Indonesian Journal of Pharma Science, June 2019
p-ISSN: 2685-6549

Test of Teratogenic Effects of Transflutrin and D-Alletrin Compounds in Mosquito Drugs against Mice Fetus (Mus Musculus L.)

Sutri Damayana [1,*], Sri Wigati [1,2], Elisma [2]

[1.] *Department of Pharmacy, Faculty of Science and Technology, University Jambi* : Jl. Jambi-Ma. Bulian KM 15 Mendalo Darat Jambi 36361

Abstract

Transflutrin and d-alletrin compounds in insect repellents that are widely used by people are classified as insecticides which can be teratogenic. This study aims to determine the teratogenic effects caused by a combination of transflutrin and d-alletrin compounds on fetus mice (Mus musculus L.) during the period of organogenesis. The design used was a randomized block design (RBD) with 4 treatments and 5 groups as replications. The treatment consisted of exposure to a combination of transflutrin and d-alletrin compounds with each dose of 4 mg and 45 mg for 0, 6, 8 and 10 hours at gestational age 6 to 15 days. On the 18th day the pregnancy was carried out laparotomy to take the fetus from the mother's uterus to the mice. The design used was a randomized block design (RBD) with 4 treatments and 5 groups as replications. The treatment consisted of exposure to a combination of transflutrin and d-alletrin compounds with each dose of 4 mg and 45 mg for 0, 6, 8 and 10 hours at gestational age 6 to 15 days. On the 18th day the pregnancy was carried out laparotomy to take the fetus from the mother's uterus to the mice. The results showed that the exposure of 6, 8 and 10 hours of the combination of transflutrin and d-alletrin compounds could cause 33.3%, 40.9% and 38.9% of fetal deaths, 19.1% of hemorrhages, respectively. 36.8% and 50%, body cancer as much as 2.38%, 2.6% and 5.6%, body humping (flexion) by 0%, 5.2% and 11.1%, bone deformities as much as 4.7%, 10, 5% and 16.7%, and abnormalities resulting from ossification of fetal mice were 7.1%, 13.2% and 19.4%. But the treatment of exposure did not affect body weight, body length and fetal morphology of mice. From the results of the study it can be concluded that the combination of transflutrin and d-alletrin compounds have mild teratogenic effects on fetus mice and the teratogenic effects increase with increasing exposure time to external abnormalities and fetal internal mice.

Keywords: *Transflutrin Compounds*, *D*-alletrin *Compounds*, *Fetal Mice, Teratogenic*

Introduction

Indonesia is a tropical country that is very suitable for breeding mosquitoes. As a result, the incidence of disease by mosquitoes increases from time to time so that it takes an insect killer compound (insecticide) such as insect repellent to eradicate it. Insect repellent has various types and forms, including mosquito repellent liquid (liquid), fuel (coil), aerosol and vaporizer (mat, electric liquid, lotion). In each type of insect repellent contains different active ingredients depending on the brand and type.

Insecticides in general can have an effect on the nervous system, disruption of the immune system, increase the incidence of breast cancer, disrupt sexual and reproductive development (Raini, 2009). In pregnant women, the frequency of repeated use of chemical compounds can cause accumulation in the fetus while the fetus does not yet have a perfectly functioning metabolic system (Manson, 1986). Pregnancy is a period that is very susceptible to various disorders of chemical compounds so that at this time careful use is needed. Not only for the mother but also the fetus, especially during organogenesis because at that time fetal cells are still active in proliferation (Robert, 1971).

A person who has grown up will partially be able to overcome the effects of poisons on him, but not so with the fetus he contains. The fetus is very susceptible to insect repellent, because its organs are not perfect, and the body's resistance is not good. The active ingredient of insect repellent will enter the body

through the respiratory tract, then it will circulate in the blood. Then this active ingredient will spread to body cells (Wahyuni, 2005). To determine the safety of a mosquito repellent against humans, especially pregnant women, it is used in the form of mice experimental animals to teratogenicity test, which is a test to obtain information on fetal abnormalities that occur due to the provision of test preparation during the fetal organ formation (BPOM RI, 2014).

