Cytostatic and genotoxic effect of temephos in human lymphocytes and HepG2 cells

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A B S T R A C T

Temephos is an organophosphorush pesticide that is used in control campaigns against Aedes aegypti mosquitoes, which transmit dengue. In spite of the widespread use of temephos, few studies have examined its genotoxic potential. The aim of this study was to evaluate the cytotoxic, cytostatic and genotoxic effects of temephos in human lymphocytes and hepatoma cells (HepG2). The cytotoxicity was evaluated with simultaneous staining (FDA/EtBr). The cytostatic and genotoxic effects were evaluated using comet assays and the micronucleus technique. We found that temephos was not cytotoxic in either lymphocytes or HepG2 cells. Regarding the cytostatic effect in human lymphocytes, temephos (10 \textmu M) caused a significant decrease in the percentage of binucleated cells and in the nuclear division index as well as an increase in the apoptotic cell frequency, which was not the case for HepG2 cells. The comet assay showed that temephos increased the DNA damage levels in human lymphocytes, but it did not increase the MN frequency. In contrast, in HepG2 cells, temephos increased the tail length, tail moment and MN frequency in HepG2 cells compared to control cells. In conclusion, temephos causes stable DNA damage in HepG2 cells but not in human lymphocytes. These findings suggest the importance of temephos bio-transformation in its genotoxic effect.

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1. Introduction

Pesticides offer numerous benefits, including crop protection and reducing the risk of diseases transmitted by vectors, and have some negative effects on humans health and the environment (Buratti et al., 2003). Because many pesticides are suspected to have mutagenic and carcinogenic activities, concern for their adverse effects on human health has increased (IARC, 1999). Temephos (O,O′,O″-tremethyl-O,O″-thiodiphenylphorothionate) is a non-systemic organophosphorus (OP) pesticide (Fig. 1) that is mainly used as a larvicide and ectoparasiticide to control mosquitoes, midges, black flies and other insects in public health. Based on the acute toxicity of temephos, the World Health Organization (WHO) has classified it in category U, but it is unlikely to cause acute damage if it is used correctly (WHO, 2011). With respect to the chronic toxicity of temephos, the Environmental Protection Agency (EPA) reported that a full assessment of its toxic potential is required due to the scarcity of information (EPA, 2008).

Regarding temephos biotransformation, little information is available. It has been described as relatively stable to metabolic degradation, and its main biotransformation route in rats is S-oxidation to generate temephos sulfoxide and temephos sulfone as well as hydrolysis by carboxylesterase to form 4,4′-thiodiphenol, 4,4′-sulfonyldiphenol and 4,4′-sulfanyl diphenol. Furthermore, it is a secondary metabolite generated by glucuronidation or the sulfation of temephos (Blinn, 1966; Ferguson et al., 1985).

On the other hand, different cells types have been used to determine the genotoxicity and cytotoxicity of pesticides (review in Bolognesi et al., 2011). Lymphocytes are easy to access cells, and they are widely reported in the literature to measure the genotoxicity of the parent compound, because it has not full battery of biotransformation (Blasiak et al., 1999; Prabhavathy et al., 2006; Eroğlu, 2009; Rojas-García et al., 2009).

Since many pesticides require metabolic activation to exert their genotoxic potential (Jokanovic, 2001), cells with a human
origin, such as hepatoma cells (HepG2), which retain the activities of xenobiotic metabolizing enzymes, can be used to study the processes in the intact liver compared to other in vitro test systems (Knasmüller et al., 1999; Wilkening et al., 2003; Young et al., 2006; Jennen et al., 2011; Wang et al., 2012a). Additionally, this model is suitable and applicable for genotoxic assays, including the MN (micronucleus) test and comet assay because it does not carry the p53 mutation and enables cells to induce the DNA damage response pathway, arrest growth and activate apoptosis (Jennen et al., 2010; Westerink et al., 2010).

Because few studies have reported the effects of temephos on mammalian cells, the aim of this study was to investigate the cytotoxicity, cytostaticity and genotoxicity of this insecticide in human lymphocytes and HepG2 cells through the comet assay and cytokinesis-block micronucleus (CBMN) assay.

