ISSN: 2754-4974

Journal of Clinical Epidemiology & Toxicology

SCIENTIFIC Research and Community

Review Article

Open Access

Assessment of Lethal Dose and Lethal Time of Diazinon in Swiss Albino Mice

Muna Salem M Solyman[1], Fatma Hassan Boshahma[2], Huda Mohamed[3], Fawzeia H Elmhalli[4] Abdalla I Mohamed[5] and Abeer H Amer[2,6*]

[1]Department of Molecular Diagnostic, Faculty of Biomedical Sciences, University of Benghazi, Benghazi, Libya

[2]Basic Medical Sciences Program, Faculty of Medical and Health Sciences, Libyan International Medical University, Benghazi, Libya

[3]Department of Environmental health, Faculty of public health, University of Benghazi, Benghazi, Libya

[4]Department of Infectious Disease, Public Health Faculty, University of Benghazi, Libya

[5]Department of Zoology, Faculty of Science, University of Benghazi, Libya

[6]Department of Histology, Faculty of Medicine, University of Benghazi, Benghazi, Libya

ABSTRACT

Approximately two thirds of deaths and millions of nonfatal cases are attributed to organophosphorus pesticides (OPP) annually. Diazinon, a prominent member of the OP pesticides, was introduced for both agriculture and public health usages during. For several decades, the compound was among the top pesticides in use in many countries, including Libya. The toxicity of this compound is based on its ability to inhibiting the acetylcholinesterase enzyme (AchE), which is vitally important for normal nerve impulse transmission. This study evaluated the acute toxicity of diazinon through acute exposure testing on female Swiss albino mice. In the acute toxicity test, 72 mice were subcutaneously injected with doses of diazinon ranging from 320 to 480 µL/kg. Mortality and symptoms were recorded over four days, with the median lethal dose found to be 403.5 µL/kg. Based on the findings, the present study suggests that stringent controls and surveillance must be placed on diazinon and similar chemicals.

***Corresponding author**

Abeer H Amer, Department of Histology, Faculty of Medicine, University of Benghazi, Benghazi, Libya.

Received: October 30, 2024; **Accepted:** November 04, 2024; **Published:** November 13, 2024

Keywords: Organophosphorus Pesticides, Diazinon, Libya, Ld50, Lt50

Introduction

Approximately 34% of pesticides manufactured and distributed for use in agriculture globally are composed of organophosphorus [1]. During the 1940's, the organophosphorus compounds appeared to represent an extremely important class of organic pesticides. They are widely used in agricultural practices to control pests on vegetable crops, fruit trees, grains, cotton, and cabbage. However, the uncontrolled usage of organophosphorus compounds causes pollution to the environment and negatively effects on plants, animals, and humans [2].

Diazinon is probably one of the most important OP pesticides that was introduced by the Geigy Company in 1952 and by the 1970s. It has a relatively low mammalian toxicity and a wide margin of safety [3]. Moreover, it was described as selective against adult and juvenile forms of insects and with moderate activity against acarina. The common spectrum of diazinon activity includes, flying insects and crawling insects; therefore, it is used as a pesticide and as a veterinary drug to control ectoparasites. Also, it has garnered attention due to its efficacy and associated health and environmental issues [4,5]. Its mode of action causes long-lasting ecological effects by endangering water systems and wildlife in addition to agricultural laborers [6-9]. WHO classified diazinon as a moderately hazardous class II OP pesticide.

The continuous use of diazinon causes it to accumulate in the environment, food chain, and animal tissues and then eventually stimulate, cause, or be a major contributing factor in a number of serious human diseases, including immune system and neurological disorders, sterility, various cancers (breast, lung, cervix, and prostate), heart diseases, Alzheimer's, and Parkinsonism[9-11].

