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Short communication

HISTOPATHOLOGICAL EFFECTS OF METHOMYL ON SPRAGUE-DAWLEY RATS AFTER REPEATED APPLICATION

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Summary

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Among pesticides, carbamates are most commonly used for the control of agricultural plagues with potential toxicity to exposed animals and humans. In the present study, methomyl (2 mg/kg b.w.) was given to Sprague-Dawley rats, three times weekly for three months. Histopathological examination of tissue specimens revealed that liver, kidneys, lungs, testicles and spleen were markedly affected after methomyl exposure. Liver showed focal and diffuse activation of Kupffer's cells, necrobiotic changes in the hepatocytes, focal areas of necrosis, apoptotic changes and increase in the mitotic figures. Kidneys had glomerular swelling, periglomerular fibrosis, degeneration of the tubular epithelial cells and dysplastic changes in renal tubules. Lesions in the lungs consisted of necrosis and sloughing of bronchiolar epithelial lining and lymphocytic perivascualr accumulations. The testicles exhibited necrosis of seminiferous tubular cells and formation of intratubular giant cells. Spleen showed congestion, haemosiderosis and lymphocytic depletion. In conclusion, methomyl was found out to be potentially toxic to liver, kidneys, lungs, testicles and spleen.

Key words: histopathology, methomyl, pesticides, rat, toxicology

Pesticides are substances or mixtures of substances that so far are still the most effective and accepted means to kill or control pests (Bolognesi, 2003). Since the agricultural modernization in the past half-century, the use of pesticides has substantially increased to improve crop yields and billions of kilogrammes of pesticide active ingredients have been distributed worldwide (Clementi *et al.*, 2007). Nowadays, most of developed countries have taken specific regulations to decrease the use of pesticides and to increase the public awareness about pesticide-related health problems (London & Rother, 2000). On the other hand, pesticide poisoning is a major health risk in developing countries (Garming & Waibel, 2008). This is due to the misuse of pesticides by concerned individuals and the

absent or weak national controlling plans regarding the safe use of these chemicals (Mansour, 2004; Ibitayo, 2006).

Methomyl, a derivative of carbamic acid, has been widely marketed since 1967 under the trade name "Lannate" 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 is classified by the Environmental Protection Agency (EPA) as a restricted-use pesticide (RUP) or class IB (Highly Hazardous) (Farré *et al.*, 2002).

Histopathological examination of different organs of test animals following toxicological studies is an important criterion to evaluate the toxicity of substances. Thus, the present study was conducted to investigate the histopathological effects induced by the pesticide methomyl, one of the most used carbamate pesticides in agriculture, on Sprague-Dawley rats.

Healthy Sprague-Dawley rats at the age of 3 months, weighing 200–300 g were obtained from the Faculty of Medicine, Assiut University, Egypt. Methomyl (S-methyl-N-[(methyl carbamoyl)oxy]thioacetimidate) is sold under the trade name Lannate 90® (Dupont Co., USA) and commercially available as water-soluble powder containing 90% of the active ingredient.

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 2 weeks of adaptation, 20 rats were treated at 2 mg/kg methomyl 3 times weekly for 3 months. The calculated dose of methomyl was dissolved in 1 mL of distilled water and given to each animal by a stomach tube. At the same time, 20 rats received only distilled water and were kept as controls.

After 3 months of methomyl exposure, tissue specimens were obtained from liver, kidneys, heart, lungs, testis and spleen for histopathological examination. Specimens were fixed in 10% neutral buffered formalin, dehydrated in a graded alcohol series, cleared with methyl benzoate and embedded in paraffin wax. Sections of 4 μm were cut and stained with haematoxylin/eosin for light microscopic examination (Bancroft & Stevens, 1990). Stained sections were examined under light microscope (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia C-5060, Japan).

