http://dx.doi.org/10.30651/jqm.v7i2.16880

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

Research Article

Changes in histopathological features of adolescent rat livers due to subchronic exposure to Chlorpyrifos, Carbofuran, and Cypermethrin

Siti Jamalia[1], Muhammad Ihwan Narwanto[2*], Yuli Hermansyah[3]

1) Faculty of Medicine, University of Jember, Jember, Indonesia
2) Department of Anatomy, Faculty of Medicine, University of Jember, Jember, Indonesia
3) *Department of Internal Medicine*, dr. *Soebandi Jember* Hospital, *Faculty* of Medicine, University of *Jember*, *Jember, Indonesia*

ARTICLE INFO

Submitted : *30th December 2022*
Accepted : *30th May 2023*
Published : *27 th July 2023*

Keywords:
carbofuran, chlorpyrifos, cypermethrin, histopathology, liver*

Correspondence:
muhammadnarwanto@unej.ac.id

ABSTRACT

Insecticides are the most widely used type of pesticide in various fields, such as agriculture, plantation, industry, and household activities. Only 1% of the insecticides used work effectively to attack the target and the remaining 99% are released freely into water, soil, and air and finally have an impact on non-target organisms. Chlorpyrifos, carbofuran, and cypermethrin were the most widely used insecticides in their respective groups, namely, organophosphates, carbamates, and pyrethroids. This study aims to determine the changes in histopathological features of adolescent rat livers due to subchronic exposure to chlorpyrifos, carbofuran, and cypermethrin. Adolescent livers have a faster regeneration rate but are more susceptible to damage than the older age group. This study used 30 male Wistar rats which were divided into 5 groups, namely, normal group (N), control group (K), chlorpyrifos group (P1), carbofuran group (P2), and cypermethrin group (P3). Subcutaneous injection of insecticide was carried out for 21 days. The scoring method used in the histopathological observations is Manja Roenigk score. Data analysis used the ANOVA test and continued with the LSD post hoc test. In this study, significant results were obtained, $P<0.05$, which indicated that there were significant differences in the histopathological features of the liver that were exposed to chlorpyrifos, carbofuran, and cypermethrin. The picture of liver damage was mostly found in the carbofuran group.

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

INTRODUCTION

Insecticides are the most widely used type of pesticide in various fields, such as agriculture, plantation, industry, and household activities. Insecticides play an important role in increasing the number and variety of agricultural and plantation products, as well as reducing the spread of diseases transmitted by insect vectors. Insecticides are not only toxic to insects, but also to non-target organisms including humans and mammals (Gupta & Milatovic, 2014). Only 1% of the insecticides used work effectively against targets and the remaining 99% escape freely into water, soil, and air and ultimately affects non-target organisms (Khan & Ahmad, 2019). Globally, insecticides are one of the main causes of poisoning cases in humans with 250,000-370,000 cases each year (Chowański et al., 2014). Exposure to insecticides can occur directly through the skin, inhalation, and ingestion. Meanwhile, indirect exposure is through the consumption of products that contain insecticide residues (Pascal et al., 2020).

Organic synthetic insecticides are divided into 4 groups based on their chemical compounds, namely organochlorines, organophosphates, carbamates, and pyrethroids (Akashe, 2018). Organochlorines have been banned from use due to long-term residual effects (Khan & Ahmad, 2019). Organophosphates are neurotoxic by inhibiting the acetylcholinesterase enzyme. In addition, organophosphates also induce cell oxidative stress, affect metabolic pathways and cause multiple organ dysfunction, including the liver (Karami-mohajeri et al, 2017). The most widely used type of organophosphate compound is chlorpyrifos (Liem et al., 2021). Carbamates have the same mechanism of action as organophosphates, the difference is in the origin of the derivatives and its binding properties to the acetylcholinesterase enzyme. Carbamate also has side effects on many organs, especially the liver (Dias et al., 2015). The most widely used type of carbamate compound is carbofuran (Casida & Bryant, 2017; Asrianti *et al.*, 2020). Pyrethroids have the main mechanism of action by modulating sodium channels resulting in the extension of nerve depolarization (Riar, 2014). In addition, pyrethroids also induce cell damage through activation of oxidative stress and expression of proinflammatory genes, especially in the liver (Aouey et al., 2017). One of the most widely used pyrethroid compounds is cypermethrin (Al-omar et al., 2020; Kayode et al., 2019).

