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The Histopathology of the Mouse Brain as a Toxic Effect of Inhalation Exposure to Prallethrin and *D*-Phenothrin Mixture

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Abstract:
Basic guidelines for the preparation of a technical work for an Test Engineering and Management Journal Publication are presented. This document is itself an example of the desired layout (inclusive of this abstract) and can be used as a template. It contains information regarding desktop publishing format, type sizes, and typefaces. Style rules are provided that explains how to handle equations, units, figures, tables, abbreviations, and acronyms. Sections are also devoted to the preparation of acknowledgments, references, and authors' biographies. The abstract is limited to 150–200 words and cannot contain equations, figures, tables, or references. It should concisely state what was done, how it was done, principal results, and their significance.

Keywords: *At least 5-6 keywords separated by comma*

I. INTRODUCTION

Prallethrin and *d*-phenothrin are pyrethroid family, which are common ingredients in mosquito repellent (MR) either in the form of spray, mats,and coils. They are neurotoxic in mammals, with sodium channels in the brain being their well-established target site [1,2]. Recently, MR contains two, three or even four compounds of pyrethroid to obtain the expected effects. However, a study that examines the toxic effects of mixture compounds of MRsin inhalation exposures has not been studied previously, even though MR as a single compound has been investigated by researchers. Grewal *et al.* [3] investigated the effects of cypermethrin orally administered in two doses. The results show that repeated administration of cypermethrin produced hemorrhages, an increase in glial cells, neuronal degeneration, an increase in sinusoidand necrosis, increased and decreased of various tissue. They concluded that repeated oral exposure of cypermethrin has considerable harmful effects on body organs in the rat. Godin *et al.* [4] studied the species differences between deltamethrin and esfenvalerate metabolism. These studies illustrated a significant species difference in the in vitro pathways of biotransformation of deltamethrin in rat and human liver microsomes due to the differences of intrinsic activities of rat and human carboxylesterases.Sheikh *et al.* [5] examined histological effects of cypermethrin on mouse lung and liver tissue. They found that cypermethrin and other pyrethroid family had hazardous effects on the nontarget of living organism through inhalation exposure.

In inhalation exposure, the mixture ofprallethrin and *d*-phenothrin is in form of particulate matters (PMs) which are a complex combination of solid particles and aerosols in the air [6] and have been becomean interesting issue since it has been well known to exacerbate human health, such as impairment

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of respiratory,cardiovascular and cellularsystem[7–11]. Two parameters of PMs that have a great role to affecthuman health disorders are chemical and physicalproperties [12]. The chemical and physical property of PMsis influenced by PMs' source, which determinesthe atmosphericbehavior of PMs. Physical characteristics include the size and shape of PMs, while chemical characteristics are a chemical constituent of PMs[13]. Both of them have an enormousresponsibility to establish toxicity onliving organisms.Lin *et al.* [14]examined the interrelationship between PMs size and in vitro toxicological effects of mainstream cigarette smoke, and they obtained that a large size of PMs induced less toxicity compared witha small size. The investigation concluded that PMs of small size were more toxic than a large size.Dieme*et al*. [14] studied the relationship between the physicochemical of $PM_{2.5}$ characteristics in Dakar city. The results show that urban $PM_{2.5}$ samples caused greater biological responses in BEAS-2B cells than the rural one due to chemical properties of *trans*ition metals (i.e. Fe, Al, Pb, Mn, Zn) and organic compounds (i.e. PAHs). They involved in a time- and/or dose-dependent toxicity, appealingto inflammatory processes.Lu*et al*. [15] examined the ambient of coarse, fine and ultrafine particles. They found that fine particles generate more free radicals than coarse and ultrafine particles. Moreover, the cell proliferation assay indicated that ultrafine particles were more cytotoxic than fine and coarse particles.

Generally, the cytotoxicity can be observed from several parameters, i.e., cell viability. The cell viability is defined as a number of healthy cells in a sample and proliferation of cells is a vital indicator for having knowledge of the functional mechanisms of particular genes, proteins and pathways involved cell survival or death as well, after exposing to toxic agents[16], such as glial cells proliferation and necrosis. Glial cells are the neuronal-specific target of the neuroanatomical region,which are affected by neurotoxicant [17], while necrotic cells are cell death which isarbitrated by multiplesignaling pathways, which act as potential targets for cellular toxicity[18].In the previous study of pyrethroid exposure,Grewal *et al.* [3] investigated cypermethrin exposure on morphological and histopathological effects of various rat tissues. They found that repeated series administration of cypermethrin produced hemorrhages, an increase in glial cells, neuronal degeneration, an increase in sinusoid and necrosis, enhancement and decreased of various tissue. Nair *et al*. [19] studied cypermethrin exposure on the various tissue of rat and obtained medium- and high-dose intoxicated groups shown necrotic changes, extensive hemorrhages,and congestion.