2. Materials and methods

2.1. Chemicals

Reagent grade temephos with 97.6% purity (CAS Number 3383-96-8) was obtained from Chem Service, West Chester, PA, USA. Ethylenediaminetetraacetic acid (EDTA-Na2) (CAS Number 139-33-3), regular agarose type IIa (CAS Number 9012-36-6), low melting point agarose (CAS Number 9012-36-6), fluorescein diacetate (FDA) (CAS Number 596-09-8), dimethylsulfoxide with 99.5% purity (DMSO) (CAS Number 67-68-5), 30% hydrogen peroxide (H2O2) (CAS Number 7722-84-1), RPMI-1640, phytohemagglutinin (PHA-M) and cytochalasin B (CAS Number 14930-96-2) were purchased from Sigma–Aldrich, St. Louis, MO, USA. Ethidium bromide (EtBr) (CAS Number 1239-45-8), Triton X-100 (CAS Number 9002-93-1) and tris (CAS Number 77-86-1) were purchased from BIO-RAD, USA. Sodium chloride (NaCl) (CAS Number 7647-14-5), methanol (CAS Number: 67-56-1) and glacial acetic acid (CAS Number 64-19-7) were acquired from JT Baker, USA. Sodium hydroxide (NaOH) (CAS Number 1310-73-2) was acquired from JT Baker, Sweden. Ethanol 96% (CAS Number 64-17-5) was purchased from Jalmek®, San Nicolás de los Garza, N.L. México. L-glutamine (100X, CAS Number 56-85-9), 100X nonessential amino acids, mitomycin-C (MMC) (CAS Number 50-07-7), DMEM medium and Trypsin–EDTA 0.25% (CAS Number 9002-07-07) were obtained from Gibco®, Grand Island, N.Y, USA. A hemacolor Kit (CAS Number: 67-561) was purchased from Merck KGaA, Darmstadt, Germany.

2.2. Cells lines and culture conditions

2.2.1. Human lymphocyte cell culture

Fresh blood was collected by venipuncture in heparinized tubes from three male volunteers who were between 18 and 23 years of age; they were non-smokers who had not been diagnosed with a chronic disease. Whole blood (0.5 mL) was cultured in Falcon tubes with 6.3 mL of RPMI-1640 medium supplemented with 1% non-essential amino acids and 1% L-glutamine. Lymphocytes were stimulated to induce cell division by adding 200 lL of phytohemagglutinin (PHA, 1 mg/mL) and were incubated at 37°C with 5% CO2. The cells were treated with temephos (0.5, 1.0, 5.0 and 10.0 µM) and 21 lL of cytochalasin-B (2 mg/mL) to inhibit cytokinesis (at 48 h post-PHA stimulation). After 72 h of incubation, the cultures were transferred into round bottom tubes, centrifuged and fixed with Carnoy’s solution (methanol:acetic acid, 3:1) and then centrifuged until a clean cell button was obtained. To clean the cell suspension, trypsin (0.0015%) was applied for 5 s; then, the cells were washed and resuspended in 0.5 mL of cytochalasin-B (2 mg/mL) to inhibit cytokinesis (at 48 h post-PHA stimulation). After 72 h of incubation, the cultures were transferred into round bottom tubes, centrifuged and fixed with Carnoy’s solution (methanol:acetic acid, 3:1) and then centrifuged until a clean cell button was obtained. To clean the cell suspension, trypsin (0.0015%) was applied for 5 s; then, the cells were washed and resuspended in 0.5 mL of fixative solution for slide preparation. The slides were stained with a Hemacolor kit according to the manufacturer’s instructions. Three independent experiments were performed in triplicate for each donor.

