

## **Dietary exposure assessment of chlorpyrifos methyl residue for some fruits, vegetables and herbs in Egypt during 2002**

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### **ABSTRACT**

The study of chlorpyrifos methyl was conducted in the central laboratory pesticide residues analysis and heavy metals in food have been set up by Egyptian Ministry of Agriculture and Finland conducts tests to evaluate the safe usage of pesticides in Egypt. Chlorpyrifos methyl one of the most widely applied organophosphate insecticide, It is classified as moderately acutely toxic by the oral route. A total of 12919 samples of 57 different types of fruits, vegetable, and aromatic medicinal plants samples were examined for chlorpyrifos methyl residues during 2002, thirty three commodities represent 36.44% were completely free of chlorpyrifos methyl residues. Twenty four commodities represent total 8211 vegetables (1592) and aromatic medicinal plants (6619) with percentages 63.56 % of total examined samples have chlorpyrifos methyl residues. All fruit samples were free from chlorpyrifos methyl residues. Only 1.57 % of total contaminated commodities (129 samples) were below limit of determination (LOD = 0.05 ppm ), 8.61 % at LOD or more However 91.39 % of those samples are not contaminated with chlorpyrifos methyl residues. Only 5.76 % of the samples exceeded MRL's of chlorpyrifos methyl residues comparing with national, codex and EU-MRL's. Chlorpyrifos methyl residues was the detected frequently in grapes leaf and cabbage samples with percentage of 2.9 % and 2.3 %, respectively also grapes leaf showed higher violation rate 1.45 % followed by dry molokhia 1.17 %, cabbage and water cress with percentages of 1.15 % . The contributors to total intake of chlorpyrifos methyl is the root vegetables 45.95 %. Data showed that the total dietary intake of chlorpyrifos methyl 0.00094 mg/kg. body weight /day is lower than ADI (0.01 mg/kg body weight ) and contributing only 9.4 % of ADI . Therefore dietary exposures to chlorpyrifos methyl are still far and not a case for Egyptian consumer concern.

**Key words:** Chlorpyrifos methyl , Exposure assessment, Residue, Monitoring, Fruits and Vegetables, Herbs, Aromatic and medicinal plants

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## INTRODUCTION

Chlorpyrifos-methyl is an organophosphate insecticide used to protect stored grain, including wheat, barley, rice, and sorghum. In addition to direct application to these grains, empty grain bins may be treated. There are no other registered uses, hence no residential exposures to chlorpyrifos-methyl are anticipated. End-use product formulations consist of dusts and a liquid concentrate. Its toxicity profile includes clinical signs and symptoms typical of other organophosphates that inhibit cholinesterase. The U.S. EPA Office of Pesticide Programs, in consultation with the FIFRA scientific advisory panel, has developed guidelines for the cumulative risk assessment of pesticides that share a common mechanism of toxicity U.S. EPA 2002a, 2002b. These new cumulative risk assessment guidelines use the finding that OP pesticides share a common mechanism of toxicity, the inhibition of cholinesterase activity (Mileson *et al.* 1998; U.S. EPA 1999, 2001).

Chlorpyrifos-methyl is moderately acutely toxic by the oral route. In mammals, chlorpyrifos methyl is rapidly absorbed and metabolized the principal metabolite being 3,5,6-trichloro-2-pyridinol. The parent compound and metabolite are excreted primarily in the urine and excretions and are not stored to any extent in the body. This metabolite has also been shown to occur in plants. Smith G.N., *et al.* 1967

Chlorpyrifos-methyl is reported to be hydrolyzed by water, the rate being dependent on temperature and pH. This hydrolysis is enhanced by traces of copper ions due to chelation. The major products of hydrolysis are 3,5,6-trichloro-2-pyridinol and O,O-dimethyl phosphate. Meikle, 1973).

Monitoring programs can contribute in improving safety of food, warning of actual and potential food contamination via food and evaluations of possible health hazards throughout providing continuous information on levels of environmental pollution in the country. Public concern over pesticide residues on vegetables and fruits has been increasing in recent years. The risk to human health is due to pesticide residues in the edible parts. The objectives of food contamination monitoring programmes are to safeguard health, to improve the management of food and agricultural resources as well as preventing economic losses.

