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Cyto- and Genotoxic Potentials of Carbamates in Human Lymphocytes

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Abstract

Carbamate pesticides are extensively used in agricultural areas due to their broad spectrum and low bioaccumulation. Although it is well-known that their toxicity occurs in the nervous system with reversible acetylcholine esterase inhibition and acetylcholine accumulation in synapses, knowledge regarding their adverse effects on nontarget systems is still limited, especially regarding their DNA-damaging properties. The toxic potential of the commonly used carbamates (aldicarb, carbaryl, methiocarb, and propoxur) have been investigated in freshly isolated human peripheral lymphocytes at the exposure concentrations of 0.5–25.0 μM. The carbamates were found to disturb the membrane permeability but were not harmful to the mitochondrial and lysosomal functions. Besides, they induced DNA damage. Except methiocarb, the DNA-damage for all carbamates was dose-dependent. The cyto- and geno-toxic effects of carbamates should raise concern about the safety associated with their applications in consumer products.

Keywords: Aldicarb, Carbaryl, Cytotoxicity, Genotoxicity, Human Lymphocytes, Methiocarb, Propoxur

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1. Introduction

Pesticides are widely used against undesirable pests, plants, and diseases that reduce the quality and safety of agricultural products. Pesticides are low cost, easy to apply and have beneficial effects; however, their wide and incorrect use might cause harmful effects on the ecosystems, humans, and wildlife (De Sousa G *et al.,* 1997). Therefore, the evaluation of toxic effects of pesticides is urgently needed (Bolognesi, 2003).

After discovery of the environmental fate of organochlorine and irreversible acetylcholinesterase (AChE) inhibition of organophosphate insecticides, carbamate pesticides have become preferred in agriculture due to their broad spectrum and low bioaccumulation (Mitra *et al.,* 2011). On the other hand, carbamates have been extensively applied in agricultural areas and are often present in low residue levels in food, groundwater, and air (Fernández *et al.,* 2000). The primary effects of carbamates are elicited by their reversible inhibition of AChE activity at the nerve terminal (Mitra *et al.,* 2011). Although it is well-known that their toxicity occurs in the nervous system, knowledge of their adverse effects on nontarget systems is still limited. In particular, there are controversial studies about their DNA-damaging properties (Gupta and Milatovic, 2011). Therefore, there are increasing concerns about their impact on the ecosystem, human health, as well as wildlife (Maran *et al.,* 2010).

Aldicarb, carbaryl, methiocarb, and propoxur, the most widely used carbamate pesticides, have been associated with many hazardous effects in the liver, kidney, lungs, and heart, as well as hormonal disorders and skin diseases (Klotz *et al.,* 1997; Samir *at al.,* 2000). They are moderately to extremely toxic via oral exposure and are probable human carcinogens by the U.S. Environmental Protection Agency (US EPA, 1997). Due to their toxic effects, activity in the last decade has sought to control or

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ban their use in Europe and elsewhere (Martínez-Haro *et al.,* 2008). However, they are still commercialized products for agricultural use in many countries including Turkey. By the Joint FAO/WHO Meeting on Pesticide Residues, it has been determined that acceptable daily intake levels are 0.003, 0.008, 0.02, and 0.02 mg/kg bw/day for aldicarb, carbaryl, methiocarb, and propoxur, respectively (JMPR, 1989; JMPR, 2015; JMPR, 2015).

In the present study, the toxic potential of four carbamate pesticides (aldicarb, carbaryl, methiocarb, and propoxur) were investigated using mitochondrial succinate dehydrogenase (MTT), Lactate Dehydrogenase (LDH) and Neutral Red Uptake (NRU) cytotoxicity and comet genotoxicity assays in human lymphocytes.

