Cytogenetic and histopathological effects of two types of fipronil formulations via inhalation exposure in albino rat

Noaishi, Mohamed A.*, and H. H. Abd alhafez
Mammalian Toxicology Department, Central Agricultural Pesticides Lab. (CAPL), Agricultural Research Center, Ministry of Agriculture, Egypt.
*Email: noaishi@gmail.com

Abstract: Fipronil (FPN) is a phenylpyrazole class of insecticides, which is used for control a wide range of agricultural, veterinary and household pests. The present work was aimed to evaluate the risk of inhalation exposure of two types of fipronil formulations which recently registered and used in Egypt. Also to evaluate which type of formulations is more hazardous than the other. The study included two toxicity criteria. The first is chromosomal aberrations assay in bone marrow and the second is the histopathological examination in lung tissues. In addition, the change in the body weights during the treatments was recorded. Thirty healthy male white rats weighing 170g±10% were used. The first group was used as a sighting study to determine the range of dose levels of the main study. The other animals were divided into five groups (a, b, c, d and e) each of has five rats. The first group (a) was retained as a negative control without exposure just ambient air. The rats were exposed to the tested formulations for 6 hours per day for 28 days continually. The high dose was the double of the low dose by putting two vials of tested formulation. Groups (b and c) were exposed to low dose (25mg/L) and high dose (50mg/L) of EC formulation respectively. Groups (d and e) were exposed to low dose (30mg/L) and high dose (60mg/L) of SC formulation respectively. The results revealed that there are no signs of toxicity like convulsions or eye bleeding observed on rats after repeated inhalation exposures of two FPN formulations. Also no decreased in body weights in animals were observed. The genotoxic effect was significant at both high doses of two formulations but low doses not induced significant changes. However the EC formulation is more extent in genotoxicity than SC formulation. The histopathological findings in this study revealed that the different types of FPN formulations induced fluctuated harmful effects on lung tissues. The EC-formulation induced hyperplasia in lung tissues while SC-formulation induced focal abscess formation with pus cells. Also the inhalation exposure at high doses led to destructive effects on the lung tissues than the low doses which led to slight effects. Although the results are based on study on experimental animals, it is not possible to predict what will happen to humans in the case of inhalation of these toxicants, because the rats are more tolerant than humans. This result is a warning or an alarm for this type of exposure and strongly imposes the need for more detailed about the toxicity resulting from occupational exposure to pesticides, especially by inhalation. Care should be taken at the time of prolonged exposure which may lead to adverse health effects.

Keywords: Fipronil, Inhalation, histopathology, cytogenetic, rats

1. Introduction

Fipronil is a pesticide that belongs to the phenylpyrazole chemical group. It is an insecticide with widespread use in the control of many agricultural and domestic pests. Over the last decade, the usage of Fipronil has increased considerably because it was developed to replace conventional pesticides, such as organophosphates, carbamates and pyrethroids insecticides, which becoming have no action against resistant pest strains **(Narahashi *et al*., 2010)**. More addition, the fipronil has a broad spectrum of action against insects, being used to control fleas, ticks, termites, mole crickets, ants, root-worms, beetles, cockroaches and other insects **(Tingle *et al*., 2003)**. Also, fipronil now is used in home applications as a public health pesticide. Fipronil toxicity is attributed to its ability to act at GABA-gated chloride channel receptor as a noncompetitive inhibitor of the GABA-receptor of neurons in the central nervous system. Fipronil act as a blocker of this receptor witch is the chief inhibitory neurotransmitter in the mammalian central nervous system. Its principal role is reducing neuronal excitability throughout the nervous system. So, any blocker of these receptors like fipronil leads to reduce neuronal inhibition, which leads to hyperexcitation of the central nervous system, convulsions, and death **(Zhao *et al*., 2004)**. As well known, the occupational exposure to pesticides has been linked to the human body by three ways: oral through the mouth, dermal through the skin or eyes, and inhalation through the lungs **(British Colombia, 2013).** Most of the pesticide workers in developing countries and Egypt may not be full protected from the pesticides when run the application because they often do not care to wear the personal protection equipment when applying the pesticides; therefore they are directly exposed to these compounds, whose toxicity is ranged from moderate to hazardous **(Mansour, 2004).** The last route of the exposure by inhalation may be the most accidental route of entry in Egypt because the Egyptian spray workers often do not care to use the safety mask of pesticides when spray the pesticides. So, the information on the inhalation toxicity and the adverse health effects of this type of exposure is very important. Furthermore, a limited number of these studies have been done to evaluate the risk of inhalation exposure **(Noaishi, *et al*., 2013).** More addition the type of formulation that composing the commercial form of the pesticide is very important, because the additives to the pesticide formulation may be increase or decrease the toxicity of the pesticide formulation **(FAO, 1995)** or solvents using in preparation of the formulations. So, the inhalation exposure effect to the pesticides may differ according to different additives of the formulation. On the hand, the toxicological parameters are many but some of its may be suitable than other. Because of the histopathology is a critical part of the toxicologic and risk assessment of drugs or chemicals **(Crissman et al., 2004).** The investigation of histopathological changes in lung tissues is a sensitive and rapid method, commonly be used in this type of the assessment **(Al-Sharqi *et al*., 2012; Al-Qudsi, and Linjawi, 2012).** Also, cytogenetic

analysis gives valuable information about mutagenic potentiality of the tested material. The inhalation exposure to mutagens may induce its effect in the blood or bone marrow in treated animals **(Kehdy *et al*., 2007)**. So, the present study was aimed to evaluate the side effect of two types of fipronil formulations which are free sale and registered in Egypt on the lung tissues as main target organ and as well as cytogenetic analysis in the bone marrow of treated rats. Also, to determine which type of them is more hazard than the other when exposed by inhalation for 28 days continually.

