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NEUROPATHOLOGIC EFFECTS OF METHOMYL ON SPRAGE-DAWELY RATS

(With 3 Figures)

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التأثير السمي الباثولوجي للميثوميل علي أدمغة الفئران البيضاء

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بالرغم من الدور المهم للمبيدات الحشرية في زيادة انتاجية المحاصيل الزراعية ومنع انتشار العديد من الاوبئة عن طريق مكافحة الحشرات الا انها احيانا قد تؤدي الي بعض الامراض العصبية في كل من الانسان والحيوان. في ضوء ذلك ، اجريت هذه الدراسة لمعرفة التاثير السمي الباثولوجي علي أدمغة الفئران البيضاء. أستخدم فيها ٦٠ فاراً ابيض قسمت الي مجموعتين تجريبيتين ومجموعة ضابطة (٢٠ فاراً للمجموعة). أعطيت المجموعة الأولي ١٠ مجم/كجم من الميثوميل كجرعة واحدة مذابة في ٢ ملي من الماء المقطر عن طريق الفم فى اليوم الاول من التجربة وأعطيت المجموعة الثانية ٢ مجم/كجم من الميثوميل مذابا في ٢ ملي من الماء المقطر عن طريق الفم ثلاث مرات اسبوعيا. اما المجموعة الضابطة فاعطيت ٢ ملي من المحلول المائي الخالي من الميثوميل عن طريق الفم. اخذت أدمغة الحيوانات للفحص الهستوباثولوجي بعد اسبوعا واحدا وثلاثة اسابيع بالنسبة للمجموعة الاولي والثانية علي الترتيب. وجد ان التعرض للميثوميل في كلا المجموعتين يؤدي الي انحلال وموت واضح للخلايا العصبية المكونة لقرن امون وكذلك بعض مناطق الدماغ الاخري كالمخ والمخيخ والانوية العصبية المحركة. ولوحظ ايضا تكوين فجوات باللبد العصبي وزيادة نشاط خلايا الابق العصبية الدقيقة والخلايا النجمية بالمخ. ولذلك تخلص هذه الدراسة الي التأثير الضار للميثوميل علي ادمغة الفئران البيضاء وتوصي بالاستخدام الامثل للمبيدات الحشرية.

SUMMARY

However pesticides contribute to a dramatic increase in crop yields and help to limit the spread of certain diseases by controlling pests, there is a strong evidence for persistent CNS damage following acute or chronic exposure to pesticides. The present study was carried out to investigate the neuropathological effects of methomyl, the common used carbamate pesticide in agriculture, on the brains of Sprage-Dawley rats. Rats were divided into two groups and treated orally with one dose of methomyl

(10 mg/kg b.w.) and (2 mg/kg b.w., three times weekly). After one week and 3 months, brains from first and second groups were taken for histopathological examination, respectively. Methomyl significantly increased the numbers of necrosed neurons in the hippocampus of both animal groups compared to untreated controls. Also, methomyl caused neuronal degeneration and necrosis in cerebral cortex, cerebellum and some motor nuclei and induced glial proliferation and vacuolation of neuropil. In conclusions, methomyl induced neuronal degeneration and necrosis particularly in the hippocampus of SD rats.

Key words: *Methomyl, rats, neurotoxicity.*

INTRODUCTION

Pesticides are a broad and rather diverse group of compounds. Among pollutants, pesticides are unique in that they are deliberately inserted into the environment rather than being accidentally released (Clementi *et al.,* 2007). They are used to support crop production through controlling pests, weeds and plant diseases (Juraske *et al.,* 2007). In this respect, pesticides are still the most effective and accepted means for protection of plants from pests (Bolognesi, 2003).

Since the introduction of DDT in the 1930s, pesticides have represented the most frequently released toxic substances into the environment and billions of kilograms of their active ingredients have been distributed worldwide (Clementi *et al.,* 2007). In recent years, many developed countries have taken specific regulations to decrease the use of pesticides and to increase the public awareness about pesticide-related health problems (London and Rother, 2003). On the other hand, developing countries are still suffering these problems due to the misuse of pesticides by concerned individuals and the lack or weakness of national controlling plans regarding the safe use of these chemicals (Mansour, 2004; Ibitayo, 2006).

