J. Agric. Sci. Mansoura Univ., 30 (6): 3459 - 3468, 2005

DETERMINATION OF METHOMYL RESIDUES IN SLUG TISSUES AND DIFFERENT ORGANS IN ALBINO RATS

El-Maghraby, H.M.E.*; I.A.A. Shaheen*; A.A. Hendawy*; A.A. Mohamed** and El-S.A. Abdallah**

* Central Agric. Pesticides Laboratory, Agric. Res. Center, Egypt.

** Pesticide Chemistry Dept., Fac. of Agric., Alex. Univ., Egypt.

ABSTRACT

High performance liquid chromatographic (HPLC) analysis of methomyl was used in order to measure the residue amount of methomyl as ng/g tissue of both terrestrial slug, *Lehmenia marginata* and male white albino rat, *Raltus norvegicus* var. *albus*. The results indicated high performance liquid chromatographic methomyl response after 48 hrs of topical application and poisoned food technique methods at different concentrations against gastropod slug, *L. marginata*. The distributed amounts of methomyl in different organs of male white albino rate, *R. norvegicus* var. *albus* due to LC_5 methomyl subcutaneously injection could be summarized as follows:

a) Cerebellum tissue contained higher amounts of methomyl than cerebrum by 1.8 folds.
b) Kidney had the highest methomyl residue of 6.99 ng/g tissue.
c) Both liver and heart had the same residual amount of methomyl; the recorded values were 4.87 and 4.82 ng/g tissue, respectively.
d) Cerebrum was the organ that contained the lowest methomyl residue.
e) It was obvious that the amount of methomyl recovered remarkably attenuated by increasing the interval after treatment.
f) Haematological investigation showed marked decrease in both red blood cells and white blood cells.

INTRODUCTION

Two important polyphagous animal pests, belonging to two different categories of animal kingdom, were selected in this study. Terrestrial gastropods (molluscus: slug and snails) are being abundantly distributed in north coast, new reclaimed lands in addition to delta region of Egypt (Kassab and Daoud, 1964; El-Okda and Khalil, 1981 and Abo-Bakr, 1997). Land slugs belong to phylum Mollusca, Class Gastropoda and Order Pulmonata. They attack plant seedlings, roots of growing trees, excrete undesirable slim material, decrease the quality of crops, causing death of young seedlings and mature buds and finally transfer plant-causing diseases such as fungi and/or nematodes from plant to another (El-Wakil and Radwan, 1991; Abdallah *et al.*, 1992 and Kassem, 1993). Methomyl is used to control land slugs, therefore the dissipation and distribution of methomyl were measured using High Performance Liquid Chromatographic analysis in terrestrial slug as well as in different organs of male white albino rat (cerebrum, cerebellum, heart, liver and kidney). Moreover, the haematological effect of methomyl was also investigated.

MATERIALS AND METHODS

1. Pesticides used

Methomyl, Lannate 90% S.P., S-methyl-N-[(methyl-carbamoyl) oxy]-thio-acetimidate.

2. Tested pests

2.1. Terrestrial slugs

L. marginata slugs were collected from ornamental nurseries and vegetable fields in Damanhour district, Behera Governorate. They were acclimatized for a week on the laboratory conditions and were fed on lettuce (*Lactuca sativa*) *ad libitum*.

2.2. White albino rats

Strain of white albino rats with an average age of 2-3 months and an average weight of 125-150 g was purchased from rodent laboratory, Extension Dept., Faculty of Agriculture, Alexandria University. The animals were housed in metallic cages in the laboratory and kept under observation at least two weeks for acclimatization.

3. Laboratory experiments

3.1. Determination of methomyl residues in land slug

The following concentrations of methomyl were prepared for lettuce leaves dipping method and topical application, 10, 30, 50, 100 and 200 ppm. Determination of the residues of methomyl in land slug, *Lehmania marginata* (Müller) was performed after 48 hrs and compared with the control groups (El-Wakil *et al.*, 1999 and Lokman and Harpy, 1999).