2. MATERIALS AND METHODS

2.1 The tested insecticides:

The tested formulations of the fipronil were commercial formulations which are free sale and registered in Egypt. The first formulation was emulsifiable concentrate (EC) with active ingredient 5% and the second formulation was suspension concentrate (SC) with active ingredient 20%. The formulations were purchased from local distributors.

2.2 Animals:

A total of 30 apparently healthy male albino rats (Wistar strain) weighing 160–180g were used throughout the whole study. The animals were obtained from the laboratory animal house of the Modern Veterinary Office, Giza, Egypt. The animals were housed of five rats per cage, had free access to fresh water and fresh well-balanced diet, and then kept under supervision for two weeks before commencing the experimental work.

2.3 Experimental design:

The rats were randomly divided into six groups each of it has a five rats per cage. The first group was used as a sighting study to determine the range of dose levels of the main study. The other animals were divided into five groups (a, b, c, d and e). The first group (a) was kept as control without exposure just an ambient air. The doses were calculated according to the volume of pesticide formulation which is evaporated in 6 hrs per day and the chamber size which is approximately 15 Liters. The high dose was the double of the low dose by putting two bottles of tested formulation. Group (b) was exposed to low dose (25mg/L) and group (c) to high dose (50mg/L) of **EC** formulation. Group (d) was exposed to low dose (30mg/L) and group (e) to high dose (60mg/L) of **SC** formulation. The rats were exposed to a whole body by using inhalation plastic room which is local made. The room is consists of an enclosed plastic cage with two fans for inlet and outlet airflow and supplied in one side with bottle contained the tested pesticide and tissue paper that help to spread the vapor of the pesticide. This enclosed cadge or room is suitable for liquid formulation only. The inhalation room is illustrated in figure (1) which clears the design and working process of it. This chamber was used in previous work **(Noaishi, *et al*., 2013).** The rats were exposed to the tested formulations for 6 hours per day for 28 days continually. The signs of toxicity and the body weights of rats were recorded weekly. The treatments were according to guidelines of **(OECD 412, 2009).**

2.4 Cytogenetic assay

Cytogenetic analysis in the present study was a chromosome aberrations assay in bone-marrow of treated rats. The metaphase of cells was performed according to the technique described earlier by **(Adler, 1984)** with some modifications was recommended by authors. In brief the animals were injected intraperitoneally with colchicine (6mg/kg b.wt.) 2hr before the harvest of the cells, in order to accumulate metaphase cells and provide more readily analyzable chromosomes. The rats were sacrificed after 24hrs of the last dose exposure. Both femurs were excised out and cleaned of any adhering muscle, the bone-marrow cells were collected from both femurs by flushing with Hank's balanced salt solution (HBSS 1X) the tubes were kept in the refrigerator at 4°C for 45-min. then centrifuged at 1100×g for 10min and discard the supernatant and the pellets were resuspended very well with a potassium chloride hypotonic solution (0.075M KCl). After that the tubes were kept in the refrigerator at 4°C for 45-min. This step is very important and recommended by the author. The cell suspension was centrifuged at 1100×g for 10min, and then fixed with cold fixative freshly prepared (methanol: glacial acetic acid, 3:1 v/v). Centrifugation and fixation were repeated three times and the tubes were kept in the freezer at overnight. In next day the tubes centrifuged again, discard the supernatant and the pellets resuspended in a small volume of fixative, dropped on cleaned slides, dried, and stained with 20% Giemsa for 45-min. One hundred good-quality metaphases containing 42 chromosomes were examined per animal to score different aberrations.

2.5. Histopathological examination:

Histopathological alterations in tissue may be used as a rapid method to evaluate the toxic effects of chemicals in different tissues and organs **(Bernet *et al*., 1999).** In our study the lung specimens were obtained from sacrificed rats and fixed in 10% formalin for 24 hrs and decalcification was performed on formic acid then washed in tap water. Serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain, and examination was done through the light electric microscope **(Banchroft *et al*., 1996).**

2.6 Statistical analysis

Statistical analysis was performed using SigmaPlot statistics software, Ver.11. All data were represented as mean ± standard error (SE). Statistical Significance between control and treated values were compared using Studen's t-test and P values less than 0.05 were considered significance.