The liver is one of the main organs that has the potential to be damaged by exposure to insecticides. This is due to the main function of the liver as a place for activation and detoxification of toxic substances. Substances that enter the body will be detoxified by the liver with the result being other compounds that are more water soluble for easy excretion (Pratama, 2019). Hepatic damage due to exposure to insecticides can be proven through changes in hepatic biomarkers, such as serum aminotransferase, direct bilirubin, and indirect bilirubin (Ismail et al., 2021). Histopathologic observations are more clear and specific to detecting damage to liver cells (Tanvir et al., 2016).

In the young age group, the liver has a high ability to regenerate. However, age only affects the rate of regeneration, not the capacity or ability to restore the original organ volume. In addition, the liver organs in the young age group are also prone to damage compared to the old age group. There are studies showing induction of hepatocyte proliferation factors and gene expression in the cell cycle that are not inhibited in young rats (Schmucker & Sanchez, 2011). However, if from a young age, a person has experienced liver damage, it will potentially reduce productivity when entering

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

the next age group.

There are previous studies that observed liver histopathology due to exposure to insecticides. However, the number of studies is very limited and does not cover all types of insecticides to be compared. Therefore, this study aims to determine the changes in histopathological features of adolescent rat liver due to subchronic exposure to chlorpyrifos, carbofuran, and cypermethrin.

METHODS

This study used experimental male Wistar rats (*Rattus novergicus*) aged adolescents who were exposed to the insecticides chlorpyrifos, carbofuran, and cypermethrin. Rats were obtained from the Laboratory of Animal Physiology of UIN Maulana Malik Ibrahim Malang, East Java. The research design used a post-test-only control group design and the sampling technique used a completely randomized design (CRD). This research was conducted at the Experimental Animal House, Biochemistry Laboratory and Histology Laboratory, Faculty of Medicine, University of Jember and Biomedical Laboratory, Faculty of Dentistry, University of Jember. The characteristics the rats used were male Wistar rats, adolescents 30-60 days old, body weight 45-115 grams, active moves, and good appetite, and had never been used as research samples before (Kirschmann et al., 2018; Sengupta, 2013).

In this study, there were 5 treatment groups, namely the normal group without exposure (N), the control group with 5% DMSO (K), the chlorpyrifos treatment group (P1), the carbofuran treatment group (P2), and the cypermethrin treatment group (P3). The sample size of each group was calculated using Federer's formula and 5 rats samples were obtained in each group (Madiyono, 2014; Irmawartini, 2017). The insecticide exposure dose used was 10% LD50, namely 20 mg/kgBW chlorpyrifos, 0.2 mg/kgBW carbofuran, and 20 cypermethrin. mg/kgBW (Sachana *et al.*, 2001; Giri & Sharma, 2003; Rai & Sharma, 2007; Abdou & Sayed, 2019; Kayode *et al.*, 2019). Exposure is carried out by subcutaneous injection with 5% DMSO solvent (Deborah & Rowland, 2000; Uzun & Kalender, 2013).

The stages of the study consisted of acclimatization for 7 days, exposure to insecticides for 21 days, cervical dislocation termination method and ending with preparation and observation of liver histopathological preparations. During the study, rats were given food and drink ad libitum method. In addition, the rats were also given normal lighting 12 hours of light - 12 hours of darkness every day. After the termination process, the liver was taken and then fixed using 10% BNF, and histopathological preparations were made using Mayers Hematoxylin Eosin staining. The finished histopathological preparations were observed using an Olympus CX31 microscope with a magnification of 400x at 5 fields of view. In each field of view, 20 hepatocyte cells were randomly selected with the criteria of being in zone 3 of the liver or centrilobular parenchyma. The scoring method used is the Manja Roenigk score. The assessment criteria were normal cells (score 1), parenchymal degenerated cells (score 2), hydropic degenerated cells (score 3), and necrotic cells (score 4) (Kumar et al 2013; Yuningtyaswari & Mega, 2020). Observations were carried out by blinding method by 1 anatomical pathologist. Data analysis used the ANOVA test (one-way analysis of variance) and the LSD post hoc test. This research has received ethical approval from the Faculty of Medicine, University of Jember with number 1584/H25.1.11/KE/2022.