2. Materials and Methods

2.1 Reagents

Aldicarb, carbaryl, methiocarb and propoxur, Pestenal^{Ò}, are analytical standard (purity 99%), were obtained from Riedel-de Haën (Seelze, Germany). Their stock solutions were prepared by dissolving in Dimethyl Sulfoxide (DMSO). Histopaque® - 1077, and other chemicals were analytical reagent grade obtained from Sigma-Aldrich Chemicals Co (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Cell culture media and all other supplements were purchased from Wisent Bioproducts (Canada), and sterile plastic materials were purchased from Greiner (Frickenhausen, Germany).

2.2 Lymphocytes Preparation and Exposure

Heparinized whole blood was collected from four 25-45 years old, nonsmoking, healthy female donors. The blood samples were diluted 1:1 with cold Phosphate Buffer Solution (PBS, Ca-Mg free, pH 7.2), and lymphocytes were isolated based on density gradient principles via Histopaque® - 1077. The isolated lymphocytes were diluted in RPMI 1640 medium and used immediately under dim light. The diluted lymphocytes (105 cells/mL) in culture medium incubated in various concentrations of aldicarb, carbaryl, methiocarb, and propoxur. The exposure concentrations were determined to be 0.5, 2.5, 5.0, and 25.0 µM in 0.1% DMSO. After 30 min of incubation at 37°C, the cyto- and geno-toxic potentials of the substances were evaluated with various assays.

2.3 Cytotoxicity (Mitochondrial Succinate Dehydrogenase, Neutral Red Uptake and Lactate Dehydrogenase Assays)

Cytotoxicity was determined via several methods based on different cellular mechanisms depending on the damaged region of cells. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) is a water soluble yellow-colored salt reduced by the mitochondrial succinate dehydrogenase to form an insoluble purple formazan product. Mitochondrial succinate dehydrogenase is only active in viable cells; thus, color changes by activity of the enzyme can be used as a cytotoxicity endpoint (Alley *et al.,* 1988; Van Meerloo *et al.,* 2011). Neutral red is a weak cationic dye that accumulates in lysosomes by nonionic passive diffusion and binds to anionic and/or phosphate groups of the lysosomal matrix by electrostatic hydrophobic bonds. In the NRU assay, lysosomal integrity is used as an indicator of cell viability by up taking neutral red dye into cells (Repetto *et al.,* 2008). In the LDH assay, extracellular LDH activity is measured as a surrogate for membrane integrity. In damaged cells, extracellular medium contains LDH, and in the presence of LDH, NAD is reduced to NADH via conversion of lactate to pyruvate. Thus, formation of NADH in damaged cells causes a change in absorbance that allows us to determine cell viability (Fotakis and Timbrell; 2006). Optical density was read at 590, 540, and 500 nm for MTT, NRU, and LDH, respectively, using a microplate spectrophotometer system (Epoch, Germany).

In every assay, the unexposed cells were evaluated as negative control. It was calculated the inhibition of enzyme activity observed in cells compared to negative control. Then, the half maximal inhibitory concentration (IC_{50}) was expressed as the concentration of sample caused an inhibition of 50% in enzyme activities in cells. About 1% DMSO and 1% Triton X-100 were used as the solvent and positive controls, respectively. The final concentrations of Triton X-100 were 0.15, 1.5, and 15 µM. The final concentration of DMSO was 0.1%. All concentrations were tested in triplicate in every donor. Each assay was repeated twice.

2.4 Comet Assay

The assay principle is to measure DNA strand breaks in eukaryotic cells lysed with detergent and high salt and embedded in agarose on a microscope slide (Collins,

