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Sub-lethal Toxic Effects Induced by Pirimiphos-methyl in The Japanese Quail (*Coturnix coturnix japonica*)

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ABSTRACT

Pirimiphos-methyl (ACTELLIC®) is recommended pesticide for control of stored product insects. In this study, pirimiphos-methyl was administered to adult Japanese quail (*Coturnix coturnix japonica*) for 21 days at dietary levels of 1/10, 1/100 and 1/1000 LC_{50}. At 1/10 LC_{50}, quail body weight was significantly decreased by 16.86% than that of the control. However, absolute weight of liver from quail exposed to 1/10 and 1/100 LC_{50} showed significant increases than that of the control. Kidney weight was also increased at the lower concentration, 1/1000 LC_{50}, while showed a significant decrease at the higher concentration, 1/10 LC_{50}. Data of liver function showed a significant increase in levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) at concentrations of 1/10 and 1/100 LC_{50}. Also total protein levels were increased at these concentrations. In kidney function parameters, data showed an obvious increase in concentrations of uric acid at 1/10 and 1/100 LC_{50}. However, creatinine concentration was significantly decreased at 1/10 and 1/100 LC_{50}. Moreover, data of sexual hormones showed significant increase in female progesterone and male testosterone at 1/10 and 1/100 LC_{50}, while no significant changes were observed at 1/1000 LC_{50}. The number of egg laying showed significant reduction by 58.6 and 44.8 % at 1/10 and 1/100 LC_{50}, respectively. Based on these results, body weight, clinical signs, and serum biochemical profile findings may be useful parameters for detecting the dietary toxicity associated with the organophosphate pesticides, pirimiphos methyl. In addition, Japanese quail could be an excellent bird model for monitoring the toxicological risks of pesticides in Egypt.

Keywords: Primiphos-methyl; 21-Day dietary exposure; Japanese quail; Serum biochemistry.

INTRODUCTION

The increased use of insecticides as means of crop protection led to many health problems in human and ambient environment. (Benbrook, 1991). Studies were conducted to few last decades included some clinical effects such as embryo immune toxicity, serum enzymes and biochemical indicators (Walker, 2003). Abrams, 1995 investigated sublethal doses of some pesticides. He claimed that these doses caused some changes in neurotransmitters, immunity, physiology, morphology and behavior of tested animals. Grue *et al.* (1991) reported that the exposure of birds to certain organophosphate (OP) and carbamate (CB) pesticides caused reduction in food consumption and symptoms of gastrointestinal. A little work has been done to investigate the possible histopathological and serum biochemical changes induced by sub-chronic doses of organophosphorus pesticides in the Japanese quail. Ghaffar *et al.* (2015) investigated sub-lethal effects induced by triazophos in the Japanese quail. They found that kidneys, lungs, spleen and heart of birds received higher dose of triazophos (8 mg/kg BW) were swollen and congested. In contrast, the relative weight of spleen, kidneys, lungs was significantly increased while the relative weight of heart significantly decreased as compared to untreated control group. Also, they found that serum urea, creatinine, cardiac enzymes were significantly increased opposite total proteins decreased in treated birds. In the same context, Hussain *et al.* (2013) studied the effects induced by malathion in male Japanese quail. They observed significant decrease in serum albumin and serum total protein and significant increased in serum creatinine, alanine and aspartate transaminases. Recently, Hamidipoor *et al.* (2015) demonstrated that feeding deltamethrin-contaminated grains to the Japanese quail caused also significant changes in some blood biochemical parameters.

In order to detect and evaluate the toxicities of pirimiphos-methyl, Japanese quail were chosen as a test species because they are small enough to handle, and are easily obtained from local suppliers. The purposes of the present study were to verify the toxic hazard of pirimiphos-methyl on Japanese quail and to provide new data for Japanese quail using the 21-day dietary toxicity test that is based on the OECD workshop report (Anonymous, 1996a), and EPA guidelines (Anonymous, 1996b). In this respect the effect on the body weight and some internal organs, egg production, concentrations of sexual hormone and some biochemical parameters were investigated.