3.2. Toxic effect against albino rats

a) Albino rats were injected subcutaneously with LC_5 (1/10 of LC_{50}) of methomyl (1 mg/kg body weight) dissolved in corn oil in DMSO (Dimethyl sulphoxid). The animals were killed by decapitation after different time intervals (12, 24, 72, 120 and 168 hrs) of treatment. Blood samples were obtained for biochemical studies as well as different organs; brain (cerebellum & cerebrum), liver, kidney and heart to determine the pesticide residues of methomyl (El-Nabarawy *et al.*, 2003 and Zidan *et al.*, 1998).

4. Determination of tested pesticide residues in slug tissue and different organs of rats

4.1. Extraction process

At each time interval for rat and one time for slug after treatment by methomyl, the soft tissues were pooled and homogenized as 1:10 (w/v) with acetone for five minutes. The homogenate was filtered through a Whatman filter paper No. 1, then the filter paper was washed several times with acetone (10 ml portion). The filtrate was collected and made up to 100 ml with acetone, then dried over anhydrous sodium sulphate at 40°C. The pesticide residue was dissolved in 3 ml of methanol (Rotary vacuum evaporator, Yamato model RE-46 with flask 500 ml).

4.2. Cleanup process

Cleanup was carried out using a chromatographic column filled with silica gel (2 g), florisil (2 g) and anhydrous sodium sulphate (1 g). The sample extract was transferred to the column, preconditioned by passing 10 ml of acetone through it. The column was eluted with 50 ml of methanol and the elute was collected and concentrated to 3 ml too. The samples were subjected to HPLC analysis.

4.3. HPLC analysis

The running conditions for High Performance Liquid Chromatography of methomyl was standardized and the optimum conditions were presented in Table (1). However, the chromatographic column in the present work was Ultrasphere Si-Column (ODS) 25 cm x 4.6 mm and the solvent system was methanol. The detector involved in this study was UV 245 nm wavelength with sensitivity of 0.02 Aufs. The chart speed was 0.5 cm/min and the injection volume was 10 µl. Identification was accomplished by retention times and compared with the standards of methomyl at the same conditions. For quantification, the peak areas corresponding to each injected sample were compared with that of standard solutions of the insecticide. Detection limits for methomyl using the employed method were determined according to the analyte concentrations that produce a chromatographic peak equal to three times of base line noise.

4.4. Determination of residues using HPLC instrument

1. The HPLC instrument involved in the present work was Beckman HPLC system consisting of:
    a) Beckman 110 B Solvent Delivery.
    b) Beckman analog interface module 406.
    c) Beckman programmable UV detector module 166.
2. Hamilton 50 micro syringe fixed needle.
3. HPLC grade methanol.
4. Stock solutions for methomyl (90% purity) were prepared in HPLC grade methanol.

Table (1): **Running condition of high performance liquid chromatographic analysis of methomyl.**

Item	Value
Column	Ultra sphere-Si-column (ODS) 25 cm x 4.6 mm
Solvent system	Methanol
Flow rate	1 ml / min
Detector	245 nm wave length
Sensitivity	0.02 Aufs
Chart speed	0.5 cm / min
Inject volume	10 µl
Att.	32

RESULTS AND DISCUSSION

Haematological effects of methomyl

The haematological effects of LC_5 methomyl subcutaneously injection on adult male white albino rat, *Rattus norvegicus* var. *albus* were investigated. Haemoglobin, creatinine, white blood cells (WBCs) and red blood cells (RBCs) counts were recorded. The following observations could be noticed (Table 2):

a) Methomyl decreased both blood cell counts by 46.49 and 57.69% for RBCs and WBCs, respectively.
b) Methomyl treatments induced insignificant changes in both haemoglobin and creatinine contents.

Residues analysis

High performance liquid chromatographic (HPLC) analysis of methomyl was used in order to measure the residue amount of methomyl as ng/g tissue of both terrestrial slug, *Lehmania marginata* and male white albino rat, *Rattus norvegicus* var. *albus*. The use of HPLC is a potentially useful technique for insecticide residue analysis (Strait *et al.*, 1991 and El-Nabarawy *et al.*, 2003). Beckman HPLC system was used for analysis of methomyl. The optimum conditions of methomyl analysis is recorded in Table (1).