Histopathological examination of HE-stained hepatic sections constantly showed congestion and dilatation of blood vessels (Fig. 1A). In most cases, there were necrobiotic changes of hepatocytes and focal and diffuse activation of Kupffer's cells (Fig. 1B and 1C). Many of intoxicated animals showed apoptotic changes either in the form of early apoptosis where hepatocytes exhibited cytoplasmic and nuclear condensation or apoptotic bodies which were recognized as condensed eosinophilic structures with basophilic nuclear fragments (Fig. 1D). In some cases, there were focal areas of necrosis which appeared infiltrated with Kupffer cells (Fig. 1E) and mid-zonal vacuolar degeneration (Fig. 1F). Hepatocytes in different stages of normal mitosis were increasingly observed in most cases (Fig. 2). These include prophase; condensation of nuclear chromatin and absence of the nucleolus and nuclear membrane (Fig. 2A), metaphase; condensed chromosomes aligned in the middle of the cell before separation into each of the two daughter cells (Fig. 2B), anaphase; each chromatid appeared moving to opposite

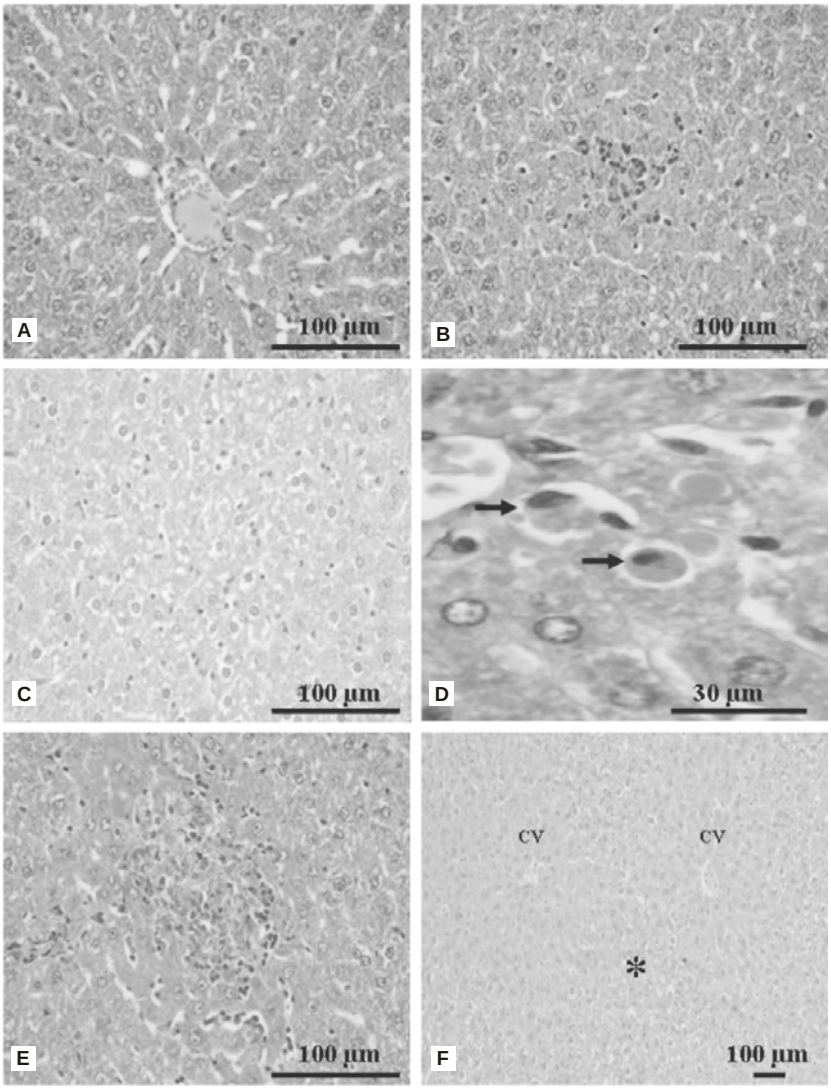

Fig. 1. Representative micrographs for some histopathological changes in the liver as the result of methomyl treatment. A. Dilatation of blood sinusoids and congestion of central vein (CV). B. Focal activation of Kupffer's cells. C. Diffuse activation of Kupffer's cells. D. Apoptotic bodies appeared as condensed eosinophilic structures with basophilic nuclear fragments (arrow) E. Focal area of necrosis infiltrated with Kupffer cells F. Midzonal vacuolar degeneration (asterisk). Haematoxylin/eosin staining; bar=100 µm for 1A, B, C, E and F; bar=30 µm for 1D.

poles of the cell (Fig. 2C) and telephase; separate groups of chromosomes at each pole were formed (Fig. 2D).

Exposure of rats to methomyl (2 mg/kg) markedly affected glomeruli, tubules and interstitium. Glomeruli appeared swollen and filled Bowman's spa-

