

# Evaluation of geno-toxicity of methyl parathion and chlorpyrifos to human liver carcinoma cell line (HepG2)

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**Abstract** Insecticides and their residues are known to cause several types of ailments in human body. An attempt had been made to assess digitally the geno-toxicity of methyl parathion (MP) and chlorpyrifos (CP) to in vitro-grown HepG2 cell line, with Hoechst 33342 staining, comet, and micronucleus assays. Additionally, “acridine orange/ethidium bromide” (AO/EB) staining was done for the determination of insecticide-induced cytotoxicity, in corollary. Hoechst 33342 staining of cells revealed a decrease in live cell counts at 8–40 mg/L MP and 15–70 mg/L CP. Moreover, nuclear fragmentations in ranges 8 to 40 mg/L MP and 15 to 70 mg/L CP were recorded dependant on individual doses, increasingly with concomitant increases in comet tail length values. DNA fragmentation index measured in comet assays was  $94.3 \pm 0.57$  at 40 mg/L MP and  $93.3 \pm 2.08$  at 70 mg/L CP. Average micronuclei number was  $59.0 \pm 2.00$  at 40 mg/L MP and  $62.6 \pm 1.52$  at 70 mg/L CP, per 1000 cell nuclei, in micronucleus assay. Minimum inhibitory concentration (MIC) values with AO/EB staining for monitoring cytotoxicity were 4 and 10 mg/L for MP and CP, respectively. Lethal concentration<sub>50</sub> (LC<sub>50</sub>) values were 20.89 mg/L MP and 79.43 mg/L CP in AO/EB staining, for cytotoxicity with probit analyses. It was concluded that MP was comparatively more geno-toxic than CP to HepG2 cell. It was discernible that at lower levels of each insecticide, geno-toxicity was recorded in comparison to cytotoxicity.

**Keywords** Methyl parathion · Chlorpyrifos · HepG2 cell line · Geno-toxicity · Comet assay

## Introduction

Methyl parathion (MP) and chlorpyrifos (CP) are organophosphorous insecticides (OPIs) with both narrow and broad spectrum uses in place of carbamates, in controlling pests on vegetables, fruits, crops, and cotton, to state curtly (Nair et al. 2014; Khalil 2015). Usually, recalcitrant residues of OPIs enter into hydrological system (Tariq et al. 2004) with eventual concentration in food crops (Bai et al. 2006; Tabassum et al. 2014). As it is known, human exposure to OPIs and their residues causes neurologic, hematopoietic, cardiovascular, and reproductive adversities and several degenerative diseases, viz., Parkinson’s disease, Alzheimer’s disease, and amyotrophic lateral sclerosis (Betarbet et al. 2000). Additionally, oxidative stress had been reported as a possible mechanism of OPI toxicity in humans (Edwards et al. 2013).

Furthermore, the use of MP exceeds the maximum residue limits in fruits and vegetables, worldwide (Bai et al. 2006; Chowdhury et al. 2014; Sapbamrer and Hongsibsong 2014). MP contaminates dairy products (Mallatou et al. 1997; Melgar et al. 2010; Srivastava et al. 2011) and human milk (Sanghi et al. 2003), ending at serious toxic impacts (Wu et al. 2007). Moreover, humans are usually exposed to MP through respiratory tract, skin, and gastrointestinal tract (Mircioiu et al. 2013). The recalcitrant residue of MP, methyl paraoxon, is toxic (ATSDR 1999) that contaminates the total environment (Sakellarides et al. 2003). Moreover, MP was reported to cause DNA damage and the brain was maximally affected (Ojha et al. 2013).