Furthermore, the sources of human exposures are accidental, occupational, or through diet [12]. On the other hand, there are several ways that pesticides, and their harmful effects can come into contact with people [13]. The two possible routes of exposure are oral consumption (food and drink) or absorption via skin by exposure to the pesticide-containing dust [12,14]. After being absorbed, organophosphorus chemicals cause excessive cholinergic stimulation by primarily blocking the enzyme acetylcholinesterase (AChE), which interferes with normal nervous system function

in both target and non-target organisms [15-17]. According to the Centers for Disease Control and Prevention the amount of organophosphorus pesticides found in the urine and blood serum of humans varies according to the extent of their exposure to these pesticides [18-20].

Symptoms of acute toxicity manifest as headaches, dizziness, nausea, respiratory distress, and potentially severe outcomes like seizures and coma [12]. Furthermore, it leads to decreased MCHC and MCV levels along with increased leukocyte and erythrocyte values [21]. Importantly, even low-level exposures can pose long-term health risks [22]. Regulatory agencies have set safety standards to reduce risks associated with diazinon usage; however, reports of acute poisoning incidents continue to highlight the need for caution [23].

Ongoing research into the mechanisms of diazinon toxicity and its overall environmental impact is essential for enhancing safety protocols and public health guidelines. This introduction establishes a foundation for understanding the acute toxicity of diazinon and emphasizes the necessity for further investigation. Therefore, this work was designed to evaluate the available formulation of Neocidol in the local market on vertebrate animal models. Neocidol, a common widely used diazinon formulation, was chosen to be evaluated in terms of its acute toxicity to the Swiss albino female mice as a vertebrate animal model. Acute toxicity was expressed as LD_{50} value within 24, 48 or 72 hours.

Materials and Methods

Test Animals

The total number of animals used in the experiments were 72 female Swiss albino mice Mus musculus produced from several parents that were obtained from the animal house of the Faculty of Medicine University of Benghazi. The animals were kept under laboratory conditions, where the temperature was 7±2°C and the relative humidity was 71±11% . The electrical current regulator was set to regulate the laboratory photoperiod at 14 hours of light and 10 hours of dark throughout animal rearing and experiment duration. The animals were housed in plastic cages; their sizes were 50 x 30 x 19cm, 4 to 6 animals per cage. A little wooden sawdust was placed at the bottom of each cage before putting the mice in for additional care. The animals were supplied with the diet two to three times daily to ensure their feeding demands. Clean bottles approximately 350 cc size were used for the watering. Cages were washed with soap and water, every other day, and fresh wooden sawdust was layered.

Chemicals

The main chemical is the organophosphorus pesticide, diazinon, manufactured by Novartis Inc Basle, Switzerland under the trade name Neocidol. This commercial preparation was designated as "60" emulsifiable concentrate and was obtained from a local pesticide market. The other chemicals used in this study were of a technical grade with known structures and functions.

Acute Toxicity Experiment

Five concentrations of Neocidol were selected for this study, these were 320, 360, 400, 440, and 480 µl/kg (ppm) body weight. These concentrations, however, were based on a preliminary test, where two and four mice were tested by way of subcutaneous injection with an aqueous diluted emulsion of the Neocidol and on the information's concern with toxicity found in the literature for the determination of the mortality range of the dosages. A digital laboratory balance, (Mettler PM 3000) was used to measure the weight of the animals and the other chemicals.

Neocidol concentrations were prepared using distilled water in a conical flask. Twelve female mice with approximate age and weight in three replicates (four per replicate) were selected randomly for each concentration.

Injection was subcutaneously performed by an ultrafine sterile and pyrogen free disposable syringe, where the calculated dose (ul/kg body weight) was delivered in volumes ranging from 0.1 to 0.2 ml/ mice according to weight in the inner upper side of the hind leg of each animal. Every care was taken to bring the temperature of the injected suspension close to the body temperature before the time of the injection. Twelve animals (three replicates of four animals each) were injected similarly, but with distilled water alone to serve as control. All treated animals, along with the control, were then kept in their rearing cages under the laboratory conditions, (temperature, humidity, and light) and supplied with the rearing diet and water. Ad libtium observations were made for the treated animal behavior while mortalities were recorded on an hourly basis, whereas postmortem examinations were conducted for the dead animals. The median lethal dose (LD50) was calculated for the compound, according to Finney, whereas the LT50 was calculated for selected dosages.