2004; Speit and Hartmann; 2006). In the study, the final concentrations of hydrogen peroxide (H_2O_2, positive control), DMSO (solvent control), and PBS (negative control) were 50 µM, 0.1%, and 1%, respectively. After 30 min incubation at 37°C, the lymphocytes were centrifuged at 2100 rpm for 10 min and washed with PBS. The viability of cells was determined with trypan blue dye exclusion test, and cell viability was > 80% in all concentrations. Briefly, 100 µL of cells were mixed with 100 µL prewarmed low-melting point agarose (0.65% in PBS). Then, this mixture was layered on conventional microscope slides precoated with normal melting point agarose (1.5% in distilled water) and covered with a cover slip. The slides were placed in the refrigerator for solidification. The cover slips were removed, and the slides were incubated for 1 h at 4°C in lysis solution (2.5 M NaCl, 100 mM EDTA, and 10 mM tris-HCl, pH 10) extemporarily added with 10% DMSO and 1% Triton X-100. The slides were denatured for 20 min in cold fresh electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) at 4°C to unravel the DNA stands, and electrophoresis was performed at 4°C for 20 min (20 V/300 mA). After electrophoresis, the slides were neutralized with 0.4 M tris-HCl buffer (pH 7.5) 3 times for 5 min. One hundred cells were scored per concentration under a fluorescent microscope (Olympus BX53, Olympus, Tokyo, Japan) at ×400 magnification via an automated image analysis system (Comet Assay IV, Perceptive Instruments, Suffolk, UK) just after DNA staining with ethidium bromide (20 mg/mL). The DNA damage to individual cells was expressed as a percentage of DNA in the comet tail (tail intensity, T_{DNA}%). All concentrations were tested in every donor in triplicates to ensure reproducibility.

2.5 Statistical Analysis

The significance of the differences between control and the carbamates-treated lymphocytes was calculated via one-way analysis of variance and Dunnett's *t*-test. The analysis was performed with Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The results were expressed as a mean ± standard error and standard deviation $P < 0.05$ was defined to be significant.

3. Results and Discussion

Carbamates are capable of inducing oxidative stress by overproduction of reactive oxygen species. Oxidative stress causes damage to cellular macromolecules and is also involved in the intracellular signaling mechanisms that determine the cell's final fate (Abdollahi *et al.,* 2004). Lipid peroxidation has been suggested to be one of the molecular mechanisms involved in the cytotoxicity of carbamates (Seth *et al.,* 2000). Similarly, we observed that carbamates disturbed the membrane permeability and were not harmful to the mitochondrial and lysosomal functions in human lymphocytes. The IC_{50} values of aldicarb, carbaryl, methiocarb, and propoxur were 4.20, 3.58, 2.89, and 13.88 µM, respectively, by LDH assay. As shown in Table 1, the LDH assay appeared less sensitive for propoxur than the other carbamates. Furthermore, carbaryl-induced cell death could involve mitochondrial and lysosomal pathways as well as membrane damage.

Maran *et al.* (2010) reported that aldicarb and propoxur had cytotoxic effects via the loss of lysosomal activity and the alteration of the energetic cell metabolism via mitochondrial reductase enzymes, respectively, in Chinese hamster ovary (CHO) cells. The IC_{50} values for aldicarb and propoxur by the NR assay were 116.1 and 234.8 µM, respectively. The IC_{50} values via the MTT assay were 153.9 and 200.9 µM, respectively (Maran *et al.*, 2010). Similarly, Schmuck and Mihail (2004) observed that propoxur exerted moderate effects on the inner mitochondrial membrane potential in primary cells from brain tissue of fetal rats. Wang *et al.*, (1998) reported that aldicarb was not cytotoxic to Chinese hamster lung fibroblast V79 cells at ≤2.10 µM. However, treatment with aldicarb enhanced proliferation of V79 cells from 2% to 4% at every dosage except 0.26 µM. The IC_{50} value was 250 µM for carbaryl in human hepatocytes (De Sousa G *et al.,* 1997). The IC_{50} values of carbaryl were 264, > 100, and > 100 µM in hepatocytes, HepG2, and HaCa T cell lines, respectively, by MTT assay after 48 h of exposure (Delescluse *et al.,* 1998). These studies show that *in vitro* assays give different responses in a toxicological evaluation of cells isolated from different tissues and organs (Ruiz *et al.,* 2007).