Table (2): Effect of LC_5 methomyl treatment on blood parameters of male white albino rat (*Rattus norvegicus* var. *albus*) after different time intervals.

Blood parameters	Control ±S.D	Time intervals (hrs)					Mean ±S.D	$L.S.D_{0.05}$
		12	24	72	120	168		
RBCs $x10^6$	3.85±0.270	2.15	2.25	2.70	1.95	1.75	2.06±0.190	0.251
WBCs $x10^3$	9.10±0.620	9.90	7.20	2.90	1.80	2.95	3.85±0.340	0.469
Hb, mg%	10.50±0.940	9.20	10.30	9.50	7.30	9.20	9.10±0.770	1.110
Creatinine, mg/dl	0.61±0.046	0.66	0.72	0.76	0.84	0.78	0.75±0.067	0.091

Values are means of 4 replicates.

The results in Table (3) show the high performance liquid chromatographic methomyl response after 48 hrs of topical application and poison food technique methods at different concentrations against gastropod slug *L. marginata*. Untreated slugs exhibited very minute amount of methomyl 0.003 ng/g tissue. Treatments by topical application and poison food technique showed insignificant difference in methomyl residue due to the method of application. In addition, increasing concentrations exhibited a remarkable increase of methomyl amount recovered. Both methods of treatment exerted a degree of fortification of the methomyl recovered that increasing concentration 3 times increased methomyl recovered by 18.7 and 26.5 times higher due to topical application and poison food tichnique, while increasing concentration 20 times enhanced the amount of methomyl found by 81.12 and 182.4 times higher due to topical application and poison food technique, respectively. These results are in agreement with those reported by Andrews (1989), Kassem (1993), Abdallah, *et al.* (1998) and Hanafy *et al.*(1998).

Table (3): Methomyl residues in land slug, *Lehmania marginata* (Müller) after 48 hrs at different concentration treatments, shown as ng/g tissue.

Treatment	Concentration (ppm)					Mean ± S.D.
	10	30	50	100	200	
Control	0.003	0.003	0.003	0 003	0.003	0 003 ± 0 0
Topical application technique	0.062	1.160	1.870	7.930	5 030	3.210 ± 1.60
Dipping poison food technique	0.054	1.430	1.140	3 120	9.850	3.118 ± 1.46

- Data represent means of 3 replicates ± S.D.
- Retention time (RT) = 2.66 min.

The distributed amounts of methomyl in different brain regions and organs of male white albino rat, *R. norvegicus* var. *albus* due to LC_5 methomyl subcutaneously injection are summarized in Table (4) as well as Figure (1).

The results of methomyl residue analysis in different organs of rat revealed that:

a) Cerebellum tissue contained higher amounts of methomyl than cortex by 1.8 folds.
b) Kidney had the highest methomyl residue of 6.99 ng/g tissue.
c) Both liver and heart had nearly the same amount of methomyl recovered, the values were 4.870 and 4.826 ng/g tissue for liver and heart, respectively.
d) The organ that contained the lowest methomyl residue was the cortex (cerebrum) of the rat brain.
e) It was obvious that increasing time of measurements after treatments attenuated the amount of methomyl recovered remarkably, the amount of methomyl was decreased descendingly by increasing time interval. This might be due to detoxification and/or rapid excretion of the methomyl of the body corresponding to increasing time.

These results are in agreement with those reported by Strait *et al.*(1991) and El-Dashlouty *et al.*(2000).

Table (4): Methomyl residues in different organs of male white albino rat, *Rattus norvegicus* var. *albus* treated with LC_5 at different time intervals, shown as ng/g tissue.

