²
Vladmila BOJANIĆ³
Zoran BOJANIĆ⁴

Stevo NAJMAN¹
Katarina KATIĆ⁵
Ester MRČARICA¹
Boris ĐINĐIĆ⁴

¹INSTITUTE OF BIOLOGY, MEDICAL FACULTY NIŠ, SERBIA AND MONTENEGRO

²INSTITUTE OF CHEMISTRY, MEDICAL FACULTY NIŠ, SERBIA AND MONTENEGRO

³INSTITUTE OF PATHOPHYSIOLOGY, MEDICAL FACULTY NIŠ, SERBIA AND MONTENEGRO

⁴INSTITUTE OF PHARMACOLOGY AND TOXICOLOGY, MEDICAL FACULTY NIŠ, SERBIA AND MONTENEGRO

⁵INSTITUTE OF PATHOLOGY, MEDICAL FACULTY NIŠ, SERBIA AND MONTENEGRO

Effect of chronic phenol intoxication on fertility and life span of *Drosophila melanogaster*

KEYWORDS: *Drosophila melanogaster*; Phenol; Fertility; Longevity

ABSTRACT

Phenol has an important role among environmental pollutants. The aim of this work was to investigate the effect of phenol (C_6H_5OH) in concentration of 0.1% in the standard nourishing medium on fertility and life span of the fruit fly *Drosophila melanogaster*. We made comparative analysis of control line of fruit flies cultivated in standard nourishing medium with the lines of flies of the first and the fifth generation cultivated in phenol medium and with lines of flies which were transplanted after one generation and after five generations from phenol in phenol free standard nourishing medium. In all experimental fly lines the average number of adult descendants per one female *Drosophila melanogaster* was counted and results were compared with control lines of flies. The life span of all lines of flies was registered in days and compared as well. Our results showed a significant toxic effect of phenol in nourishing medium in concentration of 0.1% on fertility and life span of *Drosophila melanogaster* compared to control line of flies. The average number of flies in the fly lines nourished in medium with phenol during one and during five generations (372.25 ± 41.32 ; 250.80 ± 36.19) respectively was significantly decreased ($p < 0.001$) compared to control line (581.74 ± 65.92). After transplantation of *Drosophila melanogaster* from phenol into standard nourishing medium the recovery was present in the first generation. Recovery of the average descendants number after transplantation from phenol to standard nourishing medium was effective for lines of fruit flies cultivated on phenol medium during one generations (583.38 ± 59.04) and during five generation (524.84 ± 60.32) and it was not statistically different from average number of flies of control line. The average life span of control fly line was 40.40 ± 15.36 days, what was significantly longer ($p < 0.001$) than life span of fly lines cultivated in phenol medium during one generation (23.02 ± 6.87 days) and during five generations (23.58 ± 9.45 days). After transplantation to phenol free medium, the average life span of flies cultivated in phenol medium during one generation was 40.34 ± 15.93 days and it was not statistically different compared to controls.

Address correspondence to:
Prof. dr Tomina Jasmina, Bulevar Nemanjića 58/29, 18000 Niš, Serbia and Montenegro, E-mail: mile@ni.ac.yu

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The average life span after transplantation to phenol free medium for flies cultivated in phenol medium during five generations was 33.92 ± 9.81 what was statistically significantly shorter compared to controls. The results of this investigation showed that chronic intoxication by phenol in nourishing medium induced decrease of fertility and shortening of life span of *Drosophila melanogaster*. The recovery of fertility in phenol free medium was registered in the first generation. The shortening of life span of fruit flies lines cultivated in phenol medium during one and during five generations was of the same level.

INTRODUCTION

Modern life and the great progress in science, technology, industry and medicine are followed with increased number of air, water, and soil pollutants. Among them phenol and its derivatives play a very important role. Some of phenol's synonyms are: benzol, carbolic acid, and hydroxybenzene. Phenol derivatives are less toxic than pure phenol, but their toxicity depends of the sort of the compound. Oral human toxic dose is from 200 mg to 300 mg and lethal dose is from 3g to 30 g, but it may be as low as 1 g (1). Phenol is manufactured substance found in a number of consumer products: medicinal and cosmetic preparations (mouth wash and sore throat lozenges, lotions, ointments), disinfectants, and antiseptics. Phenol is primarily used in the formation of phenolic resins. It is also used in the manufacture of nylon and other synthetic fibers.