ces. Glomerular swelling was primarily caused by congestion of glomerular capillaries (Fig. 3A) and thickening of glomerular basement membranes (Fig. 3B). There were also mild proliferation of the glomerular epithelial cells and thickening of the Bowman's capsule (Fig. 3C) and periglomerular fibrosis (Fig. 3D). Renal tubular epithelium appeared swollen and sometimes showed hyaline droplet degeneration and certain degree of necrobiotic changes (data not shown). In some cases, renal tubules showed dysplastic changes characterized by abnormal mitotic figures and nuclear pleomorphism (Fig. 3E). There was also fibroblastic proliferation in the interstitium in some cases (Fig. 3F).

Prominent findings in the lungs as a result of methomyl treatment were necrosis and sloughing of bronchiolar epithelial cells (Fig. 4A) and lymphocytic perivascular accumulations (Fig. 4B). There were also alveolar emphysema, congestion of alveolar capillaries and thickening of alveolar septa as well as haemosiderin-laden macrophages in the alveolar walls and lumens (data not shown).

Methomyl appeared to damage severely testicular tissues. Seminiferous tubules showed extensive necrosis in the seminiferous tubular epithelium with only the basement membrane and Sertoli cells were left (Fig. 4C). Moreover, the lumina of some seminiferous tubules contained

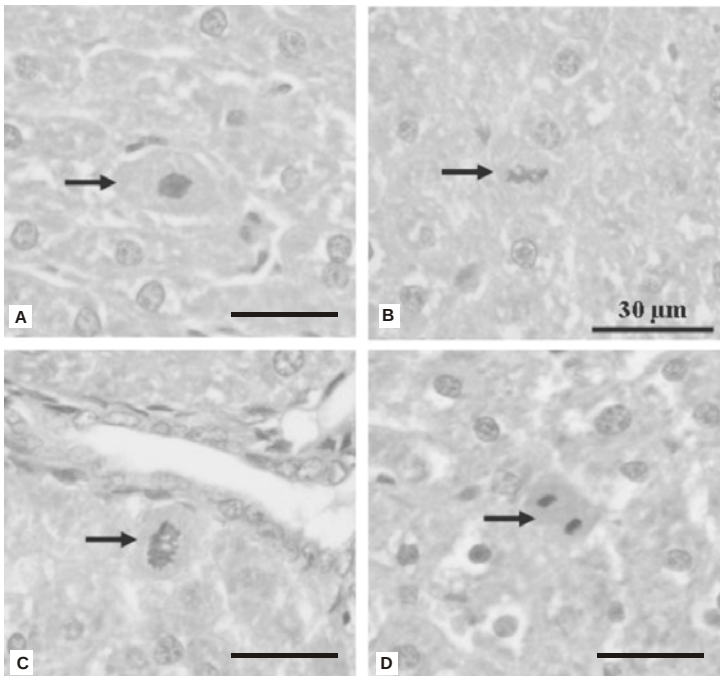

Fig. 2. Representative micrographs for normal mitotic figures in the liver of methomyl-treated animals. A. Prophase; condensation of nuclear chromatin and absence of the nucleolus and nuclear membrane (arrow). B. Metaphase; condensed chromosomes aligned in the middle of the cell before separation into each of the two daughter cells (arrow). C. Anaphase; each chromtid appeared moving to opposite poles of the cell (arrow). D. Telephase; separate groups of chromosomes at each pole were formed (arrow). Haematoxylin/eosin staining; bar=30 µm.

