The Effects of Polyethylene Glycol (PEG-600) On the Acute Neurotoxicity of Different Types of Pesticides

Author’s Detail: 1) Sayed M Rawi: Biology Department Faculty of Sciences and Arts, Khulais, King Abdulaziz University, Jeddah 
2) Mansour A Al-Hazmi: Biology Department Faculty of Sciences and Arts, Khulais, King Abdulaziz University, Jeddah 
3) Abeer M Waggas: Biology Department, Faculty of Science and Arts, Women Branch Khulais, King Abdulaziz University Jeddah

Abstract

Pesticides intoxication has been implicated in many toxic diseases, and agricultural adjuvants including polyethylene glycols are used in a variety of applications including industrial manufacturing. The aim of this study was to analyze the effect of PEG-600 on the biochemical toxic effects of malathion, imiprothrin and imidacloprid on the basis of the determined LD50 values on the quails (Coturnix coturnix). In the present study the toxic effect of the tested pesticides was characterized by a clear disturbances in the activity of plasma alkaline phosphatase (ALP), transaminases (AST, ALT), acetylcholinesterase (AChE) and total contents, as well the concentrations of brain glutamate, aspartate, GABA, glycine, norepinephrine and dopamine concentrations. PEG-600 treatment did not change blood biochemical parameters but significantly increases the concentrations of whole brain studied amino acids and catecholamines. In addition, the combination of PEG-600 with the tested pesticides acts synergistically on reducing the recorded LD50 values of malathion, imidacloprid and imiprothrin in Coturnix coturnix by -32.247, -41.529 and -11.026; attenuates more toxic symptoms, modulates more changes in the activity of plasma AChE, ALP, AST, and ALT and attenuates more decreased effect in the concentration of the brain dopamine and norepinephrine, and potentiate more toxic effects of the studied pesticides on the concentration of the free studied amino acids. These results could indicate the toxic effect of polyethylene glycol to birds and is also potentiate the neuronal toxic effect of pesticides exposure.

Keywords: Pesticides; PEG-600; Amino acids; Monoamines

Introduction

Most efforts in toxicology are devoted to safety evaluation, so considerations must be given to maximize the biological efficiency of the existing pesticide formulations by certain additives including adjuvant that decreases the rates of insecticide application (Hofstee and Gaskin, 2003; Bjorklund et al., 2009; Nobels et al., 2011). Agricultural adjuvants are used to perform different specific functions including wetting, spreading, sticking, and the tested pesticide spray drift (Paveglio et al., 1996; Sharma and Singh 2001). On the other hand, the use of adjuvant nowadays is not concerned only with agricultural application but it also extends to include additives for different types of medications (Lussier et al., 2004). Polyethylene glycol (PEG) and polyether are used in a variety of applications including industrial manufacturing (Barish and Goddard, 2011; Abdel-Mohsen et al., 2012), and pesticides formulations (Krogh et al., 2003, Kumar et al., 2010). PEG is currently the only water soluble polymer, widely accepted in therapeutics with market approval for different drugs (Veronese and Mero, 2008; Banerjee et al., 2012). Lewis (1991) and Gorzerino et al. (2009) confirm that pesticides and pharmaceutical adjuvant interaction has not been regulated under the US Federal pesticides because little is known about their bioavailability, environmental fate and physiological effects. Regarding their side effects, results of previous studies have indicated that some of agricultural adjuvants are toxic to various species (Lewis, 1991; DelValls et al., 2002), including fish (Parr, 1982), daphnia (Comber et al., 1993), frogs (Edgington et al., 2004) and birds (Rogers, 1974). Other investigators remark that adjuvant may cause adverse effects to laboratory animals and humans (Fata et al., 2002; Stills, 2005). On the other hand, it is known that PEG esters, which are widely used for medicinal treatments or pesticidal applications or agricultural adjuvant, have severe clinical (Bendele et al., 1999), behavioral (Rodrique et al., 2011), biochemical (Wieder and Davis 1983) and histopathological effects (Ishida et al., 2006). Little data has been known about the neurotoxic effect of PEG adjuvant which is administered individually or in
combination with the commonly-used pesticides. The present study focuses on the effect of PEG-600 on the acute neurotoxicity of malathion, imiprothrin or imidacloprid, on the basis of 1/4 LD$_{50}$ for each of the adult quail (\textit{Coturnix coturnix}).