Time Intervals (hrs)	Brain		Heart	Liver	Kidney	Total amount
	Cortex	Cerebellum				
Control	0.18	0.09	0.006	0.08	0.19	0.346
12	1.32	1.79	1.65	1.62	1.21	7.59
24	0.87	1.44	1.36	1.04	1.22	5.93
72	0.71	2.59	1.13	1 15	1.24	6.82
120	0.34	0.62	0.40	0.78	2.56	4.70
168	0.30	0.18	0.28	0.20	0.57	1.53
Total amount	3.72	6.71	4.826	4.87	6.99	27.116

* Data represent means of 3 replicates.
* Retention time (RT) = 2.66 min.

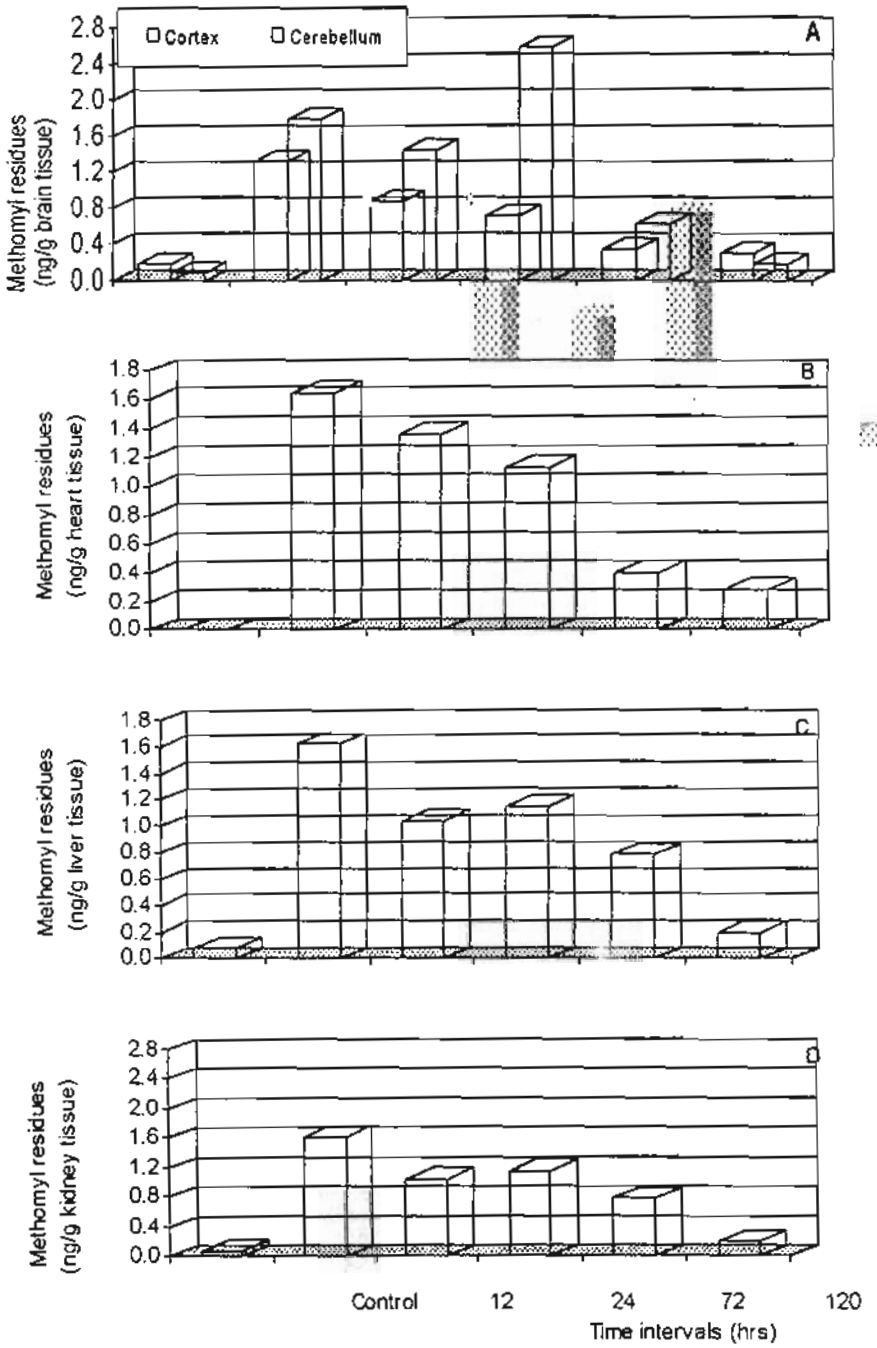

Fig. (1): Methomyl residues in different organs: A) Brain, B) Heart, C) Liver, and D) Kidney of male white albino rat, *Rattus norvegicus* var. *albus* treated with LC_5 at different time intervals, shown as ng/g tissue.

