**Mdr65 decreases toxicity of multiple insecticides in Drosophila melanogaster**

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**ABSTRACT**

ABC transporters are ubiquitous membrane-bound proteins, present in both prokaryotes and eukaryotes. The major function of eukaryotic ABC transporters is to mediate the efflux of a variety of substrates (including xenobiotics) out of cells. ABC transporters have been widely investigated in humans, particularly for their involvement in multidrug resistance (MDR). Considerably less is known about their roles in transport and/or excretion in insects. ABC transporters are only known to function as exporters in insects. *Drosophila melanogaster* has 56 ABC transporter genes, including eight which are phylogenetically most similar to the human *Mdr* genes (ABCB1 clade). We investigated the role of ABC transporters in the ABCB1 clade in modulating the susceptibility to insecticides. We took advantage of the GAL4/UAS system in *D. melanogaster* to knockdown the expression levels of *Mdr65*, *Mdr50*, *Mdr49* and *ABC6* using transgenic *UAS-RNAi* lines and conditional driver lines. The most notable effects were increased sensitivities to nine different insecticides by silencing of *Mdr65*. Furthermore, a null mutation of *Mdr65* decreased the malathion, malaoxon and fipronil LC50 values by a factor of 1.9, 2.1 and 3.9, respectively. Altogether, this data demonstrates the critical role of ABC transporters, particularly *Mdr65*, in altering the toxicity of specific, structurally diverse, insecticides in *D. melanogaster*. 

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**1. Introduction**

The ATP-binding cassette (ABC) transporters are one of the largest families of transmembrane proteins that have been characterized in all organisms from microbes to humans (Dassa and Bougie, 2001; Higgins, 1992). ABC proteins not only mediate the efflux of a large diversity of substrates, including amino acids, peptides, sugars, polysaccharides, lipids, inorganic ions, metals, waste compounds and xenobiotics (Dassa and Bougie, 2001; Dermauw and Van Leeuwen, 2014; Porretta et al., 2008; Sarkadi et al., 2006), but also participate in diverse biological processes, such as ribosome biogenesis and translation regulation (Andersen and Leevers, 2007; Paytubi et al., 2009). Due to their ability to transport drugs, some ABC transporters are involved in multidrug resistance (MDR) in cancer cells (Vasiliiou et al., 2009), and have been well studied for their roles in the absorption, distribution and excretion of drugs *in vivo* and *in vitro* (Glavinas et al., 2008; Sarkadi et al., 2006; Zhang et al., 2003). A typical ABC transporter consists of two highly conserved nucleotide-binding domains (NBDs), a characteristic feature of this family, and two highly hydrophobic transmembrane domains (TMDs), which are diverse and mediate substrate translocation (Higgins et al., 1986). The NBDs bind and hydrolyze ATP, thus providing energy to transport substrates out of the cell (Hollenstein et al., 2007). There are two types of ABC transporter genes: full transporters (FTs) contain all four domains, while half transporters (HTs) possess only one NBD and one TMD and need to homodimerize or heterodimerize to form a functional unit (Higgins, 1992; Higgins and Linton, 2004). Based on sequence similarity, domain structure and organization, ABC proteins have been divided into eight subfamilies (ABCA to ABCH), with the *Mdr* genes (those commonly found to play a role in transport of xenobiotics, such as drugs) being in the ABCB subfamily (Dean et al., 2001; Dermauw and Van Leeuwen, 2014). 

Insecticide poisoning can be divided into five general processes: penetration (through the exoskeleton or the gut epithelium), distribution (movement within the insect), interaction with the target site, metabolism and excretion (Welling, 1977). There have been tremendous advances in understanding how insecticides interact...
with their target sites and in determining the enzymes involved in metabolism. However, comparatively little progress has been made in understanding the processes involved in penetration, distribution and/or excretion. Given the role that ABC transporters play in the movement of xenobiotics, they have the potential to be key regulators of one or more of these processes.

_Drosophila melanogaster_ has 56 ABC transporter genes, including eight (four full transporters and four half transporters (Dermaw and Van Leeuwen, 2014)) that are phylogenetically most similar to the human _Mdr_ genes (Dean et al., 2001). _Mdr65_ was found to have an important role in preventing drugs and dyes from penetrating the blood-brain barrier of _D. melanogaster_ (Mayer et al., 2009). Few studies have looked at the potential of these ABC transporters to act as a barrier to, or alter the distribution of, insecticides. A previous study found that RNAi suppression of _Mdr50_ and _Mdr65_ resulted in a small (but significant) decrease in the LT95 of DDT, while RNAi of _Mdr49_ had no effect. (Gellati et al., 2015).