2.2.2. Human hepatoma (HepG2) cell culture

HepG2 cells obtained from ATCC (American Type Culture Collection) were cultured in 10 mL of DMEM supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine and 1% antibiotic–antimycotic. Cell culture was purchased from Gibco®.
maintained in 75 cm² culture flasks under sterile conditions in an incubator at 5% CO₂ and 37 °C. When cultures reached 100% confluence, the cells were passaged, the culture medium was removed and the cells were incubated with 2 mL of 0.25% trypsin–EDTA at 37 °C for 5 min. Afterwards, the culture flasks were gently moved to allow the cells to completely detach. Subsequently, supplemented DMEM culture medium was added (8 mL) for a homogenized cell suspension that was placed in a fresh culture flask. Cultured HepG2 cells (5 × 10⁵ cell passage 4) were treated with different concentrations of temephos (0.5, 1.0, 2.0, 5.0 and 10.0 μM) and with 1.5 μL of cytochalasin-B (3 μg/mL) to inhibit cytokinesis (48 h after the treatment). As a negative control, cells were treated with incubation medium containing 0.05% (v/v) DMSO. To harvest the HepG2 cells, the medium was removed and 150 μL of trypsin was added (0.25%), resuspended and neutralized. The cells were prefixed with 500 μL of Carnoy’s solution and resuspended slightly, and the content of each well was transferred to round bottom tubes; 1 mL of fixative solution was added and centrifuged (1200 rpm/10 min). The washing procedure was repeated 4 times; then, the cell pellet was resuspended in 500 μL of fixing solution to prepare the slides. The slides were stained as described for the lymphocytes. Three independent experiments, each in triplicate, were performed.

2.3. Evaluation of cytotoxicity

Cell viability in human lymphocytes and HepG2 cells was evaluated using simultaneous staining with fluorescein diacetate (FDA) and ethidium bromide (EtBr) according to the technique described by Jones and Senft (1985).

Cells were resuspended, and 100 μL aliquots of each treatment were transferred into new tubes and centrifuged at 3000 rpm for 2 min; the supernatants were removed, and the cell pellets were kept on ice until use. For analyses, the cell pellets were resuspended in 20 μL of an FDA/EtBr solution, placed on slides and covered with coverslips. Blinded cell counts were performed with a fluorescence microscope Carl Zeiss Axio Scope A1 (Göttingen, Germany) and a 20X objective. Living cells were observed in green and dead cells in red; 200 cells were counted for each treatment.

2.4. Comet assay

Human lymphocytes: The comet assay was performed according to Tice et al. (2000). Briefly, 20 μL of whole blood was treated with temephos (0.5–10 μM) for 3 h. Button cells were mixed with 150 μL of low melting point agarose (0.5%) at 37 °C, placed on slides that had been precoated with a layer of regular agarose (0.5%) and allowed to polymerize at 4 °C. Another layer of low melting point agarose was added and allowed to solidify. Slides were placed in coplin jars for 24 h with 50 mL of lysis solution (2.5 M NaCl, 100 mM EDTA-Na₂ and 10 mM Tris, pH > 10, with 5 mL of DMSO and 0.5 mL of Triton X-100) at 4 °C. Prior to electrophoresis, the slides were incubated for 20 min in alkaline buffer (10 M NaOH, 200 mM EDTA-Na₂, pH > 13), which allows the detection of DNA single-strand breaks (SSB), alkali-labile sites (ALS), and DNA–DNA/DNA–protein cross-links, among other DNA damage. After alkali unwinding, the slides underwent electrophoresis at 25 V and 300 mA for another 20 min. Subsequently, the slides were rinsed twice with 400 mM tris buffer (pH 7.5) and were immediately fixed twice with ethanol 96%. The slides were stained with 10 μL of ethidium bromide solution (1:10 in water) and analyzed with a fluorescent microscope Carl Zeiss Axio Scope A1 using the software of the Comet Imager of Meta System version 2.2. The tail length and tail moment parameters were used as indicators of DNA damage.

2.5. Cytostaticity

To evaluate the possible effect of temephos in the mitogenic response in lymphocytes and HepG2 cells, the Nuclear Index (NI) was evaluated according to the method described by Eastmond and Tucker (1989). Viable cells were scored (n = 200) to determine cell viability of more than 80% were used for analysis. HepG2 cell suspensions (1 × 10⁵) were mixed with 150 μL at 37 °C in low melting point agarose (LMP) and transferred to regular melting point agarose-coated slides. Two slides were studied for each compound concentration. The slides were covered with a coverslip and the agarose was allowed to solidify at 4 °C; thereafter, the coverslips were removed and another layer of agarose LMP was added. They were then allowed to solidify again at 4 °C. The coverslips were removed, and the slides were kept in a lysis solution (100 mM Na₂EDTA, 10 mM Tris and 2.8 M NaCl pH >10), 1% Triton-X 100 and 10% DMSO over 1 h at 4 °C. After 20 min of alkali unwinding (200 mM Na₂EDTA and 10 M NaOH, pH > 13.5), the slides were electrophoresed at 25 V and 300 mA for another 20 min. Subsequently, the slides were rinsed twice with 400 mM tris buffer (pH 7.5) and were immediately fixed twice with ethanol 96%. The slides were stained with 10 μL of ethidium bromide solution (1:10 in water) and analyzed with a fluorescent microscope Carl Zeiss Axio Scope A1 using the software of the Comet Imager of Meta System version 2.2. The tail length and tail moment parameters were used as indicators of DNA damage.