**Materials and Methods Experimental Animals**

The quails (\textit{Coturnix coturnix}) of both sexes weighing (100-150 g) were used in the present study. The animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (45±5%), temperature (22±1°C), and controlled room with 12 h day and night cycle with free access to food and water. Experiments took place between 10:00 and 15:00 h. All the experimental protocols for the use of animals in this study were reviewed and approved by Institutional Animal Care and Use Committee (IACUC). Efforts were made to minimize suffering and reduce the number of animals used.

**Pesticides and adjuvant**

Malathion: An organophosphate pesticide, has the chemical formula C$_{10}$H$_{19}$O$_6$PS$_2$ (diethyl dimethoxyphosphinothiol) thio butanedioate). It was denoted by Novartis Egypt (95% pure). The indicated dose level was 145.7 mg/kg dissolved in corn oil.

Imidacloprid: A nitromethylene ompound has the chemical formula (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine). It was donated by Novartis Egypt (35% pure). The indicated dose level was 4.5 mg/kg dissolved in corn oil.

Imiprothrin: Pyretheroid insecticide, has the chemical formula (2,5-dioxo-3-(2-propynyl)-1-imidazolidinyl) methyl 2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropane carboxylate), (50% pure). It was donated by Novartis Egypt (50% pure). The indicated dose level was 456 mg/kg dissolved in corn oil.

PEG-600: Polyethylene glycol 600 dilaurate (PEG-600) was available in emulsifier form. It was donated by the Egyptian Company of Starch, Yeast and Detergents in emulsifier form with an average molecular weight of 600. It is a clear viscous liquid at room temperature.

**Adjuvant-pesticides dose preparation**

Adjuvant-pesticide mixtures were prepared by mixing the tested dose level of the selected pesticides dissolved in 1 ml of corn oil with PEG-600 glycol (5g %) on the basis of (1:1) volume.

**Toxicity testing**

One hundred and eighty mature quails were used for toxicity testing studies (LD$_{50}$ values). The experimental animals were divided into six groups; each was divided into five subgroups consisting of 6 animals each. The animals of each group were orally administered with different doses of the selected pesticides individually or in combination with PEG-600 adjuvant. The number of the dead animals was counted 24 hours post oral application. The probit analysis was used to calculate the lethality percentiles of the studied toxicants by aid of NCSS 2007 software. After LD$_{50}$ stock solutions were prepared on the basis of 1/4 LD$_{50}$ values of the studied toxicants.

**Animal grouping and treatments**

After 2 weeks of acclimatization, 80 animals of nearly a similar weight were selected and animals were separated into 8 groups once daily of treatment: Control group (1 ml corn oil); PEG-600 group (1ml 5%); malathion alone group (145.7 mg/kg in 1 ml corn oil); malathion+PEG-600 (145.7 mg/kg malathion in 1 ml corn oil + 1 ml 5% PEG); Imidacloprid alone (4.5 mg/kg in 1ml corn oil); Imidacloprid+PEG600 (4.5 mg/kg imidicloprid +1 ml 5%PEG); Imiprothrin alone (456 mg/kg in 1 ml corn oil) and Imiprothrin+PEG-600 (456mg/kg in 1ml corn oil+1ml 5% PEG).