As a first step towards understanding the role of ABC transporters in insecticide distribution and/or excretion, we focused on the orthologs of the human _Mdr_ genes in the ABCB clade (Dean et al., 2001) for which RNAi lines were available. This included three full transporters (_Mdr49_, _Mdr50_ and _Mdr65_) and two half transporters (_ABCB6_ and _ABCB7_). We first used in vivo RNAi and the Ga4/UAS system to knock-down the expression of these transporters and then evaluated the resulting impact on sensitivity to insecticides. We determined the mortality of these knocked down flies to nine diverse insecticides and found that _Mdr65_ is a significant regulator of toxicity to multiple insecticides in _D. melanogaster_. We further confirmed these results using an _Mdr65 _null mutant line showing that _Mdr65 _is a key ABC transporter involved in limiting toxicity to some, but not all insecticides, primarily due to effects in the nervous system, Malpighian tubules (MTs) and muscles.

2. Materials and methods

2.1. Fly stocks and rearing

Five transgenic _UAS-RNAi_ lines from the DRSC/TRIP collection were employed to target 5 ABCB subfamily member genes. The transgenic _UAS-Mdr65-IR, UAS-Mdr50-IR, UAS-Mdr49-IR _and _UAS-ABCB7-IR_ lines were constructed using the _Attp2_ landing site, and a _MEF2-Gal4_ driver was used to target 5 ABCB subfamily member genes. The _Mdr_ genes in the ABCB clade (Dean et al., 2001) were expressed in a _MEF2-Gal4_ driver, _Mdr49_ was inserted within the _MEF2-Gal4_ gene and a matching background control was inserted within the _Mdr65_ gene and a matching background control line (yw) were also used.

2.2. RT-qPCR experiments

Total RNA was extracted from adult _F1_ females (5–7-day-old) for each of our crosses. Briefly, 10 flies were crushed in 500 μL TRizol reagent (Invitrogen, Carlsbad, CA, USA) with an MP FastPrep 24 bead beater (MP-Biomedicals, Santa Ana, CA, USA) and total RNA was extracted according to manufacturer’s instructions. RNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and genomic DNA was eliminated by DNase treatment (TURBO DNA-free kit, Invitrogen). The cDNA was synthesized using 1 μg total RNA using the Promega GoScript™ Reverse Transcription System with random primers according to the manufacturer’s instructions. The original solution of cDNAs was diluted 5 times with ddH2O before proceeding to RT-qPCR.

The gene-specific primer sets for four ABC transporter genes used in the RT-qPCR reactions are shown in Supplementary Table 1. The reaction volume (20 μL) contained 10 μL of 2 × IQ™ SYBR® Green Supermix, 7.4 μL of ddH2O, 8 μM of each specific primer, and 1 μL of first-strand cDNA template. The program was followed by a typical 2-step RT-qPCR protocol, which included an initial denaturation and enzyme activation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and an automatic dissociation step cycle was added for melting curve analysis. Reactions were performed in a Bio-Rad Real-Time PCR system (Bio-Rad, Hercules, CA, USA). Three biological replicates and two technical replicates were conducted for each treatment. The target gene expression was normalized relative to the reference genes _RP49, RpL32_. One-way ANOVA with Tukey’s test (overall significance level _P_ ≤ 0.01) was used to determine

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Stock#</th>
<th>Gene targeted</th>
<th>Transporter type</th>
<th>CG#</th>
<th>Genetic background</th>
<th>Length of dsRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAS-Mdr65-IR</td>
<td>28664</td>
<td>Mdr65</td>
<td>FT</td>
<td>10181</td>
<td>Attp2</td>
<td>509bp</td>
</tr>
<tr>
<td>UAS-Mdr50-IR</td>
<td>35034</td>
<td>Mdr50</td>
<td>FT</td>
<td>8523</td>
<td>Attp2</td>
<td>21bp</td>
</tr>
<tr>
<td>UAS-Mdr49-IR</td>
<td>32405</td>
<td>Mdr49</td>
<td>FT</td>
<td>3879</td>
<td>Attp2</td>
<td>21bp</td>
</tr>
<tr>
<td>UAS-ABCB6-IR</td>
<td>53284</td>
<td>ABCB6</td>
<td>HT</td>
<td>4225</td>
<td>Attp2</td>
<td>21bp</td>
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<tr>
<td>UAS-ABCB7-IR</td>
<td>51696</td>
<td>ABCB7</td>
<td>HT</td>
<td>7955</td>
<td>Attp2</td>
<td>21bp</td>
</tr>
</tbody>
</table>

*Full transporter (FT) or half transporter (HT). See Introduction for details.
significant statistical difference between treatments.