Fig. 2. Cell viability in (a) human lymphocytes and (b) HepG2 cells treated with temephos for 48 h. Cell viability was measured with the fluorescent dyes fluorescein diacetate (FDA) and ethidium bromide (EtBr). Each value represents the mean ± SEM of three separate experiments (n = 3), which were performed in triplicate. Cultures treated with MMC (1 μM) were used as a positive control and with DMSO (<0.05%) as a negative control. "p < 0.05 with respect to the negative control; statistical analysis was carried out by ANOVA and the Bonferroni post hoc test on data obtained from human lymphocytes and by the Mann Whitney U-test and Dunn’s multiple comparison test for data obtained from HepG2 cells.
the frequency of mononucleated (Mono), binucleated (BN) or polynucleated (Poly) cells. The NI was calculated using the formula: $NI = (Mono + 2(BN) + 3(Poly))/N$, where $N$ is the total number of viable cells scored.

2.6. Scoring the parameters in the cytokinesis-block micronucleus assay (CBMN)

The evaluation of the MN, nuclear buds (NB), nucleoplasmic bridges (NPB) and apoptotic and necrotic cells was performed with the CBMN technique according to the criteria established by Fenech et al. (2003) in human lymphocytes and HepG2 cells. The slides were coded for their analysis. The frequency of MN in 1000 binucleated cells per slide was scored at 1000X magnification using a light microscope Carl Zeiss Axiostar plus (Göttingen, Germany). The MN frequency is expressed as the number of MN in 1000 binucleated cells. The NB, NPB and apoptotic and necrotic cells were expressed as the relative frequency in 1000 counted cells. Each experiment had positive controls (MMC 1 μM) and negative controls (DMSO < 0.05%).

2.7. Statistical analyses

For each type of assay, three separate experiments were performed in triplicate. The data were expressed as mean ± standard error of the mean (SEM).

The effects of temephos on the cell viability and NI were analyzed using one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. The results of the genotoxicity parameters were analyzed with the Mann Whitney $U$-test and Dunn’s multiple comparison tests. The $p$ values <0.05 were considered statistically significant. Statistical analyses were conducted using the Stata 8.0 program (Stata statistical software, Stata Corporation, College Station, Texas) and GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Cytotoxic and cytostatic effects

Temephos treatments did not affect the cell viability in either human lymphocytes (Fig. 2a) or in HepG2 cells (Fig. 2b). However, a significant cytostatic effect, measured as NI, was observed in lymphocytes that were treated with 10 μM temephos (Fig. 3a and b).

3.2. Comet assay

The DNA damage measured by the comet assay was reported as the tail length (the migration of DNA in the tail) and tail moment (product of the tail length and the percentage of DNA in the tail). Temephos in human lymphocytes significantly increased the tail length at concentrations from 1 μM to 10 μM (Fig. 4a) as well as the tail moment at concentrations above of 0.5 μM (Fig. 4b). The results of DNA damage in human lymphocytes by donors are presented in Table 1. Cells from donor 1 showed an increase in the tail length at concentrations of 1, 2, 5 and 10 μM, and there was an increase in the tail moment at all concentrations. Similarly, the
temephos. DNA damage in human lymphocytes following in vitro exposure to the insecticide temephos.