The genotoxicity of carbamates has been studied by many colleagues. It is of great concern that long-term carbamate exposure causes certain types of cancer via carcinogenic and mutagenic properties including DNA damage (Kornuta *et al.,* 1996; Waters *et al.,* 1982). Investigations using different endpoints, however, often give contradictory results. Surprisingly, there has been no investigation into the effects of these four carbamates on human lymphocytes. Therefore, this study might be useful in determining their toxicological effects and taking precautions regarding their consumption. As indicated in Table 2, four carbamates caused an increase in T_{DNA}% at a concentration of 0.50-25.00 μM. This induced DNA damage in up to 75% of viable cells. Except methiocarb, the DNA-damage for all carbamates was observed in a dose-dependent manner. For methiocarb and propoxur, the increase was statistically significant ($P < 0.05$) at the studied concentration. In Figure 1, DNA damage occurred in human lymphocytes exposed the carbamates can be observed under fluorescent microscope via Comet Assay IV automated image analysis system.

Table 1. Cell death in human lymphocytes exposed to the carbamates

Concentration (μM)	Cell death %* (±SD)			
	Aldicarb	Carbaryl	Methiocarb	Propoxur
MTT assay				
0.5	≤5.0	≤5.0	≤5.0	≤5.0
2.5	≤5.0	≤5.0	≤5.0	≤5.0
5.0	≤5.0	≤5.0	≤5.0	≤5.0
25.0	≤5.0	42.1 (±1.6)	23.9 (±2.1)	≤5.0
LDH assay				
0.5	46.8 (±1.5)	47.6 (±1.8)	46.2 (±2.4)	46.8 (±1.8)
2.5	44.6 (±2.5)	44.6 (±2.1)	47.8 (±3.2)	48.8 (±2.3)
5.0	48.2 (±2.9)	52.6 (±1.6)	51.4 (±1.6)	50.1 (±3.1)
25.0	51.2 (±3.6)	74.4 (±3.2)	83.4 (±2.9)	72.8 (±3.6)
NRU assay				
0.5	22.6 (±1.2)	13.9 (±2.1)	≤5.0	15.1 (±1.3)
2.5	20.1 (±1.6)	18.3 (±2.2)	12.3 (±1.2)	21.2 (±1.4)
5.0	23.3 (±1.9)	21.2 (±2.1)	14.3 (±1.1)	18.3 (±2.1)
25.0	25.6 (±2.1)	33.1 (±2.6)	21.3 (±1.5)	16.1 (±1.8)

*The viability value was 95% for negative control (PBS 1%). The percentage of cell death was ≤80% for positive control. PBS: Phosphate buffer solution, MTT: Mitochondrial succinate dehydrogenase assay, LDH: Lactate dehydrogenase assay, NRU: Neutral red uptake assay, SD: Standard deviation

Concentration (μM)	Aldicarb		Carbaryl		Methiocarb		Propoxur	
	T_{DNA}% (±SE)	P	T_{DNA}% (±SE)	P	T_{DNA}% (±SE)	P	T_{DNA}% (±SE)	P
0.5	26.5 (±4.6)	0.335	4.7 (±1.8)	0.979	58.3 (±8.8)	0.003	30.8 (±9.2)	0.001
2.5	18.6 (±6.1)	0.772	19.6 (±4.9)	0.930	51.8 (±11.6)	0.003	38.5 (±8.6)	0.001
5.0	33.8 (±5.9)	0.120	36.4 (±11.3)	0.581	51.3 (±10.7)	0.006	48.6 (±11.2)	0.001
25.0	34.6 (±4.9)	0.199	65.9 (±15.7)	0.035	46.4 (±9.8)	0.001	64.9 (±10.7)	0.001
				T_{DNA}% (±SE)				
PBS (1%)				0.6 (±0.26)				
DMSO (0.1%)				1.8 (±0.45)				
H_2O_2 (50 μM)				24.7 (±2.8)				