Results

The probit mortality curve (LD_{50}) for female Swiss albino mice subcutaneously injected with the organophosphorus insecticide Neocidol (diazinon) is presented in (Figure 1,2,3). The median 72h-LD_{50} was found at 403.5 µl Neocidol per kg of mice body weight. The lower and upper limit values and the correlation between used dosages and mortalities are shown in Figure 1. Neocidol at 280 µl/kg, the lowest tried dose in this study did not reveal any mortality, although signs of poisoning were observed during the early period of injection. Diazinon dosages and corresponding mortalities, clearly showed a dose-dependent relationship. The higher the dose, the greater the mortality. Furthermore, as diazinon dosage increased, a shorter time was required for 50% mortality (LT_{50}) of the tested females. The severity of symptoms and time of death varied with the used dose. Thus, the median lethal time LT_{50} for 400 µl/kg was 33.84 hours post injection (LT_{50}= 33.48), with a range of 12.18 to 48.99 hours (Figure 2). As the dose increased to 440 µl/kg, a shorter time of LT_{50} 32.6 hours (range 21.5 to 41.94) was noted (Figure 3).

Also, low mortality of 8% was observed at 320 µl/kg with more pronounced ill symptoms, when Neocidol dose was increased to 360 µl/kg. An increase in mortality was also observed, where, 16% of female mice were found dead.

Besides, mortalities were then suddenly increased to 50% at Neocidol 400 µl/kg and up to 91% at 440 µl /kg. The highest mortality up to 100% was recorded for the highest used dose, 480 µl /kg mice body- weight.

On the other hand, the most obvious symptoms observed in female mice treated with Neocidol were excessive sweating, constriction of pupils, "depression" exhaustion, slow movement, reduction of food and water intake, failure of response, and paralysis at the final stages prior to death. These symptoms, however, were mainly in those mice exposed to the higher dosages, which often died. At the lower dosages, most treated mice suffered sweating, depression, and slow movement with initial reduced food and water intake. However, the majority of affected females recovered gradually to normal after 48 to 72 hours' post-treatment.

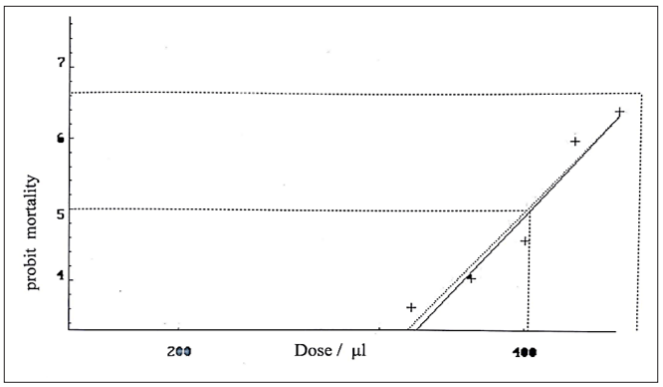

Figure 1: Probit Mortality of Female Mice Treated with Neocidol 320,360,400,440 and 480 µl /l (g (ppm) 72h - LD_{50} = 403.5 (range 381.5-426.6 correlation= 0.953).