The benefits to be derived from national monitoring programmes are improved food safety, warning of problems of contamination, provision of intake data for evaluation of health hazards, better management and use of natural resources and a series of measures of good agricultural practice (GAP). The current study was conducted as a part of the main national food contamination monitoring program that has been carried out in Egypt since 1988, Dogheim *et al.*, (1988,1990,1991,1999, 2001, 2002).

## **MATERIALS AND METHODS**

### **Sampling**

A total of 12919 samples of Local and imported vegetables, fruits and some aromatic medicinal plants were collected from different local markets representing 5 Egyptian Governorate include (Qalyubiya- Giza – Ismailia – Minufiya – Beni suef throughout 2002. The vegetables ,fruit and medicinal plant samples that selected for the survey were, demonstrated in table (1). Two kg from vegetables, fruit and 500g from aromatic medicinal plant for each commodity was thoroughly homogenized and prepared according to Codex Alimentarius Guidelines, 1993. Chlorpyrifos methyl residues are subjected for analysis in all samples.

### **Pesticide Residues Analysis**

Official method of AOAC, 1995 was followed with some modifications which were, the sample and the solvent amounts are only half of those in AOAC method. Rotary evaporator and air below are used instead of kuderna- Danish concentrators. The total volume of acetone extract is measured for result calculations. However, the AOAC method uses tabulated water percentages of commodities. After drying, aromatic phase is concentrated just to dryness. The dried samples were dissolved in hexane /acetone containing 0.3 ug/ml of ditalimphos as an internal standard for GC determination.

### **GC Determination**

The detection and confirmation of presence of residues in the samples depends on the use of two chromatography columns of different polarities equipped with nitrogen-phosphorus detectors (NPD) installed in one GC instrument with one injector. Quantitative determinations are made using ditalimiphos as an internal standard.

### **Quality Assurance:**

The analytical method and instruments were fully validated as part of a laboratory quality assurance system and are accredited by Finnish Accreditation Service FINAS (center of metrology and accreditation) Finland (1997). The criteria of quality assurance of the codex committee are followed to determine the performance of the multiresidue method. This quality system is referred to ISO/IEC Guide 17025.

The average recoveries percentages of the tested pesticides on chamomile were ranged between 80-102% at spiking levels 0.07 -0.1 mg/kg, with coefficient of variation (CV%), 2.5-13%. However, the average recoveries, coefficient of variation (CV%) of the tested compounds on pepper samples at spiking levels 0.05-0.5 mg/kg were 80-106%, 2-19% respectively. The reproducibility expressed as relative standard deviation was less than 20%. The limit of quantification was 0.02 mg/kg. The measurement uncertainty including random and systematic error at 95% confidence level is less than 10%. Blank sample is fortified with the pesticides mixture and analyzed as normal sample with each set of samples. The results are recorded on control charts. Repeated analysis of old samples is regularly followed to control reproducibility

◆ **Apparatus**

(a) Gas chromatography: HP 5890 equipped with double Nitrogen Phosphorus

Detector (NPD) with two capillary columns, injector 225°C, detector 280°C. Operating conditions: Hydrogen 3.5 ± 0.1 ml/min, Air 100-110 ml/min, and Nitrogen carrier gas 25 ml/min.

**Chromatography columns are:**

- (1) PAS-5 NPD tested ultra 2 silicon, 25m x 0.32 mm, and film thickness 0.52 um
- (2) PAS - 1701 NPD tested 1701 silicon, 25 m x 0.32mm, film thickness 0.25 um.

Temperature programmes of both GC instruments are; initial temperature 90°C for 2 min, ramp (1) 20 (°C / min) to 150 °C, ramp (2) 6 (°C/min) to 270 °C hold 15min.

◆ **Reagents**

- **Solvents and chemicals**

- (a) Acetone, dichloromethane, n-hexane, petroleum ether, (Pestiscan chromatography grade or similar quality).
- (b) Anhydrous sodium sulphate (Riedel-de Haen), sodium chloride

◆ **Pesticide reference standards**

Chlorpyrifos methyl reference material is certified standard provided by Dr. Ehrenstorfer laboratories GmbH, Gogginger Str. 78 D-8900 Augsburg and financed by FAO (Food and Agriculture Organization of the United Nations), Rome, Italy.