Low levels of phenol are found in some foods (smoked summer sausage, fried chicken, some species of fish, cheese) and in tobacco smoke. Phenol can be present in low levels in the air and in drinking water. Following small single release, phenol does not remain in the air, soil and water a long time. Large and repeated releases can remain in the air, water and the soil for much longer periods of time. Small amounts of phenol may be found in organisms that live in contaminated water (2) and certain biological species can achieve adaptation even to sublethal and lethal phenol concentration (3).

Phenol is readily absorbed by ingestion, inhalation and skin contact. This substance is systemic poison and it constitutes a serious health hazard. Acute phenol poisoning is characterized by liver damage, renal damage, diarrhea, kidney damage with dark urine, hemolytic anemia and it may lead to death (4, 5). Phenol also can induce a local corrosive effect and it can induce denaturation of proteins, burns and anesthetic effects.

A low level of phenol pollution of our environment may be the long lasting influence on exposed living beings. The aim of this study was to investigate effect of tolerable concentration of phenol in nourishing medium on fertility and life span of *Drosophila melanogaster*. A short life cycle (12 days) and high fertility of fruit fly gave us opportunity to study toxic effects of phenol on six generations of this insect for short time.

MATERIAL AND METHODS

The experiment was carried out on wild fruit fly *Drosophila melanogaster* taken from nature and cultivated in optimal conditions in standard nourishing medium and temperature of 25°C in the glass vessels. Because phenol is highly toxic substance we had to carry out preliminary experiments and to find out the concentration of phenol in nourishing medium that allows growth and reproduction of fruit flies. Phenol in concentration of 0.1% dissolved in ethanol was added to standard nourishing medium. During experiment five lines of fruit flies were simultaneously analyzed: 1) the control line of flies cultivated in standard medium; 2) lines of flies cultivated in 0.1% phenol medium during one generation; 3) lines of flies cultivated in 0.1% phenol medium during five generations; 4) descendants of flies cultivated in phenol nourishing medium and transplanted to phenol free standard nourishing medium; 5) descendants of flies cultivated in phenol medium during five generations and transplanted into standard phenol free nourishing medium. In each of five experimental lines there were 52 flies (26 male and 26 female). In each glass vessel was

one pair of flies: male and female. During experiment flies were transplanted to an identical nourishing medium every day, to avoid competition. The fertility was estimated from average number of adult descendants per one female *Drosophila melanogaster*. We compared the average number of descendants per one female from experimental lines with average number of control line. The average duration of life span of *Drosophila* (in days) was measured for each experimental line of flies and compared with control line. Student's t test was used to determine level of significance of analyzed data.

RESULTS

The average number of descendants of fly lines cultivated on nourishing medium with 0.1% phenol during one generation and during five generations was 372.25 ± 41.32 and 250.80 ± 36.14 respectively, and compared to average number of control line (581.74 ± 65.92) it was significantly decreased ($p < 0.001$).

After transplantation of *Drosophila melanogaster* on standard nourishing medium the recovery was present in the first and the fifth generation (583.38 ± 59.04 ; 524.84 ± 60.32) and the number of fly descendants did not differ from the control line ($p > 0.001$).

Exposure to 0.1% of phenol in nourishing medium induced significant shortening of average life span of fruit flies in the first generation compared to control line of flies ($p < 0.001$). Average life span of control line of fruit flies cultivated in standard nourishing medium was 40.40 ± 15.36 days. The life span of fruit flies line cultivated in phenol medium during one generation was 23.02 ± 6.87 days and it was not statistically different from life span of fruit flies line cultivated on phenol medium during five generations (23.58 ± 9.45 days). The life span of fruit flies cultivated on phenol medium during one generation after transplantation on standard nourishing medium was 40.34 ± 15.39 days what was not statistically different compared to life span of fruit flies from control line. The life span of descendants of fruit flies nourished on phenol medium during five generations after transplantation on standard nourishing medium was 33.92 ± 9.81 days and it was statistically significantly shorter than average life span of control line of fruit flies ($p < 0.001$).