From production till use, pesticides hazards can be encountered in workers and laborers in pesticide factories, farmers and farm workers and populations that live in areas of intensive pesticide use or production (Mansour, 2004). Also, agriculturally-applied pesticides that never reach the target organisms (85-90%) can move through air, soil and water reaching living tissues where they can bioaccumulate up the food chain into human diets. Th , they can affect wide rang of populations (Moses *et al.,* 1993).

Methomyl, a derivative of carbamic acid, is a commonly used carbamate insecticide. It has been widely marketed since 1967 as a

broad-spectrum insecticide to control ticks and spiders (WHO, 1996). It is also used for foliar treatment of vegetables, fruits and field crops (Farré *et al.,* 2002). Methomyl was classified by the Environmental Protection Agency (EPA) as a restricted-use pesticide (RUP) or class IB (highly Hazardous) (Farré *et al.,* 2002). Methomyl causes reversible carbamylation of acetylcholinestrase (AChE) with accumulation of acetylcholine (ACh) in the synaptic clefts leading to over-stimulation of ACh receptors and appearance of neurotoxic symptoms (Makrides *et al.,* 2005; Yi *et al.,* 2006). In addition to cholinergic toxicity, methomyl can induce over-production of reactive oxygen species (ROS) in both intra and extra cellular spaces resulting in disturbance of the pro-oxidant/antioxidant balance of the cells and thereby inducing oxidative stress and cellular damage (El-Khawaga, 2005).

Nowadays, there is strong epidemiological evidence linking between exposure to pesticides and persistent CNS damage which could underlie for the occurrence of neurodegenerative diseases. Accordingly, the present study is conducted to investigate the neuropathological effects of methomyl, one of the most used carbamate pesticides in agriculture, on SD rats.

MATERIALS and METHODS

Materials

Sprage-Dawley (SD) rats of three months old and 200-300g weight were obtained from Faculty of Medicine, Assiut University (Assiut, Egypt). Methomyl (S-methyl-N-[(methyl carbamoyl) oxy] thioacetimidate) is sold under trade name "Lannate 90®" and available in the markets as water soluble powder contains 90% of active ingredients. It was obtained from Dupont Co., U.S.A.

Methods

Treatment of rats with methomyl

Rats were handled in accordance with the EC Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the member States regarding the protection of animals used for experimental and other scientific purposes (Anonymous, 1986). After two weeks of adaptation, rats were divided into two groups (20 rats each). In the first group, animals were treated with one dose of methomyl (10 mg/kg. b.w.) and kept for one week. Calculated dose of methomyl was dissolved in 2ml of distilled water and given to rats by stomach tube. In the second group, animals were received methomyl (2 mg/kg b.w.) three times weekly for three months.

Calculated dose of methomyl was dissolved in 1ml of distilled water and given by stomach tube. In parallel, 20 rats were received only 2 ml of distilled water by stomach tube and kept as untreated controls.

Tissue specimens

At the end of each treatment, animals were sacrificed by cervical dislocation and brains were rapidly and gently released from skulls and fixed in 10% neutral buffered formalin for histopathological examination. Brains were cut in sagittal sections and then dehydrated in a graded alcohol series, cleared with methyl benzoate and embedded in paraffin wax. Four micron thick-sections were cut and stained with hematoxylin & eosin and cresyl fast violet (Bancroft and Stevens, 1990). Stained sections were examined under the light microscopy (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia C-5060, Japan).

Counting of necrosed neurons in hippocampus

In cresyl fast violet stained brain sections, necrosed neurons in hippocampus which showed cell shrinkage, dark cytoplasm and pyknotic nuclei were counted in three sections/animal after both short and long-term treatments.

Statistics

Data were expressed as mean ± standard error of mean (SEM). 100% corresponds to the mean values of controls. Comparisons were made using ANOVA and post hoc Duncan's test (S.A.S., 1998). $p<0.05$ was considered as statistically significant.

