Toxic Effects of Residue Amounts of Chlorpyrifos-methyl in Tomato to White Albino Rats

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ABSTRACT

Extensive use of insecticides contaminated the agricultural products and environment. Current study aimed to (1) Determine residue quantities of chlorpyrifos-methyl (CPM) in tomato fruits and (2) Evaluate the toxicity of the residue amounts of CPM in tomato to white albino rats. Results revealed that the residue of CPM (after 1 h of application) was 4.05 mg kg\(^{-1}\) and decreased from 3.73-1.74 mg kg\(^{-1}\) after 1-21 days post application. The half-life time of CPM in tomato fruits was 11.99 days. The toxicological effects of residue quantities were studied through administering rats with residue amounts that were detected after 1, 7, 14 and 21 days post application. Highlighted toxic effects were reduction in activities of lactate dehydrogenase (-58.13% of control) and aspartate transaminase (-39.8% of control) and increase in alkaline phosphatase (61.6% of control) and \(\gamma\)-glutamyl transferase (35.6% of control) enzyme activities. Pesticides is a requirement to control plant pests, so, it is crucial to follow safety guidelines of pesticides usage and to harvest plants after the time that bring no harmful effects to people.

Key words: Pesticide residue analysis, toxicological assessments, hematological parameters, heart, liver, kidney function

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill.) is the second most produced food commodity in Egypt after sugarcane and it provides consumers with 48 Kcal capita\(^{-1}\) day\(^{-1}\) (FAO., 2014). Whitefly, *Bemisia tabaci*, is one of the most serious insect pests attacking tomato (El-Khawalka *et al.*, 1997a). It transfers the Tomato Yellow Leaf Curl Virus (TYLCV) (Zeid and Herakly, 1972; Darwish *et al.*, 1989) and causes an estimated annual loss of several hundred million dollars worldwide (Perring *et al.*, 1993; Ellsworth *et al.*, 1999; Oliveira *et al.*, 2001). Application of synthetic insecticides is necessary to control insect pests including the whiteflies. Chlorpyrifos-methyl is registered in Egypt under many trade names including, Ictan 50% EC, Pyrodan 50% EC, Reldan 50% EC and Relozed 50% EC to control the Egyptian cotton leaf worm and aphids (MALR., 2010) and it was broadly used to control the whitefly on tomato plants (Omar *et al.*, 1994; Schuster *et al.*, 1996; El-Khawalka *et al.*, 1997b; MALR., 2010).

Numerous restrictions have been enforced by various regulatory agencies to ensure the safe use of pesticide and to protect consumers from their adverse effects. Toxicity of pesticides that accumulate in plants and fruits after single or multiple application(s) is governed by their degradation and disappearance and is measured by their half-life values. For example, the residues
of chlorpyrifos were dependent on the frequency, rate of application and weather conditions immediately following spray (Liu et al., 2005). When the Chinese cabbages were treated 4 times at maximal rates at 5 days intervals, the half-life of chlorpyrifos was 3.6 days. Based on the recommended pre-harvest intervals, the residual levels of chlorpyrifos were less than the national standards for pollution-free vegetables (Liu et al., 2005; Zhang et al., 2006). Based on that, the half-life time value of pesticides must be short to reduce their possible adverse effects.

Residue amounts in food samples and sub-lethal doses of pesticides were reported to have alterations in the biochemical parameters of lab animals. After 4 weeks of oral administration, the acute oral toxicity of chlorpyrifos and other insecticides to white rats at 0.1 of LD₅₀ dose exerted significant decrease in aspartate transaminase (AST), alanine transaminase (ALT), γ-Glutamyl transferase (GGT) and lactate dehydrogenase (LDH) (Enan et al., 1982). Similarly, feeding rats with the initial deposited amount of chlorpyrifos-methyl (13 mg kg⁻¹) that was detected in wheat grains significantly reduced the serum acetylcholinesterase activity and elevated the activities of phosphatases (acid; ACP and alkaline; ALP) and transaminases (ALT and AST) (Abbassy et al., 2000). Also, when rats administered a single oral dose of each of 0.1 LD₅₀, 0.25 LD₅₀ and the LD₅₀ quantities of pirimiphos-methyl, chlorpyrifos-methyl and fenitrothion showed significant increase in the activity of ALT, AST, ALP, creatinine, urea and total cholesterol and a significant decrease in total protein concentration compared with the control (El-Nabarawy et al., 2005). Moreover, chlorpyrifos-methyl caused significant increase in WBCs counts, ALT and AST activities of treated rats after 15 and 30 days of treatment compared to control (Soliman et al., 2007).