Moreover, 3,5,6-trichloro-2-pyridinol is the leading residue of CP, posing health hazards to animals and human

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from crops (Marasinghe et al. 2014). Neurotoxicity is a common characteristic feature of exposure to CP (Mattson et al. 1996). Previous studies of in vivo toxicity of CP include neurotoxicity, carcinogenesis, and reproductive toxicity. Several other studies demonstrate that the exposure to CP interferes with the development of mammalian nervous system (Aldridge et al. 2005; Slotkin et al. 2006). The exposure to CP had caused in utero in low birth weights and reduced head circumference of newborns especially for individuals, who have low levels of serum paraoxonase/arylesterase 1 activity (Whyatt et al. 2004; Furlong et al. 2006). However, no evidence of CP induced carcinogenicity had been found by the US EPA, and several human epidemiology studies have suggested possible links between CP and lungs or rectal cancers (Lee et al. 2007). It was reported that male mice treated with 25 mg/kg CP for 4 weeks before mating with untreated females was associated with decreased number of live fetuses (Farak et al. 2010).

The use of cell lines along with bio-monitoring data could enable a proper understanding of environmental metal/chemical toxicity, holistically (Nakadai et al. 2006). Several toxicology test animal systems are undependable, as the exemplary mouse or rabbit or guinea pig systems are not true representative of human body. Therefore, there are large gaps in the mirror of toxicity models with animals and humans (Tzimas et al. 1997); nonetheless, all popular animal models used in toxicology are mammals. Further, the requirement of a high number of animals in toxicity studies is so much that, for myriad of test chemicals like 30,000 or more in number, discourages the use of whole animals in assay systems (Gilbert et al. 2010). Therefore, the use of cell lines in toxicology had been well recognized (Rosler et al. 2004). It is consensus that the animal system has a holistic approach in toxicity studies that is unavailable in cell cultures—a fact that supports the use of whole animal models, which have physiological homeostasis that would suppress an expression of toxicity, on the other hand. When the toxic event crosses a threshold concentration of the chemical in focus only, the toxicity is expressed in animal models. But, in cell line systems, each toxicity level at and beyond the minimum inhibitory concentration (MIC) level is detected. However, whole animal tests can never be adequately standardized like human cellular systems, as the experiment should have growing cells under controlled conditions, for example, in vitro-cultured human cell lines.

Considering toxic effects of insecticides in human body, an attempt had been made to assess digitally the geno-toxicity of MP and CP to in vitro-grown HepG2 cell line, using Hoechst 33342 staining, comet assay, and micronucleus assay. Additionally, acridine orange/ethidium bromide (AO/EB) staining was done for determination of concomitant insecticide-induced cytotoxicity. Such a work would give due explanations to published work on adverse effects of MP and CP on human health. The liver cell line was selected

for work, as liver is the detoxifying organ in human body. Secondly, liver carcinoma cell line grows readily in vitro, lending themselves to assay studies (Plant 2004), and this cell line was used as a model to study effects of xenobiotic activity (Gasnier et al. 2009). The human liver cell line has been chosen since it constitutes the best characterized liver cell line and too is used as a model system to study the xenobiotic activities of OPIs (Westerink and Schoonen 2007). The defined phase I and phase II metabolisms covering a broad set of enzyme forms in HEPG2 cells offer the best hope for reduced false-positive responses in geno-toxicity testing of pesticides (Kirkland et al. 2007). Along with HepG2 and Hep3B cells, several other human liver cell lines have been isolated, without details on geno-toxicity studies; Hep3B cells were compared with HepG2 cells in toxicity studies and were less sensitive in general (Knasmuller et al. 2004).

## Materials and methods

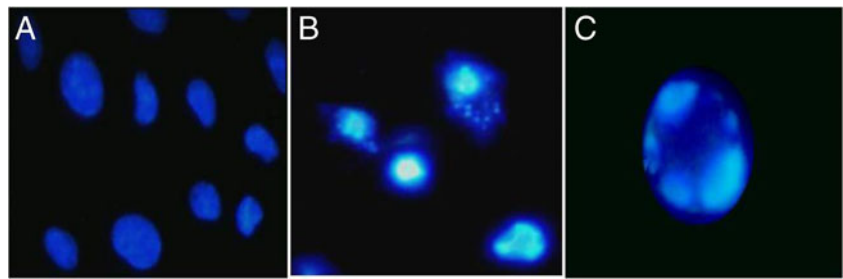
### Pesticides

Insecticides, methyl parathion (*O,O*-dimethyl-*O*-4-*p*-nitrophenyl phosphorothioate; MP), and chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate; CP) used were of commercial formulations, Methyl Parathion WP (Bayer India), and Classic Super EC 50 (Cheminova, Mumbai), respectively. Stock solutions were prepared by mixing/dissolving 100 mg of individual products in aliquots of 100 mL triple-distilled water, for the concentration of 100 mg/L each, and stock solutions were stored at 4 °C, for further use.