The results in Table (5) and Figures (2 and 3) show the high-performance liquid chromatographic response of methomyl when 50, 100, 150, 200 and 250 µg of such insecticide were injected under the previous conditions. These results indicate the linearity of response within the range of 50 µg to 250 µg of such compound. The values of peak area were calculated to be $1.9x10^4$, $3.6x10^4$, $6.8x10^4$, $11.3x10^4$ and $15.6x10^4$ (BC) when 50, 100, 150, 200 and 250 µg, respectively were injected into the high-performance liquid chromatography as shown in Table (5).

Table (5): Concentration-response relationship for methomyl using High Performance Liquid Chromatography.

No.	Concentration of methomyl (µg)	Peak area (BC)
1	50	1.9×10^4
2	100	3.6×10^4
3	150	6.8×10^4
4	200	11.3×10^4
5	250	15.6×10^4

- Retention time (RT) = 2.66 min.
- Each value is an average of 3 replicates.

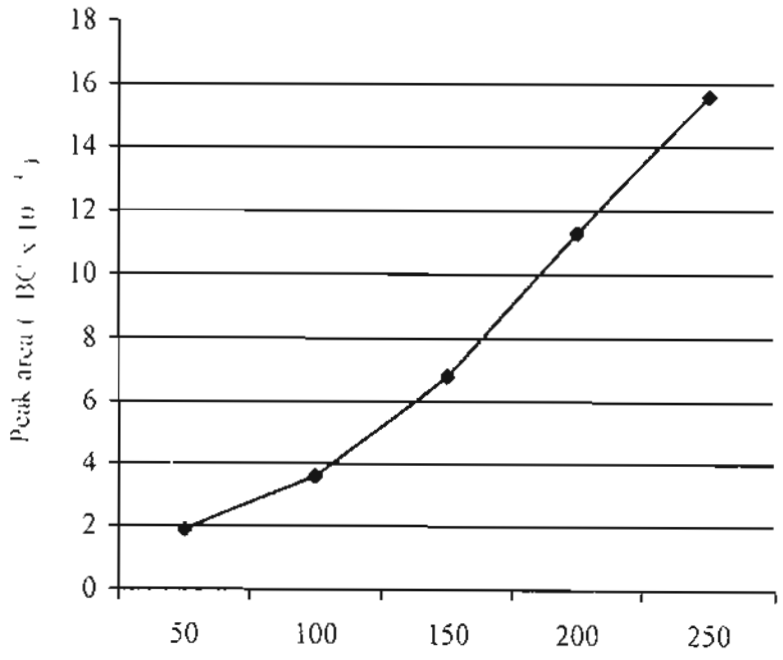

Fig. (2): Standard curve of methomyl using high performance liquid chromatography peak area (BC).