Therefore, the present study aimed to (1) Determine the residue quantities of chlorpyrifos-methyl in tomato fruits after different time intervals of last application and (2) Assess the possible side effects of the residue levels, as daily oral doses for 45 days on hematology, heart, liver and kidney biochemical traits of rats.

MATERIALS AND METHODS

Insecticides and chemicals: Chlorpyrifos-methyl (O, O-Dimethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate; Reldan® 50% EC) was purchased from National Agricultural Chemicals Company (Agrochemicals Industries, Ltd.). All solvents: acetonitrile, ethyl acetate, hexane and methylene chloride were HPLC grade and were obtained from local distributors in Egypt.

Field layout and fruit sampling: The experiment was designed as a Completely Randomized Design (CRD). Each treatment had three replicates (each replicate was a 42 m² plot; approx. 6 rows in 7 m length; each plot has about 100 tomato plants). Plots were sprayed 10 consecutive times once every 7 days using, chlorpyrifos-methyl (Reldan® 50% EC) at the recommended rates of 250 cm³/100 L of water to control whitefly (MALR., 2010). The insecticide was applied on young tomato plants; starting from the 1st infestation with whiteflies after plantation. Control plants were sprayed with water. Agricultural practices were done following the commercial production program of tomatoes. Five kilograms of tomato fruits were randomly collected from each plot including the control after 0, 1 h, 1, 3, 5, 7, 10, 14 and 21 days of the last insecticide spray. Fruits were homogenized and a sample of 0.5 kg of each replicate were placed in polyethylene bags and stored at -20°C until analysis.

Extraction and clean-up: Chlorpyrifos-methyl residues were analyzed following the method developed by MWHCA (1988). Approximately 50 g of the whole tomato fruits were homogenized in
150 mL ethyl acetate and 50 g of anhydrous sodium sulfate in a Warring blender for 5 min at the
maximum speed. The extracts were filtered through a dry pad of cotton and anhydrous sodium
sulfate and then concentrated to dryness using a rotary evaporator (Unipan vacuum rotary
evaporator type 350P, Poland) at <35°C. The dried residue was re-dissolved in 10 mL hexane. The
extract was cleaned-up using a chromatographic column filled with: A plug of glass wool, 10 g
activated Florisil (60-100 mesh) and 2 g sodium sulfate anhydrous successively from the bottom.
The column was pre-washed with 40 mL hexane and then the pesticide residues were extracted
with 200 mL of methylene chloride:hexane:acetonitrile (50:48.5:1.5) solvent mixture (Mills et al.,
1972). Extracts were filtered into a 2 mL dark HPLC glass vial using a 1 mL syringe and a 0.2 µm
nylon filter (Fisher Scientific, Ottawa, Canada).

**Residue measurement using Gas Chromatography (GC):** Chlorpyrifos-methyl residues were
quantified using a GC system (Pye Unicam 4500 Gas Chromatography System, Philips, UK) that
was equipped with a Flame Photometric Detector (FPD) and an auto sampler and operated in the
phosphorus mode (525 nm filter). Approximately 20 µL of samples were injected into a Pyrex glass
column 1.5 m×4 mm id packed with 4% SE-30+6% OV-210 on gas Chromosorb Q (80-100 mesh).
Running conditions were as follow: injection port temperature was 280°C, the column temperature
was 240°C and the detector temperature was 240°C. Hydrogen and air gases flow rate were 75 and
100 mL min⁻¹, respectively and the carrier gas was nitrogen at a flow rate of 3 mL min⁻¹. The
retention time of chlorpyrifos-methyl was 4.47 min. The external standard was used for calibration;
standard curve was prepared from 6-8 different concentrations of standard solutions of 95%
technical material of CPM.