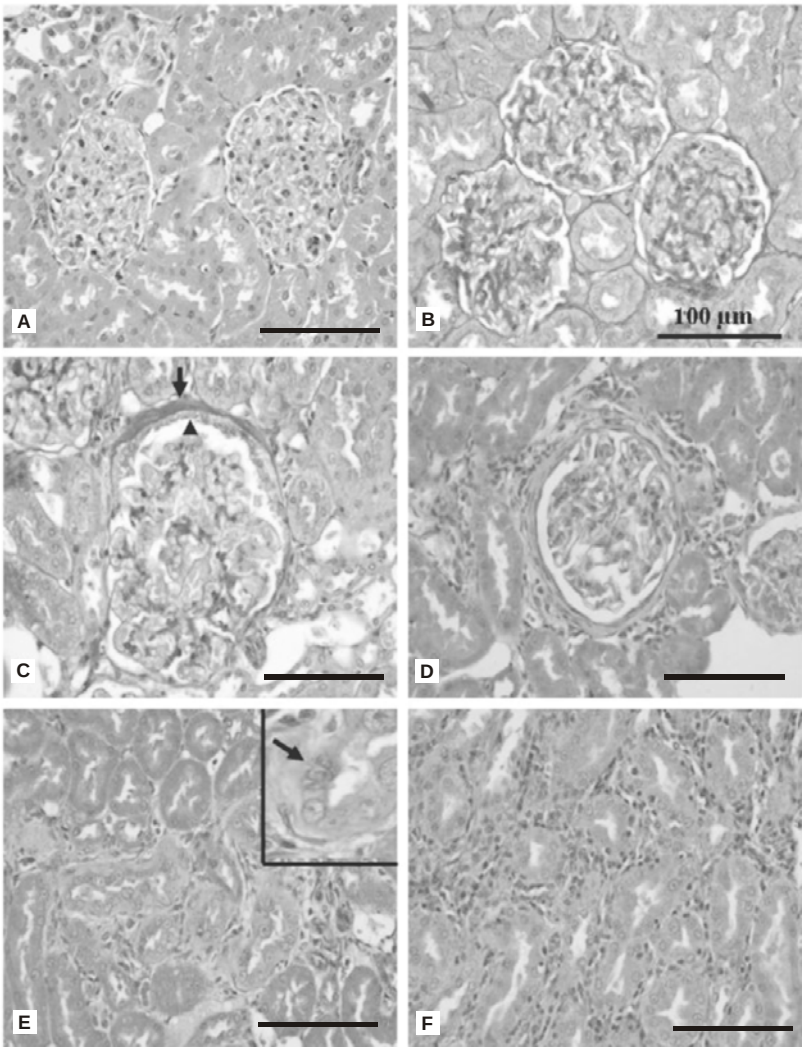

Fig. 3. Representative micrographs for some histopathological changes in the kidney of methomyl-treated animals. A. Swollen glomeruli filled Bowman's spaces due to congestion of glomerular capillaries. B. Swollen glomeruli as the result of thickening of glomerular basement membrane. C. Thickening of Bowman's capsule (arrow) and proliferation of glomerular epithelial cells (arrowhead). D. Peri-glomerular fibrosis. E. Dysplastic changes in some renal tubules characterized by abnormal mitotic figures and nuclear pleomorphism (arrow). F. Fibroblastic proliferation in the interstitium. (A, D, E and F stained with haematoxylin/eosin; B and C stained with PAS; bar=100 µm.

necrosed and sloughed cells (Fig. 4D) and large spermatid giant cells (Fig. 4E).