2.3. PCR validation of Mdr65-null mutant line

We confirmed the null mutation in the Mdr65−/− line by PCR. DNA from the heads of ten female adult flies was isolated with an alkaline extraction method (Montero-Pau and Muñoz, 2008). Three separate DNA samples were prepared from Mdr65−/− and yw lines respectively. PCR reactions were carried out using the forward primer (5’-CGAGGTCAGAAACACC-3’) and reverse primer (5’-GTGTCTCCTATGCTGTC-3’). This pair of primers amplified a 341bp product in Mdr65 wild-type flies. The reaction volume (25 μL) contained 12.5 μL of GoTaq master mix (Promega, Madison, WI, USA), 9.5 μL of ddH2O, 1 μL of each specific primer, and 1 μL of DNA template. PCR reactions were carried out using an iCycler (Bio-Rad) with the following program: 94°C, 3 min; 33 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 20 s; 72°C for 5 min. Each PCR product was verified by electrophoresis on a 1% agarose gel following by 0.5 μg/mL ethidium bromide staining.

2.4. Insecticides and bioassays

The following insecticides were used: chlorantraniliprole (97.8%, DuPont, Wilmington, DE, USA), chlorpyrifos (100%, Dow Agro-Sciences, Indianapolis, IN, USA), cypermethrin (99%, Chem Service, West Chester, PA, USA), deltamethrin (100%, Roussel UCLAF, Paris, France), diazinon (99.5%, Chem Service), esfenvalerate (82%, DuPont), fipronil (98%, Chem Service), malaoxon (99.4%, American Cyanamid, Wayne, NJ, USA), malathion (99.2%, Chem Service), oxamyl (99%, Chem Service), parathion (99.3%, Chem Service), permethrin (100%, Chem Service), phenthoate (100%, DuPont), and spinosad (98.6%, Chem Service). These were selected to represent a diverse group of insecticides (structures, mechanisms of action, metabolism, etc.).

Bioassays were carried out by residual contact application. Test insecticides were dissolved in acetone and 0.5–1.0 mL of the dilution was applied evenly to the inside of a scintillation vial (Wheaton, Millville, NJ, USA) of 38.6 cm2 and allowed to evaporate on a hot dog rolling machine (Gold Medal Products Co., Cincinnati, West Chester, PA, USA), deltamethrin (100%, Roussel UCLAF, Paris, France), diazinon (99.5%, Chem Service), esfenvalerate (82%, DuPont), fipronil (98%, Chem Service), malaoxon (99.4%, American Cyanamid, Wayne, NJ, USA), malathion (99.2%, Chem Service), oxamyl (99%, Chem Service), parathion (99.3%, Chem Service), permethrin (100%, Chem Service), phenthoate (100%, DuPont), and spinosad (98.6%, Chem Service). These were selected to represent a diverse group of insecticides (structures, mechanisms of action, metabolism, etc.).

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3. Results

3.1. Viability of RNAi lines

Act5c-Gal4 driven suppression of ABCB7 did not generate any viable RNAi flies, suggesting that this gene is required for D. melanogaster development. All other F1 progeny developed properly and no aberrant anatomical features were observed. RNAi in the four viable F1s resulted in specific silencing of their target gene (Fig. 1). Use of the Act5c-Gal4 driver resulted in significant RNAi-mediated gene suppression, ranging from 74% (Mdr49) to 91% (Mdr50). As expected, the mRNA levels of the four ABCB subfamily genes were quite similar in the progeny of Act5c-Gal4 crossed with two control lines (Attpr2 and Attpr40, Fig. 1). Altogether, our data demonstrate that using the Act5c-Gal4 driver we can achieve specific and potent suppression for four ABC transporter genes: Mdr49, Mdr50, Mdr65, and ABCB6.