In this study, we examined the toxic effect of prallethrin and *d*-phenothrinthat analyzedas PMs on mouse brain tissuethrough inhalation exposure.

II. Materials and Methods

Chemicals and reagents

Prallethrin and *d*-phenothrin were obtained from Sigma Aldrich with catalognumber ofprallethrin 32917 and *d*-phenothrin 36193. The doses of prallethrin and *d*-phenothrin mixturesconsisted of thelower and higher doses. The lower dose was a mixture of 0.0001 mg/L prallethrin and 0.104 mg/l *d*-phenothrin, while the higher dose was a mixture of 0.001 mg/l prallethrin and 1.04 mg/l *d*-phenothrin.The lower dose was based on the NOAEL value of prallethrin and *d*-phenothrin of 28 days exposure [20], while the higher one was ten times larger. Theseactive ingredients were diluted many times to obtain the expected concentration.

Mechanism of inhalation exposures

Mice were exposed to prallethrin and *d*-phenothrinthrough inhalation exposure. The mixture was dissolvedin acetonitrile then diluted several times withdistilled water[4]. The solution was volatilized using adiffuser, inwhich the air supply was derived from an air pump (RC-Q6) dischargedat 4 L per minute into the solution[21].The aerosol produced was inserted into the whole-body exposure chamberwhich containedthree mice. Mice were exposedfor four hours a day [20]for 60 days observation with a mixture of prallethrin and *d*-phenothrin, which was conducted at temperatures of 31.5^{o}C ± 0.5^{o}C and RH of 94% ± 2.5%, and was carried out in a chamber as a continuous system.

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Animal husbandry and maintenance

Six-week-old male BALB/c mice were purchased from the Airlangga University pharmacology laboratory and acclimatized for 14 days. The room was maintained at 30.3°C ± 1.4°C with relative humidity (RH) of 63.08% ± 2. 87% and light: dark cycles of 12:12 hours. The mice were housed togetherwith12 mice in stainless steel wire mesh cages (W 350 mm × L 400 mm × H 180 mm), *ad libitum* provided for tap water and a commercial diet from PT. Charoen Pokphand Indonesia. After acclimatization, three mice were placed into a chamber forexposure toprallethrin and *d*-phenothrin mixture for four hours a day for 60 days. The study was approved by the Animal Care and Use Committee(ACUC) of the Veterinary Faculty of Airlangga Universitywith certificate number 716-KE.

Experimental group

Mice weredivided into four groups, i.e., negative control, positive control and treatment (lower and higher dose)groups (Figure 1). The negative control (NC) groups were the mice without any treatment, positive control (PC) groups were mice with exposure tosolvent only, whereas lower-dose treatment groups were mice exposed to amixture of 0.0001 mg/L prallethrin and 0.104 mg/l *d*-phenothrin exposures and higher-dose treatment groups were mice exposed to a mixture of 0.001 mg/l prallethrin and 1.04 mg/l *d*-phenothrin. There were three replicates (R) foreach group.

