Effects of environmentally-relevant mixtures of four common organophosphorus insecticides on the honey bee (Apis mellifera L.)

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A B S T R A C T

We assessed whether exposure to environmentally-relevant mixtures of four organophosphorus insecticides (OPs) exerted adverse effects on honey bees. Adult and worker bees were orally exposed for five days under laboratory conditions to mixtures of four insecticides, diazinon, malathion, profenofos and chlorpyrifos at two concentrations. Concentration in the mixtures tested were equivalent to the median and 95th centile concentrations of the OPs in honey, as reported in the literature. Effects on survival, behavior, activity of acetylcholinesterase (AChE), and expression of genes important in detoxification of xenobiotics and immune response were examined. Survival of worker bees was not affected by exposure to median or 95th centile concentrations of the OPs. Activity of AChE was significantly greater in worker bees exposed to the 95th centile concentration mixture of OPs compared to the median concentration mixture. Expression of genes involved in detoxification of xenobiotics was not affected by treatment, but the abundance of transcripts of the antimicrobial peptide hymenoptaecin was significantly greater in worker honey bees exposed to the median concentration mixture. Results suggest that short-term exposure to environmentally relevant concentrations of a mixture of OPs do not adversely affect worker honey bees.

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1. Introduction

There is concern that exposure to agricultural pesticides is compromising the vigor of colonies of the honey bee, Apis mellifera L. Organophosphorus insecticides (OPs) are one of the most widely used class of pesticides, which in 2008 comprised 22% of world market share of insecticides (Lazonby and Waddington, 2015), and have been implicated in incidents in which bees were poisoned (Fletcher and Barnett, 2003; Kamler et al., 2003). Residues of OPs have frequently been detected in honey bee colony matrices, and their potential hazard to colonies have been previously studied (Al Naggar et al., 2015a,b; Chauzat et al., 2011; Cutler et al., 2014; Mullin et al., 2010; Wiest et al., 2011). While results of some studies suggest that OPs might not directly cause colony failure (Al Naggar et al., 2015a,b), OPs might interact with others stressors or in combination with each other to compromise the fitness of individual bees or colonies (Corman et al., 2012).

Most studies of toxic effects of pesticides on bees have focused on effects of single compounds. Even though honey bees in the field are rarely exposed to single compounds, few studies have examined toxic effects of mixtures of pesticides on bees (Gill et al., 2012; Johnson et al., 2013; Pilling and Jepson, 1993). Results of surveys in the USA and Canada (Al Naggar et al., 2015b; Mullin et al., 2010), Europe (Chauzat et al., 2011; Wiest et al., 2011) and North Africa (Al Naggar et al., 2015a) have shown that colonies of bees might concurrently be exposed to dozens of different compounds including multiple OPs. In some cases, mixtures of pesticides have been shown to be synergistic, with reported increases...
in toxicity as great as 100-fold relative to that which would be predicted from a strictly additive model (Thompson, 1996). For example, exposure to field-relevant concentrations of the neonicotinoid imidacloprid and the OP coumaphos impaired olfactory learning and memory formation in honey bees (Williamson and Wright, 2013). In a study with four common pesticides (flumethalin, coumaphos, chlorothalonil, and chloropyrifos), exposure to field-relevant concentrations either individually or in mixtures caused significant increases in larval mortality. Synergistic effects were observed with certain binary mixtures, but a combination of the OPs chlorpyrifos and coumaphos resulted in only additive toxicity (Zhu et al., 2014).

The objective of the present study was to assess whether exposure to environmentally relevant concentrations of a mixture of OPs exerts adverse effects on honey bee workers. Effects of the mixture of OPs on survival, behavior, activity of AChE, and expression of genes involved in detoxification and immune response, were quantified. It was hypothesized that exposure of bees to a mixture of OPs that act via the same mode of action could exert adverse additive effects on the endpoints examined in individual bees.

2. Materials and methods

2.1. Pesticides

Diazinon, malathion, profenofos and chloropyrifos were used in experiments. These four OPs were chosen because they were the most frequently detected OPs in honey bee colony matrices in a recent Egyptian study (Al Naggar et al., 2015a), frequently detected in other studies (Chauzat et al., 2011; Mullin et al., 2010; Rissato et al., 2007; Wiest et al., 2011), and are potentially hazardous to honey bee colonies (Al Naggar et al., 2015a; Cutler et al., 2014; Johnson et al., 2010; Mansour, 2004).