*P≤0.05 were selected as the levels of significance by one-way ANOVA Dunnett t-test. SE: Standard error, PBS: Phosphate buffer solution, DMSO: Dimethyl sulfoxide, H_2O_2: Hydrogen peroxide

Aldicarb was neither mutagenic in *Salmonella* nor in *Escherichia coli* WP2 uvrA; however, it induced significant increase in Sister Chromatid Exchange (SCE) and chromosomal aberrations in human lymphocytes (Bolognesi *et al.,* 2004; Dulout *et al.,* 1985; Dunkel *et al.,* 1985; Cid and Matos, 1984; Cid and Matos, 1987; Zeiger *et al.,* 1988). Bolognesi *et al*. (2004) showed that aldicarb caused no increase in micronuclei (MN) frequencies in the peripheral blood lymphocytes of floriculturists versus healthy control subjects. Carbaryl was found to enhance SCE but not hypoxanthine:guanine phosphoribosyl transferase gene mutation (Onfelt and Klasterska, 1984) or chromosome aberration induction (Dzwonkowska and Hübner, 1986).

A study performed in a Mexican floriculture worker group indicated carbaryl was genotoxic in MN (Gómez-Arroyo *et al.,* 2000). As to methiocarb, the *in vitro* study of Murli (1990) is to date the only study showing positive results for inducing chromosomal aberrations in CHO cells at the dose levels tested under both activated and nonactivated assay conditions. Methiocarb induced chromosomal aberrations in the peripheral lymphocytes of 29 male agricultural workers occupationally exposed to several pesticides (Carbonell *et al.,* 1995). Gonzales *et al*. (1990) reported that propoxur and its nitroso derivative increased the frequencies of SCE and MN in human lymphocytes *in vitro*. However, propoxur showed no significant induction of chromosome aberrations and MN in mice (Vasudev and Krishnamurthy, 1994).

Undeger and Basaran (2005) observed that propoxur at 250.0, 500.0, and 1000.0 μM significantly increased DNA damage in human lymphocytes. Pandey and Guo (2014) used the comet assay to show weak genotoxic effects and statistically significant DNA damage in flounder gill cells