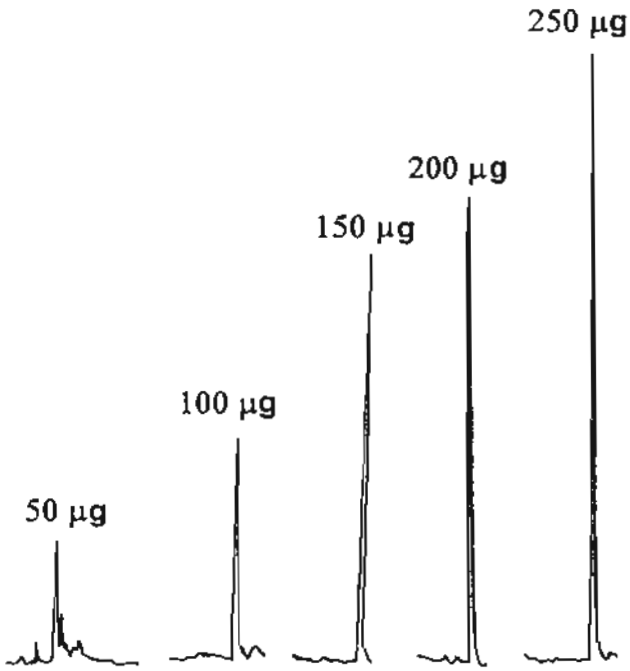

Fig. (3): High performance liquid chromatographic response for different concentrations of methomyl.

REFERENCES

Abdallah, E.A.M.; F.A. Kassem and E.A. Kadous (1992). Laboratory and field evaluation of local bait formulations of certain pesticides against mollusca species. J. Pest Control & Environ. Sci., 4 (2): 179-192.

Abdallah, E.M.A.; F.A. Kassem; H.B. El-Wakil and Y. Abo-Baker (1998) Molluscicidal potentiality of several pesticides against *Eobania vermiculata* and *Theba pisana* terrestrial snails. Annals Agric. Sci, Dec. 15-17, 1: 263-276.

Abo-Bakr, Y.A. (1997). Toxicological and environmental studies on some terrestrial gastropods. M.Sc. Thesis, Faculty of Agric., Univ. of Alex., Alexandria, Egypt.

Andrews, K.L. (1989). Slug pests of dry beans in Central America Monog. British Crop Protec. Council, 41: 85-89.

El-Dashlouty, M.S.; Z.H. Zidan; N.R. Abdel-Rahman; B.L. Wilson and E.M. El-Kholie (2002). Elimination and disappearance of carbamate residues in contaminated fish through technological processes. The First Conf. of the Central Agric. Pesticide Lab., 3-5 Sept., 1: 180-186.

El-Nabarawy, I.M.; S.G. El-Banna; A.B. Okab and A.M. Attia (2003). Diazinon residue in liver and kidney with special references to their function in red Baladi rabbits. J. Pest Cont. & Environ. Sci., 11 (1): 53-66.

El-Okda, M.M.K. and K.A. Khalil (1981). Land mollusca as economic pest attacking fruit orchards at Alexandria Governorate. Proc. 4th Arab Pesticide Conf., Tanta Univ., II: 289-302.

El-Wakil, H.B. and M.A. Radwan (1991). Biochemical study on the terrestrial snail, *Eobania vermiculata* (Muller) treated with some pesticides. J. Environ. Sci. & Heal. Part. Pest. Food Cont. & Agric. Was., 26 (5-6): 479-490.

El-Wakil, H.B. and A.M. Attia (1999). Effect of selected Insecticides on Terrestrial Snails Eobania Vermiculata (Muller) and *Theba pisana* (Muller) with respect to some Morphological changes in Egypt. J. Environ. Sci. Health, 334 (1), 47-60.

Hanafy, A.H.A.; M.M. Youssef and M.S. El-Shahaat (1998). Preparation of methomyl baits and efficacy against certain land mollusca in different vegetations. Adv. Agric. Res., 3(3):435-441.

Kassab, A. and H. Daoud (1964). Notes on the biology of land snails of economic importance in UAR. Agric. Rev., Cairo Min. of Agric., 42: 77-88.

Kassem, F.A. (1993). Molluscicidal efficiency of locally formulated methomyl and monocrotophos baits under field condition. Com. In Sci. & Dev. Res., 654: 81-94.

Lokma, H.E. and F.N. Al-Harpy (1999). Molluscicidal effect of some pesticides on land snails under field conditions at Riyadh area in Saudi Arabia. Zagazig J. Agric. Res., 26 (2): 437-444.

Paliwal, J.K.; R.C. Gupta and P.K. Grover (1989). High performance liquid chromatographic determination and pharmacokinetics of methyl N-[5-[4-(2-pyridinyl)-1-piperazinyl] carbonyl]-1H-benzimidazol-2-yl] carbamate (CDRI compound 81-470), a new anthelmintic agent in rats. Pharmaceutical Research, 6 (11): 991-993.