MATERIALS AND METHODS

1. Experimental Birds

Sexually mature (males and females), 5-6-week-old Japanese quail (*Coturnix coturnix japonica*) were purchased from a local supplier (Poultry farm, Sakha, Kafr-Elsheikh, Egypt). All the birds were healthy and kept for 14 days in wire cages for acclimatization purpose before the experiment commenced at room temperature (25 ±2 °C), 60 ± 10% relative humidity, and received ~ 10-h light/14-h dark cycles. The birds received commercial layer feed obtained from commercial local supplier and tap water *ad libitum*.

2. Toxicity assays

Commercially formulation of pirimiphos-methyl (ACTELLIC®, 50% EC, Sygenta), was used in this study. To estimate LC_{50}, the up-and-down procedure described by Bruce (1985) and Hill (1994) were used. At least, five concentration levels were used, spaced appropriately to produce test groups with a range of toxic effects and mortality rates (ranged between 20-100% mortality). Adult quails males were randomized and assigned to the treatment groups. Five animals were used at each concentration level. The test pesticide was diluted in corn oil and thoroughly mixed into the layer feed. Animals had been fasted prior to test substance administration for about 4 hrs. Quails in control group were fed on fresh untreated diet mixed with only corn oil. Mortality and signs of intoxication were monitored at least twice daily. The LC_{50} and its 95% confidence limits, expressed as milligram of active ingredient per kilogram of feed (or parts per million) and the slope and error of the dose-response curve were derived by Probit analysis (Finney, 1971).

3. 21-day subacute dietary assay

Sub-Acute dietary Toxicity tests were carried out based on procedure described by The Office of Prevention, Pesticides and Toxic Substances (OPPTS) guideline (Anonymous, 1996c). In this experiment, the birds were divided into four groups with three pairs (males and females, weighing 130-150 g) for each group. The birds in groups I, II and III were fed on diet mixed with selected sublethal concentrations of pirimiphos-methyl. These concentrations were equal to 1/10, 1/100, and 1/1000 of the determined LC_{50}. The test concentration was diluted in corn oil and thoroughly mixed this solution into the layer feed as mentioned above. Quails in the IV-group were fed on fresh untreated diet mixed with only corn oil and served as control. The birds were fed the treated diets *ad libitum* daily for 21 days. Each group was observed for an additional week to detect any potential recovery from the pesticide treatment. The birds were monitoring daily for clinical signs and toxic symptoms. Body weight and growth gain were also recorded every 3 days. The average number of eggs set during the treatment period was recorded for each treatment. On the 21th day of treatment, the birds were sacrificed by cervical dislocation (2-4 hrs after the last exposure). Selected internal organs, liver, and kidney, were surgically removed and cleaned free from blood and other tissues. The relative weight of each organ was calculated by dividing organ weight with body weight and quotient being multiplied by 100.

4. Blood serum biochemical parameters

Blood samples were collected by heart puncture using a syringe. Serum was obtained by centrifugation for 15 min at ~1000-2000 RCF. Serum samples were analyzed for different parameters, like; liver function Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and renal function (creatinine, urea) according to standard procedure described by Benjamin (1978) and Coles (1986). Alkaline phosphatase (ALP) activity in serum samples was determined based on the assay described by Wenger *et, al.,*(1984). Total protein in serum was determined colorimetrically according to Henry *et al.* (1974). All parameters were analyzed spectrophtometrically by using commercially available kits. The chemical-kits were purchased from ALKAN-Pharma Co. S.A.E., Egypt. The levels of serum steroid hormones, testosterone and progesterone, were also determined in both males and females according the methods described by Selzsam *et al.* (2005).

5. Statistical analysis

One-way analysis of variance (ANOVA) was used to analyse different parameters followed by Duncan' multiple(1925) ranged test at $p < 0.05$. The statistical data have been represented in tables and significant differences have been marked with different letters.

RESULTS

1. Mortality and clinical signs of pesticide toxicity

The 24 h-toxicity data of pirimiphos-methyl administered to adult quail at different concentrations showed that LC_{50} and LC_{90} values were 206.5 and 763.2 mg a.i. /kg diet (Table 1). Quail in the control group did not show any apparent signs of toxicity or death .The acute toxicity was characterized by cholinesterase inhibition symptoms which observed within 15-20 minutes to four hours. The toxicity severity following pesticide administration was related to test concentrations. The clinical signs of pirimiphos-methyl toxicity appeared to be similar either in acute toxicity or repeated dietary administration assays, showing huddling, depression; conjunctivitis, mild tremor, diarrhea, and dyspnea. Such observed signs were related to cholinergic crisis, a consistent sign in acute organophosphate poisoning.