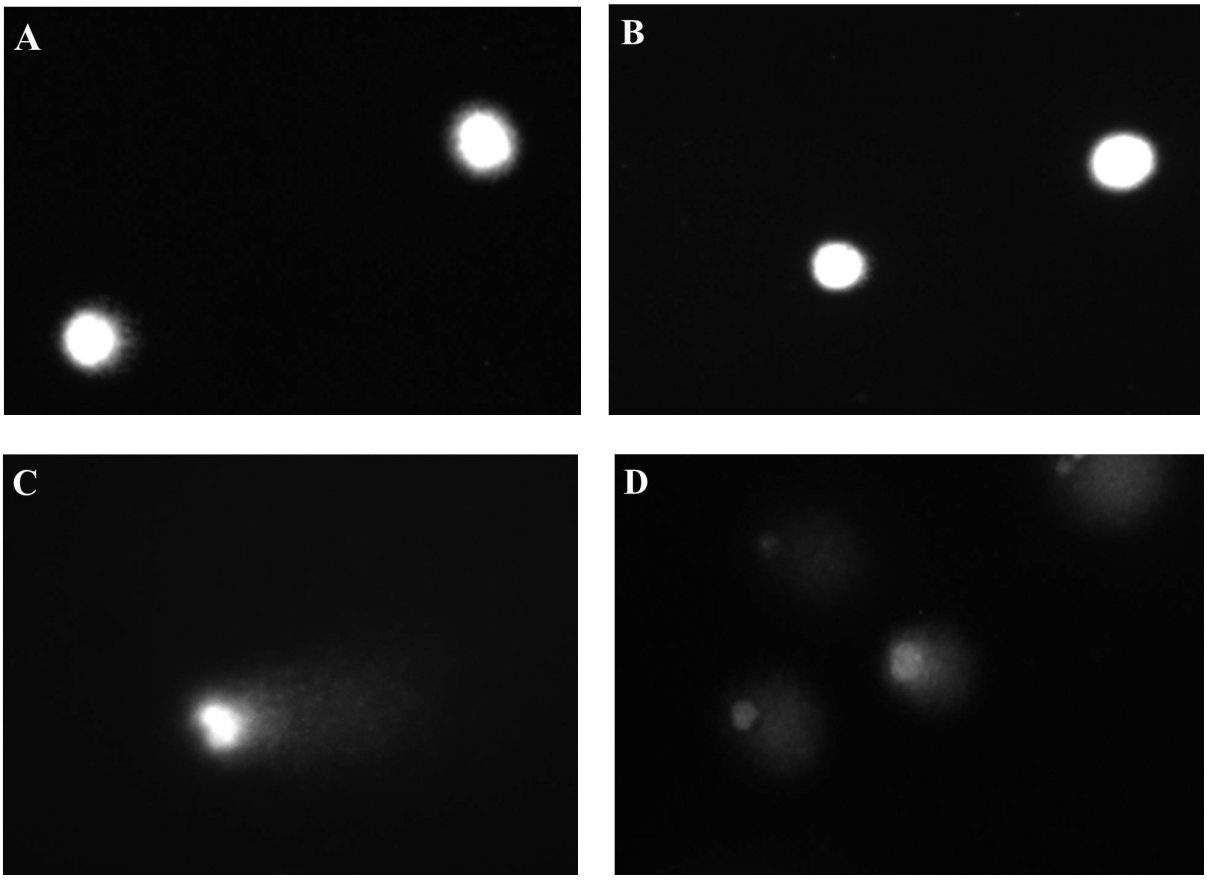


Figure 1. Comet images of human lymphocytes exposed the carbamates at ×400 magnification under fluorescent microscope via Comet Assay IV automated image analysis system after staining by ethidium bromide. **(a)** Solvent control (dimethyl sulfoxide, 0.1%). **(b)** negative control (phosphate buffer solution, 1%). **(c)** The low level of DNA damage observed (aldicarb, 2.5 μM). **(d)** The high level of DNA damage observed (carbaryl, 25 μM).

exposed to the highest tested concentration of 358.85 μM propoxur ($P < 0.05$). Propoxur exerted obvious acute toxic effects on the survival, spontaneous movement, hatching, heart rate as well as the development (yolk and pericardial sacedema) of zebrafish embryos in both time- and concentration-dependent manner (≥480.0 μM).

Recently, the sub-lethal toxicity and *in vivo* genotoxicity of propoxur in *Cyprinus carpio* were evaluated by hematological/biochemical biomarkers and histopathological studies (Gül *et al.,* 2012). Wang *et al.* (1998) found that propoxur has a lower cytotoxic effect than its N-nitroso derivative in V79 and primary rat tracheal epithelial cells. However, no mutagenic effects of propoxur were recorded on either of these cells. Maran *et al.* (2010) reported that propoxur inhibited the growth of the CHO cell line.

4. Conclusion

Our results suggest that carbamate pesticides induce cyto- and geno-toxicity. There is an urgent need for safety measures to prevent toxic exposure to humans and agricultural species.