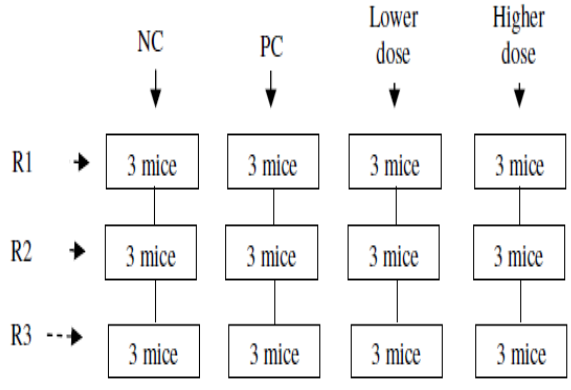

Figure 1.Illustration of Experimental Groups

Analysis of morphological change of cerebrum

Necropsy was carried out on all animals to observe gross morphological changes. The cerebrumwas dissected out, cleaned with physiological saline solution (phosphate buffer PH-7) and sucrose 10% to make them blood-free. Tissues were put in 10% buffered formalin for subsequent processing and histopathological studies. The formalin-fixed tissues were dehydrated in ascending grades of alcohol, cleared in benzene, and embedded in paraffin at 58°C. 5 μ-thick sections from paraffin-embedded tissues were stained by hematoxylin and eosin (H and E) method[22]. Slides were examined by the Research Photomicrographic Microscope System of dr. Sutomo hospital Surabaya-Indonesia.The morphological change of cerebrum tissue was analyzedusingthe histochemistry method.

Measurement of particulate matter

PMs measurements were executed at the third hour of exposures using an Aerocet 531S Particle Mass Profiler and Counter in the breathing zone of mice of 7 cm in height from the bottom of the chamber to represent inhaled PMs at day 20^{th}, 40^{th}, and 60^{th}.

Statistical analysis

All statistical analyses wereconducted using Minitab 16.0. The homogeneity of variance test was carried out using a Levene test, while normality was determinedwith a Kolmogorov Smirnov test. One-way ANOVAswere conducted to determinedifferences between groups, whereasindependent t-testswereused to analyze differences between the two groups. The correlation between the number concentration and morphological changes was analyzed using linear regression.

III. Results and Discussions

Particulate matter in number concentration

PMs number concentration (particles/L) in the breathing zone (Figure 2) illustratesthe polydispersed distribution and the major contribution was ultrafine PMs of the largest concentration of diameter 0.3 µm, followed by 0.5 µm, 1 µm,5 µm,and 10 µm. The same pattern occurred during all-day measurements of 20^{th}, 40^{th} and 60^{th}. In the breathing zone, particles with size 5 µm have approached zero, even particles in size of 10 µm were not measurable. The existing particles in the breathing zone were99.9% of ultrafine PMs (≤1µm)which reported to establish cytotoxicity mechanism [23,24]. There were no significant differences of PMs number concentration (particles/L) breathing zone of PC, higher and lower dose group in size of 0.3 µm (p-value = 0.355), 0.5 µm (p-value =0.227), 1.0 µm(p-value = 0.185), 5.0 µm (p-value = 0.161), and 10.0 µm (p-value = 0.124) in all replicate of

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breathing zone of PC, higher and lower dose groups in the same dose in all day measurements.

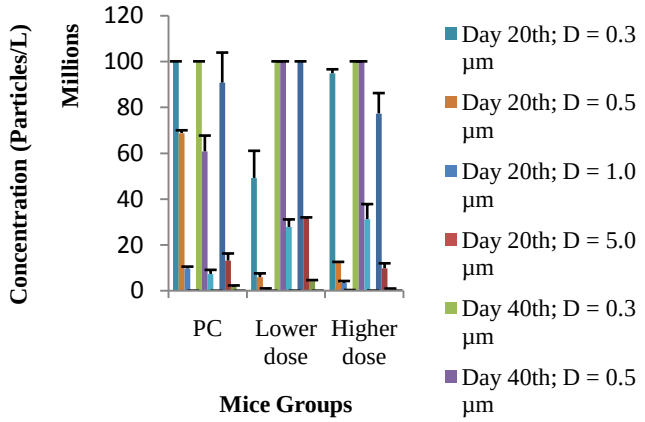

Figure 2**.** PMs Number Concentrations of Prallethrin and *d*-phenothrin Mixture in The Mice Breathing Zone.

Figure 3 Photomicrographs of a section of the cerebrum of the mice group showing necrosis and a marked increase in glial cells.
NC group day 20th; (b) NC group day 40th; (c) NC group day 60th; (d) PC group day 20th; (e) PC group day 40th; (f) PC group day 60th; (g) Lower dose group day 20th; (h) Lower dose group day 40th; (i) Lower dose group day 60th; (j) Higher dose group day 20th; (k) Higher dose group day 40th; (l) Higher dose group day 60th