The Health Legal Aid Institute reported the manufacturer of the HIT mosquito drug related to poisoning experienced by Setiawan, a housemaid from the Sucipto and Rahayu couples where Setiawan experienced dizziness, nausea and vomiting after breathing the air that had just been sprayed with HIT insect repellent. After an inspection by the Department of Agriculture's pesticide commission, the product contained propoxur compounds and dichlorvos which had been banned for a long time because of the harmful effects on human health such as nerve, respiratory, blood, body cell, liver cancer and gastric cancer disorders (Tempo, 2006)

The teratogenic effects of some active compounds such as transflutrin and propoxur in insect repellent have been reported to reduce maternal body weight, reduce fetal weight, affect fetal body length, reduce the percentage of live fetuses, increase the percentage of intrauterine deaths, resorption, hemorrhage, morphological abnormalities, anencephaly , skeletal structural abnormalities, delayed ossification, thrombus to death when dilaparaktomi (Almahdy et al. 2014; Rahayuningsih, 2006).

However, the teratogenic effects of the active ingredients contained in insect repellents related to the way they are applied are still little studied. So this study aims to examine how much the teratogenic effect of exposure to the combination of transflutrin and d-alletrin compounds on fetus mice (Mus musculus L.) during the time of organogenesis.

Material and Methods

Material

The materials used in this study were transflutrin compounds, d-alletrin compounds and mice test animals (Mus musculus L.). The dose used in transflutrin compounds is 4 mg and d-alletrin is 45 mg. Mice used have female sex with ages 6-8 weeks and body weight 20-30 g. In addition, mice that have male sex with a age of ± 3 months and a body weight of ± 30 g are also used. Male and female mice were obtained from the Jambi University Animal Husbandry Cage. Other materials used were mineral water, mice feed, 95% alcohol, Allizarin red S solution, Bouin's solution, ether, 1% KOH, acetone, glycerin and aquadest.

Instrument

The tools used in this study include long faceted tubs as animal cages accompanied by eating and drinking places, tub cover wire, surgical instruments, mice scales, digital scales, petri dishes, measuring cups (pyrex), glass beakers (pyrex), rulers, boards surgery, a set of insect repellent devices, cameras, paper millimeter blocks and surgical needles.

Methods

Preparation of Test Animals

Test animals in the form of acclimatized mice in the laboratory for 1 week were then given food and drink in ad libitum. Forty adult female mice in the estrus phase are grouped into several groups and then mixed with male mice, the comparison between the two is 1: 4.

The next day a vaginal plug was examined, if there was a vaginal plug which indicated that the mice had experienced copulation (intercourse) then that day was determined as the beginning of pregnancy (day 0). Pregnant mice are placed in a separate cage and those who are not pregnant are mixed with male mice (Almahdy, 2004).

Treatment of Test Animals

The experimental design used was a randomized block design with four treatments and each treatment was repeated five times.

Twenty mice that have been shown to be pregnant are divided randomly into four treatment groups (0, 6, 8, 10).

0: Control group (only given food and drink).
6: Treatment group given transflutrin and d-alletrin compounds with exposure for 6 hours.
8: Treatment group given transflutrin and d-alletrin compounds with exposure for 8 hours.
10: The treatment group was given transflutrin and d-alletrin compounds with exposure for 10 hours.

Pregnant mice were placed in a maintenance cage measuring 30x30x30 cm3 and the exposure space was 3x3 m2 with a distance of exposure to ± 75 cm from the maintenance cage. Mice are given food and drink in ad libitum.

Submitted: Mei 2, 2019

Revised: June 2, 2019

Accepted: June 10, 2019

*Corresponding author: elisma@unja.ac.id

Before the exposure of the compound to mice, the mice were weighed first to determine the initial weight The exposure was carried out on the 6th day to the 15th day of the pregnancy which period was the time of organogenesis and then the mice were kept up to days 18 pregnancies, namely ± 3 days before giving birth and then undergoing surgery.

Surgery For Test Animals

On the 18th day of pregnancy, surgery is carried out using ether inhalation. Surgery is done Caesar (laparotomy) using a surgical instrument to take the fetus from the uterus of the mouse. The fetuses are cleansed from the placental membrane, blood and mucous fluid which covers it with tissue and is then observed.

Observation of Fetus

Fetuses that have been cleaned, fetal abnormalities are externally and internally observed. Observation objects of external abnormalities observed are:

a. Number of implants (number of living, dead, and absorbing fetuses).
b. Fetusmencit body weight and body length.
c. Congenital abnormalities in the fetus such as hemorrhage, dwarf body, kinkey (circular tail), hunched body (flexion).
d. Fetal morphology such as eyes, ears, fingers, tail, fingers, etc.