5. Acknowledgments

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6. References

1. De Sousa G, Fontaine F, Pralavorio M, Botta-Fridlund D, Letreut Y, Rahmani R. Insecticide cytotoxicity and CYP1A1/2 induction in primary human and rat hepatocyte cultures. Toxicol in Vitro. 1997; 11:451–7.
2. Bolognesi C. Genotoxicity of pesticides: A review of human biomonitoring studies. Mutat Res. 2003; 543:251–72.
3. Mitra A, Chatterjee C, Mandal FB. Synthetic chemical pesticides and their effects on birds. Res J Environ Toxicol. 2011; 5:81–96.
4. Fernández M, Picó Y, Mañes J. Determination of carbamate residues in fruits and vegetables by matrix solid-phase dispersion and liquid chromatography-mass spectrometry. J Chromatogr A. 2000; 871:43–56.
5. Gupta RC, Milatovic D. Toxicity of organophosphates and carbamates. Marrs TC, editor. Mammalian Toxicology of Insecticides. Cambridge, United Kingdom: Royal Society of Chemistry Publication; 2011. p. 104–36.
6. Maran E, Fernández-Franzón M, Font G, Ruiz MJ. Effects of aldicarb and propoxur on cytotoxicity and lipid peroxidation in CHO-K1 cells. Food Chem Toxicol. 2010; 48:1592–6.
7. Klotz DM, Arnold SF, McLachlan JA. Inhibition of 17 beta-estradiol and progesterone activity in human breast and endometrial cancer cells by carbamate insecticides. Life Sci. 1997; 60:1467–75.
8. Samir AMZ, Eman GEH, Talaat EIA, Somaia ZAR. Carbamate toxicity and protective effect of vit. A and vit. E on some biochemical aspects of male albino rats. Egypt J Hosp Med. 2000; 1:60–77.
9. US EPA (U.S. Environmental Protection Agency), 1997. Reregistration Eligibility Decision (RED). EPA 738-R-97-009. US EPA, Washington, DC, USA. Available from: http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showRedDocs
10. Martínez-Haro M, Mateo R, Guitart R, Soler-Rodríguez F, Pérez-López M, María-Mojica P, et al. Relationship of the toxicity of pesticide formulations and their commercial restrictions with the frequency of animal poisonings. Ecotoxicol Environ Saf. 2008; 69:396–402.
11. JMPR (The Joint FAO/WHO Meeting on Pesticide Residues). Evaluations of Pesticide Residues in Food-1989 (Part II, Toxicology). FAO Plant Production and Protection Paper 100/2, FAO, Rome; 1989.
12. JMPR (The Joint FAO/WHO Meeting on Pesticide Residues). Pesticide Residues in Food 2006, Aldicarb. Available from: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/JMPRrepor2006.pdf
13. JMPR (The Joint FAO/WHO Meeting on Pesticide Residues). Pesticide Residues in Food 2006, Methiocarb; 2006. Available from: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/JMPR05report.pdf
14. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, et al. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 1988; 48:589–601.
15. Van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: The MTT assay. Methods Mol Biol. 2011; 731:237–45.
16. Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat Protoc. 2008; 3:1125–31.
17. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett. 2006; 160:171–7.
18. Collins AR. The comet assay for DNA damage and repair: Principles, applications, and limitations. Mol Biotechnol. 2004; 26:249–61.
19. Speit G, Hartmann A. The comet assay: A sensitive genotoxicity test for the detection of DNA damage and repair. Methods Mol Biol. 2006; 314:275–86.
20. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: A review. Med Sci Monit. 2004; 10:RA141–7.