Strait, J.R.; G.C. Thornwall and M. Ehrich (1991). Sensitive high-performance liquid chromatographic analysis for toxicological studies with carbaryl. J. Agric. & Food Chem., 39 (4): 710-713.

Youssef, H.M.; A. El-Sebai and M.A. Desheesh (1998). Susceptibility of roof-rats (*Rattus rattus*) to anticoagulant rodenticides baits in comparison with *Rattus norvegicus* var. *albinos*. Alex. Sci. Exch., 19 (3): 497-506.

Zidan, Z.H.; A.K. Mashhour; A.A. Zidan; A.A. Fawzy and Y.A. Okasha (1998). Toxicological effect of long-term administration of minimal doses of certain insecticides on white albino rats. Abstracts, 7th Conf. Agric. Dev. Res., Fac. Agric., Ain Shams, Univ. Cairo, Egypt. Annals Agric. Sci. Sp. Issue.

تقدير متبقيات الميثوميل فى أنسجة البزاقات والأعضاء المختلفة فى الفئران البيضاء

حمدى محمد المغربى*، إسماعيل عوض شاهين*، علاء الدين عبدالفتاح هنداوى*، عادل عبدالعال محمد**، السيد أحمد عبدالله**

* المعمل المركزى للمبيدات، مركز البحوث الزراعية، مصر.

** قسم كيمياء المبيدات، كلية الزراعة، جامعة الأسكندرية، مصر.

إشتملت الدراسة على دراسة التأثير على قياسات الدم فى الفئران البيضاء وأوضحت النتائج أنه عند المعاملة بـ LC5 لذكور الفئران البيضاء حقنا تحت الجلد وكذلك دراسة تركيز الهيموجلوبين والكرياتينين وعدد خلايا الدم الحمراء والبيضاء وقد لوحظ الآتى:

١) نقص فى كلا من كرات الدم الحمراء والبيضاء بمعدل ٤٦,٤٩%، ٥٧,٦٩% على التوالى عند المعاملة بمبيد الميثوميل.

٢) المعاملة بالميثوميل أحدثت تغيرات غير معنوية فى كل من محتوى الهيموجلوبين والكرياتينين.

كذلك إشتملت الدراسة على دراسة إختفاء وتوزيع مبيد الميثوميل بإستخدام جهاز الكروماتوجرافى السائل ذو الكفاءة العالية وذلك فى البزاقات الأرضية والأنسجة المختلفة من ذكور الفئران البيضاء إشتملت على الفص الأمامى والفص الخلفى للمخ وكذلك القلب والكبد والكلى. ومن نتائج الدراسة وجد أنه عند تقدير كميات متبقيات مبيد الميثوميل معبراً عنها بالنانو جرام لكل جرام من النسيج (ng/g tissue) فى البزاقات الأرضية لم تظهر نتائج معنوية بعد ٤٨ ساعة على تركيزات مختلفة بإستخدام الطريقتين وهما التطبيق السطحى والطعم السام لمبيد الميثوميل، أما الكميات المنتشرة من المبيد فى مناطق المخ والأعضاء لذكور الفئران البيضاء المعاملة بـ LC5 من المبيد حقناً تحت الجلد تتلخص فى الآتى:

١- أنسجة الفص الخلفى Cerebellum تحتوى على كمية أعلى من الميثوميل أكثر من الفص الأمامى Cerebrum بـ ١,٨ أضعاف.

٢- الكلى بها متبقيات عالية حوالى ٦,٩٩ نانوجرام من المبيد/جرام من النسيج.

٣- تساوت كميات الميثوميل تقريباً فى كلا من الكبد والقلب حيث القيم هى ٤,٧٨٠، ٤,٨٢٦ نانوجرام/جرام نسيج من الكبد والقلب على التوالى.

٤- بزيادة زمن القياس بعد المعاملات إنخفضت كمية الميثوميل المقدرة تنازليا بمرور الزمن.