While the object of observation of internal abnormalities observed was the skeleton system of fetal mice (bone shape and the results of the reinforcement process). As Inouye explained in 1976 for observing the shape of the mouse skeleton, mice were needed to make Wholemount preparations using the coloring method. The way to make the preparation is as follows:

a. The fetus is preserved in 95% alcohol for 3 days.
b. Performed Visceration, namely the skin, viscera, and fat tissue is removed, this process is carried out very carefully so that the position or position of the fetal limb does not change.
c. White mouse mice are included in acetone for 1 day to dissolve the fat.
d. The fetus was stained on day 4 after fixation with the Alizarin RedS dye solution for 1-3 days at 370C.
e. The fetus is washed with running water several times until it is clean.
f. The fetus is cleared with a 1% KOH solution in water for 2 days until the tissue that wraps the body becomes transparent and the red one is hard bone tissue while the non-colored tissue is cartilage.
g. The fetus was transferred to 20% glycerin solution in 1% KOH for 1-4 days.
h. The fetus was entered in a row in 50% and 80% glycerin solution in 1% KOH for 1 hour, then stored in 100% glycerin for observation.

Data Analysis

Data in the form of morphological abnormalities, congenital abnormalities, number of implants, number of live fetuses, absorbed fetal numbers, number of dead fetuses, deformities and results of fetal mouse ossification were analyzed descriptively, while data in fetal body weight and length were analyzed using covariance analysis to compare body weight fetus (BBF) on the body weight (BBI) and Litter Size (LS) as well as the effect of fetal body length (PBF) on the parent body length (PBI) and Litter Size (LS).

Result

1. Effects of a combination of transflutrin and d-alletrin compounds on fetal development

The combination of transflutrin and d-alletrin compounds was exposed to the mother of pregnant mice during organogenesis (6th to 15th day pregnant). Then the 18th day of pregnancy (peri partus) was carried out laparotomy (Caesarean section) in the mother of mice to determine the number of implants, the number of live fetuses, the number of fetuses absorbed and the number of fetuses dead. The results of measuring these parameters are presented in Table 2.

The results of the observations show that exposure to the combination of transflutrin and d-alletrin compounds can cause death and the number of deaths increases with increasing exposure time (Table 2). The observations of these parameters are similar to the results of Rahayuningsih (2006) which stated that there was a decrease in the percentage of living fetuses and an increase in intra-uterine deaths caused by a combination of propoxur and transflutrin compounds found in mosquito drugs. Only at 8 hours of exposure to fetal resorption, that is as much as 4.5%. The absorbed fetus is a fetus that does not develop into a normal fetus at the implantation site. This is caused by morphological errors with various bodily defects that end in death (Peters and Berkvens, 1996). The absorbed fetus is shown in Figure 11C and has the characteristic of a blackish red blood clot. The absorbed fetus oc-

curs in the organogenesis phase and carries a lethal (death) effect of intra-uterus in the fetus of mice. Embryo death (resorption) is caused by the entry of high-dose teratogens at the beginning of embryo development (Goldstein et al., 1974).

Absorbed fetus or dead fetus (Figure 11b) occurs because the embryo has a low body resistance to foreign substances and can not make repairs (recovery) with new cells, causing the death of the embryo / fetus, and then the mother responds by absorbing it again. The opposite occurs in living fetuses (Figure 11a) where the embryo has a high body resistance to foreign substances and a damaged cell recovery process occurs against new cells so that it allows the fetus to survive (Rahayuningsih, 2006). If there is teratogen exposure in the embryo during pre-differentiation, both in all or most embryonic cells, it will cause damage to the embryo or can cause embryonic death (Ramelan and Shahrun, 1994). The high percentage of intra-uterine fetal deaths in this study is suspected because the combination of translutin and d-palletrin compounds in insect repellents is teratogenic.