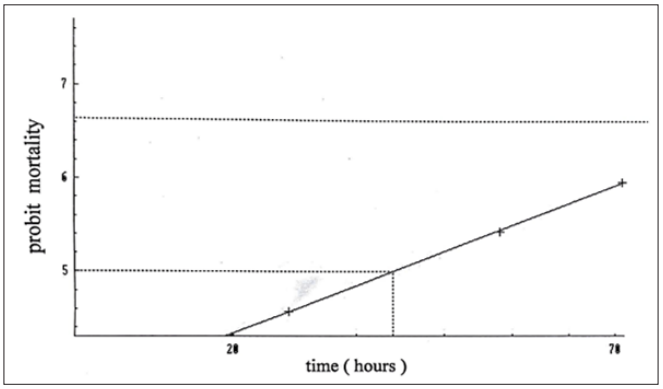

Figure 2: Lethal time (LT_{50}) of female mice treated with Neocidol LT_{50} (400 ppm)= 33.84 hours (range 12.18 - 48.99 hours) (LT_{50} - mean time required for 400 ppm to kill 50% of tested animals)

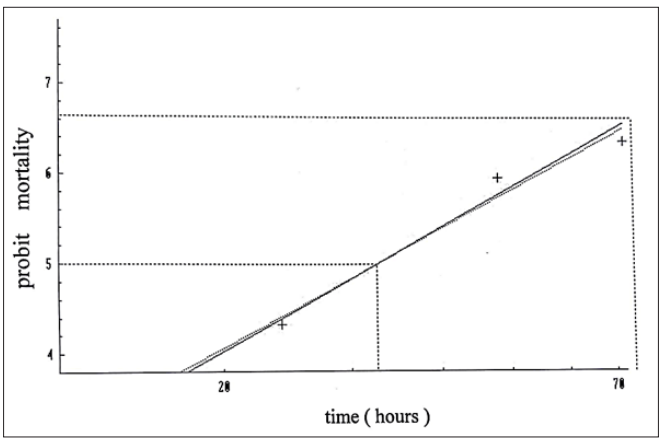

Figure 3: Lethal Time LT_{50} of 440 ppm Neocidol Treated Female Mice

LT_{50} =32.6 hours (range 21.5 -41.94 hours). Note, the higher concentration, the shorter period required to kill 50% of treated mice.

Discussion

The results of this experiment found clear support for the negative impact of diazinon on female Swiss albino mice, which at low doses of diazinon, poisoning signs were observed and the mortality increased with increase its dose. Diazinon, likes all other organophosphates, is a cholinesterase inhibitor. The clinical signs of diazinon toxicity are the results of acetylcholine accumulation which eventually causes an over-stimulation of the parasympathetic and central nervous systems and skeletal muscles [24-27]. Inactivation of AChE causes a blockage of the cholinergic transfer of nerve signal, paralysis, and death due to asphyxia [27].

In line with previous studies reported that diazinon produces depression effects on the respiratory centers and leads to impairment of respiratory muscle efficiency [28]. Acute toxicity of diazinon varies among different animals, and among different formulations. The acute oral 72hour LD50 of the commercial Neocidol reported in this test was found to be 403 5 µl/kg female body weight. This value confirms the ranking of diazinon among the moderately toxic compounds listed by Matsumura and Loomis and Hays [29,30]. Overall, these findings are in accordance with findings reported by WHO, which it that confirms the diazinon classification was being among the moderately hazardous pesticides WHO [31].

In line with previous studies, acute diazinon exposure induced oxidative stress indicators in a time-dependent manner that matched the effects on the pattern of hepatic antioxidant gene expression. Also, it had an influence on the gene expression of antioxidant enzymes even at non-lethal doses [32].

Other confirming reports of diazinon acute toxicity include that of Howard who reported repeated single dose LD_{50} range from 2.75mg/kg to nearly 450 mg/kg/day for rats, and that of the National Library of Medicine, who reported repeated single dose LD_{50}, as high as 720 mg/kg/day [33]. Acute toxicity in animals other than mammals was also reported. The LD50 values for birds range from 2.75 mg/kg/day to 40.8 mg/kg, and for the rainbow trout the LC_{50} values range from 2.6 to 3.2 mg/L [34]. As it can be seen, both birds and fish (wildlife) were found quite susceptible to diazinon poisoning when compared to most mammals, including humans. The toxicity of diazinon to all animal species, however, is dose-dependent, the higher the dose, the higher the mortality rate accompanied by shorter mortality time. The results reported here, showed that diazinon at 440 µl/kg mice body weight inflicted 50% mortality among the tested animals within 32.6 hours (LT_{50}=32.6h) which was a shorter time than that reported for diazinon 400µl/kg with 33.84 hours (LT_{50} 33.84 hours). The symptoms of acute toxicity of diazinon treated female mice reported in this study were clearly indicating OP-poisoning. These symptoms included excessive sweating, depression, slow movement, and exhaustion, these were followed by failure to response, and then paralysis, then coma before death.