The size was the physicalproperties of PMs that have a great influence to affect human health disorders[12]. When particle size was degraded from micrometer to nanometer range, it would be raised its toxicity due to the enhancement of the particle surface area [13]. Moreover, the second properties of PMs that determined toxicity werethe chemical constituent, which contained in the compounds. In this study, MR consisted ofa mixture ofprallethrin and d-phenothrin.Prallethrin is a synthetic pyrethroid with fast knock-down activity against household insect pests and also used in household insecticide products against mosquitoes, houseflies and cockroaches [25]. Prallethrin, as defined in the accompanying specification, consists mainly of [1R,*trans*; S], and [1R,*cis*; S] isomers in a ratio of approximately 4:1. The chemical names (CAS) is (S)-2-methyl-4-oxo-3-(2-propynyl)-2-cyclopentaneane-1-yl(1R)-*cis*,*trans*-2,2- dimethyl-3-(2-methyl-1-propenyl)-cyclopropanecarboxylate[20]. While *d*-phenothrin is a synthetic pyrethroid with high lethal activity against household insect pests. It is used in public health against mosquitoes, houseflies and cockroaches [26]. *d*-phenothrin is the 4:1 mixture of the [1R,*trans*] and [1R,*cis*] and the proposed CAS name is (3-phenoxyphenyl)methyl (1R)-*cis*-*trans*-2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylate[20]. Pyrethroids that having the 1R,*cis*configuration (e.g. [1R,*cis*]-d-phenothrin) are both insecticidal and toxic to mammals, whereas the pyrethroids having the 1R,*trans*configuration (e.g. [1*R*,*trans*]-d-phenothrin) though similar in insecticidal potency, obscure measurable acute toxicity to mammals. The low toxicity of 1*R*,*trans*compounds to mammals was derived from rapid hydrolytic detoxication by liver esterases[27],nevertheless,thedosing experiments of intracerebral demonstrated that these compounds had very low intrinsic toxicities even when the effects of biotransformation were omitted. This dependence on the acute toxicity of cyclopropanecarboxylate esters of primary alcohols on the stereochemical configuration at C-3 is commonlyappropriate to this class, but two significant exceptions are known: [1*R*,*cis*]-phenothrin lacks measurable toxicity to mammals. *d*-Phenothrin isomer did not implicate as the causative agent of microgranulomatous lesions in various tissues, following a stereospecific *trans*esterification reaction that results in the formation of a cholesterol ester [28].

Morphological changes of mouse cerebrum

The mixture doses (lower and higher dose) produced a varying degree of mild to moderate toxic symptoms in mice at day 20th, 40th,and 60th that characterized by increasing the percentage of necrosis area (Figure 4) and gliosis(Figure 5). The lower dose group produced mild toxicosis (Figure 3), while the moderate toxicosis was generated by the higher dose group on day 60th. However, there were no dying mice in all groups at day 20th, 40th,and 60th. In the NC group, there were no morphological changes of necrosisappearance in the brain tissue, while glial cells were a mild produced of 5% on day

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20th and stayed steady state until day 60th(Figure 3). The PC groupcreated 5.5% on average of necrosis percentage and there wasa slight decrease of 2.5% on day 60th(Figure 3). Similar to PC, the higher dose group a mild declined of necrosis and gliosis percentage at day 60th compared to day 40th and 20th, however, it did not happen on the percentage of gliosis of the lower dose group (Figure3). The statistical analysis shows that there wereno significant differences in percentage gliosis and necrosis area (p-value = 0.000) between NC, PC, lower and higher dose with a confidencelevel of 95% at all day measurement.

These findings are in agreement with Grewal *et al*. [29]who found extensive neuronal damage and glial cell proliferation with repeated oral doses of cypermethrin and Nair *et al*. [19]that reported intoxicated groups which shown necrotic changes, extensive hemorrhages and congestion. Many pesticide agents were described to affect variable changes in the brain on repeated series exposure which have been correlated to hypoxia, hypoglycemia, and/or cell ion homeostasis disorder[30].

The statistical results illustrate that there were no correlation between number concentration and the percentage area of necrosis at all day measurements of 0.3 μm (p-value = 0.665), 0.5 μm (p-value = 0.493), 1 μm (p-value = 0.776) and 5 μm (p-value = 0.707). The same results were indicated by the correlation between number concentration and the percentage of gliosisareaat all day measurements of 0.3 μm (p-value = 0.), 0.5 μm (p-value = 0.493), 1 μm (p-value = 0.776) and 5 μm (p-value = 0.707).