Insecticides used in experiments were technical grade (>98% purity, Accu Standard, New Haven, CT). The peer-reviewed literature was examined to determine concentrations of each OP in honey, from which representative median and 95th centile concentrations of each OP was calculated (Table 1). Because honey represents all the nectar sources collected, whether these are treated crops or wild flowers, and represents food consumed by adults and juvenile bees inside the colony, we focused on residues found in honey to derive test concentrations used in our experiments. The median and 95th centile concentrations of each OP were determined. Stock solutions (1000 ppm) of each insecticide were prepared in acetone according to guidelines for efficacy and side effect testing from the European and Mediterranean Plant Protection Organization (EPPO, 1992) and the International Commission for Plant-Bee-Relationships (ICPBR) and then required dilutions were made for the median and 95th centile concentrations of each OP. Insecticides were collectively added to a 500 g L⁻¹ sucrose solution. The final concentration of acetone in solutions was 1% (v/v). For all experiments, bees were exposed to: (1) a mixture of the median concentrations of the four OPs; or (2) a mixture of the 95th centile concentrations of the four OPs. A solution of 1% acetone (vol/vol) in 500 g L⁻¹ of sucrose was used as a control. Fresh solutions were prepared for each bioassay replicate (i.e. repeat of the experiment).

2.2. Exposure protocol and effects on survival

Experiments were conducted in August 2014 with A. mellifera workers of indeterminate age obtained from hives maintained in an apiary near the Dalhousie University Agricultural Campus (Truro, NS, Canada). Adults collected from frames without brood were then returned to the laboratory and placed in refrigerator at 4 °C for approximately 10 min to slow movement of bees. Adult workers were then transferred to ventilated transparent round plastic cages (9 cm H × 7 cm diam.), with 20–30 bees per cage. Bees were deprived of food for 4 h prior to commencement of exposures and thereafter fed treatment or control sucrose solution. Access to sucrose solution was through a cotton dental wick that was inserted through a hole in the bottom of the container, which was soaked in sucrose solution via a 50 mm diameter Petri dish placed under the container holding the bees. The Petri dish was covered with a lid and sealed with parafilm to minimize loss through evaporation. Bees in containers were held in an incubator (24 h darkness; 32 °C; ~60% RH) for up to five days and mortality was recorded daily. Each bioassay consisted of three replicate containers per treatment, and the bioassay was done on three separate occasions (i.e. blocks in time). On the fifth day, subsamples of surviving bees were collected from each container to quantify gene expression (Section 2.3) and AChE activity (Section 2.4). Heads for quantification of activity of AChE and alimentary canals for quantification of gene expression were removed from bee specimens by dissection on ice and samples were stored at −80 °C.

2.3. Gene expression

Real-time quantitative PCR (RT-qPCR) was performed to determine the effect of exposure to the mixture of OPs on expression of genes important for detoxification of pesticides and immunity. Total RNA was isolated from alimentary canals of worker bees by use of an RNeasy Lipid Tissue Mini Kit (Qiagen, Toronto, ON, Canada) according to the protocol provided by the manufacturer. Immediately after extraction, the concentration of RNA was determined by use of a Nanodrop ND-1000 spectrometer (Nanodrop Technologies, Wilmington, DE) and first strand cDNA was synthesized from 0.5 µg of RNA by use of a Quantitect cDNA Synthesis Kit (Qiagen). To perform RT-qPCR, samples of cDNA were diluted 1:5 in water that was free of nucleases. Reactions were performed using Quantitect SYBR Green Reagent (Qiagen). Briefly, a separate 50 µL PCR reaction consisting of 2 × SYBR Green master mix, an optimized concentration of gene-specific primers, nuclease free

### Table 1

Concentrations of organophosphorous insecticides (OPs) (ng/g, wm) in honey reported in the peer-reviewed literature, nominal (regular font) and dosed (bold font)⁣