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

RESULTS

Obtained observational data is the result of microscopic assessment in the form of scoring using the Manja Roenigk method. The results of the average liver histopathological score for each group can be seen in Table 1. Based on data on the average liver histopathological score for each group, it was found that the lowest average score was group N, which means the degree of damage to hepatocyte cells was the mildest. Then followed by group K where the difference in the values of groups N and K is not much different. Then, group P1 and group P3. The highest mean rat liver histopathology score was in the P2 group, which means the degree of hepatocyte cell damage was the most severe. The results of histopathological observations can be seen in Figure 1.

After obtaining the scores for all samples using the Manja Roenigk method, it was followed by a comparative test analysis. The normality test uses the Shapiro-Wilk test and the homogeneity test uses the Levene test. The results of the Shapiro-Wilk test can be seen in Table 2. Meanwhile, the results of the Levene test can be seen in Table 3. The results of the normality test and homogeneity test show a significance value, which means that the Manja Roenigk score data in this study is normally distributed and the data is homogeneous. Therefore, it is continued with the One Way Anova test in Table 4.

Table 1. The average results of Manja Roenigk's liver histopathology score

Group	Average hepatic histopathology score (Average ± SD)
N (Normal)	195.00±2.000 (n=5)
K (Control)	195.40±1.140 (n=5)
P1 (Chlorpyrifos)	202.00±3.162 (n=5)
P2 (Carbofuran)	216.60±4.159 (n=5)
P3 (Cypermethrin)	210.40±3.847 (n=5)