Table 1. Mortality percentage of pirimiphos-methyl in male Japanese quail.

Pesticide concentrations (mg AI/kg diet)	24-hrs Mortality (%)	Estimated LC_{50}, LC_{90} (mg a.i. /kg) [a] (95% Confidence Limits), Slope ± SE
600	85.0	LC_{50} = 206.53
400	75.0	(181.46 - 235.18)
200	50.0	LC_{90} = 763.218
100	20.0	(615.20 - 1012.44)
50	10.0	Slope = 2.258 ± 0.181
0	0	

[a]LC_{50}, LC_{90}, expressed as mg a.i. /kg (Finney, 1971). Five adult males were used for each concentration.

2. Body and organ weights

Administration of pirimiphos-methyl to quail adults caused a significant decrease in the mean body weights, after 21-days dietary treatment (Table 2). Feeding on diet mixed with concentrations of 1/10 and 1/100 LC_{50} resulted in 16.8 and 13.5 % reduction in the mean body weights of quail, respectively, in comparison with the control. However, the decrease in mean body weights of quail in the group received 1/1000 LC_{50} was not significant than that of the control.

After treatment with sub-lethal concentrations of pirimiphos-methyl, the quail exhibited a statistically significant increase in absolute and relative liver and kidney weights compared with controls (Table 2). The data revealed a significant increase in the relative values of liver from quails fed on 1/10- and 1/100 LC_{50}-treated diet indicating values of 2.71 and 2.25 %, respectively, compared to 1.62 % for the control treatment. For kidney weights, the data also indicated that feeding on the lowest concentration, 1/1000 LC50, of pirimiphos-methyl caused a significant increase in both absolute and relative kidney weights compared to the control (Table 2). In contrast, feeding on the higher concentration, 1/10 LC_{50} , caused a significant decrease in the relative weight of kidney (1.41%) compared to relative weight to the body weight in the control (1.75%).

Table 2. Effect of sublethal concentration of pirimiphos-methyl on absolute and relative weights of liver and kidney of male quail

Treatment 1/x of LC_{50}	Body weight (g ± SD)	reduction %	Absolute weight of liver and kidney (g ± SD)		Relative to body weight %	
			Liver	Kidney	Liver	Kidney
Control	210.5 ± 7.6 ^{a}	0.00	3.40 ± 2.60 ^{a}	3.70±1.98 ^{a}	1.62	1.75
1/10	175 ± 3.2 ^{b}	16.86	4.75 ± 2.05 ^{b}	2.47±1.50 ^{c}	2.71	1.41
1/100	182 ± 3.2 ^{b}	13.53	4.10 ± 2.26 ^{b}	3.36±2.00 ^{a}	2.25	1.84
1/1000	187 ± 4.6 ^{a}	11.16	3.90± 2.19 ^{a}	4.71± 2.20 ^{b}	2.08	2.51

Values are means of 4 adult males for each treatment after 21 day-dietary treatment.
Values in parentheses refer to reduction % of body weight relative to the control.
Means within each column followed by the same letter are not significantly different at *P*< 0.05.

3. Effect of pirimiphos-methyl on serum enzymes

Data in Table 3 show the activities of some serum enzymes from the quail subjected to 21-days dietary treatment with sublethal concentrations of pirimiphos-methyl. Data showed a significant increase in levels of ALT (alanine aminotransferase) at the higher concentrations tested, 1/10 and 1/100 LC_{50}, in comparison with that of the control. No significant differences were observed for AST (aspartate aminotransferase) levels between pesticide treatments and the control. Data also indicated that the ALP (alkaline phosphatase) levels were significantly increased at concentrations of 1/10 and 1/100 LC_{50} compared to that of the control.

Data also showed a significant increase in uric acid in a concentration dependent manner. In contrast, a significant decrease in serum creatinine level was observed in both 1/10 and 1/100 LC_{50}-pesticide treatments compared to the control. On other hand, no significant difference was observed in serum protein between different treatments and the control.

Table 3. Effect of pirimiphos-methyl on serum biochemical parameters in Japanese quail.