### Cell culture

HepG2 cells, obtained from National Centre for Cell Science (NCCS), Pune, were washed with 0.025 % trypsin and 0.02 % EDTA dissolved in phosphate-buffered saline (PBS). The supernatant was removed, and the cells were cultured in Dulbecco's modified Eagle's medium (DMEM low glucose, HiMedia), which contained 15 % fetal bovine serum (FBS, Sigma), 1 % penicillin-streptomycin, and 1 % sodium pyruvate and were loaded onto a six-well culture plate (Tarson); HepG2 cells were seeded at the final level,  $1 \times 10^6$  cells/mL. Plates were incubated at 37 °C in 5 % CO<sub>2</sub> with 85–95 % humidity (Patnaik and Padhy 2015). After an incubation for 48 h, the medium was changed and the cells were exposed to graded concentrations of MP (0, 4, 8, 12, 16, 20, 24, 28, 32, 36, and 40 mg/L) and CP (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 mg/L). The volume of 2 mL was maintained in each well of the plate. Geno-toxicity was studied by Hoechst staining, comet assay, and micronucleus

**Fig. 1** Hoechst staining. Control cells (a). Cells after treated with 20 mg/L MP (b). Cells after treated with 45 mg/L CP (c)



assay. Cytotoxicity was also determined by AO/EB staining as detailed (Patnaik and Padhy 2015).

### Morphological analysis of cell nuclei

Nuclear morphologies of control-, MP-, and CP-treated cells were assessed by fluorescence microscopy after staining with Hoechst 33342 stain (100  $\mu$ L of 5 mg/mL stock solution). Control and treated cells were centrifuged separately, at 1000 rpm for 10 min. The supernatant was removed. Each pellet was treated with the stain at 37  $^{\circ}$ C and 5 % (v/v)  $\text{CO}_2$  for 30 min. The slides were observed. A total of 1000 cells per sample were analyzed, and the percentage of fragmented and condensed nuclei was calculated (Fig. 1). Apoptotic cells were characterized by nuclear condensation of chromatin and/or nuclear fragmentation (Dubrez et al. 1996).

### Comet assay

Single-cell gel electrophoresis was carried out to study DNA damage of the treated HepG2 cells. Cultured cells were harvested and used in the alkaline comet assay technique. After coating slides with 1 % agarose, the slides were allowed for air drying. HepG2 cells treated with different concentrations of MP and CP were centrifuged, and pellets were washed with PBS, and the washed cells were mixed with three times the cell volume with the low-melting-point agarose (LMPA) 1 % in solution state. The mixture of cells and LMPA solution was placed over the agarose-coated slide that was dried at 4  $^{\circ}$ C for 10 min. The slides were further treated with 1 % Triton X-100 and 10 % DMSO, individually, and were placed in the lysing solution of the mixture of 100 mM  $\text{Na}_2\text{EDTA}$ , 10 mM Tris, 2.5 mM NaCl (pH, 10), at 4  $^{\circ}$ C for 1 h. The slides were subsequently removed and placed in the electrophoretic buffer consisting of 1 mM  $\text{Na}_2\text{EDTA}$  and 300 mM NaOH (pH, 13)

for 30 min. The slides were subjected to electrophoresis which was carried out at 1.0 V/cm for 30 min, and the slides were placed in the neutralizing solution (0.4 M Tris HCl, pH, 7.5) for 5 min (Tice et al. 2000). The slides were stained with an aliquot of 40  $\mu$ L of 10  $\mu$ g/mL EB solution. Comets were scored with a fluorescence microscope at  $\times 400$ , and the DNA fragmentation index (DFI) as percent values was presented. Hydrogen peroxide 100- $\mu$ M solution was used as the positive control. Values of comet tail length were measured with the help of an ocular micrometer (Fig. 2).