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

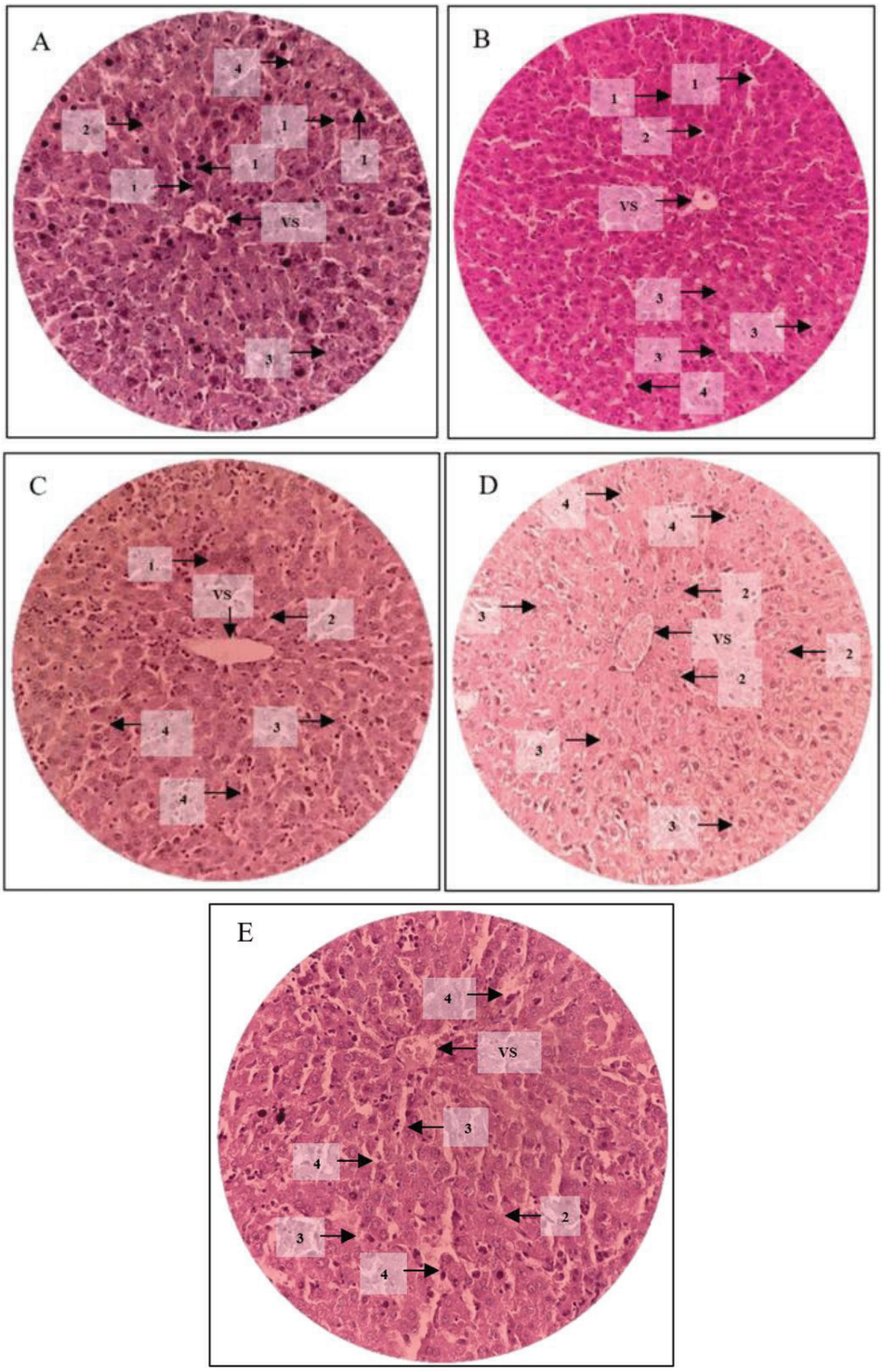

Figure 1. Hepatic Histopathology 400x (A: Normal Group, B: Control Group, C: Chlorpyrifos Group, D: Carbofuran Group, E: Cypermethrin Group). VS: Vena centralis, 1: Normal Hepatocyte, 2: Parenchymal Degenerated Hepatocyte, 3: Hydropic Degenerated Hepatocyte, 4: Necrotic Hepatocyte

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

Table 2. The normality test results of the liver histopathology scores for each group

Group	*Shapiro-Wilk*		
	Statistics	df	Sig.
N (Normal)	0.905	5	0.440
K (Control)	0.961	5	0.814
P1 (Chlorpyrifos)	0.987	5	0.967
P2 (Carbofuran)	0.933	5	0.617
P3 (Cypermethrin)	0.829	5	0.137

Table 3. Results of liver histopathology score homogeneity test

Levene statistic	df1	df2	Sig.
2.484	4	20	0.077

Table 4. One Way ANOVA test results liver histopathology score

	Sum of squares	df	Mean square	F	Sig.
Between groups	1793.040	4	448.260	47.285	0.000
Within groups	189.600	20	9.480		
Total	1982.640	24			

Table 5. Post Hoc test results liver histopathology score

	K0	K1	P1	P2	P3
K0 (Normal)		0.839	0.002*	0.000*	0.000*
K1 (Control)	0.839		0.003*	0.000*	0.000*
P1 (Chlorpyrifos)	0.002*	0.003*		0.000*	0.000*
P2 (Carbofuran)	0.000*	0.000*	0.000*		0.005*
P3 (Cypermethrin)	0,000*	0,000*	0,000*	0,005*	

Based on the One Way Anova test, a significance result of 0.000 was obtained. A significance result of <0.05 indicates a significant difference between groups in this study. Furthermore, to find out the differences in each group, then proceed with the LSD Post Hoc test.