RESULTS

Methomyl produced seizures on SD rats

Treatment of SD rats with a single dose of methomyl (10mg/kg b.w.) produced mild seizures within 1-2 min and lasted for half an hour. Seizures were not produced by treatment with methomyl in long-term toxicity (2mg/kg b.w., three times weekly for 3 months).

Neuropathology induced by methomyl

Neuropathological changes as a result of methomyl treatment consisted primarily from neuronal degeneration and necrosis in hippocampus, cerebellum, cerebral cortex and some motor nuclei and appeared more pronounced in long-term toxicity. In the hippocampus, necrosed neurons appeared shrunken with dark-stained cytoplasm and pyknotic nuclei (Fig. 1B and C). Upon counting, necrosed hippocampal neurons as a result of methomyl treatment were higher in numbers by 7 and 11 folds in short and long-term treatments, respectively, compared

to untreated controls (Fig. 2). In the other brain regions, treatment of rats with single dose of methomyl caused neuronal chromatolysis, vacuolation of neuropil and mild microglial reaction (Fig. A, B). Long-term methomyl treatment resulted in neuronal swelling, necrosis and microglial reaction with evidences of neuronophagia (Fig. 3C and D).

Fig. 1

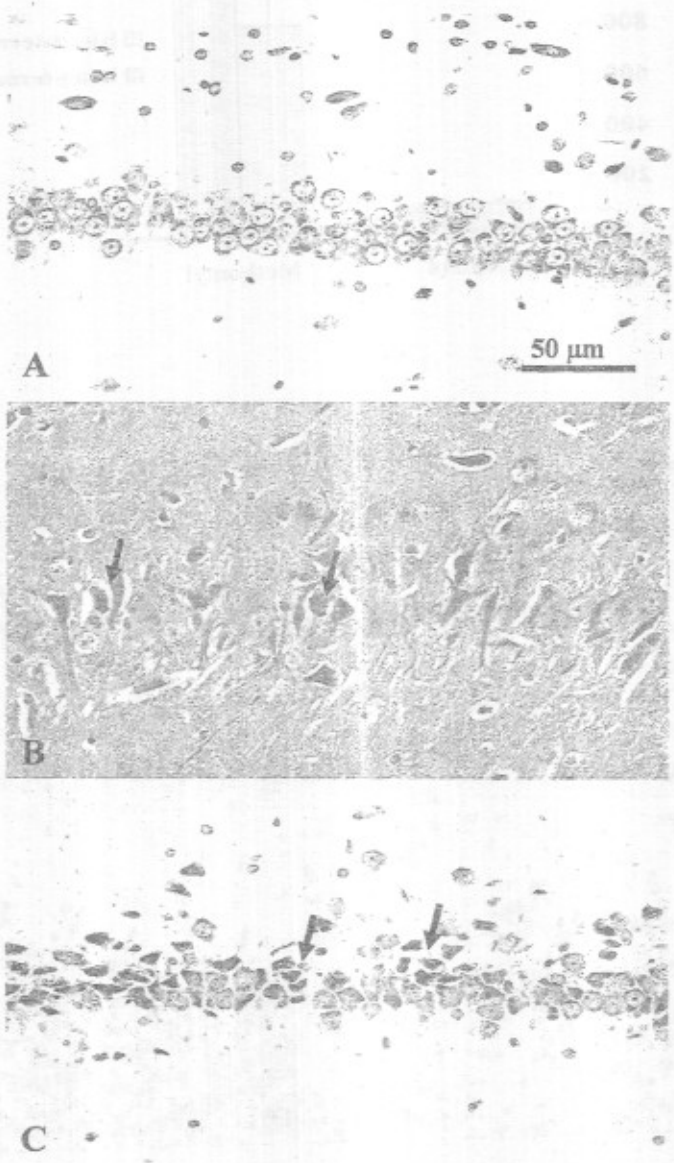


Fig. 2

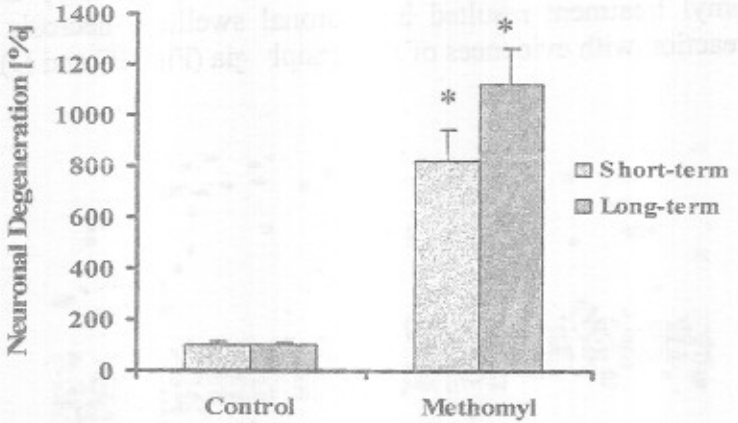
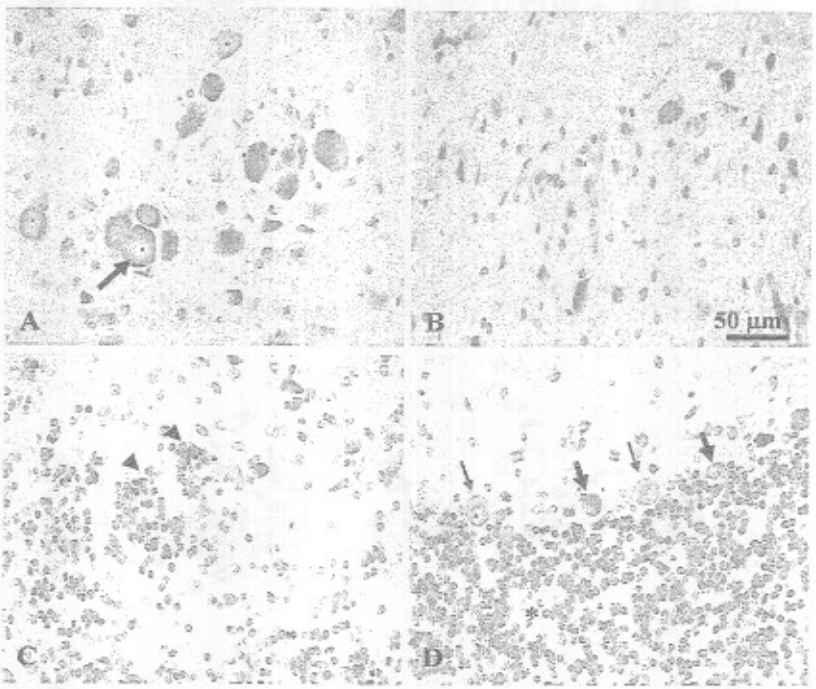