**Recovery studies:** Untreated tomato fruits were spiked with 3 different amounts of 95% technical
grade of chlorpyrifos-methyl (0.5089, 1.3332 and 5.0106 µg) prior to extraction and clean-up. Three
replicates of each concentration were passed through the entire process of extraction, clean-up and
analyzed as described, previously. The recovery values were calculated according to the following
formula and the obtained results were corrected according to the recovery rate:

\[
\text{Recovery %} = \frac{(\mu g \text{ pesticide residue/g sample found})}{(\mu g \text{ pesticide residue/g sample added})} \times 100
\]

**Calculation of insecticide residue disappearance and half-life time values:**

\[
\text{Loss of residue (%) } = \frac{(\text{Initial residues} - \text{found residues at different time})}{(\text{Initial residues})} \times 100
\]

Half-life times (t₁/₂) in days were calculated according to the equation of Moye et al. (1987).
\[
t_{1/2} = \ln_2/K = 0.693/K \text{ and } K = 1/t \times \ln a/m, \text{ where: } K = \text{ apparent rate constant, } t = \text{ time in days,}
\]
\[
m = \text{residue at x time and a = initial residue.}
\]

**Limits of detection and quantification:** Limits Of Detection (LOD) and Limits Of Quantification (LOQ) were estimated statistically using a standard curve of chlorpyrifos-methyl (95% technical grade). Dilution series of CPM from 0.5-25 µg mL⁻¹ were repeated 3 times each and Standard Error (SE) was calculated. A calibration curve was plotted and the Slope (S) was calculated from the regression equation (Y = 3,000,000*X - 1713.2; R² = 0.9997) and then
LOD = 3.3*SE/S and LOQ = 10*SE/S (Ermer, 2005). Results of detection and quantitation limits of chlorpyrifos-methyl were 4.02 and 8.45 μg kg\(^{-1}\), respectively. All residue levels in tomato fruits were greater than the LOD and LOQ values.

**Tested animals and insecticide treatment:** Male white albino rats (*Rattus norvegicus*) weighing 110±10 g were used. Rats were supplied from the Animal Health Research Center (Cairo, Egypt). Rats were housed 4 per cage and acclimatized for 1 week with free access to water and food. Rats were divided into 5 groups each of 4 rats. The rats of each group received daily oral doses equal to the detected residue amounts of chlorpyrifos-methyl in tomato fruits after 0, 1 h, 7, 14 and 21 days of last application for 45 days. Oral doses were adjusted from mg kg\(^{-1}\) to mg g\(^{-1}\) according the weight of rats. Control group was given daily 0.5 mL of corn oil (equal to the volume of the residue doses). Animals were decapitated after 24 h of the last oral treatment. Blood samples were collected into serum separation and heparinized tubes. All experimental procedures using the laboratory animals were done according to the approved animal care and use protocol of Damanhour University, Egypt.

**Blood hematological and biochemical parameters**

**Blood picture:** Red blood cells and white blood cells were counted according to the method of Britton (1963) and Seiverd (1964). The number of RBC's was multiplied by 10\(^4\) to obtain the RBC count for each cm\(^3\). The counts of WBC's were multiplied by 50 to obtain the number of WBC's cm\(^{-3}\). Haematocrit value (PCV\%) was determined according to the method reported by Bull et al. (2001) using a Micro-Haematocrit Centrifuge (Bench top High Speed Micro Haematocrit Centrifuge model Sh120-I, Microfield Instrument, UK). The obtained data were expressed as percentages of haematocrit value to the total blood volume. Hemoglobin measurements were done by following Wintrobe (1981).

**Measurement of biochemical parameters of blood serum:** Blood glucose, total protein, creatinine and uric acid and enzyme activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined following the directions of Boehringer Mannheim GMBH Diagnostics Kits (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were determined using Diamond Diagnostics Kits (Rec, 1972). Determination of γ-Glutamyl Transferase (GGT) activity was done using Linear Chemicals, SL Kits (Whitfield et al., 1973). Serum Creatine Kinase (CK) activity was measured using Stanbio Laboratory Kits (Nealon and Henderson, 1977).