Data from the LSD post hoc test results obtained a p-value <0.05 which indicated that there was a significant difference in the average rat liver histopathological scoring between groups. The results of all treatment groups, namely groups P1, P2, and P3 showed significant differences when compared to the normal and control groups. In addition, groups P1, P2, and P3 had significant differences when compared with each other. The normal group showed no significant difference when compared to the control group.

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

DISCUSSION

Chlorpyrifos, carbofuran, and cypermethrin are included in the group of toxic foreign compounds. Therefore, if these compounds enter the body, they must be immediately detoxified and excreted. The liver is the main site of the detoxification mechanism or xenobiotic biotransformation and is the ultimate target of insecticides. Detoxification of chlorpyrifos, carbofuran, and cypermethrin occurs in the liver which is played by several oxidation mechanisms of specific cytochrome P450 mixtures through several reaction pathways (Tanvir et al., 2016).

Chlorpyrifos is converted into its active metabolite, CPF-Oxon, through phase I metabolism. At the end of phase II, residual diethyl compounds are produced which then bind to (OH-) groups to form new Reactive Oxygen Species (ROS). CPF-Oxon can inhibit the action of the acetylcholinesterase enzyme which can cause accumulation of the neurotransmitter acetylcholine resulting in an influx of calcium ions (Ca2+) in cells. In addition, CPF-Oxon can also cause cell mitochondrial dysfunction. This can interfere with the calcium ion transport mechanism (Ca2+) so that the ion is persistently retained in the cell. Increased calcium ions (Ca2+) can stimulate the production of Nitric Oxide (NO) which in turn also increases Reactive Oxygen Species (ROS) free radicals. In addition, there was a decrease in endogenous antioxidants such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx). An imbalance of free radicals and antioxidants can cause oxidative stress. It is this oxidative stress that ultimately induces lesions in body cells, especially the liver (Eleršek & Filipi, 2006; Uzun & Kalender, 2013; Pearson & Patel, 2016; Tanvir *et al.*, 2016).

Carbofuran produces active metabolites in the form of 3-hydroxy carbofuran and 3-keto carbofuran through an oxidation mechanism in the phase I metabolism stage. This key metabolite is produced through a hydroxylation mechanism on a benzylic carbon to produce 3-hydroxy carbofuran which is then oxidized to 3-keto carbofuran. After that, substances in the form of 3-hydroxy-7-phenol, 3-keto-7-phenol, and 7-phenol are produced through a hydrolysis mechanism. At the end of phase II, most of the metabolites formed in the form of glucuronide or sulfate conjugates will be excreted through the urine (Song & Point, 2014; Jaiswal *et al.*, 2015). The specific mechanism of biochemical cell damage due to exposure to carbofuran has not yet been discovered. However, it can be ascertained that it is caused by oxidative stress based on several previous studies (Patel, 2017; Purushothaman & Kuttan, 2017).

Cypermethrin is converted to its active metabolite product, namely 3-phenoxybenzoic acid in stage I hepatic metabolism. Cypermethrin's main site of action is not only in the cell's sodium channels, but can also affect chloride and calcium channels. Cypermethrin can cause mitochondrial dysfunction which in turn causes an increase in Reactive Oxygen Species (ROS) free radicals and decreases antioxidant activity. This is what triggers cell damage through the mechanism of oxidative stress due to an imbalance in the production of free radicals and antioxidants. In addition, cypermethrin can also increase tissue damage by increasing levels of pro-inflammatory cytokines and decreasing anti-inflammatory cytokines (Gupta & Milatovic, 2014; Abdou & Sayed, 2019; Al-omar *et al.*, 2020).

Based on the results of the average liver histopathological score using the Manja Roenigk method, it is known that the treatment group exposed to chlorpyrifos, carbofuran, and cypermethrin (P1, P2, and P3) got a higher

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

score compared to the normal and control groups (N and K). This result indicated that the damage to the hepatocyte cells in the treatment group was more severe than in the normal and control groups. Based on the results of statistical tests in three treatment groups (P1, P2, and P3), there was also received significant difference between the normal (N) and control (K) groups. This is in accordance with research conducted by Uzun and Kalendar (2013) and Tanvir et al. (2016) in the chlorpyrifos group of rats, El-Damaty et al. (2012) in the carbofuran group of rats, and Mahna et al. (2019) in the cypermethrin group of rats (El-Damaty et al., 2012; Uzun & Kalender, 2013; Tanvir *et al.*, 2016; Mahna et al., 2019). However, the rats used as samples in this study were not adolescent, but adult rats. There has been no research related to liver histopathology due to insecticide exposure in adolescent rats.