<table>
<thead>
<tr>
<th>OP</th>
<th>Conc. (ng/g, wm)</th>
<th>References</th>
<th>Median⁣</th>
<th>95th centile⁣</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>14</td>
<td>Wiest et al. (2011)</td>
<td>14.0</td>
<td>33.0, 32.9</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>Johnson et al. (2010)</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Al Naggar et al. (2015a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>243</td>
<td>Johnson et al. (2010)</td>
<td>122.0</td>
<td>231.0, 217.2</td>
</tr>
<tr>
<td></td>
<td>0.423</td>
<td>Rissato et al. (2007)</td>
<td>112.5</td>
<td></td>
</tr>
<tr>
<td>Profenofos</td>
<td>0.27</td>
<td>Al Naggar et al. (2015a)</td>
<td>0.30</td>
<td>0.30, 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.15</td>
<td>Al Naggar et al. (2015a)</td>
<td>9.0, 7.7</td>
<td>70.0, 68.8</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>Rissato et al. (2007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Paraja et al. (2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Johnson et al. (2010)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁣Dosed concentrations were different from nominal concentrations because it had been measured after treatment sucrose solutions were prepared as a mixture at field relevant median and 95th centile concentrations.

Median and 95th centile concentration were calculated from concentrations detected for each OP in honey in the peer-reviewed literature.
water, and an optimal volume of cDNA was prepared for each sample and gene of interest. Then, 20 μL of reaction mix was transferred to two wells of a 96-well PCR plate. Reactions were performed in an ABI 7300 Real-Time PCR System (Applied Biosystems, Burlington, ON, Canada). Primers were either from Boncristiani et al. (2012) or were designed by use of Primer 3 software (Rozen et al., 2000) with sequences available in the NCBI nucleotide database (Table 2).

The PCR reaction mixture was denatured at 95 °C for 10 min before the first PCR cycle. The thermal cycle profile was as follows: denature for 15 s at 95 °C and extension for 1 min at 60 °C for a total of 40 PCR cycles. The RT-qPCR cycle was followed by a dissociation step to validate that only a single product was amplified in each reaction. For each target gene, abundance of transcripts was quantified according to the Mean Normalized Expression (MNE) method of Simon (2003), and actin-related protein-1 (AMActin) was used as a reference gene. Efficiency of each set of primers was determined by use of a standard curve of serial dilutions of cDNA. Reactions conditions were optimized so that the coefficient of determination ($R^2$) was at least 0.99 and efficiencies were 1.9–2.1, where efficiency = 10$^{-1/slope of standard curve}$. In total, qPCR was performed on RNA isolated from a total of six alimentary canals per treatment; two randomly selected from each set of treatments from each of the three bioassays (blocks).

### 2.4. AChE activity

A total of five heads of bees from each of the three bioassays (blocks) that survived each exposure scenario were randomly selected, pooled and homogenized in 0.1 M phosphate buffer (pH 7.0). Homogenates were then centrifuged at 5000 g for 20 min at 4 °C and supernatants were collected for quantification of activity of AChE using the Amplex Red Acetylcholine/Acetylcholinesterase (AChE) Assay Kit (A-12217) (Life Technologies, Carlsbad, CA, USA), according to protocol provided by the manufacturer. Fluorescence was measured at an excitation wavelength of 560 nm and emission wavelength of 590 nm. All samples were quantified in duplicate. Specific activity of AChE was expressed as activity/mg protein. Concentrations of protein in supernatants were determined with the Bio-Rad Protein Assay (Bio-Rad Laboratories, Mississauga, ON, Canada) with bovine serum albumin used as a standard. The protocol is based on the method of Bradford (Bradford, 1976).

### 2.5. Proboscis extension reflex (PER)

In a separate experiment, effects of mixtures of OPs on the proboscis extension reflex (PER) (Frost et al., 2013) of forager bees of indeterminate age were determined after a 24 h exposure, using the procedure described above. Returning forager worker bees were collected into glass jars using a vacuum at the hive entrance. Bees were placed in a refrigerator at 4 °C until their movement slowed, and then transferred to exposure cages.