**Statistical design and analysis:** Field experiment was designed as a Completely Randomized Design (CRD) with one main factor, the insecticide treatment and three replicates. Results of insecticide residues were analyzed using the General Linear Model (GLM) of Statistical Analysis System (SAS). Toxicological results were analyzed using GLM procedure of SAS. Means were compared using, LSD significant difference test (p≤0.05) (SAS., 2013).

**RESULTS**

**Recovery of Chlorpyrifos-methyl:** Fortified tomato samples were used for calculation of recovery percentages of chlorpyrifos-methyl (Table 1). Recovery percentage of chlorpyrifos-methyl in tomato fruits ranged from 92.96%. Similar results were reported by Abbassy et al. (2000), who found that recovery % for chlorpyrifos-methyl was 95% in wheat grain.
Chlorpyrifos-methyl

$t_{1/2} = 11.99$ days

$R^2 = 0.9892$

**Table 1:** Recovery percentages of chlorpyrifos-methyl insecticide from tomato fruits (average of 3 replicates)

| Amount (µg) | Measured±SD | Recovery (%) |
|-------------|-------------|--------------|
| 0.5089      | 0.4681±0.04 | 92.0         |
| 1.3332      | 1.2842±0.05 | 96.3         |
| 5.0106      | 4.7254±0.27 | 94.3         |

**Table 2:** Degradation pattern of chlorpyrifos-methyl represented by the amount of residue and the percentage of loss of residue in tomato fruits after 1 h, 1, 3, 5, 7, 10, 14 and 21 days of the last application

| TAA$^a$ (days) | Residues (mg kg$^{-1}$) | SE | Loss of residues (%) |
|----------------|-------------------------|----|----------------------|
| Initial (after 1 h) | 4.054 | 0.032 | 0.00 |
| 1              | 3.731 | 0.277 | 7.96 |
| 3              | 3.256 | 0.167 | 19.68 |
| 5              | 2.953 | 0.120 | 27.15 |
| 7              | 2.784 | 0.071 | 31.32 |
| 10             | 2.510 | 0.104 | 38.08 |
| 14             | 2.179 | 0.150 | 46.25 |
| 21             | 1.739 | 0.296 | 57.10 |

$^a$TAA: time after application, n = 3 replicates

**Fig. 1:** Disappearance pattern and half-time values of chlorpyrifos-methyl after 1 h, 3, 5, 7, 10, 14 and 21 days of last spray application, $t_{1/2} = \ln 2/K = 0.693/K$, $K = 1/t \times \ln a/m$, where, $K$ is apparent rate constant, $t$ is time in days, $m$ is residue at $x$ time and $a$ is initial residue.

**Residue analysis in tomato fruits:** Results of the residue levels of chlorpyrifos-methyl in tomato fruits that were collected from the tomato field after different time intervals of the last foliage application were shown in Table 2. The initial deposit (residue level after 1 h of application) was 4.05 mg kg$^{-1}$ and it was decreased gradually to 3.731, 3.256, 2.953, 2.784, 2.510, 2.179 and 1.739 mg kg$^{-1}$ after 1, 3, 5, 7, 10, 14 and 21 d post application, respectively. The disappearance pattern as percentages of loss of residue were 7.96, 19.68, 27.15, 31.32, 38.08, 46.25 and 57.10%, respectively and the half-life value of chlorpyrifos-methyl was 11.99 days (Table 2 and Fig. 1).

**Toxicological side effects of residue levels to male rats:** The adverse effects of repetitive oral doses equal to the residue amounts that were measured in tomato fruits after 1 h, 7, 14 and 21 days of the last application of chlorpyrifos-methyl to male rats were studied in a 45 days sub-chronic toxicity experiment. These doses were equal to 4.054, 2.784, 2.179 and 1.739 mg kg$^{-1}$, which were greater than the maximum residue limits (MRL; 0.5 mg kg$^{-1}$) in tomato fruits (CAC, 2010). Chlorpyrifos-methyl sub-lethal doses did not cause any acute toxicity and/or mortality in the treated rats.
In vivo effects of chlorpyrifos-methyl residues on hematological parameters: Results in Table 3 showed that the oral administration of doses equal to residue levels of chlorpyrifos-methyl after 7 (2.784 mg kg\(^{-1}\)), 14 (2.179 mg kg\(^{-1}\)), or 21 (1.739 mg kg\(^{-1}\)) days of last application had no effect on the Packed Cell Volume (PCV) % of blood compared to control. Treatments significantly reduced the RBC’s to 25, 20 and 16% compared with the control (4.47×10\(^6\) mm\(^3\)), respectively. On the other hand, the doses 2.784 and 3.731 mg kg\(^{-1}\) body weight per day (b.wt. day\(^{-1}\)) increased the WBC counts compared with control.