**Tissue Preparation and biochemical assays**

After 96 hours of administration, the animals were sacrificed by decapitation in the morning to avoid diurnal variations of the endogenous amines, enzymes, and other biochemical molecules. The brain tissue was rapidly removed and homogenized in 0.1 M phosphate buffer (pH 7.8), using a glass-Teflon homogenizer. Tissue homogenates were centrifuged at 10,000 g for 60 min at 4°C and the homogenates were used for the determination of both free amino acids according to Heinrikson and Meredith (1984).
and monoamines, according to Pagel et al. (2000) using HPLC (Perkin-Elmer). At the time of scarification, blood was collected into clean heparinized centrifuge tubes, plasma were separated by centrifugation and used for determination of total protein (Gornall et al., 1949), transaminases (ALT and AST) (Reitman and Frankel 1957), acetylcholinesterase (AChE) by modified Ellman method (Gorun et al., 1978) and alkaline phosphatase (ALP) (Ellis et al., 1971) by using appropriate reagent kits purchased from Biodiagnostic.

**Statistical analysis**

The data is presented as Mean± SE. Statistical analysis is evaluated by One-way analysis of variance (ANOVA) followed by LSD (Least significance difference) for comparison between groups using SPSS program version 17.

Joint action analysis Mansour’s formula (Mansour et al. 2008) was used to identify the type of interaction in terms of interaction index \( II = \frac{M + C}{A + B} \). M, C, A and B represent the mean values obtained; M for the mixture value; A and B for the values of the individual compounds in that mixture, and C for the control value. Based on the above-mentioned authors view, the rating of interaction is either potentiating \((II \geq 1)\), additive \((II=1\pm0.05)\) or antagonistic \((II \leq 1)\).

**Results Toxicity Testing**

The values describing the mortality response to a range of concentrations of the tested pesticides in the presence or absence of PEG-600 are given in Table (1). It is obvious that the potentian is highly recommended; the toxicity of the tested insecticides when administered in combination with PEG-adjuvant is more toxic than do the insecticides alone. In comparison, the recorded LD\(_{50}\) values have been decreased by -32.247, -41.529 and -11.026 more than malathion, imidacloprid and imiprothrin when they are administered individually.

**General toxic observations**

Animals treated with the selected insecticides, which are administered individually or in combination with PEG-adjuvant, display noticeable behavioral changes during the experiment. General weaknesses, salivation, sometimes excitation, variable sequence of motor symptoms involving pawing and tremor of the body and convulsions have been developed. These symptoms are observed in all treated groups. The addition of PEG-adjuvant to the selected insecticides has showed a highly-toxic potential more than the insecticide alone.

**Plasma biochemical analysis**

The present study has revealed that the disturbance resulted in serum enzyme activities and different biochemical parameters of quails is in response to the dispensing of malathion, imiprothrin and imidacloprid pesticides when they are administered individually or in combination with PEG-600 adjuvant.The recorded data has showed significant increase in the activities of ALP, AST and ALT (Fig.1). On the other hand, total protein content and AChE activity has been significantly decreased (Fig.2). The maximal effects, however, have been attained post treatments with imiprothrin-PEG-600 for total protein content, AST, ALT and AChE and with imidacloprid-PEG-600 for ALP.

**Brain neurotransmitters Amino acids**

Data in Figs.(3,4) shows for the animals which are exposed to 1/4 LD\(_{50}\) of the tested pesticides which is administered individually or in combination with PEG-600 has higher levels of whole brain amino acids more than those of the control groups. The maximal concentrations of glutamate, aspartate and GABA, however, are attained when PEG-6000 is administered individually. On the other hand, maximal glycine concentration is recorded post exposure to imidacloprid when it is administered individually. According to the overall results, antagonistic effect represents 83.33% of the total number of analyzed mixtures towards the four amino acids and potentiative effect accounted only 16.67%.