Table 2. Average fetal life, death and absorption in each treatment group

Parameter	Lama pemaparan (Jam)			
	0	6	8	10
Jumlah implantasi (ekor)	6,8 ± 0,8	8,4 ± 0,5	8,8 ± 1,1	7,2 ± 0,8
Jumlah fetus hidup (ekor)	6,8 ± 0,8	5,6 ± 1,7	4,8 ± 1,6	4,4 ± 0,6
Jumlah fetus mati (ekor)	0,0 ± 0,0	2,8 ± 1,6	3,6 ± 2,2	2,8 ± 1,4
Jumlah fetus terresorpsi (ekor)	0,0 ± 0,0	0,0 ± 0,0	0,4 ± 0,2	0,0 ± 0,0
Jumlah fetus mati & teresorpsi (ekor)	0,0 ± 0,0	2,8 ± 1,6	4,0 ± 2,4	2,8 ± 1,4
Persentase fetus hidup (%)	100	66.7	54.5	77.8
Persentase kematian intrauterus:				
a. Fetus mati (%)	0,0	33,3	40,9	38,9
b. Fetus terresorpsi (%)	0,0	0,0	4,5	0,0
c. Fetus mati & teresorpsi	0,0	33.3	45,4	38,9

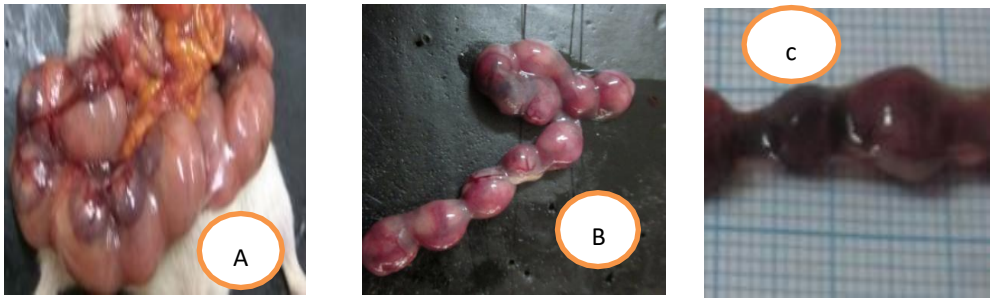

Figure 1. Fetal morphology exposed to transflutrin and d-allethrin compounds and parent control. (a) a uterus containing a normal fetus (b) a uterus containing an absorbed fetus and a dead fetus (c) fetus absorption.

2. Effects of the combination of transflutrin and d-alletrin compounds on fetal mice body weight and length

Fetal mice body weight and body length is one of the most important parameters in knowing the effect of a substance on embryo development. According to Ryan et al. (1991), a decrease in fetal body weight and length is one of the mildest forms of the effect of compounds that are teratogens and include parameters that are sensitive to malformation and fetal death. The average body weight and length of the fetus in each treatment is presented in Table 3

Table 3. Mean fetal body weight and length of mice exposed to a combination of transflutrin dand-alletrin compounds.

Parameters	exposure (hours)			
	0	**6**	**8**	**10**
Average BB	1,1 ± 0,4	1,2 ± 0,4	1,2 ± 1,2	1,6 ± 0,24
Average PB	2,0 ± 0.6	1,9 ± 0,3	1,7 ± 0,7	2,1 ± 0,2

Description: BB = Weight, PB = Body Length

3. The effect of the combination of transflutrin and d-alletrin compounds on fetal morphology of mice

Fetal morphology is the most important part in observing teratogenic effects in a compound. Macroscopic observations of fetal morphology are presented in Table 4. Among the fetal morphologies observed were the eyes, legs, fingers, skeleton, tail, palate and ears. The results showed that the transflutrin and d-alletrin compounds did not cause abnormalities in fetal morphology of mice.

Table 4. Observations of fetal morphology of mice macroscopically

Organ yang diamati	Exposure Length (Hours)																			
	0					**6**					**8**					**10**				
	Deuteron-omy					**Ulangan**					**Ulangan**					**Ulangan**				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Kaki	n	n	n	n	n	n	N	n	n	n	n	n	n	n	n	n	n	n	n	n
Jari –jari	n	n	n	n	n	n	N	n	n	n	n	n	n	n	n	n	n	n	n	n
Rangka	n	n	n	n	n	n	N	n	n	n	n	n	n	n	n	n	n	n	n	n
Ekor	n	n	n	n	n	n	N	n	n	n	n	n	n	n	n	n	n	n	n	n
	n	n	n	n	n	n	N	n	n	n	n	n	n	n	n	n	n	n	n	n
Langit-langit																				
Telinga	n	n	n	n	n	n	N	n	n	n	n	n	n	n	n	n	n	n	n	n
Mata	n	n	n	n	n	n	N	n	n	n	n	n	n	n	n	n	n	n	n	n

Description: n = normal

4. The effects of the transflutrin and d-alletrin compounds on fetal external abnormalities of mice

External abnormalities are abnormalities that occur because the entry of teratogens into the pregnant mother's body during the organogenesis period is observed macroscopically. Among the abnormalities observed were hemorrhage, dwarf body, hunchback body (flexion) and circular tail (kinkey). The results of observations of fetal external abnormalities are presented in Table 5.