Neuronal Degeneration [%]
1400
1200
1000
800
600
400
200
0
*
*
Short-term
Long-term
Control
Methomyl

Fig. 3


A
B
50 µm
C
D

LEGENDS TO FIGURES

Fig. 1: Representative micrographs for the hippocampus (CA1). A) Normal hippocampus. (Cresyl fast violet) B) Hippocampus showing neuronal necrosis (arrows) as a result of treatment with one dose (10mg/kg b.w.) of methomyl. (H&E) C) Hippocampus showing neuronal necrosis (arrows) as a result of treatment with methomyl (2mg/kg b.w.) three times weekly for three months. (Cresyl fast violet)

Fig. 2: Numbers of necrosed neurons in the hippocampus as a result of treatment with methomyl. Data were expressed as the mean ± S.E.M (standard error of means). 100% corresponds to the mean value of necrosed neurons in the hippocampus of untreated control animals. (*$p<0.001$)

Fig. 3: Single dose of methomyl induced: **A)** Neuronal chromatolysis (arrow). (Cresyl fast violet) **B)** Vacuolation of neuropil and mild microglial reaction. (Cresyl fast violet); Treatment of rats with methomyl for three months caused: **C)** Neuronal necrosis, microglial reaction and evidence of neuronophagia (arrow heads) in cerebrum. (Cresyl fast violet) **D)** Degeneration (thin arrow) and necrosis (thick arrow) of Purkinje cells and decrease the intensity of granule cell layer in cerebellum (asterisk).