3. Results and Discussion

3.1. Effect on the body weights

The present study revealed that there are no signs of toxicity like convulsions or eye bleeding observed on rats during inhalation exposure period (28 days) of two FPN formulations. These observations are in agreement with **Chodorowski and Anand, (2004)** who reported that a 50-year-old man was exposed by inhalation to the fipronil solution when he was spraying his field, there were no seizures, other neurological deficits, signs of conjunctivitis or skin irritation were observed. Physical

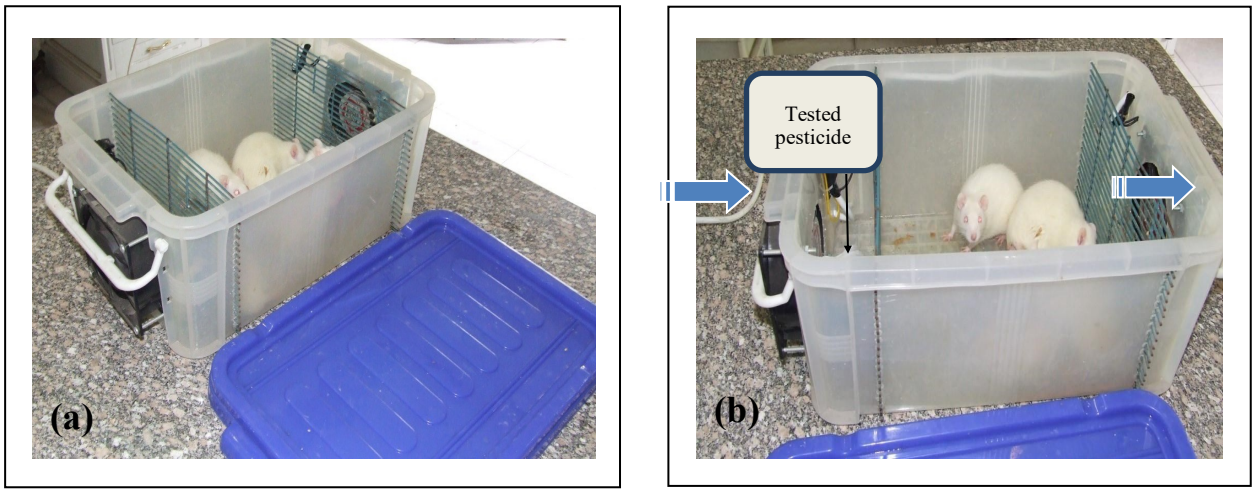


Fig (1) The view of the inhalation room (a), the inlet and outlet air flow and location of tested pesticide (b).

examinations and biochemical results were normal. The patient just complained of a headache, nausea, vertigo and weakness. All symptoms resolved spontaneously after about 5h. Also our results revealed that there was no decrease in body weights after 28 days from repeated inhalation exposure in the both treatments of two formulations see (Fig 2). The results is apparently in agreement with previous findings but by another route entry **Hughes *et al*., (1997)** found that slight loss of weight was seen after treatment with an oral single dose 25 mg/kg bwt of FPN (purity, 97.9%). Also our results are in agreement with **Badgujar *et al*., (2015)** who reported that there was no significant change in the body weight of mice treated orally with fipronil 2.5, 5 and 10 mg/kg b.wt. respectively for 28 days as compared to control. So, according to our result it can be propose that the inhalation exposure of both treatments did not induce significant effect in the body weights of the treated rats.

3.2. Effects on the cytogenetic:

The detection of chromosomal damage serves as a tool for the verification of the genotoxic effects of chemical substances in vitro or in vivo **(Ficová and Galdíková, 2017)**. The present results of genotoxicity presented in table (1) and Fig (3) revealed that, only the high doses of two formulations induced significant effect in chromosomal aberrations CAs. EC-formulation induced high significant effect ($p < 0.001$) but SC-formulation induced moderate significant effect ($p < 0.01$). Low doses for both formulations did not induce any significant effect. These findings are agreements with **Badgujar *et al*., (2017)** who found significant increase in CAs in bone marrow cells and DNA damage in the lymphocytes in the male and female rats were gavaged with various doses of fipronil (2.5, 12.5, and 25 mg/kg b.wt. as compared to their respective controls. Also **Çelik et al., (2014)** reported that FPN induced a statistically significant increase in the micronuclei (MN) and sister chromatid exchanges (SCEs) frequency and DNA damage in a dose-dependent manner in human peripheral blood lymphocytes cultures in high and medium doses (0.7 and 0.3 µg/mL) but there is no significant difference in low dose (0.1 µg/mL) when compared