And this is coming in agreement with the present study which found that the most noticeable signs seen in female mice given Neocidol were increased perspiration, narrowed pupils, "depression" fatigue, sluggishness, decreased consumption of food and liquids, inability to react, and paralysis in the final moments before death. However, the mice exposed to the larger dosages exhibited these symptoms primarily, and these mice frequently died. These symptoms confirm the previously reported symptoms of OP including diazinon by Matsumura, Hodgson and Levi and Ganie who stated that OP compounds induce a broad spectrum of clinical effects that are indicative of over stimulating the cholinergic system [29,35,36]. The toxicity of diazinon as an OP compound was described by Hodgson and levi [35]. These compounds are toxic because of their inhibition of the enzyme acetylcholinesterase found in the nervous system. This enzyme inhibition results in the accumulation of acetylcholine in nerve tissue and effector organs, primarily at muscarinic and nicotinic synapses in the periphery. This leads to cholinergic hyperstimulation, which is characterized by bradycardia, bronchospasm, diarrhea, hypersalivation, loss of bladder control, and pupillary miosis and lacrimation.

Hyperstimulation of nicotinic receptors causes hypercontraction and spasms in the muscles, which eventually result in paralysis. Confusion, nausea, and dizziness, as well as a possible coma, seizures, and centrally mediated cardiac and respiratory arrest, are additional impacts of the central nervous system [37].

Conclusion

Diazinon, one of the most prominent OP compounds, was proved to be moderately toxic to female Swiss Albino mice with an LD50 of 403.5 ppm. The observed trend of toxicity is similar to other toxic compounds, where, a positive correlation between the dose and percent mortality was very evident. Vital organs (lung and heart) did not reveal weight differences between control and treated groups. However, these organs were relatively less in weight in the treated females. No gross histological abnormalities were observed in any of the vital organs studied, however, minor to moderate histopathological effects were evident in these organs. Such effects included distorted bronchioles and disrupted alveolar septa in the treated lung. No sign of histopathological abnormalities, however, was observed in the treated heart as compared to that control group. Even though the results of this investigation do not offer adequate evidence to conclusively establish the detrimental effects of diazinon independently, given the precautionary principle, the authors advocate for immediate control and surveillance on the importation, sale, and distribution of diazinon and other pesticides that could harm human health or the environment [38-40].

Compliance with Ethical Standards

No conflict of interest between the authors

Animal experiments were designed and carried out in compliance with the guidelines set by the institutional animal ethics committee.