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>Tail length (µm)</th>
<th>Tail moment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (−)</td>
<td>18.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Control (+)</td>
<td>32.4</td>
<td>3.5</td>
</tr>
<tr>
<td>0.5</td>
<td>20.3</td>
<td>1.4</td>
</tr>
<tr>
<td>1.0</td>
<td>21.2</td>
<td>1.7</td>
</tr>
<tr>
<td>2.0</td>
<td>23.9</td>
<td>2.0</td>
</tr>
<tr>
<td>5.0</td>
<td>25.4</td>
<td>1.9</td>
</tr>
<tr>
<td>10.0</td>
<td>26.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Donor 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (−)</td>
<td>18.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Control (+)</td>
<td>51.1</td>
<td>10.0</td>
</tr>
<tr>
<td>0.5</td>
<td>18.6</td>
<td>1.4</td>
</tr>
<tr>
<td>1.0</td>
<td>22.5</td>
<td>1.6</td>
</tr>
<tr>
<td>2.0</td>
<td>25.7</td>
<td>2.4</td>
</tr>
<tr>
<td>5.0</td>
<td>23.3</td>
<td>1.5</td>
</tr>
<tr>
<td>10.0</td>
<td>27.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Donor 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (−)</td>
<td>15.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Control (+)</td>
<td>49.2</td>
<td>9.4</td>
</tr>
<tr>
<td>0.5</td>
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<td>1.7</td>
</tr>
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<td>1.0</td>
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<tr>
<td>2.0</td>
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<td>5.0</td>
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<tr>
<td>10.0</td>
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<td>1.5</td>
</tr>
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</table>

Tail length: migration of DNA in the tail. Tail moment: product between the tail length and percentage of DNA in the tail. Values are presented as the geometric means of three separate experiments (n = 3), which were performed in triplicate. Cultures treated with H2O2 (30%) were used as a positive control and with DMSO (<0.05%) as a negative control. *p < 0.05, with respect to the negative control.

Fig. 5. DNA damage in HepG2 cells treated with temephos as measured by the tail length (a) and tail moment (b). The tail length is the migration of DNA in the tail. The tail moment is the product of the tail length and percentage of DNA in the tail. Each value represents the mean ± SEM of three separate experiments (n = 3), which were performed in triplicate. Cultures treated with DMSO (<0.05%) were used as the negative control. *p < 0.05 with respect to the negative control; statistical analysis was carried out with the Mann Whitney U-test and Dunn’s multiple comparison test.

lymphocytes obtained from donor 2 showed a significant increase in tail length following exposure to 1, 2, 5 and 10 µM of temephos, and the tail moment increased with 2 and 10 µM of temephos.

Fig. 6. The micronuclei (MN) frequency in (a) human lymphocytes and (b) HepG2 cells treated with temephos for 48 h. Each value represents the mean ± SEM of three separate experiments (n = 3), which were performed in triplicate. Cultures treated with MMC (1 µM) were used as a positive control and with DMSO (<0.05%) as a negative control. *p < 0.05 with respect to the negative control; statistical analysis was performed with the Mann Whitney U-test and Dunn’s multiple comparison test.

Table 1
DNA damage in human lymphocytes following in vitro exposure to the insecticide temephos.

Table 2
CBMN parameters in human lymphocytes and HepG2 cells.

Slightly different results were observed in lymphocytes from donor 3, who showed a significant increase in the tail length with 1 and 5 µM of temephos, and there was a significant difference in the tail moment with 5 µM treatment compared to controls. Our results might suggest a different susceptibility to DNA damage from exposure to temephos, at least under in vitro conditions.
Fig. 7. Representative photomicrographs of nuclear structures observed in the CBMN test in human peripheral blood lymphocytes. (a) Mononuclear cell, (b) binucleated cell, (c) polynucleated cell, (d) binucleated cell with micronuclei, (e) necrotic cell, (f) apoptotic cell with micronuclei, (g) nucleoplasmic bridge, and (h) nuclear buds.

Fig. 8. Representative photomicrographs of nuclear structures observed in the CBMN test in HepG2 cells. (a) Mononuclear cell, (b) binucleated cell, (c) polynucleated cell, (d) binucleated cell with micronuclei, (e) apoptotic cell with micronuclei, (f) nucleoplasmic bridge, and (g) nuclear buds.
In HepG2 cells, temephos significantly increased the tail length at 10 μM concentrations (Fig. 5a); with respect to the tail moment, we observed an increase at 0.5 and 10 μM (Fig. 5b).

3.3. Micronucleus assay

Fig. 6a and Table 2 show that temephos treatment does not increase either the frequency of MN in binucleated lymphocytes or the frequency of NPB, NB, necrotic cells or MN in mononuclear cells. However, exposure to 10 μM temephos increased the frequency of apoptotic cells and decreased the percent of binucleated cells compared to the negative control. In contrast, in metabolically competent cells, such as HepG2, temephos increased the frequency of MN 1.5 more times than control cultures (Fig. 6b), but it did not increase the frequency of apoptotic cells, percent of binucleated cells or other CBMN parameters (Table 2). Representative photographs of nuclear structures observed in the CBMN test in human peripheral blood lymphocytes and HepG2 cell are shown in Figs. 7 and 8.