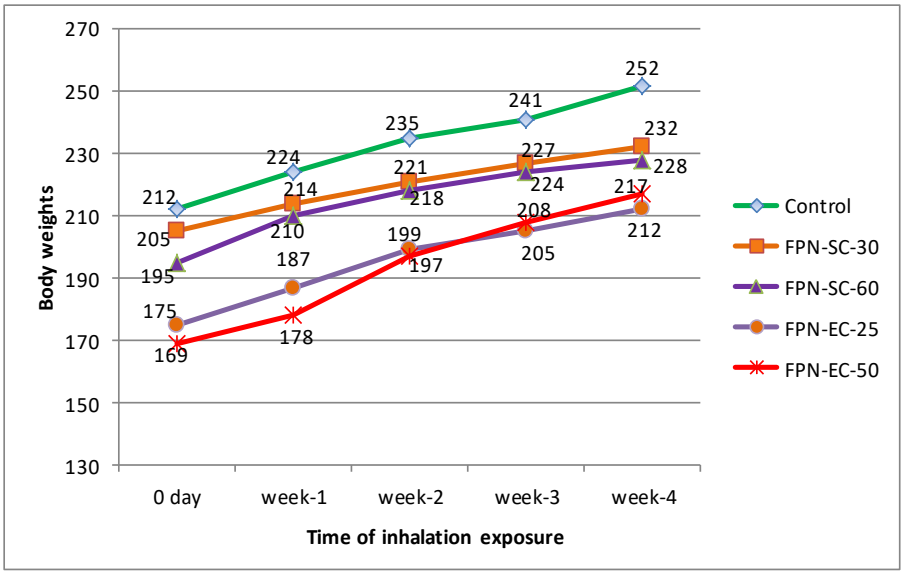


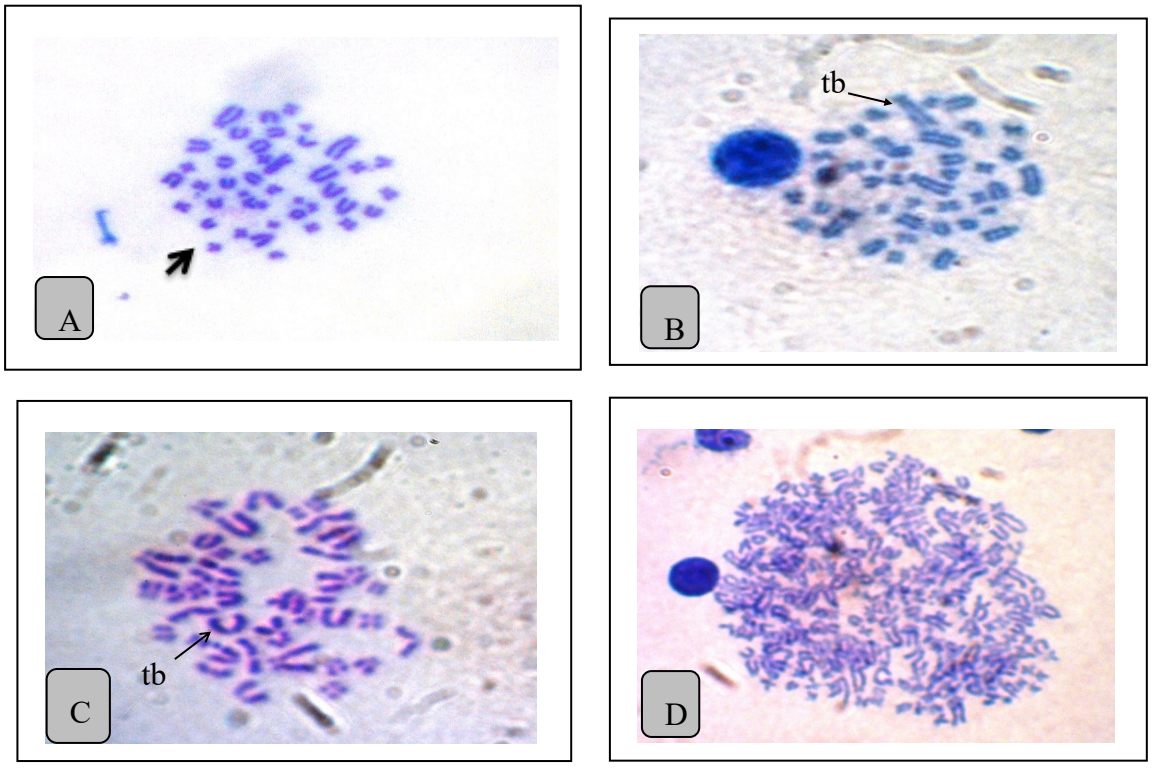
Fig.(2): The correlation between the time of exposure of FPN and the mean of body weights of control and treatment groups.

Table (1) : Chromosomal aberrations induced in rat bone marrow after inhalation exposure period of two types of fipronil formulations

Treatments		Types of structural aberrations				Numerical aberration Polyploidy >(2n)	Total aberrant cells/ 500 scored metaphases	Mean ± S.E.
		tg	tb	e-e	c-a			
Control group		2	0	0	2	2	6	1.2 ± 0.2
EC formulation	Low dose 25mg/L	2	1	1	1	5	10	2.0 ± 0.316
	High dose 50mg/L	2	5	3	4	7	21	4.2 ± 0.583 ***
SC formulation	Low dose 30mg/L	1	1	2	2	6	12	2.4 ± 0.51
	High dose 60mg/L	1	2	4	3	5	15	3.0 ± 0.316 **

Values are from five replicates in each treatment and the last column represent mean ± S.E. of aberrant cells per 500 spread metaphases/treatment; * Significant at $p < 0.001$; ** Significant at $p < 0.01$**

Abbreviations: tg, chromatid gap; tb, chromatid break; e-e, end to end chromatid association; c-a, centromere attenuation



Fig(3). Rats bone marrow metaphases, showing (a) normal metaphase; (b) chromatid break(tb); (c) centromeric attenuation & (tb); and (d) polyploidy(>2n).

with a negative control. On the other hand, if we take in consideration the different between species **Ziliotto et al., (2017)** reported that a single dose (6.7mg/kg) of FPN on the dorsal neck region of dogs after 3, 8 and 24 hours does not induce systemic genotoxic effect. So the last report was in parallel with our results which indicated only high doses of two fipronil formulations induced genotoxic effect. Furthermore our data revealed that genotoxic effect of EC formulation is more extent than SC formulation.