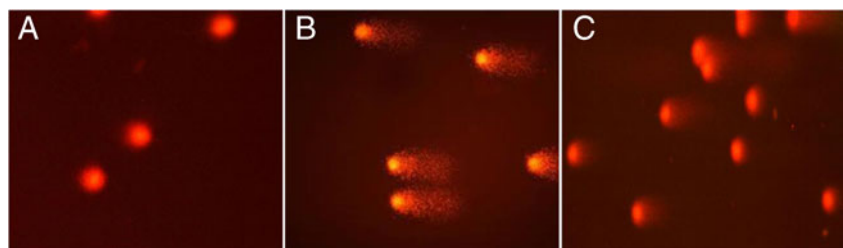
### Micronucleus assay

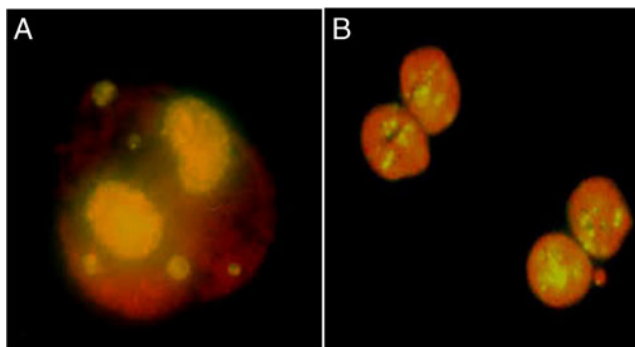
After 24 h, the cells were treated with 100  $\mu$ L of cytochalasin B (6  $\mu$ g/mL) in each well of culture plate. Graded concentrations of MP and CP were simultaneously added, individually. After 44 h, the cells were washed with PBS and trypsinized with 0.025 % trypsin and 0.02 % EDTA solution, and the cells were centrifuged at 1000 rpm for 10 min. The pellet was mixed with 0.075 % KCl solution and incubated at 37  $^{\circ}$ C for 15 min. After centrifugation of the mixture at 1000 rpm for 10 min, the pellet was fixed with glacial acetic acid (acetic acid and methanol in 1:3). The cells were re-centrifuged at 1000 rpm for 10 min. Cells in the pellet were placed in glass slide and stained with AO 40  $\mu$ g/mL. The slides were observed. The micronuclei were counted in 1000 binucleated cells (Fig. 3). The diameter of micronuclei should be under one third that of the main nucleus (Kirsch-Volders et al. 2000) and was clearly distinguishable from the main nucleus with the same staining as the main nucleus.

### AO/EB staining

The AO/EB solution was prepared in PBS at the concentration of 100  $\mu$ g/mL, and HepG2 cells were grown in the presence of

**Fig. 2** Comet assay. Control cells (a). Cells after treated with 20 mg/L MP (b). Cells after treated with 45 mg/L CP (c)





**Fig. 3** Micronucleus assay. Cells after treated with 20 mg/L MP (a). Cells after treated with 45 mg/L CP (b)

graded concentrations of MP and CP. When observed under the fluorescent microscope (Magnus at  $\times 400$ ) after 24 h of incubation, green color indicated live cells, whereas cells with orange and red color were apoptotic and necrotic cells, respectively (Fig. 4). Toxicity values were obtained with concentrations of MP and CP. Percent lethal values were transformed to Finney’s probit values, which were potted against corresponding  $\log_{10}$  values of MP and CP concentrations, detailed (Rath et al. 2011). Probits of observed lethality percentage values are from statistical tables of probit transformation.