References

1. Ajiboye TO, Oladoye PO, Olanrewaju CA, Akinsola GO (2022) Organophosphorus pesticides: Impacts, detection and removal strategies. Environmental Nanotechnology, Monitoring & Management 17: 100655.
2. Fu H, Tan P, Wang R, Li S, Liu H, et al. (2022) Advances in organophosphorus pesticides pollution: Current status and challenges in ecotoxicological, sustainable agriculture, and degradation strategies. Journal of Hazardous Materials 424: 127494.
3. Semple RL (1992) Towards integrated commodity and pest management in grain storage https://www.fao.org/4/x5048e/x5048e00.htm.
4. Cao F, Souders CL, Li P, Pang S, Qiu L, et al. (2018) Biological impacts of organophosphates chlorpyrifos and diazinon on development, mitochondrial bioenergetics, and locomotor activity in zebrafish (Danio rerio). Neurotoxicology and teratology 70: 18-27.
5. Glinski DA, Purucker ST, Van Meter RJ, Black MC, Henderson WM (2018) Analysis of pesticides in surface water, stemflow, and throughfall in an agricultural area in South Georgia, USA. Chemosphere 209: 496-507.
6. Hajirezaee S, Mirvaghefi A, Farahmand H, Agh N (2017) NMR-based metabolomic study on the toxicological effects of pesticide, diazinon on adaptation to sea water by endangered Persian sturgeon, Acipenser persicus fingerlings. Chemosphere 185: 213-226.
7. Al-Otaibi AM, Al-Balawi HFA, Ahmad Z, Suliman EM (2018) Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, Clarias gariepinus. Brazilian Journal of Biology 79: 326-336.
8. Mena F, González-Ortegón E, Solano K, Araújo CV (2020) The effect of the insecticide diazinon on the osmoregulation and the avoidance response of the white leg shrimp (Penaeus vannamei) is salinity dependent. Ecotoxicology and Environmental Safety 206: 111364.
9. Wu X, Li J, Zhou Z, Lin Z, Pang S, et al. (2021) Environmental occurrence, toxicity concerns, and degradation of diazinon using a microbial system. Frontiers in Microbiology 12: 717286.
10. Bedi JS, Gill JPS, Aulakh RS, Kaur P (2015) Pesticide residues in bovine milk in Punjab, India: spatial variation and risk assessment to human health. Archives of environmental contamination and toxicology 69: 230-240.
11. Nath A, Vendan SE (2013) Carcinogenic pesticides residue detection in cow milk and water samples from Patna, India. Current Trends in Biotechnology and Chemical Research https://www.researchgate.net/publication/338385790_Carcinogenic_Pesticides_Residue_Detection_in_Cow_Milk_and_Water_Samples_from_Patna_India_Current_Trends_in_Biotechnology_and_Chemical_Research.
12. Casida JE, Durkin KA (2013) Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annual review of entomology 58: 99-117.
13. Tudi M, Li H, Li H, Wang L, Lyu J, et al. (2022) Exposure routes and health risks associated with pesticide application. Toxics 10: 335.
14. Gbadamosi MR, Abdallah MAE, Harrad S (2021) A critical review of human exposure to organophosphate esters with a focus on dietary intake. Science of the Total Environment 771: 144752.
15. Marrs TC (1993) Organophosphate poisoning. Pharmacology & therapeutics 58: 51-66.
16. Jokanović M (2018) Neurotoxic effects of organophosphorus pesticides and possible association with neurodegenerative diseases in man: A review. Toxicology 410: 125-131.
17. Sarlak Z, Khosravi-Darani K, Rouhi M, Garavand F, Mohammadi R, et al. (2021) Bioremediation of organophosphorus pesticides in contaminated foodstuffs using probiotics. Food Control 126: 108006.
18. Soares S, Rosado T, Barroso M, Vieira DN, Gallardo E (2019) Organophosphorus pesticide determination in biological specimens: bioanalytical and toxicological aspects. International Journal of Legal Medicine 133: 1763-1784.
19. Akramipour R, Golpayegani MR, Sedighi M, Fattahi F, Fattahi N (2020) Determination of common organophosphorus pesticides in the blood of children with acute leukaemia using a double-solvent system as a novel extractant for dispersive liquid–liquid microextraction. RSC advances 10: 44736-44742.
20. Thompson HM, Walker CH (2020) Blood esterases as indicators of exposure to organophosphorus and carbamate insecticides. In Nondestructive biomarkers in vertebrates. CRC Press 37-62.
21. Ibrahim L, Solyman M, Omami M, Amer A, Abdalla I Mohamed A (2024) The Impact of Neocidol on Hematological Parameters in Swiss Albino Mice. Open Access Research Journal of Life Sciences 7: 038-045.
22. Roberts TR, Hutson DH, Jewess PJ, Lee PW, Nicholls PH (1999) Pt. 2: Insecticides and fungicides 1-1475.
23. Environmental Protection Agency EPA (2008) Registration review—preliminary problem formulation for ecological risk and environmental fate, endangered species and drinking water assessments for diazinon, edited. Available online

at https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-057801_26-Mar-08_a.pdf.