3.3. Effects on the lung tissues

Lung is a target organ for detoxification and is prone to various disorders as a consequence of exposure to environmental pollutants **(Castell al., 2005**). Unfortunately, there are little data about the side effect of FPN exposure on the lungs. The present study may add some information about the FPN inhalation exposure effect in mammals. The histopathological examination of specimens taken from control group showed normal histological structure of the bronchioles and surrounding air alveoli as well as the blood vessels (Fig.4, 1-A) and (Fig.5, 2-A). The specimens taken from low dose group of EC-formulation showed just infiltration in air alveoli with focal lymphoid inflammatory cells (1-C). But the high dose group showed hyperplasia in the bronchiolar lining epithelium cells as well as collapse in the air alveoli. But still the main effect in bronchiolar tissues is associated with hyperplasia which is the enlargement of tissues caused by an increase in the reproduction rate of its cells; often happen as an initial stage in the development of cancer. On the other hand, SC-formulation groups showed other type of lung tissues damage. The low group showed massive number of lymphoid cells proliferation with infiltration in the air alveoli. But the high dose group showed focal abscess formation with pus cells; sever congested blood vessels and collapse of the air alveoli in the peribronchiolar tissues. These results are agreements with **Merkowsky *et al*., (2016)** who found that the airway epithelial cells were enlarged and domed in mice treated intranasally with fipronil (8 mg/kg/day) for 7 days. There was an increase and accumulation of inflammatory cells in the alveoli and increase the expression of Toll-like receptor 4 (TLR4 receptor) which is responsible for activating the innate immune system. Also in review article by **Lee *et al*., (2010**) reported that the most Illness cases(89%) of spray workers in the USA associated with occupational exposure to fipronil products from 2001 to 2007 had mild and temporary health effects. Neurological symptoms such as headache, dizziness, and paresthesia were the most common symptoms (50%). So, previous findings indicate that exposure to fipronil can pose a risk in health effects in various body systems. But our findings indicated that the different types of FPN formulations induced different harmful effects on lung tissues. The EC-formulation induced hyperplasia in the bronchiolar lining epithelium cells because this type of formulation usually contains a liquid active ingredient and one or more petroleum-based solvents which give EC formulations their strong odor. So, may these solvent induced these types of damage. While SC-formulation induced focal abscess with pus cells. This may be due to the active ingredient content in the SC formulation three times of the EC formulation. So, the content of active ingredient evaporate in SC-formulation is more than EC-formulation. This increase of active ingredient content may leads to impair in immunity system and formation of focal abscess and pus cells as a consequence of malfunction of the defense system.

Conclusion

There are no signs of toxicity observed on rats after repeated inhalation exposures for 28 days of two tested fipronil formulations. Also no decreased in body weights in animals were observed. Furthermore the genotoxic effect was observed significantly only at high doses of two formulations but low doses not induced significant changes. However the EC formulation is more extent as genotoxic agent than SC formulation. The histopathological findings in this study revealed

References

Adler I.D. (1984), Cytogenetic tests in mammals, in Mutagenicity Testing, A Practical Approach, Venitt, S. and Parry,J. M, Eds, IRL Press, Oxford, PP. 275-306.

Al-Qudsi F., and Linjawi S., 2012. Histological and hormonal changes in rat endometrium under the effect of camphor. Life Science Journal, 9: 348–355.

Al-Sharqi S.A.H., Alwan M.J. and Al-Bideri A.W., 2012. Histological changes induced by the action of Actara 25 wg insecticides in mice. International Journal of Advanced Biological Research, 2: 760–765.

Badgujar P.C., Pawar N.N., Chandratre G.A., Telang A.G., and Sharma A.K., 2015 Fipronil induced oxidative stress in kidney and brain of mice: Protective effect of vitamin E and vitamin C., Pesticide Biochemistry and Physiology 118, 10–18.

Badgujar P.C., Selkar N.A., Chandratre G.A., Pawar N.N., Dighe V.D., Bhagat S.T., Telang A.G., Vanage G.R., 2017 Fipronil-induced genotoxicity and DNA damage in vivo: Protective effect of vitamin E., Volume: 36 issue: 5, pp: 508-519.

Banchroft J.D., Stevens A., and Turner D.R., 1996., Theory and practice of histological techniques. Fourth ed. Churchil Livingstone, New York.

Bernet D., Schmidt H., Meier W., Burkhardt-Holm P., and Wahli T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. Journal of Fish Diseases, 22: 25–34.

British Colombia (2013): Human Pesticide Exposure. Available at: http://www.agf.gov.bc.ca/pesticides/k_2.ht

Castell J.V., Donato M.T., Gomez-Lechon M.J., 2005. Metabolism and bioactivation of toxicants in the lung. The in vitro cellular approach., *Exp Toxicol Pathol.* 57 (1):189-204.