**Results and discussion**

**Morphological analysis of cell nuclei**

Nuclear condensation of chromatin and/or nuclear fragmentation was found at 8 mg/L MP and at 15 mg/L CP. Nuclear fragmentation had increased from 8 to 40 mg/L MP and from 15 to 70 mg/L CP (Tables 1 and 2). HepG2 cells had a higher value of nuclear fragmentation at the level of 40 mg/L MP as compared to 70 mg/L CP.

**Comet assay**

Five hundred cultured HepG2 cells were counted for tail length and DFI analysis with hundred cells in one field of view. Average comet tail length was  $1.1 \pm 0.26 \mu\text{m}$  at 8

**Table 1** Lethality values during methyl parathion toxicity to HepG2 cells growing in DMEM, assessed by two methods, AO/EB staining and Hoechst 33342 staining with mean  $\pm$  standard deviation

Methyl parathion (mg/L)	Lethality of cells by AO/EB staining (%)	Lethality of cells by Hoechst staining (%)
0	0	0
4	$4.6 \pm 2.08$	0
8	$12.7 \pm 1.61$	$2.73 \pm 0.57$
12	$19.4 \pm 2.54$	$12.6 \pm 1.16$
16	$34.4 \pm 3.74$	$22.9 \pm 4.80$
20	$48.9 \pm 2.17$	$41.4 \pm 5.15$
24	$58.6 \pm 0.97$	$55.9 \pm 3.27$
28	$66.1 \pm 1.71$	$66.5 \pm 2.17$
32	$75.3 \pm 2.72$	$73.4 \pm 5.46$
36	$87.4 \pm 0.70$	$83.1 \pm 2.68$
40	$94.5 \pm 0.70$	$89.0 \pm 1.70$

*HepG2* human liver carcinoma cell line, *AO/EB* acridine orange/ethidium bromide, *DMEM* Dulbecco’s modified Eagle’s medium

mg/L MP, which increased to  $23.3 \pm 0.56 \mu\text{m}$  at 40 mg/L MP. Also for CP, comet tail average length increased from  $1.4 \pm 0.21$  at 15 to  $23.9 \pm 0.26 \mu\text{m}$  at 70 mg/L concentration. DFI value (%) increased gradually from 8 to 40 mg/L MP (Table 3) and 15 to 70 mg/L CP (Table 4).

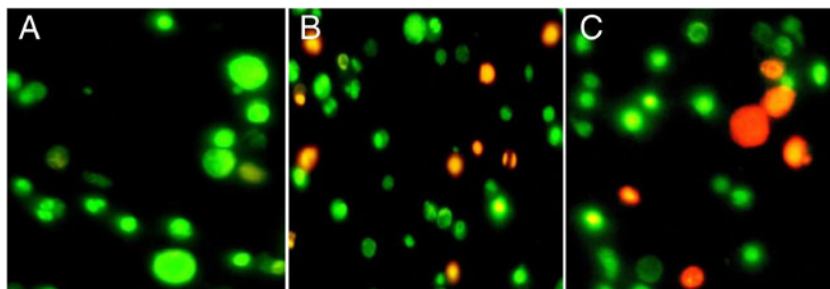
**Micronucleus assay**

One thousand cells were counted to analyze the formation of micronucleus for cells of each gradation of each insecticide. The formation of micronuclei increased accordingly as a function of the used concentrations of MP, with the average value of numbers as  $3.6 \pm 2.08$  at 8 and  $59 \pm 2.0$  % at 40 mg/L MP (Table 3). Similar gradually increasing numbers of the formation of micronuclei at CP gradations recorded were  $4.6 \pm 1.52$  at 15 and  $62.6 \pm 1.52$  % at 70 mg/L CP (Table 4). Thus, CP was found to be less toxic in this assay than MP.