Serum biochemical parameters	Control	Concentrations of pirimiphos-methyl		
		1/10 LC_{50}	1/100 LC_{50}	1/1000 LC_{50}
ALT (IU/L)	263 ± 2.60^{c}	644.0 ± 2.05^{a}	484.0 ± 2.26^{b}	319.0 ± 2.19^{c}
AST (IU/L)	7.0 ±1.98^{a}	5.0 ±1.56^{a}	6.0 ± 2.15^{a}	7.0 ± 2.20^{a}
ALP (IU/L)	541.0 ±3.15^{b}	678.0 ± 6.33^{a}	587.0 ± 3.75^{ab}	520.0 ± 3.56^{b}
Total protein (IU/L)	2.06 ± 0.20^{a}	2.35 ±1.45^{a}	2.52 ± 1.23^{a}	2.48 ± 1.10^{a}
Uric Acid (mg/dl)	3.79 ± 1.60^{b}	7.47 ± 2.05^{a}	7.30 ± 2.26^{a}	4.08 ± 2.19^{b}
Creatinine (mg/dl)	0.10 ± 0.11^{a}	0.06 ± 0.02^{b}	0.06 ± 0.03^{b}	0.09 ± 0.04^{ab}

Values are means ± SD of 4 adult males for each treatment. Means within each row followed by the same letter are not significantly different at *P*< 0.05.
ALT, alanine aminotransaminase (sGPT); AST, aspartate aminotransferase (sGOT), ALP, alkaline phosphatase

4. Effect of pirimiphos-methyl on progesterone and testosterone in Japanese quail

Concentrations of hormones, progesterone in females and testosterone in males, were estimated at study termination (Table 4). A significant increase in progesterone and testosterone levels in serum from the quail fed on diet treated with the higher concentrations, 1/10 LC_{50}, was observed compared to that of the untreated control. Non significant difference was observed in case of birds received the lower concentration tested, 1/1000 LC_{50}.

Table4. Effect of pirimiphos-methyl on progesterone and testosterone in Japanese quail

Treatment 1/x of LC_{50}	Progesterone (mg/dl)	Testosterone (mg/dl)
Control	0.10 ± 0.04 ^{b}	0.25 ± 0.01 ^{b}
1/10	2.53 ± 0.14 ^{a}	0.72 ± 0.22 ^{a}
1/100	0.21 ± 0.05 ^{b}	0.61 ± 0.11 ^{a}
1/1000	0.18 ± 0.03 ^{b}	0.29 ± 0.08 ^{b}

Values are means ± SD of 4 adults for each treatment. Means within each column followed by the same letter are not significantly different at *P*< 0.05.

5. Effects on egg laying rate

It was obvious that all sublethal concentrations caused significant decrease in egg laying rates in a concentration dependent manner. Administration of concentration of 1/10 and 1/100 LC_{50} led to severe reduction in egg laying rate by 58% and 44%, respectively, compared to the untreated control, while the lowest concentration of 1/1000 LC_{50} reduced egg laying by only 17% (Table 5).

Table 5. Effect of pirimiphos-methyl on the eggs laid by Japanese quail

Treatment 1/x of LC_{50}	Mean number of eggs ± SD/ female	% Reduction
Control	29.0 ± 2.0 [a]	0.00
1/10	12.0 ± 1.0 [d]	58.6
1/100	16.0 ± 3.0 [c]	44.8
1/1000	24.0 ± 3.0 [b]	17.21

Values are means of 4 females for each treatments. Means followed by the same letter within column are not significantly different at *P* < 0.05).