**Results and Discussions:**

A total of 12919 samples of 57 different types of fruit, vegetable, and aromatic medicinal plants samples were examined for chlorpyrifos methyl residues during 2002, results are shown in table (1) & (2). Thirty three commodities represent 4708 samples with percentages 36.44 % were completely free of chlorpyrifos methyl residues. Chlorpyrifos methyl residues, usually analyzed by multiresidue method capable of detecting up to 82 or more pesticides. Twenty four commodities represent total 8211 samples of vegetables (1592) and aromatic medicinal plants (6619) with percentages 63.56 % of total examined samples have chlorpyrifos methyl residues. All fruits samples examined (2913) were free of chlorpyrifos methyl residues.

Table (1) & (2) showed the levels of chlorpyrifos methyl detected in fruit, vegetable and aromatic medicinal plant samples. European union (EC), Egyptian Organization of Standardization (EOS) and codex maximum residue limits were followed and due to lack of chlorpyrifos methyl codex MRL's on such these commodities combinations. The EC maximum residue limits was used followed by EOS then codex to evaluate the results. In few cases extrapolation was followed in such crops that didn't include in codex or at any guidelines and produced locally such as grape leaf and molokhia and larkspur flower. The contaminated commodities have detectable chlorpyrifos methyl residues below limit of determination (LOD) were found in 129 sample with 1.57 %, contaminated samples at LOD or more were 578 sample with 7.04 %, meaning the total contaminated samples were 707 sample (129 + 578 sample) with 8.61 %, However the samples not contaminated with chlorpyrifos methyl residues were 7504 sample with 91.39%. Only 473 sample represent 5.76 % exceeded MRL's of chlorpyrifos methyl residues comparing with national, codex and EU-MRL's.

Chlorpyrifos methyl residues was the detected frequently in grape leaf and cabbage samples with percentages of 2.9 % and 2.3 %, respectively also grape leaf showed higher violation rate 1.45 % followed by dry molokhia 1.17 % , cabbage and water cress with percentages of 1.15 % .

In case of aromatic medicinal plants chlorpyrifos methyl residues was the detected frequently in green and dry mint samples with percentages of 18.87 % and 17.69 %, respectively and also showed higher violation rate for the same commodities 18.87 % and 14.99 % which indicates that we need to put a extensive plane to apply good agricultural practices for chlorpyrifos methyl use for both commodities.

Watercress, dry molokhia and green beans showed the lowest contamination rates 1.15 %, 1.16 % and 1.24 %, respectively with slightly violation percentages 1.15, 1.17 and 0.24 % .No violation observed in tomatoes green molokhia and dry coriander However, in case of aromatic medicinal plants Basil and fennel samples showed the lowest contamination rate than all herbs 0.41 and 0.38 % .

A person exposed to a pesticide at a level that is considered safe may in fact experience harm if that person is also exposed to other substances that cause a common toxic effect by a mechanism common with that of the subject pesticide, even if the individual exposure levels to the other substances are also considered safe.

Chlorpyrifos methyl is a member of the organophosphate (OP) class of pesticides. All pesticides of this class contain phosphorus and other members of this class of pesticides are numerous. EPA considers the organophosphate pesticides should be considered as a group when performing cumulative risk assessments.

In the framework it is stated that a cumulative risk assessment of substances that cause a common toxic effect by a common mechanism will not be conducted until an aggregate exposure assessment of each substance has been completed. This framework is need more studies to be finalized. The risk to human health is due to pesticide residues in the edible parts of the crop and the consequent daily intake of these residues. The acceptable daily intake (ADI) of the chlorpyrifos methyl derived from the chronic toxicity data. The established ADI value of

chlorpyrifos methyl in Egypt are indicated in codex published by Food and Agriculture Organization of united nations (FAO) which is 0.01 mg/kg body weight. The EDI (Estimated daily intake) of a chlorpyrifos methyl by Egyptian people is calculated from the Middle Eastern food consumption GEMS / food data and chlorpyrifos methyl residue monitoring data using an average body weight of 60 kg.

The current exposure assessment addressed only the risks posed by chlorpyrifos methyl residue in vegetables and herbs . The contributors to total intake of chlorpyrifos methyl are in descending order as follows root vegetables 45.95 %, fruiting vegetables 31.72 %, dry herbs 9.56 %, green herbs 7.93 % and Leafy vegetables 4.84 %. The major contributors crops to total intake of chlorpyrifos methyl are potatoes 45.95 % followed by pepper 11.31 %, tomato 11.12 %, green beans 9.29 % Green and dry mint 5.86 and 5.27 % respectively.