**Monoamines**

Regarding the effect of imidacloprid, when it is administered individually or in combination with the
tested adjuvant, recorded data (Table 2) reveals a great depletion in the concentrations of brain dopamine and serotonin. The potent highly-significant effect has been attained post exposure to malathion and PEG. Conversely, the acute toxicants administration has led to a moderate increase of effect in brain norepinephrine concentration. Moreover, the maximal highly significant change has been attained post exposure to imidacloprid when it is administered individually or in combination with PEG-600. Regarding the interaction indices for monoamines concentration in brain of quails, antagonistic effect represents 66.66 % of the total number of analyzed mixtures towards the three tested monoamines whereas additive effect accounts for only 33.33 %.

Discussion

Many chemicals are regarded as pollutants, ranging from simple inorganic ions to complex organic molecules. Among the pollutants that animals encounter daily are the pesticides which occupy special position. Furthermore, many of the agricultural adjuvant used in the formulations of pesticides present a high likelihood of exposure (Stark and Walthall 2003). Several investigators (Liu and Stansly 2000; Sharma and Singh 2001), have reported that some adjuvants are toxic to certain species and others increase the toxicity of pesticides. The first data reported in this study is concerned with the toxicity syndromes. The observed signs reveal general weakness, salivation, sometimes excitation, tremors and convulsions. These results coincide with Costa (2008) using malathion; Verschoyle and Barnes (1972) and Verschoyle and Aldridge (1980), who have studied the effect of different types of Pyretheroid, and Azevedo-Pereira et al. (2011) who have conducted their experiment on the effect of imidacloprid. The mode of actions, however, is mainly attributed to their inhibition to AChE in the nervous system, resulting in the accumulation of the neurotransmitter acetylcholine at the postsynaptic receptor. In the present study, the potential health effects depend upon the kind of the tested pesticide and are in parallel contact with the activity of AChE which shows variable inhibition. The reason for such variability is mainly attributed to pesticide structural differences as well as their differences in affinity for the AChE active sites. This hypothesis is also well confirmed in terms of the dose level, (LD50) and the determined AChE activity. Imidacloprid shows both the higher toxicity symptoms, higher toxic effect and higher potent effect on plasma AChE as compared with malathion or imiprothrin. Moreover, the characteristic toxicity syndromes of imidacloprid, which belong to a new chemical family of chloronicotinyl compounds, include emaciation, tremors and convulsions. These findings coincide with the results declared by Azevedo-Pereira et al. (2011). According to the same author, after 96-hour exposure to imidacloprid, AChE activity, behavior parameters, ventilation and locomotion were reduced and the mode of actions was through action on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system. On the other hand, the clinical symptoms, which are observed during imiprothrin acute exposure, include hypersensitivity, tremors, and motor ataxia which they are mainly due to the higher potency and selectivity of the tested compound against the nervous system of the studied animal. Our data, however, correspond to the observations made by other researchers reporting on the toxicity of pyretheroid pesticides (Dobsíková et al. 2006; Velisek et al. 2009). According to David et al. (2005), Pyretheroid acts on a variety of putative biochemical and physiological target sites, like voltage-sensitive sodium, calcium, and chloride channels. Moreover, the combination of PEG-600 as adjuvant to the tested pesticides has enhanced their potency and revealed more obvious toxicity syndromes. Little is known about the biochemical neurotoxicity effect of PEG-600. The combination of PEG-600 with the tested pesticides might lead to structural modification and potentiating their mode of actions on the central nervous system depression, acidosis, and nephrotoxicity. According to DeSantis and Jones (1999) and Thorardarson et al. (2006), modification of the protein surface synthetic polymers like PEG is a major problem. In this study, the deleterious effects of PEG, when it is administered individually on the activity of the studied enzymes, are not well-recommended and the prevalence is well-recognized in their combination with the tested pesticides. With the exception of AChE, the activities of ALP, AST and ALT have markedly been increased post treatments with the combined effect. According to Chang et al. (1998), PEG stabilizes the enzyme activity to some extent. Andersson and Hahn-Hägerdal (1988) have also reported that high
concentrations of PEG are proton motive force for α-
proline. Therefore, pesticide exposure could indicate elevated plasma transaminases and ALP activities by means of a transient damage of different body tissues, whereas PEG allows a state of enzyme release stability. In addition, the more toxicity symptoms produced by pesticide-PEG mixtures may be attributed to the availability of more AChE inhibition. Many researchers postulate that the more Ach accumulation due to AChE inhibition resulted in more tremors and convulsions (Worek et al. 2005; Mohebbi et al. 2001). The mechanism, which is involved in pesticide-PEG 600, is unknown at present. It may suggest that pesticide-adjuvant treatment has more neuronal oxidative stress leading to more free radical formation. Brain cells seem to be vulnerable to this toxic effect since more inhibition in AChE activity and more toxicity symptoms have been recorded.