DISCUSSION

Pirimiphos methyl (PMP-M) that belongs to the organophosphate (OP) pesticides' group has been used extensively in controlling the stored-product insect pests. Even though OP pesticides, including PMP-M degrade rapidly in the environment, these chemicals may exert lethal and sub lethal effects on birds immediately after chemical application (White *et al.,* 1982). The sublethal effects induced by organophosphate pesticides in the Japanese quail have been studied recently by some researchers. Ghaffar *et al.* (2015) investigated sub-lethal effects induced by triazophos in the Japanese quail. They found that kidneys, lungs, spleen and heart of birds received higher dose of triazophos (8 mg/kg bw) were swollen and congested. In contrast, the relative weight of spleen, kidneys, lungs was significantly increased while the relative weight of heart significantly decreased as compared to untreated control group. Also, they found that serum urea, creatinine, cardiac enzymes were significantly increased opposite total proteins decreased in treated birds. Similarly, Hussain *et al.,* (2013) fed the quail for 51 day on diet mixed in corn oil with sublethal concentrations of malathion(50, 75, 100 and 125 mg/kg b.w). The results indicated a significant decrease in serum albumin and serum total protein compared to the control group. Except the concentration of 50 mg/kg b.w, the remained concentrations caused significantly increased of serum creatinine, aspartate and alanine transaminase at 34 and 51 day posttreatment.

In the present study, toxicity assay showed that LC_{50} of primiphos-methyl (PMP-M) in the Japanese quail was 206.5 mg/kg food. This results are in agreement with Gage, (1971) who ascribed the relative high toxicity of pirimiphos-methyl to the deficiency of esterases accountant of hydrolysis of pirimiphos–methyl oxon. 21- day dietary sub-acute tests OPPTS (Anonymous, 1996c), were carried out by feeding the quail on diet mixed in corn oil with sublethal dose levels that equal 1/10, 1/100 and 1/1000 LC_{50} of pirimiphos methyl. Our results revealed that 21-day dietary sub-acute exposure caused a comparatively lower body weight gain compared to the control groups. It seemed that the rate of decrease in body weight was dose- and exposure time-dependant. These results were in accordance of Seok *et al.* (2008) where they found that the 21-day LC_{50} of organophosphate pesticides, isazofos and pyraclofos significantly decreased the body weight of Japanese quail than that of the untreated control.

The present study also indicated that administration of PMP-M at sub-acute dietary assay significantly increased the liver weights at the higher doses used, 1/10 and 1/100 LC_{50}. However, the kidney weights were significantly reduced after 21 day- sub acute dietary test at 1/10LC_{50}, and increased significantly at the lower dose, 1/100 LC_{50}. These findings were in agreement with results obtained by Grote *et al.* (2006). The effects on liver weight in treated quail might be an indication of either the induction of OP pesticide metabolism as a result of increased substrate availability or minor hepatotoxic effects of pesticide on birds. Similar explanation has been mentioned by Ambali *et al.,* 2007; Ambali, 2009; and Mansour and Mossa, 2010.

Concerning the biochemical profile of blood serum from both treated and control groups of quail, the present study showed that administration of 1/10 LC_{50} of primiphos-methyl caused non-significant increase in protein level compared to that of the control. However, the total serum protein concentration was significantly lower in Wister rats exposed to sub chronic-chlorpyrifos treatment (Ambali *et al.* 2011). Also, a significant increase in serum uric acid concentrations was observed by administration of 1/10 and 1/100 LC_{50}-pirimiphos methyl compared to the control. The increase in serum uric acid concentration is demonstration of impairment of kidney function since the organ primarily excrete urea in the urine. These findings are in agreement with those reported previously by Ambali *et al.* (2007, 2010, and 2011).

The present study showed that 21-day subacute pirimiphos-methyl administration, at 1/10 and 1/100 LC_{50}, caused a significant increase in the activities of serum alanine amino-transferase (ALT) and alkaline phosphatase (ALP). However, no significant difference was observed in the activity of aspartate aminotransferase(AST). These findings suggest that plasma ALT and ALP may be a good marker of prolonged sub-lethal pirimiphos-methyl treatment. Similar results has been obtained by Hussain *et al.* 2012, and Hussain *et al.* 2013 where they noticed significant increase in serum alanine amino-transferase (ALT) activity following prolonged pesticide exposure.

Reproductive performance of the quail was also clearly affected by 21-day dietary sub-acute treatment with sub-lethal doses of pirimiphos methyl. The reduction rate in egg production was dose-dependant. The most reduction percent (~58.6%) in egg reduction was observed at 1/10 LC_{50} of pirimiphos methyl.