Table 5. Observation of external abnormalities in the fetus

Jenis abnormalitas eksterna	**Lama pemaparan (Jam)**			
	0	**6**	**8**	**10**
Jumlah total fetus	34	42	38	36
Hemoragi (ekor)	0	8 (19,05 %)	14 (36,84 %)	18 (50 %)
Tubuh kerdil (ekor)	0	1 (2,38 %)	1 (2,63 %)	2 (5,56 %)
Fleksi/bongkok (ekor)	0	0	2 (5,26%)	4 (11,11 %)
Kinkey	0	0	0	0

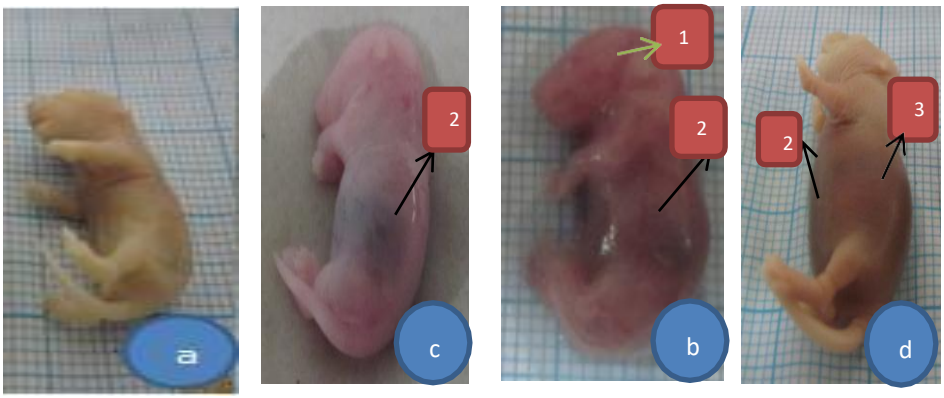

Figure 2. Comparison of normal fetal morphology with fetuses experiencing hemorrhage. (a) exposure to 0 hours (b) exposure to 6 hours (c) exposure to 8 hours (d) exposure to 10 hours (1) hemorrhage in the head (2) hemorrhage in the abdomen (3) hemorrhage on the back.

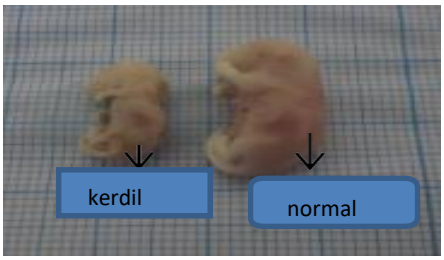

Figure 3. Comparison of normal fetal morphology (right) with a fetus that has a dwarf body (left)

Figure 4. Comparison of normal (left) fetal morphology and fetus that has a hump body (right)

5. Efek senyawa transflutrin dan d-alletrin terhadap abnormalitas interna fetus mencit

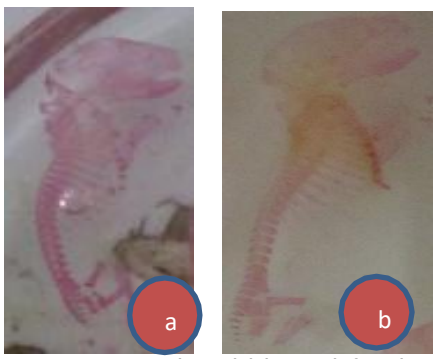

Figure 5. Comparison of normal fetuses as a comparison (a) bone deformity and (b) ossification abnormalities