21. Seth V, Banerjee BD, Bhattacharya A, Chakravorty AK. Lipid peroxidation, antioxidant enzymes, and glutathione redox system in blood of human poisoning with propoxur. Clin Biochem. 2000; 33:683–5.
22. Schmuck G, Mihail F. Effects of the carbamates fenoxycarb, propamocarb and propoxur on energy supply, glucose utilization and SH-groups in neurons. Arch Toxicol. 2004; 78:330–7.
23. Wang TC, Chiou CM, Chang YL. Genetic toxicity of N-methylcarbamate insecticides and their N-nitroso derivatives. Mutagenesis. 1998; 13:405–8.
24. Delescluse C, Ledirac N, de Sousa G, Pralavorio M, Lesca P, Rahmani R. Cytotoxic effects and induction of cytochromes P450 1A1/2 by insecticides, in hepatic or epidermal cells: Binding capability to the Ah receptor. Toxicol Lett. 1998; 96-97:33–9.
25. Ruiz MJ, Festila LE, Fernández M. Comparison of basal cytotoxicity of seven carbamates in CHO-K1 cells. Toxicol Environ Chem. 2007; 88:345–54.
26. Kornuta N, Bagley E, Nedopitanskaya N. Genotoxic effects of pesticides. J Environ Pathol Toxicol Oncol. 1996; 15:75–8.
27. Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, et al. Study of pesticide genotoxicity. Basic Life Sci. 1982; 21:275–326.
28. Bolognesi C, Landini E, Perrone E, Roggieri P. Cytogenetic biomonitoring of a floriculturist population in Italy: Micronucleus analysis by Fluorescence In Situ Hybridization (FISH) with an all-chromosome centromeric probe. Mutat Res. 2004; 557:109–17.
29. Dulout FN, Pastori MC, Olivero OA, González Cid M, Loria D, Matos E, et al. Sister-chromatid exchanges and chromosomal aberrations in a population exposed to pesticides. Mutat Res. 1985; 143:237–44.
30. Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al. Reproducibility or microbial mutagenicity assays: II. Testing or carcinogens and noncarcinogens in Salmonella typhimurium and Escherichia coli. Environ Mol Mutagen. 1985; 7:1–51.
31. Cid MG, Matos E. Induction of sister-chromatid exchanges in cultured human lymphocytes by aldicarb, a carbamate pesticide. Mutat Res. 1984; 138:175–9.
32. Cid MG, Matos E. Chromosomal aberrations in cultured human lymphocytes treated with aldicarb, a carbamate pesticide. Mutat Res. 1987; 191:99–103.
33. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen. 1988; 11 Suppl 12:1–157.
34. Onfelt A, Klasterska I. Sister-chromatid exchanges and thioguanine resistance in V79 Chinese hamster cells after treatment with the aneuploidy-inducing agent carbaryl +/- S9 mix. Mutat Res. 1984; 125:269–74.
35. Dzwonkowska A, Hübner H. Induction of chromosomal aberrations in the Syrian hamster by insecticides tested in vivo. Arch Toxicol. 1986; 58:152–6.
36. Gómez-Arroyo S, Díaz-Sánchez Y, Meneses-Pérez MA, Villalobos-Pietrini R, De León-Rodríguez J. Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides. Mutat Res. 2000; 466:117–24.
37. Murli H. Mutagenicity test on H 321 in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese Hamster Ovary (CHO) Cells with a confirmatory assay. Unpublished report no. R 4980 from Hazleton Laboratories America, Inc., Kensingtonb Maryland, USA, 8 March 1990. Submitted to WHO by Bayer AG, Leverkusen, Germany, 1990 [BA; Sub: 11792, Vol. 11].
38. Carbonell E, Valbuena A, Xamena N, Creus A, Marcos R. Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides. Mutat Res. 1995; 344:127–34.
39. Cid MG, Loria D, Matos E. Genotoxicity of the pesticide propoxur and its nitroso derivative, NO-propoxur, on human lymphocytes in vitro. Mutat Res. 1990; 232:45–8.
40. Vasudev V, Krishnamurthy NB. In vivo cytogenetic analyses of the carbamate pesticides Dithane M-45 and Baygon in mice. Mutat Res. 1994; 323:133–5.
41. Undeger U, Basaran N. Effects of pesticides on human peripheral lymphocytes in vitro: Induction of DNA damage. Arch Toxicol. 2005; 79:169–76.
42. Pandey MR, Guo H. Evaluation of cytotoxicity, genotoxicity and embryotoxicity of insecticide propoxur using Flounder Gill (FG) cells and zebrafish embryos. Toxicol In Vitro. 2014; 28:340–53.
43. Gül A, Benli AÇ, Ayhan A, Memmi BK, Selvi M, Sepici-Dinçel A, et al. Sublethal propoxur toxicity to juvenile common carp (Cyprinus carpio L 1758): Biochemical, hematological, histopathological, and genotoxicity effects. Environ Toxicol Chem. 2012; 31:2085–92.