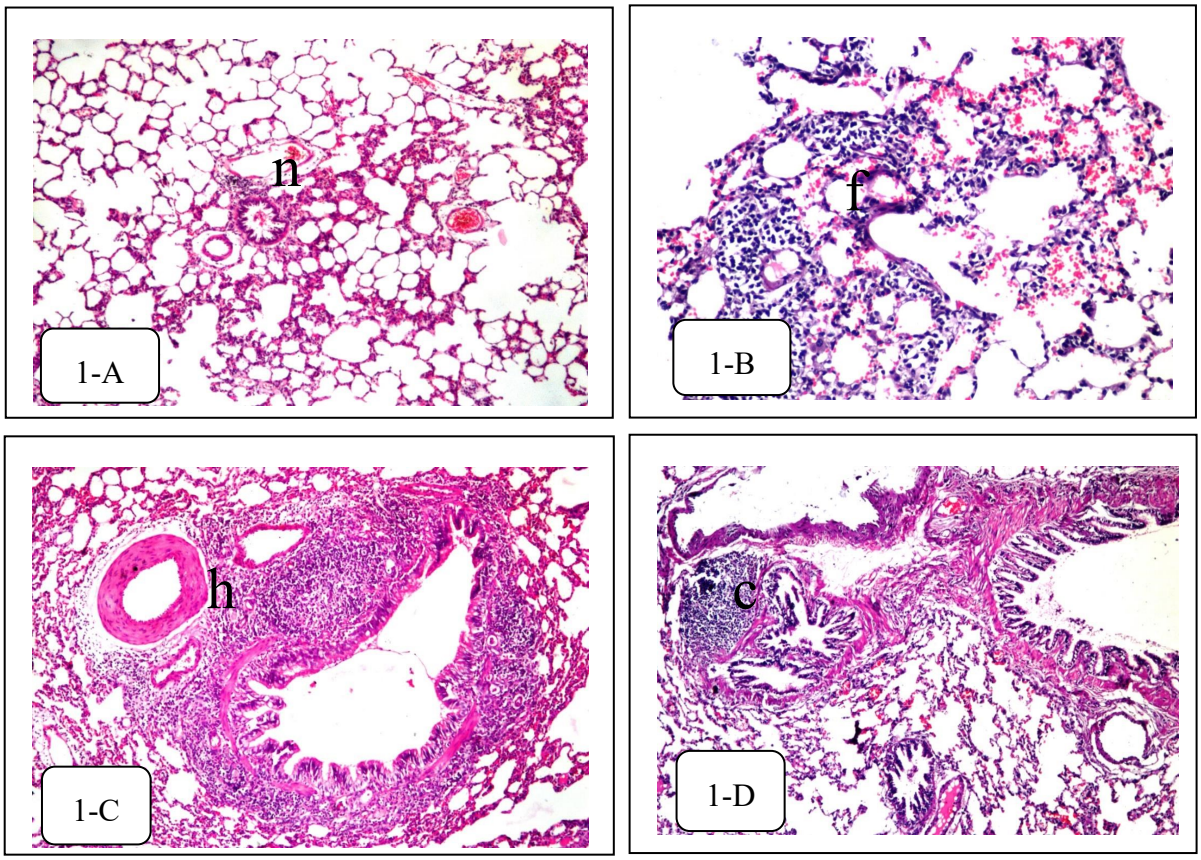


Fig (4) (1-A) rat lung tissues from control group showing normal histological structure of bronchioles and surrounding air alveoli(n); (1-B) from low dose group of EC-formulation showing infiltration in air alveoli with focal lymphoid inflammatory cells(f); (1-C), and (1-D) from high dose group of EC-formulation showing hyperplasia in the bronchiolar lining epithelium(h) as well as collapse in the air alveoli(c).