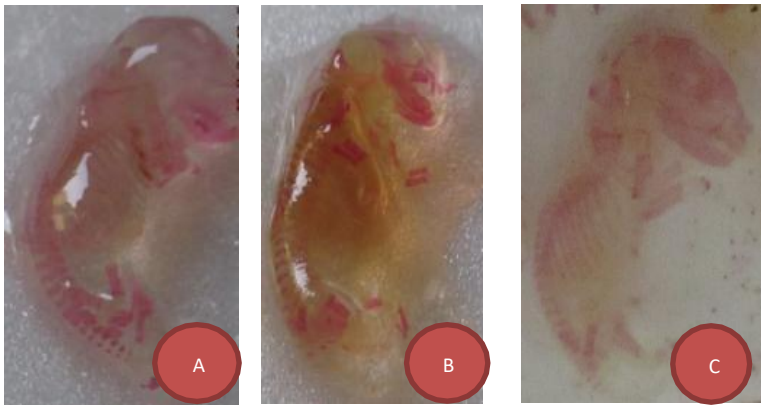

Figure 6. Comparison of bone deformities in each treatment. (a) 6 Hours (b) 8 Hours (c) 10 Hours

The results of observations of internal abnormalities are presented in Table 6.

Table 6. Results of observations of internal abnormalities in fetus fever

Parameter	Lama pemaparan(Jam)			
	0	6	8	10
Jumlah total fetus (ekor)	34	42	38	36
Kelainan bentuk tulang	0	2 (4,76 %)	4 (10,52 %)	6 (16,67 %)
Kelainan hasil osifikasi	0	3 (7,14 %)	5 (13,16 %)	7 (19,44 %)

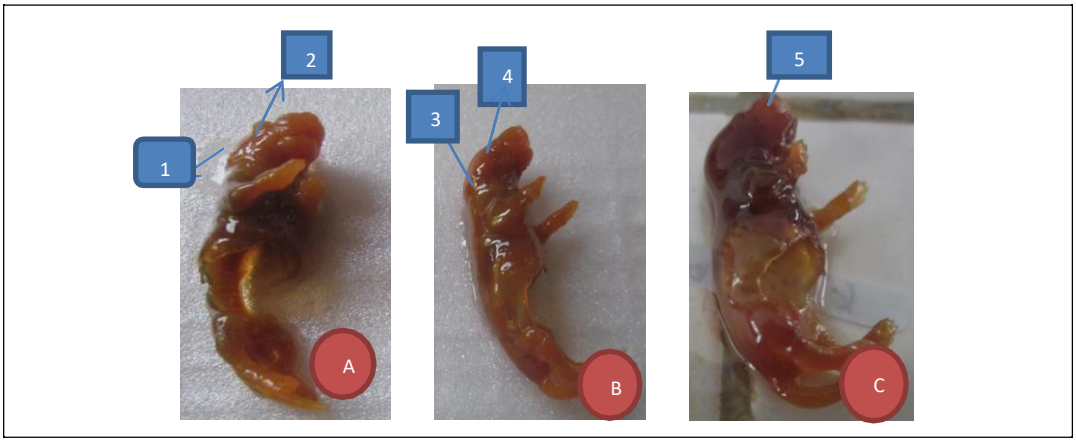

Figure 7. Comparison of results of ossification in each treatment. (A) 6 Hours (B) 8 Hours (C) 10 Hours (1) os-frontal (2) os-parietal (3) os-occipital (4) os-temporal (5) os-nasal

Discussion

The results of the observations show that exposure to the combination of transflutrin and d-alletrin compounds can cause death and the number of deaths increases with increasing exposure time (Table 2). The observations of these parameters are similar to the results of Rahayuningsih (2006) who stated that there was a decrease in the percentage of living fetuses and an increase in intra-uterine deaths caused by a combination of propoxur and transflutrin compounds contained in insect re

pellent. The results of the statistical analysis showed that the treatment had no significant effect (p> 0.05) on the body weight and length of the fetus.

Only at 8 hours of exposure did fetal resorption occur, which was as much as 4.5%. The absorbed fetus is a fetus that does not develop into a normal fetus at the implantation site. This is caused by morphological errors with various bodily defects that end in death (Peters and Berkvens, 1996). The absorbed fetus is shown in Figure 11C and has the characteristic of a blackish red blood clot. The absorbed fetus occurs in the organogenesis phase and carries a lethal (death) effect of intra-uterus in the fetus of mice. Embryo death (resorption) is caused by the entry of high-dose teratogens at the beginning of embryo development (Goldstein et al., 1974).