DISCUSSION

Since agricultural modernization at the beginning of the second half of the past century, the use of pesticides has substantially increased to improve crop yields through controlling pests and limiting certain diseases. In parallel, many hazardous compounds have been introduced into the environment and their dangerous effects are now considered one of the most important problems faced globally particularly by developing countries. In this context, Konradsen (2006) reported that acute and chronic pesticide poisoning has become a major health problem with more than 300.000 deaths each year around the world. Moreover, Ecobichen (2001) mentioned that inexpensive and older environmentally persistent pesticides are used extensively in developing countries and are creating serious acute health problems and local and global environmental contamination.

Among pesticide hazards, neurontoxicity is of great concern. Keifer and Firestone, (2007) reported that there is strong human

epidemiological evidence for persistent nervous system damage following acute intoxication, chronic exposure to low levels or in utero/or early childhood exposure to pesticides. In this context, Morretto (1998) stated that organophosphates and carbamates acutely affected central and peripheral nervous system through inhibition of AChE which results in cholinergic syndrome or even death approximately at 50% and >90% AChE inhibition, respectively. In a cohort of French elderly, Baladi *et al.* (2003) found that there was an association between the past occupational exposure to pesticides and low cognitive performance together with an increase in the risk of developing Alzheimer's or Parkinson's diseases. Moreover, Eskenazi *et al.* (1999) reported that exposure to neurotoxic compounds at levels believed to be safe for adults could result in permanent loss of brain function if it occurred during the prenatal and childhood period of brain development.

In the present study, SD rats developed seizures within 1-2 min and lasted for 30 min following treatment with a single dose of methomyl. In consistency, Dekundy *et al.* (2003) and Kaminski *et al.* (2007) reported that methomyl produced motor seizures in male Wistar rats and mice. Moreover, Gupta *et al.* (2007) found that carbamate insecticide carbofuran (1.5 mg/kg b.w) also developed similar seizures within 30-60 min and lasted for about 2 h in SD rats. These seizures were attributed to inhibition of AChE which resulted in accumulation of ACh in the synaptic clefts and subsequent stimulation of ACh receptors (DEKUNDY *et al.,* 2003). Disappearance of the seizures after half an hour returned to the reversibility of AChE inhibition by carbamates (Makrides *et al.,* 2005). On the other hand, no seizures were developed following treatment with methomyl in long-term toxicity.

Histopathological examination of brain stained sections revealed that treatment with methomyl either for short (single dose of 10 mg/kg b.w.) or long-term (2 mg/kg b.w., three times weekly for three months) produced neuronal degeneration and necrosis in the hippocampus, cerebral cortex, cerebellum and some motor nuclei. In consistency, Kaminski *et al.* (2007) reported that methomyl in combination with lithium chloride caused extensive neuronal degeneration and disruption of neuropil particularly in hippocampus and amygdala in rats. Similar to methomyl, some carbamate insecticides were reported to produce neuronal damage in experimental animals. For examples, Gupta *et al.* (2007) found that degeneration and cell death in the pyriform cortex, amygdale, hippocampus, dorsal thalamus and cerebral cortex were the most consistent pathological findings in acute carbofuran toxicity in SD

rats. El-Manakhly (1996) reported neuronal degeneration in the form of shrunken and pyknotic neurons in hippocampus as the result of carbosulfan treatment in rabbits. Tos-Luty *et al.* (2001) observed that carbaryl induced degenerative changes in hypothalamus nucleus, hippocampus and granular layer and Purkinje cells of the cerebellum in rats. Of which, degeneration and necrosis of hippocampal neurons are of great importance as hippocampus is functionally related to the vital behaviors and intellectual activities such as memory and learning (Han *et al.*, 2007). The other consistent histopathological finding produced by methomyl was microglial reaction particularly after three months of exposure. Microglial reaction was seen convenient to necrosed neurons in an attempt of neuronophagia. Using other carbamate compounds, microglial proliferation was seen by Nariman *et al.* (1995) and El-Manakhly (1996) in rats and rabbits intoxicated by thiodicarb and carbosulfan, respectively.

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