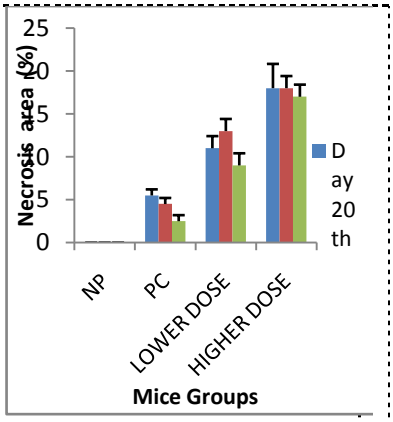

Figure 4. The percentage area of necrosis area.

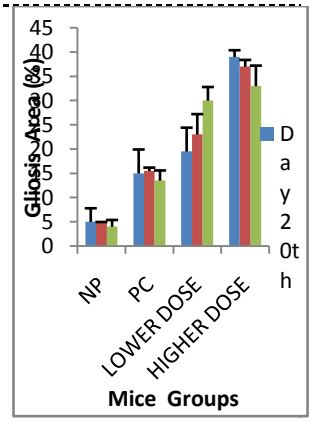

Figure 5. The percentage area of gliosis area.

Two parameters of PMs that had a significant effect on health were chemical and physical properties [12], thus the main reason for increasing of the percentage area of glial and necrosis were the chemical properties of PMs. Prallethrin contained mainly of [1R,*trans*; S], and [1R,*cis*; S] isomers in a ratio of approximately 4:1. The chemical names (CAS) is (S)-2-methyl-4-oxo-3-(2-propynyl)-2-cyclopentane-1-yl(1R)-*cis*,*trans*-2,2- dimethyl-3-(2-methyl-1-propenyl)-cyclopropanecarboxylate[20]. While *d*-phenothrinwas 4:1 mixture of the [1R,*trans*] and [1R,*cis*] and the proposed CAS name is (3-phenoxyphenyl) methyl (1R)-*cis*-*trans*-2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylate[20]. The toxicity of PMs was derived from the 1R,*cis*configuration (e.g. [1R,*cis*]-*d*-phenothrin) was toxic to mammals, whereas the pyrethroids having the 1R,*trans*configuration (e.g. [1*R*,*trans*]-d-phenothrin) though similar in insecticidal potency, obscure measurable acute toxicity to mammals. The low toxicity of 1*R*,*trans*compounds to mammals was determined by the rapid hydrolytic detoxication by liver esterases[27]. In the case of the *trans* form of prallethrin, the change of the *l* to *d* in acid moiety is generalized to be accompanied by a 75-fold increase of toxicity, and a 5,7-fold increase of toxicity with the change of the *l* to *d* in alcohol isomeric [25]. Matsunaga *et al*. [25] investigated insecticidal activities of prallethrin isomer. The results show that the *d* configuration in crysanthemic acid moietywas principallyessential for insecticidal activity, similar to allethrin. The rate of magnificationof toxic value due to the alteration of the*l* to the *d* incrysanthemic acid moiety, inprallethrinis much greater than that in allethrin. The generalization can be also possible for the *cis* form in prallethrin whereas allethrin could not be generalized for the *cis* form, though the increasing direction was the same manner as in the *trans* form[31].

IV. Conclusion

Prallethrin and *d*-phenothrin are members of the pyrethroid family which are neurotoxic to mammals and sodium channels in the brainare well established as the target site. They were analyzed as particulate matters (PMs) with respect toinhalation exposure and have been reportedgreat effects toexacerbate human health, such as effecton the brain morphological changes.The PMs are declared as number concentration, while the morphological changesareanalyzedusing histochemistry method as the percentage area of gliosis and necrosis.In this study, PMs were generated from the process of bubbles producing,illustratespolydispersed distribution by concentration andthe largest PMs is 0.3 μm in diameter. Both of percentage area of necrosis and gliosis shows a mild increasing at day 20th and 40th, however they decreased at day 60th. There is no significant correlation between the numberconcentration and the percentage area of necrosis and gliosis, thus the reason is suspected by the chemical properties of prallethrin and *d*-phenothrin. This study suggests that the mixture of prallethrin and *d*-phenothrin in inhalation exposure has hazardous effects on mammals, produces morphological

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changes of mouse brain tissue due to the chemical properties of prallethrin and *d*-phenothrin mixture.

Conflict of interest

All author declares that there is no conflict of interest.

V. Acknowledgment

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