Splenic lesions as a result of methomyl treatment consisted primarily of lymphocytic depletion. In which, many lymphocytes were lost leaving free areas of fine eosinophilic materials (Fig. 4F).

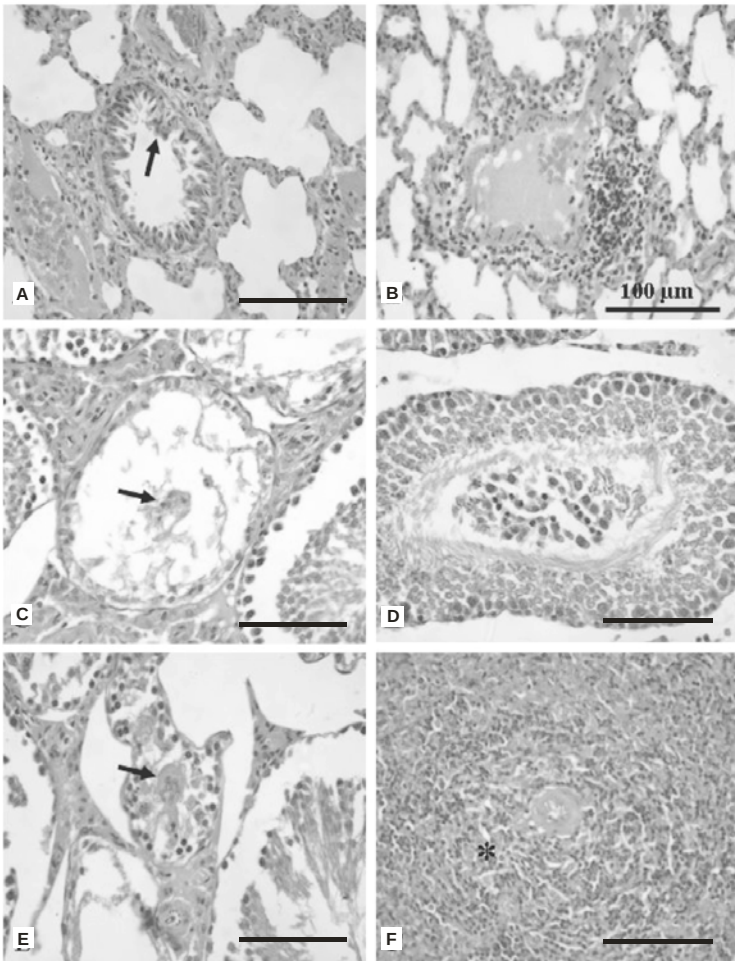

Fig. 4. Representative micrographs for some histopathological changes in lungs, testicles and spleen of methomyl-treated animals. A. Lung showing necrosis and sloughing of bronchiolar epithelial cells (arrow). B. Lung showing perivascualr accumulation of mononuclear cells. C. Testicle showing necrosis of seminiferous tubular cells, only Sertoli cells were spared (arrows). D. Testicle showing sloughing of necrosed cells in the lumen of seminiferous tubule. E) Testicle showing atrophy of seminiferous tubules and formation of giant cell (arrow) F. Spleen showing lymphocytic depletion in a splenic corpousle (asterisk). Haematoxylin/eosin staining; bar=100 µm.