In vivo effects of chlorpyrifos-methyl residues on biochemical parameters

Adverse effects on total protein and glucose:

Results in Table 4 showed that the low dose of chlorpyrifos-methyl (1.739 mg ai kg\(^{-1}\) b.wt. day\(^{-1}\)) had no adverse changes in total protein and glucose concentrations. However, the high doses of this insecticide (2.179, 2.784 and 4.05 mg ai kg\(^{-1}\) b.wt. day\(^{-1}\)) increased the levels of total protein and glucose in a dose dependent manner. Percentages of increase of total protein relative to control were 1.59, 7.14, 11.90 and 23.81%, respectively and 1.32, 8.11, 8.99 and 14.12% for glucose, respectively.

Adverse effects on cardiac function:

Results showed that residues of chlorpyrifos-methyl decreased the activity LDH enzyme by 6.42, 40.45, 54.15 and 58.13%, respectively, relative to the control. On contrary, the 1.739, 2.179, 2.784 and 4.050 mg kg\(^{-1}\) daily doses caused significant increase in the GGT activity relative to control (percentages of increase ranged from 18.92-35.59%). No side effects of the accumulated residue quantities of 1.739 mg kg\(^{-1}\) of chlorpyrifos-methyl on CK were detected. However, doses equal to 2.179, 2.784 and 4.050 mg kg\(^{-1}\) increased CK activity with 1. 31, 2.75 and 4.37% of control, respectively.

Table 3: Mean±SE values of hematological parameters; Packed Cell Volume (PCV %), Red blood cell counts and white blood cell counts after given rats daily oral doses equal to residue level of chlorpyrifos-methyl in tomato fruits for 45 days

| TAA\(^a\) (days) | Dose\(^b\) | PCV (%) | RBC (×10\(^6\) mm\(^{-3}\)) | WBC (×10\(^3\) mm\(^{-3}\)) |
|------------------|-----------|---------|----------------------------|-----------------------------|
| Control          | 0.000     | 42.00±1.97 | 4.47±1.23                 | 62.25±1.79                  |
| 21               | 1.739     | 42.25±1.68 | 3.74±1.60                 | 60.75±1.43                  |
| 14               | 2.179     | 41.50±1.88 | 3.60±1.52                 | 97.00±1.16                  |
| 7                | 2.784     | 40.25±1.82 | 3.56±1.30                 | 102.50±1.73                 |
| 1 h              | 4.050     | 39.75±1.30 | 3.35±1.26                 | 111.75±1.41                 |
| LSD (p<0.05)     |           | 2.244    | 0.086                      | 2.675                        |

\(^a\)TAA: Time after application; \(^b\)Dose: Daily oral doses in mg active ingredient kg\(^{-1}\) b.wt. (bw.t) day\(^{-1}\), n = 4

Table 4: Mean±SE values of total protein and glucose levels and cardiac enzymes; lactate dehydrogenase (LDH), \(\gamma\)-glutamyl transferase (GGT) and Creatine Kinase (CK) activity after given rats daily oral doses equal to residues of chlorpyrifos-methyl in tomato fruits for 45 days