In the study of Uzun and Kalendar (2013), histopathological liver damage was found with sinusoidal dilatation, diffuse Kupffer cell proliferation, mononuclear cell infiltration, pyknosis, and eosinophilic cytoplasm in hepatocyte cells. The research by Tanvir et al. (2016) found liver histopathological damage with infiltration and degeneration of hepatocyte cells in the central vein area and congestion with infiltrates in the central vein. The study of El-Damaty et al. (2012) found liver histopathological damage with hepatic sinusoidal congestion, large vacuolated hepatocyte cells, microvesicles, and pyknotic nuclei, and disturbance of the hepatic lobules. The study of Mahna et al. (2019) found liver histopathological damage with features of hepatocyte necrosis and vacuolar degeneration (El-Damaty et al., 2012; Uzun & Kalender, 2013; Tanvir *et al.*, 2016; Mahna et al., 2019) .

CONCLUSION

This study concluded that there were differences in the histopathological picture of the livers of adolescent rats that were given subchronic exposure to chlorpyrifos, carbofuran, and cypermethrin. Carbofuran caused the most severe liver histopathological damage compared to other types of insecticides. Suggestions that can be given by researchers are to carry out further studies related to insecticide doses that can still be tolerated by the liver, especially carbofuran insecticides. At low doses, carbofuran can already cause the most severe histopathological damage to the liver, so it is necessary to pay more attention to the use of carbofuran insecticides for agro-industry actors. In addition, further research can be carried out related to the prevention of liver damage due to exposure to insecticides.

REFERENCES

Abdou, R. H., & Sayed, N. (2019). Antioxidant and Anti-Inflammatory Effects of Nano-Selenium against Cypermethrin-Induced Liver Toxicity, 53–65. https://doi.org/10.4236/cellbio.2019.84004

Akashe, M. M. (2018). CLASSIFICATION OF PESTICIDES : A REVIEW, (May 2020). https://doi.org/10.7897/2277-4343.094131

Al-omar, M. S., Naz, M., Mohammed, S. A. A., & Mansha, M. (2020). Pyrethroid-Induced Organ Toxicity and Anti-Oxidant-Supplemented Amelioration of Toxicity and Organ Damage : The Protective Roles of Ascorbic Acid and α -Tocopherol. doi:10.3390/ijerph17176177. Website:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503327/pdf/ijerph-17-06177.pdf

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

Aouey, B., Derbali, M., Chtourou, Y., & Bouchard, M. (2017). Pyrethroid insecticide lambda-cyhalothrin and its metabolites induce liver injury through the activation of oxidative stress and proinflammatory gene expression in rats following acute and subchronic exposure. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-016-8323-4

Asrianti, A., Legowo, D., Maslachah, L., & Muhammad, E. P. Y. (2020). The Effect of Carbofuran Exposure on Mice During Lactation Period Against on Histopathology Description of The Heart of Mice (Mus Musculus) Offspring, *12*(4), 4646–4653. https://doi.org/10.31838/ijpr/2020.12.04.327

Casida, J. E., & Bryant, R. J. (2017). The ABCs of pesticide toxicology: amounts, biology, and chemistry. https://doi.org/10.1039/c7tx00198c

Chowański, S., Kudlewska, M., Marciniak, P., & Rosiński, G. (2014). Synthetic Insecticides – is There an Alternative ?, (January). Website: http://www.pjoes.com/Synthetic-Insecticides-is-There-an-Alternative-,89194,0,2.html

Deborah, S., & Rowland, J. (2000). Toxicology Chapter for Chlorpyrifos. Website: https://archive.epa.gov/scipoly/sap/meetings/web/pdf/hed_ra.pdf