3.2. Does RNAi alter toxicity of insecticides?

RNAi mediated suppression of Mdr65, Mdr50, Mdr49 and ABCB6 significantly altered the toxicity of specific insecticides, in a gene specific manner (Table 2), relative to control flies. Flies with decreased Mdr65 expression (Act5c-Gal4×UAS-Mdr65-IR) were significantly more susceptible to exposure to seven of the nine insecticides, including all of the pyrethroids. The most notable difference was for malathion in which mortality was 9% in the controls and 97% in the Mdr65 RNAi flies (Table 2). There was also increased mortality to the other two insecticides (chlorpyrifos and oxamyl), but the differences were not significant. Flies with decreased ABCB6 expression (Act5c-Gal4×UAS-ABC6-IR) showed little change in response to the insecticides, except for a significant increase in mortality in the case of malathion (less of a change than was seen with Mdr65). Small but significant decreases in mortality were seen for flies with decreased Mdr49 (parathion) or Mdr50 (malathion, esfenvalerate and permethrin) expression (Table 2). These findings indicate that RNAi mediated suppression of both full and half transporters in the ABCB subfamily can alter the toxicity of insecticides, with Mdr65 having the greatest effect against the greatest number of insecticides.

Given the importance of Mdr65 in altering toxicity to seven insecticides in our initial experiments, we expanded the characterization of Mdr65 with five additional insecticides. Two of these (malathion and phenthoate) were chosen because they were structural analogs of malathion. Malathion is the bioactivated form of malathion. The other three were selected to represent insecticides with different mechanisms of action. Suppression of Mdr65 (Act5c-Gal4×UAS-Mdr65-IR) resulted in increased mortality to malathion and fipronil, but not to the other three insecticides (Table 3). The increases in toxicity to malathion and fipronil were similar in magnitude to those observed for malathion (Table 2). Altogether, our results suggest that Mdr65 is a major regulator of the toxicity of a high number of insecticides.

3.3. Sensitivity of Mdr65 null mutants

We next sought to confirm the role of Mdr65 in insecticide tolerance, by monitoring the insecticide sensitivity of flies deficient...
in Mdr65. We obtained an Mdr65 null mutant line (Mdr65<sup>−/−</sup>) and determined the LC<sub>50</sub> values for malathion, malaoxon and fipronil. The Mdr65 mutation was generated in a yw background, we therefore used the yw line as control to examine the effects of the Mdr65 deletion. We first verified the Mdr65<sup>−/−</sup> line via diagnostic PCR. A 341 bp product was amplified in all three samples from the yw line, and no products were amplified in any Mdr65<sup>−/−</sup> samples, indicating that Mdr65 was disrupted by the P-element in the Mdr65<sup>−/−</sup> line as expected (Fig. S1). Disruption of Mdr65 resulted in significantly increased mortality to all three insecticides (1.9- to 3.9-fold based on the LC<sub>50</sub>s, Table 4), consistent with the results of the Mdr65 RNAi experiments (Tables 2 and 3). Mdr65 is clearly playing a role in determining the toxicity of malathion, malaoxon and fipronil in D. melanogaster.

Table 2
Toxicity of nine insecticides to the F1 progeny of the Gal4 driver line crossed with the UAS-RNAi and its genetic background lines.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Mortality (%) (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class of insecticide&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Name</td>
</tr>
<tr>
<td>Group 1-A</td>
<td>Oxamyl</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>1.09</td>
</tr>
<tr>
<td>Diazinon</td>
<td>2.59</td>
</tr>
<tr>
<td>Malathion</td>
<td>3.89</td>
</tr>
<tr>
<td>Parathion</td>
<td>1.62</td>
</tr>
<tr>
<td>Group 3-A</td>
<td>Cypermethrin</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>9.07</td>
</tr>
<tr>
<td>Esfenvalerate</td>
<td>10.4</td>
</tr>
<tr>
<td>Permethrin</td>
<td>25.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on the IRAC Mode of Action Classification (Sparks and Nauen, 2015). Group 1-A: carbamates; Group 1-B: organophosphates; Group 3-A: pyrethroids.