| TAA\(^a\) (days) | Dose\(^b\) | Total protein (g dL\(^{-1}\)) | Glucose (mg dL\(^{-1}\)) | LDH (UL\(^{-1}\)) | GGT (UL\(^{-1}\)) | CK (UL\(^{-1}\)) |
|------------------|-----------|----------------------------|--------------------------|------------------|-----------------|-----------------|
| Control          | 0.000     | 1.26±0.36                  | 22.80±1.74               | 448.00±1.84      | 2.22±0.28       | 792.84±1.72     |
| 21               | 1.739     | 1.28±0.96                  | 23.10±1.94               | 419.26±1.82      | 2.22±0.31       | 790.29±1.99     |
| 14               | 2.179     | 1.35±0.58                  | 24.65±1.50               | 266.78±1.84      | 2.64±0.53       | 803.25±1.97     |
| 7                | 2.784     | 1.41±0.40                  | 24.85±1.47               | 205.43±1.69      | 2.83±1.07       | 814.68±1.39     |
| 1 h              | 4.050     | 1.56±0.27                  | 26.02±1.71               | 187.58±1.99      | 3.01±0.90       | 827.48±1.40     |
| LSD (p<0.05)     |           | 0.037                      | 0.713                     | 3.561            | 0.038           | 4.562           |

\(^a\)TAA: time after application; \(^b\)Dose: daily oral doses in mg active ingredient kg\(^{-1}\) b.wt. day\(^{-1}\), n = 4
Table 5: Mean±SE values of liver enzyme function; aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) and kidney creatinine, and uric acid levels of serum of rats administered daily oral doses equal to chlorpyrifos-methyl residue in tomato after different time intervals for 45 days

| TAAa (d) | Doseb | AST (UL−1) | ALT (UL−1) | ALP (UL−1) | Creatinine (g dL−1) | Uric acid (mg dL−1) |
|----------|-------|------------|------------|------------|---------------------|-------------------|
| Control  | 0.000 | 24.11±1.43 | 10.64±0.73 | 53.63±1.58 | 2.17±0.39          | 7.95±1.60         |
| 21       | 1.739 | 24.48±1.50 | 8.95±1.35  | 57.72±1.48 | 2.18±0.36          | 7.86±1.22         |
| 14       | 2.179 | 18.75±1.37 | 8.55±1.09  | 67.51±1.77 | 2.22±0.37          | 8.32±1.47         |
| 7        | 2.784 | 16.24±1.46 | 8.42±1.47  | 74.44±1.42 | 2.27±0.42          | 8.40±1.35         |
| 1 h      | 4.050 | 14.51±1.57 | 8.17±1.41  | 86.67±1.65 | 2.30±0.34          | 9.37±1.11         |
| LSD (p<0.05) | | 1.098 | 0.123 | 3.763 | 0.097 | 0.233 |

aTAA: Time after application. bDose: Daily oral doses in mg active ingredient kg−1 b.wt. day−1, n = 4

Side effects on liver function: Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) enzyme activities were evaluated in the serum of rats that were treated with doses equal to the residue levels of chlorpyrifos-methyl in tomato fruits (Table 5). Results showed that chlorpyrifos-methyl significantly decreased activities of both AST and ALT enzymes. Percentages of the decrease of AST in comparison to control were 22.23, 32.64 and 39.82%, at 2.179, 2.784 and 4.050 mg kg−1, respectively. Percentages of the decrease of ALT relative to control were 15.88, 19.64, 20.86 and 23.21% at 1.739, 2.179, 2.784 and 4.050 mg kg−1, respectively. On contrary, these doses increased the ALP activity relative to the control by 7.63-61.61%.

Adverse effects on kidney function: The effects of chlorpyrifos-methyl on the levels of creatinine and uric acid were presented in Table 5. The results revealed that low dose of 1.739 mg ai kg−1 b.wt. day−1 had no effect on the concentrations of creatinine and uric acid. However, high doses of chlorpyrifos-methyl (2.179, 2.784 and 4.050 mg ai kg−1 b.wt. day−1) increased the levels of the tested parameters in a dose dependent manner. The percentages of increase relative to the control were 2.21, 4.52 and 6.00% for creatinine and 4.65, 5.66 and 17.86% for uric acid.