**AO/EB staining**

After treating with different concentrations of MP and CP for 24 h, it was observed that numbers of living cells gradually

**Fig. 4** AO/EB staining. Control cells (a). Cells after treated with 20 mg/L MP (b). Cells after treated with 45 mg/L CP (c)



**Table 2** Average lethality percent values during chlorpyrifos toxicity to HepG2 cells growing in DMEM, assessed by AO/EB staining and Hoechst staining methods with mean  $\pm$  standard deviation

Chlorpyrifos (mg/L)	Average lethality of cells by AO/EB staining (%)	Lethality of cells by Hoechst staining (%)
0	0	0
5	0	0
10	4.5 $\pm$ 0.94	0
15	8.3 $\pm$ 0.97	2.2 $\pm$ 1.00
20	11.5 $\pm$ 0.87	8.4 $\pm$ 0.86
25	14.4 $\pm$ 1.37	11.8 $\pm$ 1.24
30	17.5 $\pm$ 0.77	14.7 $\pm$ 1.05
35	23.8 $\pm$ 2.23	19.1 $\pm$ 1.97
40	28.9 $\pm$ 1.48	32.5 $\pm$ 3.65
45	36.6 $\pm$ 1.35	43.1 $\pm$ 1.46
50	47.1 $\pm$ 2.09	54.8 $\pm$ 2.30
55	58.3 $\pm$ 1.02	60.5 $\pm$ 1.11
60	71.0 $\pm$ 2.32	71.7 $\pm$ 2.64
65	82.1 $\pm$ 2.07	76.8 $\pm$ 1.15
70	92.3 $\pm$ 0.55	82.6 $\pm$ 0.66

HepG2 human liver carcinoma cell line, AO/EB acridine orange/ethidium bromide, DMEM Dulbecco's modified Eagle's medium

decreased. Obviously, numbers of apoptotic cells gradually increased from the level of 4 mg/L (the MIC value) to 40 mg/L (the HPC value) with MP; similar values of toxic concentrations were 10 mg/L (the MIC) to 70 mg/L (the HPC) with CP. Moreover, probit values obtained were used in the ordinate and  $\log_{10}$  values of MP and CP concentrations in the abscissa for the construction of probit plots (Fig. 5), from which it was ascertained that values of  $LC_{25}$ ,  $LC_{50}$ , and

**Table 3** DNA fragmentation index (DFI) and tail length of comets in micrometer and number of micronucleus seen after growth of HepG2 cells in the presence of graded concentrations of methyl parathion with mean  $\pm$  standard deviation

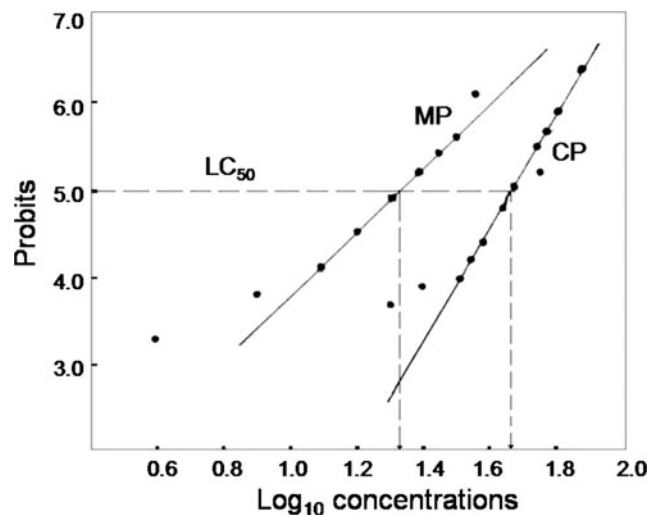
Methyl parathion (mg/L)	DFI (%)	Tail length (%)	Micronucleus seen (/1000 nuclei)
0	0	0	0
4	0	0	0
8	8.0 $\pm$ 1.00	1.1 $\pm$ 0.26	3.6 $\pm$ 2.08
12	19.6 $\pm$ 2.08	2.5 $\pm$ 0.65	7.6 $\pm$ 1.15
16	34.6 $\pm$ 3.21	4.1 $\pm$ 0.32	13.0 $\pm$ 2.00
20	41.3 $\pm$ 2.51	6.3 $\pm$ 0.40	20.6 $\pm$ 1.50
24	55.0 $\pm$ 2.64	9.2 $\pm$ 1.05	21.3 $\pm$ 3.05
28	71.3 $\pm$ 3.05	13.8 $\pm$ 0.40	31.3 $\pm$ 3.05
32	80.0 $\pm$ 1.00	19.2 $\pm$ 0.98	42.0 $\pm$ 1.00
36	91.0 $\pm$ 2.00	21.4 $\pm$ 0.20	53.0 $\pm$ 3.60
40	94.3 $\pm$ 0.57	23.3 $\pm$ 0.56	59.0 $\pm$ 2.00