After 24 h of exposure to the control diet or the diet containing the median of 95th centile mixture of OPs, bees were mounted individually in plastic tubes with only their antennae and mouth parts left free. Bees were starved for 3–4 h prior to conditioning. Bees were selected for showing a PER after stimulation of the antennae with a sucrose solution (300 g L$^{-1}$) and the number of individuals exhibiting the reflex response was recorded. Bees were then placed in an airflow (main airflow of 50 ml s$^{-1}$) added to a secondary airflow of 2.5 ml s$^{-1}$ for 15 s, to familiarize them with the mechanical stimulation. Conditioning trials were done with a conditioning stimulus of 10 μL of pure rosemary (Sigma-Aldrich, Oakville, ON, Canada), a standard floral odor, soaked on a filter paper strip inserted into a Pasteur pipette. The PER was elicited after 3 s by contacting the antennae with a sucrose solution (300 g L$^{-1}$) as the unconditioned stimulus, and the same solution was immediately given as a reward, before the odor delivery ended. Three successive conditioning trials (Cond1–Cond3) were scored as a yes-or-no response and analyzed by Chi Square ($\chi^2$) tests. Activity of AChE and abundances of transcripts of genes involved in detoxification of OPs and immune response were assessed by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. An alpha level of 0.05 was used for all tests.

### 2.6. Data analyses

Data analyses were performed with MINITAB Software (Minitab, 2013). Normality of data was assessed with a Kolmogrov–Smirnov test, and homogeneity of variance was determined with a Levene’s test. If necessary, data were log10 transformed to ensure normality and homogeneity of variance. Mortality was analyzed by a Kruskal–Wallis test. The response of forager bees to odor stimulus during conditioning trials and testing were scored as a yes-or-no response and analyzed by Chi Square ($\chi^2$) tests. Activity of AChE and abundances of transcripts of genes involved in detoxification of OPs and immune response were assessed by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. An alpha level of 0.05 was used for all tests.

### 3. Results

#### 3.1. Concentrations of OPs in sugar syrup

Concentrations of OPs were measured after treatment sucrose solutions were prepared. Greater than 90% recovery of pesticides was observed (Table 1).

#### 3.2. Honey bee survival

Exposure to either mixture of OPs did not affect cumulative five day mortality (%) of worker bees ($H = 1.41, df = 2, P = 0.49$). There
was no difference in survival of bees in response to treatments across experimental blocks ($H = 2.44, df = 2, P = 0.30$) (Fig. 1).

3.3. Gene expression

Expression of genes important for detoxification of OPs was not significantly different in alimentary canal tissues collected from worker bees fed the control solution compared to those fed a solution containing either mixture of OPs (CYP 4G11: $F = 0.82, df = 2, P = 0.45$; CYP AS14: $F = 0.56, df = 2, P = 0.58$; CYP 306A1: $F = 0.59, df = 2, P = 0.56$; GST S3: $F = 0.27, df = 2, P = 0.77$; GST D1: $F = 1.65, df = 2, P = 0.22$; GARXase: $F = 0.43, df = 2, P = 0.65$) (Fig. 2). Treatment had an effect on defensin1 gene expression ($F = 4.37; df = 2, P = 0.030$), with relative expression in worker bees given sucrose solution with the median concentration mixture of OPs being 1.6-fold greater than in bees given the diet containing the 95th centile concentration mixture; however, expression of defensin1 in control bees was not different from that of bees from either

![Fig. 1. Cumulative mortality (%) (mean ± SEM) of worker honey bees exposed to a control or sucrose solution diet containing a mixture of organophosphorus insecticides at field-relevant median or 95th centile concentrations.](image)

![Fig. 2. Fold-change in abundance of transcripts of genes involved in pesticide detoxification in worker honey bees exposed to a control or sucrose solution diet containing a mixture of organophosphorus insecticides at field-relevant median or 95th centile concentrations. Columns represent the mean concentration (±SEM) of six samples.](image)
OP treatment. There was no treatment effect on abaecin gene expression ($F = 0.22; df = 2, P = 0.802$) in worker bees fed the control solution compared to those fed a solution containing either mixture of OPs. Expression of hymenoptaecin was 8.4-fold greater in bees given sucrose solution containing the median concentration mixture of OPs than in bees given either the control solution or the solution containing the 95th centile concentration of the mixture of OPs ($F = 9.24; df = 2, P = 0.004$) (Fig. 3).