Similar finding were obtained by many authors who reported that there were significant alterations in activities of transaminase in different animal species treated with various pesticides such as organophosphates (Rao *et al,* 1991, Rajeev *et al,* 1991 and Abo Arab and Salem, 2005). Also, pirimiphos-methyl produced significant alteration in levels of uric acid in Japanese quail (Abo Arab and Salem, 2005). Form the findings of the present study it can be concluded that sub chronic exposure of quail to pirimiphos-methyl induces various gross and serum biochemical parameters like creatinine and uric acid which are indicators in effect on kidney function while the effect on total protein level and activities of AST, ALT and ALP are indicators of liver function. This study verified the 21-day dietary toxicity test that is based on the PPT workshop report and EPA guidelines. In addition, it is concluded that the Japanese quail, *C. coturnix japonica*, could be an excellent bird model for monitoring the toxicological risks of pesticides in Egypt.

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التأثيرات السامة غير المميتة لمبيد بريميفوس- ميثيل في طائر السمان

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يستخدم المبيد الفسفوري العضوي بيريميفوس- ميثيل (أكتيليك) كأحد المبيدات الموصى بها في مكافحة آفات الحبوب المخزونة , وفي هذا البحث تم تقدير بعض التأثيرات السلبية السامة لهذا المبيد في طائر السمان نتيجة تغذيته علي بيئة غذائية تم خلطها بتركيزات تحت مميتة من المبيد. والتركيزات المختبرة تعادل ١٠/١ , ١٠٠/١ ، ١٠٠٠/١ من التركيز النصفي القاتل LC_{50} للمبيد والذي تم تقديره في اختبارات سمية حادة. وأجريت التجربة بتغذية الطيور علي بيئة غذائية مخلوطة بالتركيزات المختبرة ، لمدة ٢١ يوم متصلة ، وفقا للبرتوكول الذي أصدرته منظمة حماية البيئة الأمريكية لسنة ١٩٩٦. أوضحت النتائج أنه عند تركيز ١٠/١ من الـ LC_{50} فإن وزن جسم الطائر المعامل ينخفض معنوياً بنسبة ١٦.٨٦% عن الكنترول غير المعامل. كذلك يزداد الوزن الكلي للكبد معنوياً عند تركيزات ١٠/١ و ١ /١٠٠ من الـ LC_{50} , وأيضاً يزداد متوسط وزن الكلية معنوياً عند التركيز المنخفض ١ /١٠٠٠ من الـ LC_{50} بينما ينخفض عند التركيز الأعلى ١ /١٠ من الـ LC_{50} . وبتقدير بعض الدلائل البيوكيماوية بسيرم دم ذات الصلة بوظائف الكبد في كل من الطيور المعاملة وتلك غير المعاملة بالمبيد ، أظهرت نتائج التحليل الكيماوي زيادة معنوية في مستويات إنزيمات ALT ، ALP عند تركيزات ١٠/١ و ١ /١٠٠ من الـ LC_{50} , وزيادة مستوى البروتين الكلي عند نفس التركيزات ، عن مثيلتها بالكونترول غير المعامل. كذلك سجلت نتائج التقدير الكيماوي لبعض القياسات ذات الصلة بوظائف الكلى زيادة في تركيز حمض اليوريك عند تركيزات ١٠/١ و ١ /١٠٠ من الـ LC_{50} ، ونقصاً معنوياً في مستوى الكرياتينين عند نفس التركيزات. وإضافة لذلك، فقد أظهرت نتائج التحليل لمستوي الهرمونات الجنسية، زيادة معنوية في مستوى الهرمون الجنسي لكل من ذكور وإناث الطيور المعاملة بتركيزي ١٠/١ ، ١ /١٠٠ من الـ LC_{50} عن مثيلتها في الطيور غير المعاملة. وبالنسبة لمعدل الخصوبة في الطيور المعاملة ، فقد أظهرت النتائج انخفاض معنوي في متوسط عدد البيض الموضوع خلال فترة المعاملة ، وكان معدل الانخفاض عند تركيزات ١٠/١ و ١ /١٠٠ من الـ LC_{50} ٥٨.٦ و ٤٤.٨% على التوالي ، مقارنة بالكونترول. وبصفة عامة ، فإن نتائج هذه الدراسة تؤكد علي مخاطر المبيد الفسفوري بيريميفوس ميثيل بالتركيزات المستخدمة على طائر السمان والذي يعتبر نموذجاً مثالياً لتقدير المخاطر والتأثيرات السلبية لمبيدات الآفات علي الإنسان والحيوان.