Dias, E., Garcia, F., Morais, S., & Pereira, M. D. L. (2015). A Review on the Assessment of the Potential Adverse Health Impacts of Carbamate Pesticides. doi: 10.5772/59613. Website: https://www.intechopen.com/chapters/48017

El-Damaty, E.-S. M. A. A.-R. H. F., Rowayshed, G., & Fahmy., H. M. (2012). Biochemical and histopathological effects of systemic pesticides on some functional organs of male albino rats Biochemical and Histopathological Effects of Systemic Pesticides on Some Functional, (October). Website: https://www.researchgate.net/publication/286292321_Biochemical_and_histopathological_effects_of_systemic_pesticides_on_some_functional_organs_of_male_albino_rats

Eleršek, T., & Filipi, M. (2006). Organophosphorus Pesticides - Mechanisms Of Their Toxicity. Website: https://www.intechopen.com/chapters/13231

Giri, S., Giri, A., & Sharma, G. D. (2003). Induction of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice in vivo, *18*(1), 53–58. https://doi.org/10.1093/mutage/18.1.53

Gupta, R. C., & Milatovic, D. (2014). Chapter 23. Insecticides, (Cm), 389–407. https://doi.org/10.1016/B978-0-12-404630-6.00023-3

Irmawartini, Nurhaedah. (2017). Metodologi Penelitian. Jakarta: Kementerian Kesehatan

Ismail, A. A., Hendy, O., Rasoul, G. A., Olson, J. R., Bonner, M. R., & Rohlman, D. S. (2021). Acute and Cumulative Effects of Repeated Exposure to Egyptian Adolescents. https://doi.org/10.3390/toxics9060137

Jaiswal, S. K., Gupta, V. K., Siddiqi, N. J., Pandey, R. S., & Sharma, B. (2015). Hepatoprotective Effect of Citrus limon Fruit Extract against Carbofuran Induced Toxicity in Wistar Rats, *2015*. https://doi.org/10.1155/2015/686071

Karami-mohajeri, S., Ahmadipour, A., & Rahimi, H. (2017). Adverse effects of

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

organophosphorus pesticides on the liver : a brief summary of four decades of research, (Figure 1), 261–275. https://doi.org/10.1515/aiht-2017-68-2989

Kayode, O., Adesola, F., Teslim, D., Arinola, A., & Dorcas, A. (2019). Heliyon Oxidative stress and in fl ammation following sub-lethal oral exposure of cypermethrin in rats : mitigating potential of epicatechin. *Heliyon*, *5*(July), e02274. https://doi.org/10.1016/j.heliyon.2019.e02274

Khan, M. A., & Ahmad, W. (2019). Microbes for Sustainable lnsect Pest Management (Vol. 1). Sustainability in Plant and Crop Protection. Website: https://link.springer.com/book/10.1007/978-3-030-23045-6

Kirschmann, E. K., Pollock, M. W., Nagarajan, V., & Torregrossa, M. M. (2018). Developmental Cognitive Neuroscience Development of working memory in the male adolescent rat. *Developmental Cognitive Neuroscience*, (November), 0–1. https://doi.org/10.1016/j.dcn.2018.11.003

Kumar, V. A., K. Abbas, & J. C. Aster. (2013). Robbin's Basic Pathology. 9th edition. Singapore: Elsevier. Terjemahan oleh Nasar I. M., dan S. Cornain. 2015. Buku Ajar Patologi Robbins. Edisi 9. Singapore: Elsevier

Liem, J. F., Mansyur, M., Soemarko, D. S., Kekalih, A., Subekti, I., Suyatna, F. D., … Pangaribuan, B. (2021). Cumulative exposure characteristics of vegetable farmers exposed to Chlorpyrifos in Central Java – Indonesia ; a cross-sectional study, 1–9. Website: https://scholar.ui.ac.id/en/publications/cumulative-exposure-characteristics-of-vegetable-farmers-exposed-