3.4. AChE activity

Activity of AChE was significantly greater in worker bees exposed to the 95th centile concentration mixture of OPs compared to bees given sucrose solution with the median concentration mixture of OPs, but was not different from worker bees given the control diet ($F = 4.75; df = 2, P = 0.03$) (Fig. 4).

3.5. Proboscis extension reflex

Exposure to a sucrose solution containing either mixture of OPs did not affect olfactory learning performances represented as the percentage of PER obtained during (A) the conditioning phases (Cond1–Cond3) (Cond1: $\chi^2 = 0.36, P = 0.83$; Cond2: $\chi^2 = 1.3, P = 0.51$; Cond3: $\chi^2 = 1.03, P = 0.59$), and the (B) testing phase ($\chi^2 = 2.2, P = 0.30$) of forager bees compared to bees given the control solution (Fig. 5).

Fig. 3. Fold-change in abundances of transcripts of genes involved in immune responses in worker honey bees exposed to a control or sucrose solution diet containing a mixture of organophosphorus insecticides at field-relevant median or 95th centile concentrations. Columns represent the mean concentration ±SEM of six samples. Different letters denote significant differences among treatments (one-way ANOVA with Tukey post hoc test, $P < 0.05$).

Fig. 4. Acetylcholine esterase (AChE) activity in heads of honey bee workers after exposure to a control or sucrose solution diet containing a mixture of organophosphorus insecticides at field-relevant median or 95th centile concentrations. Columns represent the mean concentration ±SEM of six samples. Different letters denote significant differences from the control group (one-way ANOVA with Tukey post hoc test, $P < 0.05$).

Fig. 5. Olfactory learning performance of honey bee forager measured as (%) of conditioned proboscis extension reflexes during (A) the conditioning phases (Cond1–Cond3) ($n = 45$ per group), and the (B) testing phase ($n = 45$ per group) when given (24 h exposure) a control or sucrose solution diet containing a mixture of organophosphorus insecticides at field-relevant median or 95th centile concentrations ($\chi^2 = 2.2, P = 0.30$).
4. Discussion

There is increasing concern that agricultural pesticides, including OPs, are causing undue stress and mortality of honey bee colonies. However, there have been few studies of toxic effects of environmentally relevant concentrations of mixtures of pesticides on honey bees (Johnson et al., 2013). In the current study, we tested the hypothesis that a mixture of prominent OP insecticides – diazinon, malathion, profenofos and chlorpyrifos – at environmentally-relevant concentrations would adversely affect worker bee survival, behavior, and expression of genes that are important for detoxification of OPs and immune-response.

Survival of worker bees was not affected by either the median or 95th mixtures of OPs we tested. These results differ from those of Zhu et al. (2014), who reported reduced survival of honey bee larvae exposed to fluvalinate, coumaphos, chlorpyrifos, and chlorothalonil at 3, 8, 1.5, and 34 mg/L, respectively, either individually or in mixtures. Two major differences between our study and that of Zhu et al. (2014) might explain differences in effects on survival. First, concentrations of OPs that bees were exposed to was much greater in the study by Zhu et al. (2014). Second, bees used in the study by Zhu et al. (2014) were at the larval stage of development whereas in the current study the bees were adults, and in general, early life stages of organisms have greater sensitivity than adults to xenobiotics (Desneux et al., 2007; Wu et al., 2011).

The primary mechanism of toxicity of OPs against insects and other organisms is through inhibition of activity of AChE (McDonough and Shih, 1997; Pope, 1999; Pohanka, 2011). Such effects on AChE are dose dependent. In the present study, mixtures of OPs at environmentally relevant concentrations did not inhibit activity of AChE in honey bees. This is consistent with the lack of effect of exposure to the same mixtures we observed on bee survival. However, activity of AChE was greater in worker bees exposed to the greater 95th centile concentration mixture of OPs compared to worker bees exposed to the median concentration of the mixture. Greater activity of AChE has been reported in-in field and laboratory studies of honey bees exposed to OPs (Badiou et al., 2008; Boily et al., 2013).