In the present study, it was found that treatment of Sprague-Dawley rats with 2 mg/kg methomyl for three months markedly affected liver, kidneys, lungs, testicles and spleen. In the liver, increased frequency of normal mitosis was the most pronounced finding in most methomyl-treated rats. In this context, Quest *et al.* (1987) reported that administration of methomyl in doses ranging from 50–800 mg/kg for thirteen weeks resulted in dose-related lesions in the liver, characterized

by proliferative changes in hepatocytes consisting of foci of cellular alteration and frequent mitosis with atypical forms. Appearance of atypical forms of mitosis in the study of Quest *et al.* (1987) may be attributed to the higher doses of methomyl (50−800 versus 2 mg/kg in our study). Another frequent liver lesion as the result of the methomyl treatment was increased apoptotic features. Banerjee *et al.* (1999) reported that methomyl exposure promoted oxidative damage of liver cells by enhancing peroxidation of membrane lipids and this might enhance apoptosis. There were also midzonal vacuolar degeneration, necrobiotic changes of hepatocytes and focal areas of necrosis. These lesions were similarly reported in other carbamate toxicities. For example, Muthuviveganandavel *et al.* (2008) and El-Manakhly (1996) observed degeneration and multiple necrotic areas of hepatocytes infiltrated with mononuclear cells as a result of carbendazim and carbosulfan treatment in rats, respectively. Degeneration and necrosis of hepatocytes appeared to be due to vascular changes particularly in the portal vessels. In the kidney, methomyl treatment damaged the glomeruli, the tubules and the interstitium. Similarly, Nariman *et al.* (1995) and Selmanoglu *et al.* (2001) observed proliferation and swelling of glomerular endothelial cells and tubular degeneration, mononuclear cell infiltration and fibrosis in thiodicarb and carbendazim-treated rats, respectively. Dysplastic changes seen in the tubules of some methomyl-treated rats are of great concern. Together with increased frequency of normal mitosis in the liver, they might suggest that methomyl is a potentially carcinogenic. However, there was no evidence of carcinogenicity in both rats and mice fed methomyl for 2 years (EPA, 1987).

Methomyl-induced pulmonary lesions consisted mainly of necrosis and sloughing of bronchiolar epithelial cells, congestion of blood vessels and perivascular accumulation of mononuclear cells. Kidd & James (1991) and El-Khwaga (2005) attributed these pulmonary lesions to the extensive storage of methomyl in the lungs and its excretion in expired air. Testicles showed extensive necrosis in the seminiferous tubular epithelium, atrophy of seminiferous tubules and formation of spermatid giant cells in the tubular lumens. Similarly, Quest *et al.* (1987) found that methyl carbamates caused testicular hypoplasia in F344 rats and Hess & Nakai (2000) reported that carbendazim caused sloughing of elongate spermatids in the tubular lumens in rats. These effects are the same as those of Bretveld *et al.* (2007) who reported that exposure to pesticides was a potential risk factor for subfertility.

Depletion of lymphocytes was the main effect observed in the spleen as the result of methomyl treatment. Similarly, Lohitnavy & Sinhaseni (1998) found that treatment of rats with a single methomyl dose (6–8 mg/kg) reduced spleen weight and splenocyte viability in the spleen.

In conclusion, methomyl was found to be potentially toxic to liver, kidney, lungs, spleen and testicles when applied repeatedly at a dose of 2 mg/kg. The observed hepatic, renal and testicular damages could predispose to hepatic insufficiency, renal failure and impaired fertility in exposed individuals.

REFERENCES

Anonymous, 1986. Council 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. *Official*

Journal of the European Communities, **L358**, 1–29.

Bancroft, J. D. & A. Stevens, 1990. Theory and Practice of Histological Techniques. 3rd edn, Edinburgh, Churchill Livingstone; 1990.

Banerjee, B. D., V. Seth, A. Bhattacharya, S. T. Pasha & A. K. Chakraborty, 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicology Letters*, **107,** 33–47.

Bolognesi, C., 2003. Genotoxicity of pesticides: A review of human biomonitoring studies. *Mutation Research/Reviews in Mutation Research*, **543,** 251–572.

Bretveld, R., M. Brouwers, I. Ebisch & N. Roeleveld, 2007. Influence of pesticides on male fertility. *Scandinavian Journal of Work, Environment & Health*, **33,** 13–28.