Madiyono, Bambang. (2014). Perkiraan Besar Sampel. Di dalam Dasar-Dasar Metodologi Penelitian Klinis, Edisi ke-5. Jakarta

Mahna, D., Puri,S., Shama, S. (2019). Cypermethrin Induced Liver Toxicity Alered Gene Expression and DNA Methylation. Biochemistry and Molecular Biology. https://doi.org/10.1096/fasebj.2019.33.1_supplement.621.9

Pascal, F., Manfo, T., Mboe, S. A., Nantia, E. A., Ngoula, F., Telefo, P. B., … Chongwa, F. (2020). Evaluation of the Effects of Agro Pesticides Use on Liver and Kidney Function in Farmers from Buea , Cameroon, *2020*. https://doi.org/10.1155/2020/2305764

Patel, J. (2017). TOXICOPATHOLOGY OF INDUCED CARBOFURAN TOXICITY IN WISTAR RATS, (January 2014). Website: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5063722/pdf/nihms-786028.pdf

Pearson, J. N., & Patel, M. (2016). The role of oxidative stress in organophosphate and nerve agent toxicity, 1–8. https://doi.org/10.1111/nyas.13115

Pratama, D. A. O. A. (2019). The Study of Organophosphate (Diazinon) Toxicity Toward Liver Histopathology ity Toward Liver Histopathology a nd Malondialdehyde (Mda) Serum Levels On Rats (Rattus norvegicus), *1*(2), 15–23. Website: https://vbcj.ub.ac.id/index.php/vbcj/article/view/12/12

Purushothaman, B. P., & Kuttan, R. (2017). Protective Effect of Curcumin Against Carbofuran- Induced Toxicity in Wistar Rats, *36*(1), 73–86. Website: https://pubmed.ncbi.nlm.nih.gov/28605332/

QANUN MEDIKA
JURNAL KEDOKTERAN FKUM SURABAYA
http://journal.um-surabaya.ac.id/index.php/qanunmedika

Rai, D. K., & Sharma, Æ. B. (2007). Carbofuran-Induced Oxidative Stress in Mammalian Brain, 66–71. https://doi.org/10.1007/s12033-007-0046-9

Riar, N. K. (2014). Chemical Profile, *1*, 449–451. https://doi.org/10.1016/B978-0-12-386454-3.01169-6

Sachana, M., Flaskos, J., Nikolaidis, E., Hargreaves, A., Alexaki-tzivanidou, E., Sachana, M., & Al, E. T. (2001). Inhibition of Rat Platelet 5-Hydroxytryptamine Uptake by Chlorpyrifos and Carbaryl, 195–200. https://doi.org/10.1111/j.0901-9928.2001.890409.x

Schmucker, D. L., & Sanchez, H. (2011). Liver Regeneration and Aging : A Current Perspective, *2011*. https://doi.org/10.1155/2011/526379

Sengupta, P. (2013). The Laboratory Rat : Relating Its Age with Human ' s, *4*(624), 2–8. Website:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3733029/pdf/IJPVM-4-624.pdf

Song, X. dan W. Point. 2014. Mechanism of toxicity. 1:673–674. Website: https://pubchem.ncbi.nlm.nih.gov/compound/Carbofuran

Tanvir, E. M., Afroz, R., Chowdhury, M. A. Z., Gan, S. H., Karim, N., Islam, M. N., & Khalil, M. I. (2016). A model of chlorpyrifos distribution and its biochemical effects on the liver and kidneys of rats. https://doi.org/10.1177/0960327115614384

Uzun, F. G., & Kalender, Y. (2013). Chlorpyrifos induced hepatotoxic and hematologic changes in rats : The role of quercetin and catechin. *FOOD AND CHEMICAL TOXICOLOGY*, *55*, 549–556. https://doi.org/10.1016/j.fct.2013.01.056

Yuningtyaswari, & Mega, S. D. (2020). The Effect of Phoenix Dactylifera Pollen on Histology Liver of Rattus norvegicus Exposed with Air Fresheners, (October), 13–14. Website: http://repository.umy.ac.id/handle/123456789/36464?show=full