Absorbed fetus or dead fetus (Figure 11b) occurs because the embryo has a low body resistance to foreign substances and can not make repairs (recovery) with new cells, causing the death of the embryo / fetus, and then the mother responds by absorbing it again. The opposite occurs in living fetuses (Figure 11a) where the embryo has a high body resistance to foreign substances and the recovery process of damaged cells to new cells occurs so as to enable the fetus to survive (Rahayuningsih, 2006). If there is teratogen exposure in the embryo during pre-differentiation, both in all or most embryonic cells, it will cause damage to the embryo or can cause embryonic death (Ramelan and Shahrun, 1994). The high percentage of intra-uterine fetal deaths in this study is suspected because the combination of transflutrin and d-alletrin compounds in insect repellents is teratogenic.

External abnormalities are abnormalities that occur because the entry of teratogens into the pregnant mother's body during the organogenesis period is observed macroscopically. Among the abnormalities observed were hemorrhage, dwarf body, hunchback body (flexion) and circular tail (kinkey). The results of observations of fetal external abnormalities are presented in Table 5. The results of the observation showed that exposure to the combination of transflutrin and d-alletrin compounds during the organogenesis period caused disabilities such as flexed bodies, hemorrhages and dwarf bodies. Special circular tail is not found in the case of exposure using a combination of these compounds.

Hemorrhage occurs in all treatments and increases in percentage as time increases exposure to a combination of transflutrin and d-alletrin compounds. Hemorrhage is caused due to the discharge of blood from the system cardiovascular which is accompanied by accumulation in body tissues (Widiyani and Sagi, 2001). In this observation hemorrhage (bleeding) occurs in the back of the head and abdomen as presented in Figure 10. Hemorrhage in the 6 and 8 hour exposure group generally occurs in the abdomen, and a little occurs in the head. Meanwhile, hemorrhage in the 10-hour exposure group generally occurs in the back and abdomen.

The dwarf body was found in all treatment groups except in the control

group. This disorder is most commonly found in the 10-hour exposure group. Wilson (1973) states that an agent can influence cell proliferation, cell interaction or biosynthesis that is associated with inhibition of nucleic acid, protein and mucopolysaccharide synthesis so that growth delays occur. In addition, this dwarf body may also be caused by the inhibition of growth hormone secretion, causing bone growth to become obstructed and the body to become stunted (midget). Comparison of normal fetal morphology with fetus with dwarf body is presented in Figure 13.

Humpback body (flexion) is one of the external abnormalities which is observed macroscopically. The humpback body was only found in the 10-hour exposure group (Table 4). The highest percentage was in the 10-hour exposure group which was then followed by the 8-hour exposure group. The form of this abnormality is probably caused by deformity of the spine (vertebrae) and the delay of ossification in the fetus of mice. Comparison of normal fetal morphology and fetuses experiencing collusion is presented in Figure 14. Internal abnormalities can be observed by looking at bone deformities and the results of ossification of the fetus. This abnormality can be observed after making Whole-mount preparations by immersing the fetus into a red alizarin dye solution which causes the fetus to become transparent and reddish purple bone (Inouye, 1976). Observations of bone deformities and abnormalities of fetal bone ossification in this study were based on the results of Allizarin staining on normal fetal skeletons (0 hours of exposure) as a comparison (Figure 15). In the normal fetus, the vertebral column shows the results of ossification of the perfect lumbar cranium and vertebrae. This is indicated by red as true bone and which is not stained by Allizarin as cartilage. Bone deformities are indicated by imperfect bone formation characterized by a bent vertebra (kyphosis flexion), as presented in Figure 16.

In groups of 6, 8 and 10 hours exposure, fetuses experience abnormalities in the vertebrae in the form of kebengkokan (kyphosis flexion). This is thought to be related to abnormalities in the form of a hunched body (flexion) which may be caused by the occurrence of cell death in certain areas so that there is a difference in the speed of growth between vertebrae segments. Guyton (2007) states that the presence of pressure on bones in certain circumstances will determine the shape of the bone. Mechanical pressure such as contraction of the uterine muscles due to exposure to chemical compounds can change the direction of bone growth. Abnormalities of bone ossification results are caused by the delay in ossification that is characterized by the colorlessness of the fetal skeleton on the frontal, parietal, temporal, occipital and nasal templates by Allizarin, as shown in Figure 15. In the 6, 8 and 10 Hour exposure groups, the fetus experiences abnormal ossification in several parts of the bone (Figure 15). It is suspected that the combination of transflutrin and d-alletrin compounds is able to enter bone cells and affect osteogenic (bone bone) causing the process of bone formation to be disrupted.