DISCUSSION

The fertility and duration of life span are good indicators of viability of organisms and they are influenced not only by genetic factors but also by many environmental factors (6,7). Phenol and different anti-inflammatory agents induced shortening of life span of *Drosophila*, or showed no effect on it (8). Our results showed toxic influence of 0.1% phenol in nourishing medium on fertility and life span in the first generation of fruit flies. Bought life span and average number of fruit flies descendants were significantly decreased due to exposition to phenol. Chronic exposition to phenol during five generations induced further decrease of fertility. However, the shortening of life span was of the same level for flies exposed to phenol during one generation and for flies exposed to phenol during five generations. This could be understood as a consequence of adaptation of *Drosophila* to low concentration of phenol in nourishing medium, what is in concordance with findings of other authors (3). Because of that, we should consider the toxic influence of phenol as pollutant on fertility of different living beings.

Phenol exerts not only toxic but also genotoxic effects. Exposure of human leukocytes from peripheral blood to different phenol concentrations induced reduction of mitotic activity and many structural and numerical aberrations occurred. The frequency of chromosomal aberrations increased with increased concentration of phenol (9,10). Our results showed that interruption of phenol exposition and transplantation on standard nourishing medium gave fast recovery registered in the first generation. Because of that we can suppose that concentration of 0.1% phenol in nourishing medium was not genotoxic.

CONCLUSION

The results of this investigation showed that chronic intoxication by phenol in nourishing medium induced decrease of fertility and shortening of life span of *Drosophila melanogaster*. The recovery of fertility was registered in the first generation. After interruption of phenol exposition, the life span of fruit flies descendants cultivated on phenol medium during one generation was in the rank of control line. The life span of *Drosophila* descendants cultivated on phenol medium during five generations after transplantation to phenol free medium was still significantly shorter compared to control lines. The chronic exposition through generations of fruit flies to same concentration of phenol induces the higher level of impairment of longevity. These data should alarm us to think about possible damage due to long lasting exposition to low level of environmental phenol pollution.

REFERENCES

1. Todorović V. Acute phenol poisoning. Med Pregl 2003;LVI Suppl 1:37-41.
2. Blinova NK, Čerkašin SA. Vlijanje fenola na hemorepciju antenul travjanog cilima. Biol nauki 1987;2(278):44.
3. Gapocka LD, Karauš GA. Osobnosti adaptacije kulturi zelenoj vodorosli *Scenedesmus quadricornis* k fenolu. Biol nauki 1986;5:64-7.
4. Dreisbach RH, Robertson WO. Trovanja: Preventiva, dijagnoza, lečenje. Beograd: Savremena administracija; 1989.
5. Dico CI, Caplan YH, Levine B, Smith DF, Smialek JE. Phenol tissue distribution in a fatality. J Forensic Sci 1989;34:1013-5.
6. Clara MJ, Luckinbill LS. The effects gene-environment interaction on the expression of longevity. Heredity 1985;55 (Pt 1):19-26.
7. Graves JL. The costs of reproduction and dietary restriction: parallels between insects and mammals. Growth Dev Aging 1993;57:233-49.
8. Massie HR, Williams TR, Iodice AA. Influence of anti-inflammatory agents on the survival of *Drosophila*. Journal of Gerontology 1985;40(3):257-60.
9. Jovičić D, Sofradžija A, Novaković M, Alečković Z. Genetička analiza mutagenih efekata fenola na humane limfocite. Genetika 1989;21:147-53.
10. Forni A. Tests for chromosome aberrations in vitro and in vivo due to industrial chemicals. Methods for carcinogenesis tests at the cellular level and their evaluation for the assessment of occupational cancer hazards. Milano: Fond Carlo Erba; 1978. p. 87-98.