The risk exposure assessment of chlorpyrifos methyl residues results are shown in Table 3 . Data showed that the total dietary intake of chlorpyrifos methyl 0.00094 mg/kg. body weight /day is lower than ADI (0.01 mg/kg body weight ) and contributing only 9.4 % of ADI . Therefore dietary exposures to chlorpyrifos methyl are still far and not a case for Egyptian consumer concern

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Table (1): Monitoring data of chlorpyrifos methyl residues in some fruits and vegetables collected from local markets in 2002

Product Name	Total No. of samples	Freq. %	contaminated samples		Min. Conc.	Max. Conc.	Mean Conc.	MRL's mg/kg	No of samples violated	Violation %
			No	%						
<b>Fruits**</b>										
	2913	0	0	0	0	0	0	--	0	0
<b>Vegetables</b>										
<b>Fruiting vegetables</b>										
Green beans	404	0.25	5	1.24	1.5	1.5	1.500	0.05 EOS	1	0.25
Pepper	166	1.20	4	2.41	0.06	3.7	1.880	0.5 CXL	1	0.60
Tomatoes	149	2.01	5	3.36	0.05	0.12	0.077	0.5 CXL	0	0.00
<b>Leafy vegetables</b>										
Cabbage	87	2.30	3	3.45	0.05	0.41	0.230	0.1 CXL*	1	1.15
Dry Molokhia	86	1.16	1	1.16	0.47	0.47	0.470	0.1 CXL*	1	1.17
Grape leaf	69	2.90	3	4.35	0.08	0.11	0.095	0.1 CXL*	1	1.45
Green Molokhia	111	1.80	3	2.70	0.07	0.08	0.075	0.1 CXL*	0	0.00
Water Cress	87	1.15	1	1.15	0.23	0.23	0.230	0.1 CXL*	1	1.15
<b>Root vegetables</b>										
Potatoes	433	0.23	2	0.46	0.44	0.44	0.440	0.05 EC	1	0.23

- (Codex) Codex Alimentarius commission, (Min) Minimum, (Max) Maximum, (EU) European union (MRL's ) Maximum Residue Limits, (Freq) Frequency, (EU) European union, (EOS) Egyptian Organization of Standardization, (ppm) part per million (\*) Extrapolated
- The commodities (apple 2342, cantaloupe 79, fig 27, grape 138, guava 77, lemon 15, orange 128, peach 31, pear 23, plum 20, pomegranate 33, cucumber 123, Egg plant 117, Green peas 79, hot chili 138, okra 69, Squash 113, Strawberry 57, lettuce 78, Spanish 71, carrot 91, green onion 116 and celery 13 ) 3978 samples are free of chlorpyrifos methyl residues.
- Total No of contaminated commodities analyzed 1592 samples (only vegetables)

Table (2): Monitoring data of chlorpyrifos methyl residues in some herbs collected from local markets in 2002.

Product Name	Total No. of samples	Freq. %	contaminated samples		Min. Conc.	Max. Conc.	Mean Conc.	MRL's mg/kg	No of samples violated	Violation %
			No	%						
<b>Herbs</b>										
<b>Dry herbs</b>										
Anise Seed	219	3.20	8	3.65	0.05	1.90	0.483	0.1 CXL*	5	2.28
Basil	737	0.68	16	2.17	0.05	0.26	0.164	0.1 CXL*	3	0.41
Chamomile	1022	3.03	37	3.62	0.06	3.90	0.784	0.1 CXL*	24	2.35
Cumin	176	11.36	19	10.80	0.06	2.50	0.493	0.1 CXL*	18	10.23
Dry Celery	39	5.13	2	5.13	0.07	6.80	3.435	0.1 CXL*	1	2.56
Dry Coriander	278	0.36	1	0.36	0.05	0.05	0.050	0.1 CXL*	0	0.00
Dry Dill	111	5.41	10	9.01	0.04	0.11	0.077	0.1 CXL*	1	0.90
Dry Mint	2442	17.69	506	20.72	0.04	24.00	1.296	0.1 CXL*	366	14.99
Dry Parsley	135	1.48	2	1.48	0.68	5.90	3.290	0.1 CXL*	2	1.48
Fennel	526	0.95	6	1.14	0.09	0.19	0.124	0.1 CXL*	2	0.38
Marjoram	619	2.42	28	4.52	0.05	4.20	0.391	0.1 CXL*	7	1.13
<b>Green herbs</b>										
Green Coriander	89	8.99	8	8.99	0.07	7.0	1.761	0.05 EC	8	8.99
Green Dill	86	13.95	19	22.09	0.05	14.0	1.538	0.05 EC	11	12.79
Green Mint	53	18.87	10	18.87	0.13	5.8	1.440	0.05 EC	10	18.87
Green Parsley	87	9.20	8	9.20	0.07	5.0	1.340	0.05 EC	8	9.20