4. Discussion

On the basis of the biomonitoring data and application of pharmacokinetic models, Buratti et al. (2007) proposed that OP concentrations lower than 10 μM in in vitro studies provide the best model of the in vivo response to exposure. In this study, we used a concentration gradient, ranging from 0.5 to 10 μM, of temephos, which may represent environmental exposure, and this gradient is also equivalent (2 μM) to that used in dengue eradication campaigns (NOM-032-SSA2-2002). We hypothesized that temephos, at low doses, did not alter cell viability, nor the citostaticity, but it did cause stable genetic damage in human lymphocytes and hepatoma cells.

In this context, the treatment with 10 μM temephos in human lymphocytes caused a decrease in the NI and in the percent of binucleated cells as well as an increase in the apoptotic cell frequency. Our results agree with those reported previously in human and animal cells from both in vivo and in vitro studies by other anticholinesterase pesticides, such as chlorpyrifos, parathion and malathion (review in Li, 2010). Although our apoptosis results agree with those reported in the literature, it is important to confirm them with specific methods, including caspace-3 activation, DNA fragmentation, TUNEL assay or Annexin-V/propidium iodide staining.

Furthermore, we observed an increase in DNA damage at concentrations as low as 1 μM of temephos in human lymphocytes, which were evaluated through the comet assay. However, the data obtained by the CBMN assay suggest that the DNA damage induced by temephos is not permanent. The CBMN findings demonstrated normal, or low, frequencies of NPB and NB in binucleated cells and a low number of MN in mononuclear cells. On the other hand, the results showed that temephos increased the DNA damage in HepG2 cells, which was evaluated through the comet assay and MN test, at concentrations of 10 μM, suggesting permanent damage in metabolically competent cells.

Our results agree with Aiub et al. (2002), who evaluated the effect of temephos in the total blood cells of Wistar rats with the comet assay and found a dose-dependent increase of severe lesions in DNA (comet type IV) with 1.34 mM of commercial temephos (granular 2% (w/v)). Our results also agree with findings by Bezerra de Mêlo et al. (2008), who evaluated the presence of MN in the bone marrow cells of mice treated with oral 27.75, 55.5 and 111.0 mg/kg of temephos through the CBMN test and found a directly proportional relationship between the dose of pesticide and MN presence.

The information reported in the literature and data obtained in this study might suggest an important role of temephos biotransformation with regard to its genotoxic potential. Thus far, little is known about temephos biotransformation, and it has been described as relatively stable to metabolic degradation (Blinn, 1966; Ferguson et al., 1985). The mechanism throughout temephos causes a genotoxic effect could be similar to that described for other OP pesticides; one of the most well-known is the production of reactive oxygen species (ROS) during the biotransformation process (review in Jokanovic, 2001). ROS are not only involved in the toxicity of OP pesticides, they are also involved in various other pesticides. It is known that ROS induce several types of lesions in DNA, including single and double strand breaks, ALS, and various species of oxidized purines and pyrimidines that are easily detected by the comet assay (Raj and Srivastava, 2014).

Studies of genotoxicity in HepG2 cells suggest that the suppression of repair genes could be another mechanism of xenobiotic-induced DNA damage (Wang et al., 2012b; Huan et al., 2014). Although the temephos biotransformation in humans is still unknown, we suggest that some products of its metabolism may cause similar effects on some critical cell regulatory and DNA repair genes, such as those described by Wang et al. (2012b) (Apel, Rad51, Bcl-2, Bax, Xpa, and Xpc) and Huan et al. (2014) (UNG, LIG1, EXO1, XRCC2, PCNA and FANCE) in HepG2 cells.

In conclusion, the data obtained in this study suggest that temephos could have a cytostatic and apoptotic effect, but it does not have cytotoxic or genotoxic potential in human lymphocytes. Nevertheless, the genotoxic potential of this insecticide was observed in metabolically capable cells, such as HepG2 cells. Additional studies are needed to evaluate the genotoxic potential of the commercial formulation of temephos and their products of biotransformation.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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