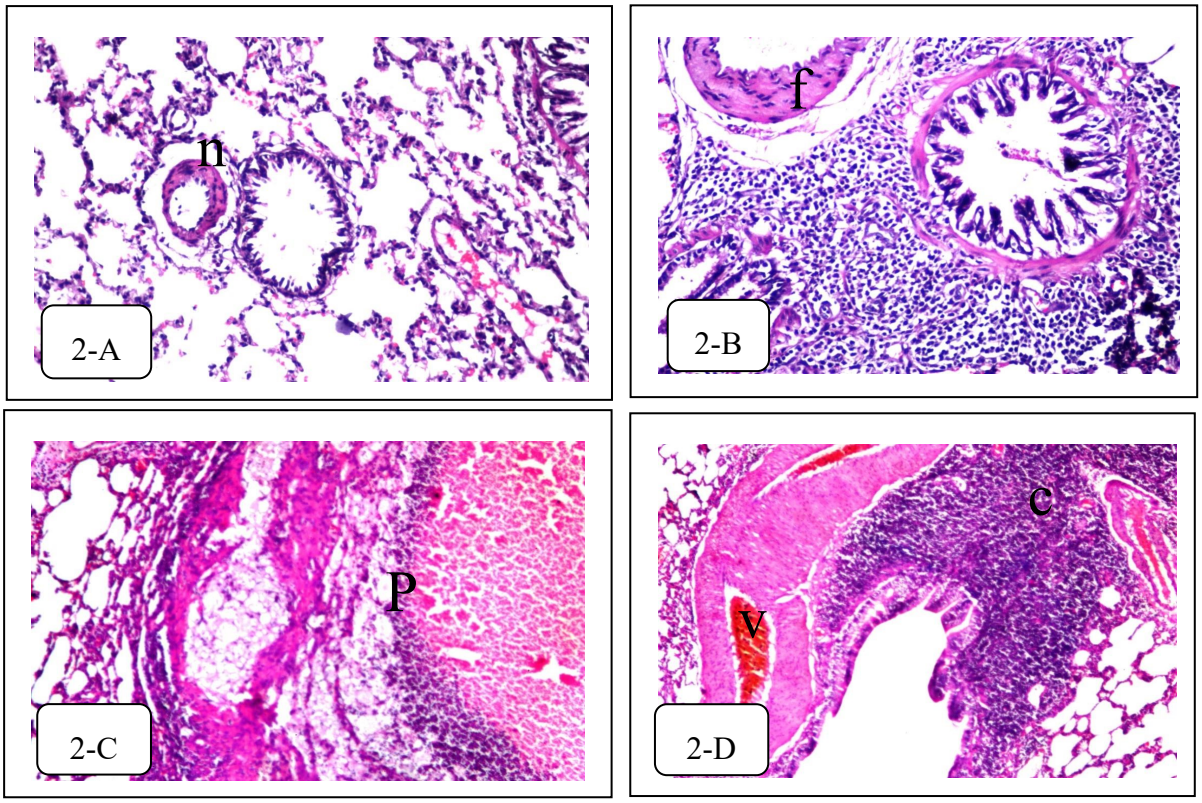


Fig (5) (2-A) rat lung tissues from control group showing normal histological structure of the bronchioles(n) and as well as the blood vessels were recorded; (2-B) from low dose group of SC-formulation showing massive number of lymphoid cells proliferation(f); (2-C), and (2-D) from high dose group of SC -formulation showing focal abscess formation with pus cells(p), sever congested blood vessels(v) and collapse of the air alveoli in the peribronchiolar tissue(c).

Çelik A., Ekinci S.Y., Güler G., Yildirim S., 2014 In vitro genotoxicity of fipronil sister chromatid exchange, cytokinesis block micronucleus test, and comet assay, DNA Cell Biol. 33(3):148-54.

Chodorowski Z., Anand J.S., 2004. Accidental dermal and inhalation exposure with fipronil-a case report ,.J Toxicol Clin Toxicol.;42(2):189-90.

Crissman J.W., Goodman D.G., Hildebrandt P.K., Maronpot R.R., Prater D.A., Riley J.H., Seaman W.J. and Thake D.C., 2004. Best practice guideline: toxicologic histopathology. Toxicologic Pathology, 32: 126–131.

FAO, 1995. Guidelines on Good Labelling Practice for Pesticides, Food and Agriculture Organization of the United Nations Rome, Available at http:// www.fao.org/fileadmin/templates/agphome/ documents/Pests_Pesticides/Code/Old_guidelines/ label.pd.

Ficová I. and Galdíková M., 2017. Testing the Potential Clastogenic/Cytotoxic Effects of Pesticide CALYPSO 480 SC, FOLIA VETERINARIA, 61, 3: 47—51, 2017

Hughes E.W., 1997. Fipronil: Neurotoxicity to rats by acute oral administration, Unpublished report No. RNP 536/973345 from Huntingdon Life Sciences. Submitted to WHO by Rhone-Poulenc, Inc., RTP, North Carolina, USA.

Kehdy, F.S.G., E.M.M. Cerqueira, M.B. Bonjardim, R.M. Camelo and M.C.L. Castro 2007, Study of the cytogenetic effects of occupational exposure to pesticides on sanitation workers in Belo Horizonte, Brazil., Genet. Mol. Res. 6 (3): 581-593.

Lee S.J., Mulay P., Diebolt-Brown B., Lackovic M. J., Mehler L.N., Beckman J., Waltz J., Prado J.B., Mitchell Y.A., Higgins S.A., Schwartz A., and Calvert G.M., 2010, acute illnesses associated with exposure to fipronil-surveillance data from 11 states in the United States, 2001–2007., Clinical Toxicology, 48, 737–744.

Mansour S.A., 2004. Pesticide exposure- Egyptian scene, Toxicology, 198: 91-115.

Merkowsky K., Sethi R.S., Gill J.P.S. and Singh B., 2016 Fipronil induces lung inflammation in vivo and cell death in vitro., Journal of Occupational Medicine and Toxicology 11:10.

Narahashi T, Zhao X, Ikeda T, Salgado VL, Yeh JZ., 2010. Glutamate-activated chloride channels: unique fipronil targets present in insects but not in mammals. Pestic Biochem Physiol. 97:149–152.

Noaishi M. A., Afify M.M. and AbdAllah A.A. 2013, Study the inhalation exposure effect of pesticides mixture in the white rat., Nat. Sci.;11(4)

OECD N°412, 2009. Guideline for Testing of Chemicals: Subacute Inhalation Toxicity: 28-Day Study.

Tingle C.C., Rother J.A., Dewhust C.F., Lauer, S., and King W.J., 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. Rev. Environ. Contam. Toxicol. 176: 1-66.

Zhao X., Yeh JZ, Salgado VL, and Narahashi T, 2004. Fipronil is a potent open channel blocker of glutamate-activated chloride channels in cockroach. neurons. J Pharmacol Exp Ther. 310(1): 192-201.

Ziliotto L., Stelio P.L.L., Filho D. A.A., Resende L.O., Aun A.G., and Braz M.G., 2017 Genotoxicity assessment of fipronil (frontline plus) in Canis familiaris., Pesq. Vet. Bras. 37(3):257-260.