- (Min) Minimum, (Max) Maximum, (EU) European union (MRL's) Maximum Residue Limits, (Freq) Frequency, (EU) European union, (EoS) Egyptian Organization of Standardization, (ppm) part per million (\*) Extrapolated.
- The commodities (calendula flower 74, calendula petals 35, caraway 228, hibiscus 219, larkspur flower 4, Rosemary 7, Tillio 4, verbascum 17 and lemon grass 142) 730 samples are free of chlorpyrifos methyl residues.
- Total No of contaminated commodities analyzed 6619 samples

**Table (3). The estimated daily intake and intake percentages of chlorpyrifos methyl residues for fruits, vegetables and herbs according to middle eastern food consumption data.**

Product Name	Chlorpyrifos methyl residues Mean	Middle eastern Food consumption gm/person/day	Estimated Daily Intake (EDI) µg/day	% Intake
<b><u>Fruits</u></b>	0	0	0	0
<b>Subtotal fruit</b>	0	0	0	0
<b><u>Vegetables</u></b>				
<b>Fruiting vegetables</b>				
Green beans	1.500	3.5	5.25	9.29
Pepper	1.880	3.4	6.39	11.31
Tomatoes	0.077	81.5	6.28	11.12
<b>Subtotal Fruiting vegetables</b>			<b>17.92</b>	<b>31.72</b>
<b>Leafy vegetables</b>				
Cabbage	0.230	5.0	1.15	2.04
Dry Molokhia	0.470	0.5	0.24	0.42
Grape leaf	0.095	7.8	0.74	1.31
Green Molokhia	0.075	7.8	0.59	1.04
Water Cress	0.230	0.1	0.02	0.04
<b>Subtotal Leafy vegetables</b>			<b>2.73</b>	<b>4.84</b>
<b>Root vegetables</b>				
Potatoes	0.440	59.0	25.96	45.95
<b>Subtotal Root vegetables</b>			<b>25.96</b>	<b>45.95</b>
<b>Subtotal Vegetables</b>			<b>46.61</b>	<b>82.51</b>

Cont. Table (3)

Product Name	Chlorpyrifos methyl residues Mean	Middle eastern Food consumption gm/person/day	Estimated Daily Intake (EDI) µg/day	% Intake
<b>Herbs</b>				
<b>Dry herbs</b>				
Anise Seed	0.483	2.3	1.11	1.96
Basil	0.164	0.3	0.05	0.09
Chamomile	0.784	0.3	0.24	0.42
Cumin	0.493	0.5	0.25	0.44
Dry Celery	3.435	0.1	0.34	0.60
Dry Coriander	0.050	0.5	0.03	0.05
Dry Dill	0.077	0.1	0.01	0.02
Dry Mint	1.296	2.3	2.98	5.27
Dry Parsley	3.290	0.1	0.33	0.58
Fennel	0.124	0.3	0.04	0.07
Marjoram	0.391	0.1	0.04	0.07
<b>Subtotal Dry Herbs</b>			<b>5.40</b>	<b>9.56</b>
<b>Green herbs</b>				
Green Coriander	1.761	0.5	0.88	1.56
Green Dill	1.538	0.1	0.15	0.27
Green Mint	1.440	2.3	3.31	5.86
Green Parsley	1.340	0.1	0.13	0.23
<b>Subtotal Green Herbs</b>			<b>4.48</b>	<b>7.93</b>
<b>Subtotal Herbs</b>			<b>9.88</b>	<b>17.49</b>
<b>Total</b>			<b>56.50</b>	<b>100</b>