DISCUSSIONS

Chlorpyrifos-methyl is being applied extensively on plants to control many insect pests in Egypt and worldwide. It is a phosphorothioate insecticide that is detoxified mainly by binding to carboxylesterases, hydrolysis by α-esterases and to some extent degradation by the glutathione system (Smegal, 2000). Chlorpyrifos-methyl is rapidly absorbed and metabolized in humans and environment. The main metabolite is 3,5,6-trichloro-2-pyridinol (3,5,6-TCP). Both chlorpyrifos-methyl and 3,5,6-TCP were excreted in the urine and feces and were not stored to any extent (Smegal, 2000).

The adverse effects of its accumulated residue levels after several times of application on fruits of tomato have not been evaluated. Current study was the first (to the best of our knowledge) to measure the residue levels of chlorpyrifos-methyl in tomato fruits after field application for more than 2 months and moreover to study side effects of the residual amount on mammals.

Many researchers evaluated the residue of chlorpyrifos-methyl and its disappearance pattern in plant crops but not tomato to the best of our knowledge. For example Abdel-Rahman (1996) reported that the initial residue of chlorpyrifos-methyl to be 27.84 mg kg−1 in lettuce and the amount of the residue was decreased to 23.08 and 1.0 mg kg−1 after 1 h and 21 days of application. El-Gohary (1998) found that the initial deposit of chlorpyrifos-methyl in cabbage leaves 1 h after application was 5.05 mg kg−1 but it decreased to 0.1 mg kg−1 after 15 days.
Abbassy et al. (1999) found that the initial deposit of chlorpyrifos-methyl in wheat grains was 12.98 mg kg\(^{-1}\) after 1 h of treatment and it decreased to 8.87 mg kg\(^{-1}\) after 24 days of treatment and the half-life was 19.6 days. It’s clear that residue disappearance of chlorpyrifos-methyl was dependent on the dose and the crop.

Results reported herein revealed that the residue level of chlorpyrifos-methyl in tomato fruits after 21 days of application (1.739 mg kg\(^{-1}\)) was more than its maximum residual limit (MRL = 0.5 mg kg\(^{-1}\) (CAC., 2010)) but did not show any adverse effects.

Toxicological results of chlorpyrifos-methyl residues in tomato fruits after 21 days of the last spray application caused some adverse effects on liver, heart and kidney functions. But it had no deleterious effect on rats’ hematological parameters; Packed Cell Volume (PCV%), Red Blood Cell counts (RBC’s) and White Blood Cell counts (WBC’s). The severe toxic effects were noticed after orally-administering rats with doses equal to the residue levels after 7 and 14 days of application. The pronounced adverse effects were reduced lactate dehydrogenase (-58.13% of control) and aspartate transaminase (-39.82% of control) and increased alkaline phosphatase (61.61% of control) and \(\gamma\)-glutamyl transferase (35.59% of control) activities. The damage to liver, heart and kidney functions that was reported herein was supported by previous studies (Amal, 1997; Abbassy et al., 2000; El-Maghraby, 2000; Dere and Polat, 2001; Adeniran et al., 2006; Soliman et al., 2007).

Abbassy et al. (2000) found that chlorpyrifos-methyl increased the activity of AST, ALT and ALP enzymes and levels of total protein, urea and creatinine in white albino rats. Soliman et al. (2007) reported that chlorpyrifos-methyl increased WBCs counts, ALT and AST activities and creatinine level and decreased RBCs counts and hemoglobin concentration.

Current study reported important information regarding the toxicity of the actual residue amounts of chlorpyrifos-methyl insecticide in tomato after different time intervals of last spray application. It was clear that residue amounts after 21 days of last application had no toxic effects on rats. Future work would be to feed rats on freeze-dried tomato samples mixed with the rats’ diet. Also, the evaluation of the insecticide residue amount in tomato on the oxidative stress systems of rats would be studied. It’s very important to measure the amount of the 3, 5, 6-TCP in tomato and investigate its toxicity. The application of pesticides to control plant pathogens is a requirement, so it’s critical to follow safety guidelines of pesticides usage and to harvest plants after the time that bring no harmful effects to people.

ACKNOWLEDGMENT
The authors would like to acknowledge the Pesticide Residues and Environmental Pollution Department, Central Agricultural Pesticide Laboratory, Agricultural Research Center, Dokki, Giza 12618, Egypt for renting the HPLC and GC-MS equipment for helping with the analysis of insecticide residues.

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