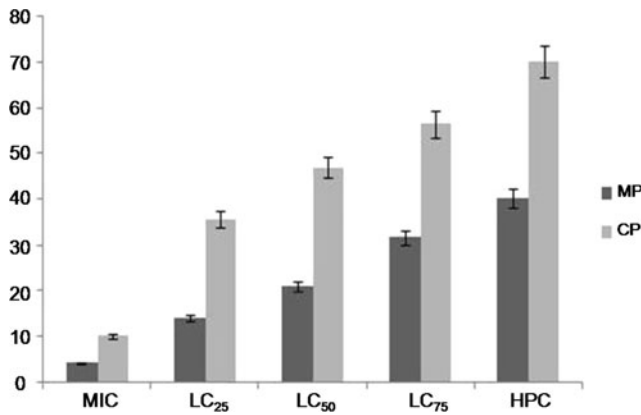
**Table 4** DFI and tail length of comets in micrometer and number of micronucleus seen after growth of HepG2 cells in the presence of graded concentrations of chlorpyrifos with mean  $\pm$  standard deviation

Chlorpyrifos (mg/L)	DFI (%)	Tail length (%)	Micronucleus seen (/1000 nuclei)
0	0	0	0
5	0	0	0
10	0	0	0
15	9.3 $\pm$ 1.50	1.4 $\pm$ 0.21	4.6 $\pm$ 1.52
20	15.3 $\pm$ 2.08	2.6 $\pm$ 0.65	10.0 $\pm$ 1.00
25	25.6 $\pm$ 3.51	3.3 $\pm$ 0.40	14.6 $\pm$ 2.08
30	35.6 $\pm$ 2.08	4.8 $\pm$ 0.41	14.6 $\pm$ 3.05
35	44.0 $\pm$ 2.64	6.4 $\pm$ 0.62	22.0 $\pm$ 1.00
40	53.0 $\pm$ 1.00	7.6 $\pm$ 0.15	29.3 $\pm$ 1.52
45	59.0 $\pm$ 2.00	8.7 $\pm$ 0.92	36.0 $\pm$ 2.64
50	66.0 $\pm$ 4.35	11.8 $\pm$ 1.05	44.3 $\pm$ 1.52
55	77.3 $\pm$ 3.51	15.9 $\pm$ 1.30	52.0 $\pm$ 1.00
60	87.0 $\pm$ 3.60	17.1 $\pm$ 0.87	52.3 $\pm$ 1.52
65	92.0 $\pm$ 2.64	21.9 $\pm$ 1.10	54.6 $\pm$ 4.04
70	93.3 $\pm$ 2.08	23.9 $\pm$ 0.26	62.6 $\pm$ 1.52

$LC_{75}$  were 13.80, 20.89, and 31.62 mg/L MP, respectively. And for CP,  $LC_{25}$ ,  $LC_{50}$ , and  $LC_{75}$  values were 35.48, 46.77, and 56.23 mg/L, respectively (Fig. 6).