الاثار الوراثية الخلوية والنسيجية المرضية بعد التعرض لاستنشاق نوعين من مستحضرات مبيد الفيبرونيل في الفئران البيضاء

د. محمد عبد الفتاح ابراهيم نويشي* ، د حسام الدين عبد الحفيظ

قسم بحوث سمية المبيدات للثدييات، المعمل المركزي للمبيدات ، مركز البحوث الزراعية، وزارة الزراعة واستصلاح الاراضي، مصر.

البريد الالكترونى*:noaishi@gmail.com

الملخص العربى

فيبرونيل (FPN)هو من مجموعة الفينيل بيرازول من المبيدات الحشرية، والذي يستخدم للسيطرة على مجموعة واسعة من الآفات الزراعية والبيطرية والصحة العامة. و تهدف هذه الدراسة إلى تقييم خطر التعرض لاستنشاق نوعين من مستحضرات الفيبرونيل المسجلة مؤخرا في مصر والمستخدمة حديثا. و أيضا لتقييم أي نوع من المستحضرات هو أكثر سمية أو خطرا من الآخر. وشملت الدراسة علي معيارين للسمية الاول هو فحص الشذوذات الكروموسومية في خلايا نخاع العظام في الفئران المعاملة، والثاني هو الفحص النسيجي المرضي في أنسجة الرئة، وكذلك تسجيل التغير في وزن الجسم للفئران المختبرة أثناء المعاملة. وفي هذه الدراسة تم استخدام ثلاثين من ذكور الفئران البيضاء الأصحاء وزنهم تقريبا 170جرام. وتم استخدام المجموعة الأولى كدراسة اولية لتحديد مدى مستويات الجرعة في الدراسة الرئيسية. ثم تم تقسيم الحيوانات الأخرى إلى خمس مجموعات (أ، ب، ج، د، هـ) كل منها بها خمسة فئران. تم الإبقاء على المجموعة الأولى (أ) كمجموعة ضابطة سلبية وتم فيها التعريض للهواء النقي فقط. ام بالنسبة للمعاملات فقد تم حساب جرعات المعاملات وفقا لكمية مستحضر المبيد الذي يتبخر في 6 ساعات وحجم الغرفة الذي هو حوالي 15 لتر. و كانت الجرعة العالية ضعف الجرعة المنخفضة وذلك من خلال وضع زجاجتين من مستحضر المبيد المختبر. تعرضت المجموعة (ب) لجرعة منخفضة (25 ملغم / لتر) و المجموعة (ج) لجرعة عالية (50 ملغم / لتر) من تركيبة مستحضر المبيد الاول وهو مركز قابل لاستحلاب .(EC)وتعرضت المجموعة (د) لجرعة منخفضة (30 ملغم / لتر) والمجموعة (هـ) لجرعة عالية (60 ملغم / لتر) من تركيبة مستحضر المبيد الثاني وهو مركز معلق .(SC)تعرضت الفئران لمستحضرات المبيدات المختبرة بكامل جسمها باستخدام غرفة استنشاق محلية الصنع. وتتكون من غرفة بلاستيكية مع اثنين من المراوح الصغيرة لمدخل ومخرج تدفق الهواء، وهي مجهزة في جانب واحد بزجاجة أو زجاجتين تحتوي على المبيدات التي تم اختبارها وتحتوي على انسجة ورقية والتي تساعد على نشر بخار المبيد. وأظهرت النتائج أنه لا توجد علامات سمية على الفئران مثل التشنجات أو نزيف العين أثناء فترة المعاملة كما لم يتم تسجيل أي انخفاض في وزن الجسم للحيوانات المختبرة طول فترة التعريض. وسجل تأثير السمية الوراثية بشكل ملحوظ في كلا الجرعتين العاليتين من مستحضرات المبيدات المختبرة. ولكن المستحضر(ECكان تأثيره الوراثي أكبر معنويا ($p < 0.001$)من المستحضر(SC) ($p < 0.01$).كذلك أظهرت نتائج الفحص النسيجي المرضي في خلايا أنسجة الرئة أن تم رصد نوعين مختلفين من الآثار الضارة على أنسجة الرئة. فبينما تسبب المستحضر(ECفي احداث تهيج لخلايا انسجة الرئة وتسبب في الجرعات العالية في احداث نمو كثيف للخلايا الليمفاوية وحدوث نمو عشوائي لها بما يسمي .(hyperplasia)لكن نجد من الناحية الأخرى المستحضر(SCتسبب في تكون خراريج وخلايا صديدية. كما أدى التعرض لاستنشاق الجرعات العالية إلى آثار مدمرة على أنسجة الرئة من الجرعات المنخفضة والتي أدت إلى آثار طفيفة. وأخيرا على الرغم من ان النتائج القائمة على أساس الدراسة علي حيوانات التجارب فإنه لا يمكن التنبؤ بما سوف يحدث للبشر في حالة التعرض لاستنشاق المبيدات. وذلك لأن الفئران أكثر تحملا عن الإنسان وهذه النتائج هي انذار او تنبيه لهذا النوع من التعرض ويفرض بقوة الحاجة إلى اختبارات أكثر تفصيلا عن السمية الناتجة عن التعرض المهني لمبيدات الآفات وخاصة عن طريق الاستنشاق، وينبغي توخي الحذر في التعامل معها في وقت التعرض لفترات طويلة والتي قد تؤدي إلى آثار صحية ضارة.