In the present study, the effect of the tested compounds was also investigated on the brain biochemical change. When the tested ester is administered individually, brain amino acids, DA and 5-HT monoamines are significantly affected and it has no or little effect on the studied plasma biochemical parameters. Moreover, while the tested parameters are highly affected as a result of the tested doses of the selected pesticides when administered individually, moderate modulations in their levels have been attained with the combined treatment with the tested pesticides and PEG-600. The recorded data are in accordance with several approaches studies using different pesticides and different experimental animals; Moulton et al. (1996) in their treatment of the freshwater mussel Elliptio complanata; Balani et al. (2011) in their experiment on imidacloprid male White Leghorn; and Al-Attar (2010) studies on the alterations induced by malathion.

The decrease of serum total protein as compared to control is thought to be due several reasons like the decrease in amino acids uptake, the increase of conversion rate of glycogenic amino acids and the reduction of protein synthesis which in turn may be due to a decrease in the amount and availability of mRNA and DNA. On the basis of the recorded increase in the level of all the studied amino acids, it has been observed that the tested pesticides which are administered individually or in combination with PEG-600 adjuvant, cause inhibition of protein synthesis which yielded high levels of free amino acids.

Regarding the effect of the tested compounds on the studied biogenic amines, the tested pesticides have variable effects. While brain norepinephrine shows a general increase, there significant reduction in the levels of DA and 5-HT as a result of the imiprothrin and imidicloprid neurotoxicity. More change was observed in the serotonin and nor-epinephrine levels in all groups post the combined effect with the tested ester. PEG-600 alone shows significant decreased change in the levels of DA and 5-HT monoamine. There are several possible mechanisms for the biogenic actions of the tested pesticides such as neuroendocrine disruption (Cooper et al., 1999), MAO inhibition (Nag and Nandi, 1987); and monoamines metabolism (Xu et al., 2012) and modify the kinetics of voltage-sensitive ion channels and calcium ion flux/homeostasis that could affect the release of several neurotransmitters. Moreover, the recorded change in the brain endogenous may result from pathologic damage changes as recorded in the present investigation. On the other hand, the combination with PEG-600 exhibited more significant catecholamine depletion as compared to pesticides treated animals. However, the combination with organophosphate malathion have a highly potent reductional effect in the 5-HT and DA. Reasonable explanation could be that PEG-600 exerts its effects due to its weak DA releasing, dopamine reuptake and NMDA inhibitory actions. Also, it may suggest that PEG-600 might modulate the membrane potential of nerve cells over a wide range of stimulus which affects synaptic release of the tested biogenic amines and such changes induce cognitive, behavioral and motor coordination defects.

In conclusion, there are clinical trials that have reported the safety and tolerability of PEG-600 in humans and signified the safety and lack of central effects but the present article provides an overview of modulation effect PEG-600 as adjuvant on neurotoxicological effects of different types of pesticides on birds. The proposed mechanisms are potentiation at most of the studied parameters that could be attributed to the influence on neurotransmitters or their metabolism.