It was recorded that each of three assay methods used for monitoring geno-toxicity of HepG2 cells yielded the same toxic range for a chemical, but MP had a higher toxic range than CP. To state in short, the percent lethality values due to MP were  $2.73 \pm 0.57$  and  $89.0 \pm 1.70$  at 8 and at 40 mg/L, respectively, with Hoechst staining. PL values due to CP were

**Fig. 5** Probits of percentage lethality values plotted against  $\log_{10}$  concentrations of MP and CP in the toxicity study of HepG2 cells by AO/EB staining; each line is fitted by eye; three pairs of  $\log_{10}$  concentration values were determined taking probit points, 4.3255 ( $LC_{25}$ ), 5.0000 ( $LC_{50}$ ), and 5.6745 ( $LC_{75}$ )



**Fig. 6** Histogram of MIC, LC<sub>25</sub>, LC<sub>50</sub>, LC<sub>75</sub>, and HPC values corresponding to AO/EB staining (see text for abbreviations) after HepG2 cells treated with MP and CP separately

2.2 ± 1.00 and 82.6 ± 0.66 at 15 and 70 mg/L CP, respectively. Increasing patterns of DFI and tail length values of cells were recorded progressively at graded concentrations 8 to 40 mg/L MP and similarly were observed at 15 to 70 mg/L CP. Similarly, micronuclei numbers gradually increased from 3.6 ± 2.08 to 59.0 ± 2.00 at 8 to 40 mg/L MP, respectively, and 4.6 ± 1.52 to 62.6 ± 1.52 % at 15 to 70 mg/L CP, respectively. During cytotoxicity study, in AO/EB staining, PL of cells was at 4 mg/L (MIC) MP and 10 mg/L (MIC) CP. Gradually, cell viability decreased in the presence of graded concentrations of MP and CP, individually with cell counts 94.5 ± 0.70 at 40 mg/L MP and 92.3 ± 0.55 at 70 mg/L CP as HPC values.

It was reported that MP at 100 µg/mL level caused a reduction of proliferation of HepG2 cells (Grimsrud and Andersen 2010), but this study recorded HPC at 40 mg/L MP. Geno-toxicity testing of 131-radioiodine, a drug used for the treatment of patients with thyroid diseases with cultured human lymphocytes, was described (Hosseinimehr et al. 2013), a work in predictive toxicology. Using 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT) assay and lipid peroxidation assay, MP was reported to reduce gradually the viability and increase in MDA level of HepG2 cells in a dose-dependent manner, with LC<sub>50</sub> value as 26.20 mM, at the 48-h growth (Edwards et al. 2013).

A considerable amount of work on animals and animal cell lines with OPs has been known. A geno-toxicity assessment of the freshwater fish *Channa punctatus* was assessed by micronucleus and comet assay with 811 µg/L CP in a 96-h exposure period (Ali et al. 2008). OPs, azinophos-methyl, MP, CP-methyl, methamidophos, and diazinon caused inhibitions of acetylcholinesterase in the fish, *Sparus aurata*, with cardiotoxicity (Tryfonos et al. 2009). A study on the evaluation of DNA damage and chronic cytotoxicity induced by MP and CP in rat liver, brain, kidneys, and spleen tissues after 24 h of treatment revealed that both insecticides applied together

did not cause the cell damage at levels more than these of individual chemicals, confirming that the insecticides had no potentiating activity on the toxicity of each other (Ojha et al. 2013). Ameliorating effects of vitamins and amino acid acetyl-L-carnitine against MP toxicity in rats were reported (Uzunhisarcikli and Kalender 2011; Chidiebere et al. 2011). It was reported that in Wistar rats, CP induced DNA damage at daily doses of 3 and 12 mg/kg body weight after 7 and 14 days of treatment, respectively (Sandhu et al. 2013). In fact, for deciphering nuclear toxicity levels due to chemicals of environmental concern that induce DNA damage and affect DNA repair and other associated genetic process, such a work in “predictive toxicology” would bring new ideas that could aid in the identification and classification of carcinogens (Hreljac et al. 2008).

**Conclusions**

It was discernible that MP is more toxic than CP to in vitro-grown HepG2 cells, particularly that both caused moderate to high levels of geno-toxicity. It was evident that MP induced apoptotic cells, at 4 and 20.89 mg/L MP as were the MIC value and LC<sub>50</sub> value, respectively. Similarly, it was evident that CP induced apoptotic cells, at 10 and 79.43 mg/L, as the MIC value and LC<sub>50</sub> value, respectively.

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