- Calculated Dietary intake for chlorpyrifos methyl =  $56.50 / 1000 / 60 = 0.00094$  mg/kg. body weight /day
- Acceptable Daily Intake of chlorpyrifos methyl (ADI) = 0.01 mg/kg body weight
- Total estimated dietary intake for chlorpyrifos methyl as a percentage of set ADI =  $0.00094 * 100 / 0.01 = 9.4 \%$

## الملخص العربي

### دراسة مخاطر السمية لمتبقيات مبيد الكلوربيريفوس في بعض محاصيل الفاكهة والخضر والأعشاب الطبية في مصر خلال عام 2002

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المعمل المركزي لتحليل متبقي المبيدات والعناصر الثقيلة في الأغذية

وزارة الزراعة - مركز البحوث الزراعية

تم إنشاء المعمل المركزي لتحليل متبقيات المبيدات والعناصر الثقيلة في الأغذية بالتعاون بين وزارة الزراعة المصرية ودولة فنلندا لعمل الاختبارات وتقييم الاستخدام الآمن للمبيدات في مصر . مركب الكلوربيريفوس مثل أحد المركبات الفسفورية المستخدمة بصورة واسعة لمقاومة الآفات . تم تصنيف مركب الكلوربيريفوس مثل كمركب متوسط السمية الحادة عن طريق الفم . تم اختبار عدد 12919 عينة من 57 نوع من أنواع الخضر والفاكهة وكذلك النباتات الطبية والعطرية لمبيد الكلوربيريفوس مثل لعام 2002 حيث وجد أن 33 نوع من أنواع المحاصيل المختبرة تمثل نسبة 36.44 % خاليه من متبقيات هذا المبيد بينما 24 نوع من أنواع هذه المحاصيل بإجمالي 8211 عينة 1592 خضر و 6619 نباتات الطبية والعطرية بنسبة إجمالية 63.56 % من مجموع العينات المختبرة ملوث بمتبقي مبيد الكلوربيريفوس وتعتبر هذه الأنواع من المحاصيل ملوثة . وجد أن جميع عينات الفاكهة خالية من متبقيات هذا المبيد . أشارت النتائج إلى أن 1.57 % تمثل 129 عينة من مجموع أنواع المحاصيل الملوثة تحت حدود التقدير ( 0.05 جزء في المليون) كما أن 8.61 % عند أو أعلى من حدود التقدير لمتبقي مبيد الكلوربيريفوس مثل بينما 91.39 % من مجموع أنواع هذه المحاصيل غير ملوث بمتبقي مبيد الكلوربيريفوس مثل . أشارت النتائج إلى أن 5.76 % من مجموع أنواع المحاصيل الملوثة تتعدى الحدود القصوى المسموح بها مقارنة بالحدود القصوى المسموح بها محليا أو حدود منظمة الكودكس وكذلك الحدود الأوروبية . تم تعيين متبقي مبيد الكلوربيريفوس مثل بصورة متكررة في عينات ورق العنب والكرنب بنسبة 2.9 % و 2.3 % على التوالي كما سجل ورق العنب أعلى نسب تعدى لحدود القصوى 1.45 % متبوعا بنبات الملوخية 1.17 % وأخيرا الكرنب والجرجير 1.15 % . أشارت النتائج إلى أن محاصيل الخضر الجذرية هي أكثر المحاصيل مشاركة في المتناول الغذائي اليومي بمتبقي مبيد الكلوربيريفوس مثل حيث وصلت هذه النسبة إلى 95 45 % كما أظهرت الدراسة أن مجموع المتناول اليومي للخضر

والفاكهة وكذلك النباتات الطبية والعطرية للمحاصيل المختبرة لهذا المبيد للإنسان المصري 0.00094 ملليجرام لكل كيلو جرام من وزن جسم الإنسان في اليوم وهذه القيمة اقل من قيمة المتناول اليومي المسموح به وهو 0.01 ملليجرام لكل كيلو جرام من وزن جسم الإنسان في اليوم ويمثل فقط 9.4 % من قيمة المتناول اليومي المسموح به وهذا يعنى أن التعرض لميتيقي مبيد الكلوربيريفوس بعيد عن مستوى الخطورة إلى الآن بالنسبة للمستهلك المصري