24. Brandenberger H, Maes RA (2011) Analytical toxicology for clinical, forensic and pharmaceutical chemists Walter de Gruyter 5: 1-11.
25. Colovic MB, Krstic DZ, Lazarevic Pasti TD, Bondzic AM, Vasic VM (2013) Acetylcholinesterase inhibitors: pharmacology and toxicology. Current neuropharmacology 11: 315-335.
26. National Research Council, Division on Earth, Life Studies, Commission on Life Sciences, Board on Toxicology, Environmental Health Hazards and Safe Drinking Water Committee, 1986. Drinking Water and Health. National Academies Press 6.
27. Gupta RC, Todd GD (2017) Toxicity of pesticides. In Lu's Basic Toxicology CRC Press. Draft Toxicological Profile for Diazinon. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry 453-485.
28. Hulse EJ, Davies JO, Simpson AJ, Sciuto AM, Eddleston M (2014) Respiratory complications of organophosphorus nerve agent and insecticide poisoning. Implications for respiratory and critical care. American journal of respiratory and critical care medicine 190: 1342-1354.
29. Matsumura F (1985) Toxicology of insecticides. plenum press, New York https://www.scirp.org/reference/referencespapers?referenceid=1211538.
30. Hayes AW, Loomis TA (1996) Loomis's essentials of toxicology. Elsevier https://www.sciencedirect.com/book/9780124556256/loomiss-essentials-of-toxicology.
31. WHO G (2020) The WHO recommended classification of pesticides by hazard and guidelines to classification 2019. Available online at https://iris.who.int/bitstream/handle/10665/332193/9789240005662-eng.pdf.
32. Hassani S, Maqbool F, Salek Maghsoudi A, Rahmani S, Shadboorestan A, et al. (2018) Alteration of hepatocellular antioxidant gene expression pattern and biomarkers of oxidative damage in diazinon-induced acute toxicity in Wistar rat: a time-course mechanistic study. EXCLI journal 17: 1-57.
33. Howard PH (1991) Fate and exposure data for organic chemicals, vol. III, Pesticides. Lewis Publishers., Chelsea 1-684.
34. Bennett SM (2001) World Health Organization and Oregon State University https://www.who.int/director-general/who-headquarters-leadership-team.
35. Hodgson E, Levi PE (1987) A textbook of modern toxicology. Elsevier, New York, NY(USA) https://research.uok.ac.ir/_Pages/FileDownloadHandler.aspx?Code=b92a2696-a6d2-4638-a5e8-5a0a0fcc267d&FileName=Image.pdf.
36. Ganie SY, Javaid D, Hajam YA, Reshi MS (2022) Mechanisms and treatment strategies of organophosphate pesticide induced neurotoxicity in humans: A critical appraisal. Toxicology 472: 153181.
37. Morris CM, Savy C, Judge SJ, Blain PG (2014) Acute toxicity of organophosphorus compounds. Basic and clinical toxicology of organophosphorus compounds 45-78.
38. Amer AH, Elhawari WA, Mohamed AI (2014) Acute Toxicity of hexavalent chromium oxide administered subcutaneously and orally to Swiss albino mice (Mus musculus). JBMAS 3: 1-3.
39. Centers for Disease Control and Prevention (2023) Biomonitoring Data Tables for Environmental https://research.uok.ac.ir/_Pages/FileDownloadHandler.aspx?Code=b92a2696-a6d2-4638-a5e8-5a0a0fcc267d&FileName=Image.pdf.
40. Ibrahim L, Solyman M, Omami M, Amer A, Abdalla I Mohamed A (2024) The Impact of Neocidol on Hematological Parameters in Swiss Albino Mice. Open Access Research Journal of Life Sciences 7: 038-045.