Clementi, M., R. Causin, C. Marzocchi, A. Mantovani & R. Tenconi, 2007. A study of the impact of agricultural pesticide use on the prevalence of birth defects in north-east Italy. *Reproductive Toxicology*, **24,** 1–8.

El-Khawaga, O. Y., 2005. Role of selenium on antioxidant capacity in methomyl-treated mice. *Journal of Physiology and Biochemistry*, **61,** 501–506.

El-Manakhly, E. M., 1996. Therapeutic effect of selenium in rabbits intoxicated with the pesticide carbosulfan: Some pathologic and biochemical studies. *Egyptian Journal of Comparative Pathology and Clinical Pathology*, **9**, 89–100.

Farré, M., J. Fernandez, M. Paez, L. Granada, L. Barba, H.M. Gutierrez, C. Pulgarin & D. Barceló, 2002. Analysis and toxicity of methomyl and ametryn after biodegradation. *Analytical and Bioanalytical Chemistry*, **373,** 704–709.

Garming, H. & H. Waibel, 2008. Pesticides and farmer health in Nicaragua: A willingness-to-pay approach to evaluation. *The European Journal of Health Economics: Health Economics in Prevention and Care,* http://www.springerlink.com/content/dw621054m3j65193/fulltext.html (12 January 2009 date last accessed).

Hess, R. A. & M. Nakai, 2000. Histopathology of the male reproductive system induced by the fungicide benomyl. *Histology and Histopathology*, **15,** 207–224.

Ibitayo, O. O., 2006. Egyptian farmers' attitudes and behaviors regarding agricultural pesticides: implications for pesticide risk communication. *Risk Analysis*, **26,** 989–995.

Kidd, H. & D. R. James, 1991. The Agrochemicals Handbook. 3rd edn, Royal Society of Chemistry Information Services, Cambridge, UK.

Lohitnavy, O. & P. Sinhaseni, 1998. Increase in lactate dehydrogenase isoenzyme-4 and splenocyte toxicity in methomyl-treated rats. *Arhiv za Higijenu Rada i Toksikologiju*, **49,** 231–238.

London, L. & H. A. Rother, 2000. People, pesticides, and the environment: Who bears the brunt of backward policy in South Africa? *New Solutions,* **10,** 339–350.

Mansour, S. A., 2004. Pesticide exposure – Egyptian scene. *Toxicology*, **198,** 91–115.

Muthuviveganandavel, V., P. Muthuraman, S. Muthu & K. Srikumar, 2008. Toxic effects of carbendazim at low dose levels in male rats. *The Journal of Toxicological Sciences*, **33,** 25–30.

Nariman, A. R., A. R. Ahmed, H. M. Amira & M. I. Dessouky, 1995. Serum biochemical and histopathological changes associated with repeated exposure of rats to Thiodicarb insecticide. *Egyptian Journal of Comparative Pathology and Clinical Pathology*, **8,** 79–85.

Quest, J. A., P. C. Chan, D. Crawford, K. K. Kanagalingam & W. C. Hall, 1987. Thirteen-week oral toxicity study of methyl carbamate in rats and mice. *Fundamental and Applied Toxicology,* **8,** 389–399.

Selmanoglu, G., N. Barlas, S. Songür & E. A. Koçkaya, 2001. Carbendazim-induced haematological, biochemical and histopathological changes to the liver and kidney

of male rats. *Human & Experimental Toxicology*, **20,** 625–630.

US Environmental Protection Agency, 1987. Health Advisory Summary. Methomyl. Washington, DC, pp. 3–40, http://www.epa.gov/iris/subst/0069.htm (12 November 2008 date last accessed).

WHO, 1996. Environmental health criteria; Methomyl insecticide. World Health Organization,Geneva, pp. 1–96. http://www.inchem.org/documents/ehc/ehc/ehc178.htm (12 November 2008 date last accessed).

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