Pesticides are well-known to affect the behavior of honey bees (Desneux et al., 2007), and it is possible that effects on individuals could transcend to the colony level. However, in the present study, exposure to a mixture of OP insecticides at environmentally relevant median and 95th centile concentrations for 24 h did not affect the PER response of honey bee foragers. These results are contrary to some other studies that examined effects of pesticides on honey bee learning. Prolonged exposure (4 days) to field-realistic concentrations (100 nM) of the neonicotinoid imidaclopid and the OP coumaphos, or these compounds in combination, impaired olfactory learning and memory formation in honeybee workers (Williamson and Wright, 2013). Exposure to sub-lethal concentrations of nine pesticides did not affect olfactory learning performances of honey bees, but four of the pesticides had effects during the PER assay (Decourtay et al., 2005). Others found that exposure to diazinon had a significant effect on odor learning of honey bees (Weick and Thorn, 2002). Alternatively, though exposure to certain concentrations of OP insecticides like dimethoate can affect survival of honey bees, the same concentrations might not alter learning performance (Decourtay et al., 2005). Effects on learning and recall of visual and olfactory discrimination tasks have also been studied in bees orally treated with the OP methyl-parathion, where recall of tasks learned with visual and olfactory discriminations were not affected (Guez et al., 2010). These discrepancies in the effects of OPs have been observed previously in honey bees (Belzunces et al., 2012).

Exposure to OP insecticides might make bees more susceptible to natural or anthropogenic stressors. One indirect effect of OPs that has been proposed is suppression of immune function. Exposure to OP insecticides has been shown to activate immune responses in insects (Dubovskiy et al., 2013), and some insecticides and miticides used for control of parasitic mites can affect immune competence, thus rendering bees more susceptible to infections (Boncrristiani et al., 2012; Di Prisco et al., 2013). Cellular immunity-mediated responses by hemocytes, and humoral immunity based on secretion of antimicrobial peptides (AMPs), are important process of the immune system of honey bees. In the present study, although there was no clear dose response effect of OP on expression of defensin1, expressions of hymenoptaecin was significantly greater in worker bees given sucrose solution containing the median concentration mixture of OPs compared to bees given the diet containing the 95th centile concentration mixture. Several studies have investigated functioning of the honey bee immune system when exposed to insecticides, but results of these studies vary depending on insecticides and methods used (James et al., 2012). Greater expressions of abaecin, defensin1, apidaecin, and hymenoptaecin have been reported in honey bees challenged by mechanical and pathogen-like stressors (Siede et al., 2012). Greater expression of these genes under mild chemical, pathogen, or physical stress might be an adaptive mechanism that allows honey bees and other insects to better cope with those stressors (Costantini, 2014; Costantini et al., 2010). It is not clear why expression of genes responsible for AMP seemed to be more affected by the median concentration OP mixture than the 95th centile concentration OP mixture.

Cytochrome P450 monoxygenases (P450s), glutathione-S transferases (GSTs), and carboxyl/cholinesterases (CCEs) are enzymes used by insects for detoxification of xenobiotics (Feyerisen, 2012). However, these enzymes appear to play a lesser role in detoxification of xenobiotics in honey bees compared to other insects. Based on abundances of transcripts quantified by use of qPCR, expression of genes that encode for these enzymes were not affected in worker bees exposed to mixtures of environmentally relevant concentrations of OPs. Results of the current study are consistent with current understanding of regulation of these enzymes in honey bees. With the exception of members of the CYP9 family (Claudianos et al., 2006), P450 enzymes in honey bees are not induced by exposure to the substrates on which these enzymes act, including phenobarbital (Glavan and Bozic, 2013; Mao et al., 2011; Yu et al., 1984). Also, studies indicate that GSTs and CCEs play only a minor role in detoxification of xenobiotics (Iwasa et al., 2004; Johnson et al., 2009).

In conclusion, short-term dietary exposure of honey bee workers to environmentally-relevant concentrations of a mixture of OPs did not adversely affect their survival or behavior, but some effects were observed on activity of AChE, and expression of genes involved in detoxification of OPs and immune response. This results align with those of Al Naggar et al. (2015a,b), in which minimal hazard from direct and dietary exposures of bee hives to OPs occurred. Results of the current study suggest that short-term dietary exposure to a mixtures of OP insecticides, as a singular stress factor, at levels found in honey within hives will not adversely affect worker bees, and probably have no effect on colony vigor.

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