References


Table 1. Lethal Dosage (LD<sub>50</sub>) Values of the tested pesticides individually or in combination with PEG600

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (mg/g)</th>
<th>Pesticide-Adjuvant</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (mg/kg)</th>
<th>% of toxicity increased value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion</td>
<td>583</td>
<td>Malathion-PEG</td>
<td>395</td>
<td>32.247</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>17.02</td>
<td>Imidacloprid-PEG</td>
<td>15.98</td>
<td>41.529</td>
</tr>
<tr>
<td>Imiprothrin</td>
<td>1823</td>
<td>Imiprothrin-PEG</td>
<td>1622</td>
<td>11.026</td>
</tr>
</tbody>
</table>

Table 2. The effect of pesticides and/or PEG600 on brain monoamines of quail (Couturnix couturnix)

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (µg/g fresh tissue)</th>
<th>Norepinephrine</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>510.57±11.49</td>
<td>1371.65±30.8</td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>511.80±17.33</td>
<td>454.92±31.40</td>
<td></td>
</tr>
<tr>
<td>Imiprothrin</td>
<td>613.28±8.77&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1019.39±42.8</td>
<td></td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>663.68±52.02&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1242.47±67.2</td>
<td></td>
</tr>
<tr>
<td>F-value</td>
<td>5.08&lt;sup&gt;##&lt;/sup&gt;</td>
<td>65.48&lt;sup&gt;##&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PEG-600</td>
<td>500.38±46.70</td>
<td>879.67±37.57</td>
<td></td>
</tr>
<tr>
<td>Malathion-PEG</td>
<td>398.68±11.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>267.99±12.17&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Imiprothrin-PEG</td>
<td>523.67±37.65&lt;sup&gt;an&lt;/sup&gt;</td>
<td>1022.43±56.5&lt;sup&gt;8*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Imidacloprid-PEG</td>
<td>690.90±18.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1050.02±33.2&lt;sup&gt;1*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>F-value</td>
<td>14.48&lt;sup&gt;##&lt;/sup&gt;</td>
<td>91.24&lt;sup&gt;##&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Data is expressed as Mean± SE (n=6). *Significant (P<0.05): Pesticide treated group as compared to control group and Pesticide-adjuvant as compared to PEG-treated group. F-value for One-way analysis ANOVA (LSD) between groups in the same column # Significant (P<0.05), an= antagonism, ad= Additive, Po= Potentiation.
Fig. 1: The effect of pesticides and/or PEG600 on the plasma AST, ALT and ALP activity of quail (Couturnix couturnix).

Data are expressed as Mean±SE (n=6). *Significant (P<0.05): Pesticide-treated group as compared to control group and Pesticide-adjuvant group as compared to PEG-treated group. an= antagonism, ad= Additive, Po= Potentiation.

Fig. 2: The effect of pesticides and/or PEG600 on the plasma total protein content and AChE activity of quail (Couturnix couturnix).

Data are expressed as Mean±SE (n=6). *Significant (P<0.05): Pesticide-treated group as compared to control group and Pesticide-adjuvant group as compared to PEG-treated group. an= antagonism, ad= Additive, Po= Potentiation.
Table 3: The effect of pesticides and/or PEG600 on brain glutamate and aspartate concentration in the brain of quail (*Coturnix couturnix*).

Data are expressed as Mean± SE (n=6). *Significant (P<0.05): Pesticide treated group as compared to control group and Pesticide-adjuvant group as compared to PEG-treated group. an= antagonism, ad= Additive, Po= Potentiation.

Table 4. The effect of pesticides and/or PEG600 on GABA and glycine concentration in the brain of quail (*Coturnix couturnix*).

Data are expressed as Mean± SE (n=6). *Significant (P<0.05): Pesticide treated group as compared to control group and Pesticide-adjuvant group as compared to PEG-treated group. an= antagonism, ad= Additive, Po= Potentiation.