In observing internal abnormalities, the results of the observation showed that the treatment had an effect on bone deformities and the results of ossification of fetal mice. Bone deformities and ossification results in a percentage increase along with the increased exposure time of the transflutrin and d-alletrin compounds. The occurrence of bone deformities and results of ossification in fetal mice given a combination of transflutrin and d-alletrin compounds indicate that the combination of these two compounds has anatogenic effect.

Conclusion

The combination of transflutrin and d-alletrin compounds described during organogenesis can cause death in the fetus, hemorrhage, dwarf body, hunchback (flexion), deformity and results of ossification of fetal mice, but does not affect body weight, body length and fetal morphology of mice , so it can be concluded that the combination of transflutrin and d-alletrin has mild teratogenic effects on fetal mice. The teratogenic effect increases with increasing exposure time.

Reference

Almahdy. 2004. Uji Aktivitas Teratogenitas Ekstrak Etanol Daun Inggu (*Rutagraveolens* Linn.) pada Mencit Putih. *Jurnal Sains dan Teknologi Farmasi*. 82-87.

Almahdy, A., Dachriyanus dan M. Rosa. 2014. Uji Efek Teratogenik Antinyamuk Bakar Yang Mengandung Transfluthrin Terhadap Fetus Mencit Putih. *Scientia.* 4(2):46-50.

BPOM RI. 2014. *Pedoman Uji Toksisitas Nonklinik Secara In Vivo,* BPOM RI, Jakarta. Goldstein., A.L. Aronow *and* S.M. Kalman. 1974. *Principle of Drug Action : The Basic of Pharmacology*, John Wiley & Sons, New York.

Guyton, A.C. 2007. *Buku Ajar Fisiologi Kedokteran*, Edisi 11, EGC, Jakarta.

Inouye, M. 1976. Differential Staining of Cartilage and Bone in Fetal Mouse Skeleton by Alcian Blue and Allizarin Red S. *Congenital Anomalies.* 16(3) :171-173.

Manson, J.M. 1986. *The Basic Science of Poisons in Casarett and Doull'sToxicology*, MC Millan Publishing Co, New York.

Peters, P.W.J. and J.M. Berkvens. 1996. *General Reproduction Toxicology* in Niensink, R.J.M., J.D Vries and M.A Hollinger. Toxicology : Principle and Application, CRC- Press, NewYork.

Rahayuningsih, T. 2006. Efek Teratogenik Asap Obat Nyamuk Bakar terhadap Fetus Mencit (*Mus musculus* L.) galur Balb-c pada Masa Organogenesis. *Skripsi*. UNS. Surakarta.

Ramelan, W., dan H.M. Syahrun. 1994. *Kelainan pada Proses Perkembangan Embrio (Teratologi), Reproduksi dan Embriologi dari Satu Sel Menjadi Organisme*, Balai Penerbit FKUI, Jakarta.

Raini, M. 2009. Artikel Toksikologi Insektisida Rumah Tangga dan Pencegahan Keracunan. *Media penelitian dan pengembangan kesehatan*. 21:10-17.

Roberts. 1971. *Veterinary Obstetricts and Genital Diseases (Theriogenology)*, Ithaca, New York.

Ryan, L.M., P.J. Catalano., C.A. Kimmel *and* G.L. Kimmel. 1991. Relationship between Fetal Weight and Malformation in Developmental Toxicity Studies. *Teratology.* 44: 215-223.

Tempo, I. 2006. Produsen HIT Dilaporkan ke

Polisi.(https://m.tempo.co/read/news/2006/06/17/05779031/produsen-hit-dilaporkan-ke-polisidiakses pada tgl 16 januari 2017 pukul 06.59WIB)

Wahyuni, T. 2005. Waspadai Efek Negatif Anti-Nyamuk. (http://www.konline.com/intisari/baygon.htm. diakses pada tgl 9 September 2016).

Widiyani, T. dan M. Sagi. 2001.

Pengaruh Aflatoksin B1 terhadap Pertumbuhan dan Perkembangan Embrio dan Skeleton Fetus Mencit (*Mus musculus* L.). *Teknosains.* 14 (3): 409-427.

Wilson, J.G. 1973. *Environment and Birth Defect*, Academy Press Inc, London