Fig. 1. Validation of the gene expression levels in each progeny of Act5c-Gal4 x UAS-RNAi/control lines. Three biological replicates of 10 F1 females each at 5-7-day-old were used for RT-qPCR analysis. Values shown are the average ± standard error. The letters above the bars indicate significant statistical difference between treatments determined by one-way ANOVA with Tukey’s test (P < 0.01). Histogram shading denotes flies of common genetic background.
Increased mortality was observed when Mdr65 was suppressed in F1 progeny of Gal4 driver lines crossed with the UAS-Mdr65-IR and Attp2 lines.

### Table 3: Toxicity of five insecticides to the F1 progeny of the Gal4 driver line crossed with the UAS-Mdr65-IR and Attt2 lines.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Classificationa</th>
<th>Name</th>
<th>Concentration (ng/cm²)</th>
<th>% Mortality (±SE)</th>
<th>Act5c-Gal4 × Attt2</th>
<th>Act5c-Gal4 × UAS-Mdr65-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1-B</td>
<td>Malaoxon</td>
<td>6.48</td>
<td>6(±2)</td>
<td>92(±3)</td>
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<tr>
<td></td>
<td>Phenthoate</td>
<td>3.24</td>
<td>80(±7)</td>
<td>77(±7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2-B</td>
<td>Fipronil</td>
<td>3.24</td>
<td>2(±1)</td>
<td>94(±1)</td>
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</tr>
<tr>
<td>Group 5</td>
<td>Spinosad</td>
<td>64.8</td>
<td>31(±6)</td>
<td>39(±4)</td>
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</tr>
<tr>
<td>Group 28</td>
<td>Chlorantraniliprole</td>
<td>90.7</td>
<td>38(±4)</td>
<td>46(±4)</td>
<td></td>
<td></td>
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</table>

*a* Based on the IRAC Mode of Action Classification (Sparks and Nauen, 2015). Group 1-B: Organophosphates; Group 2-B: phenylpyrazoles; Group 5: spinosyns; Group 28: diamides. ▲ Indicates mortality significantly increased relative to its genetic background flies (F1 progeny of Act5c-Gal4 × Attt2) (P ≤ 0.01).

### Table 4: Toxicity of malathion, malaoxon and fipronil against yw and Mdr65-null (Mdr65−/−) lines.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Line</th>
<th>LC50 (95% CI)</th>
<th>Slope (SE)</th>
<th>n</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion</td>
<td>yw</td>
<td>4.68 (4.53–4.83)</td>
<td>10.8 (0.8)</td>
<td>780</td>
<td>1.9</td>
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<tr>
<td></td>
<td>Mdr65−/−</td>
<td>2.47 (2.39–2.55)</td>
<td>11.2 (0.8)</td>
<td>780</td>
<td></td>
</tr>
<tr>
<td>Malaoxon</td>
<td>yw</td>
<td>3.96 (9.05–9.67)</td>
<td>10.6 (0.7)</td>
<td>760</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Mdr65−/−</td>
<td>4.48 (4.32–4.63)</td>
<td>9.6 (0.6)</td>
<td>760</td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>yw</td>
<td>5.16 (4.81–5.53)</td>
<td>3.8 (0.2)</td>
<td>1040</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Mdr65−/−</td>
<td>1.32 (1.25–1.40)</td>
<td>5.3 (0.3)</td>
<td>960</td>
<td></td>
</tr>
</tbody>
</table>

*a* LC50 in ng/cm².  
*b* LC50 yw/LC50 Mdr65−/− (Mdr65 deletion) strain.

### 3.4. Tissue specific knock-down of Mdr65

Mdr65 is expressed in the thoracic-abdominal ganglion, brain and MTs, with low expression levels in fat body (www.flyatlas.org). In order to locate the tissues where Mdr65 modifies insecticide toxicity, four tissue-specific driver lines were chosen to cross with UAS-Mdr65-IR and Attt2 lines: elav-Gal4 (pan-neuronal), MT1-Gal4 (MTs), c564-Gal4 (fat body and hemocytes) and MEF2-Gal4 (muscles). Single dose assays were performed on the F1 progeny of each of the crosses using fipronil, because this insecticide had the greatest difference between wild-type and Mdr65 null flies. Increased mortality was observed in when Mdr65 was suppressed in neurons (elav), MTs (MT1) and muscles (MEF2), but not fat body and hemocytes (c564) (Fig. 2). Thus, Mdr65 appears to be modulating the toxicity of fipronil via actions in multiple tissues.

### 4. Discussion

Altogether, our results demonstrate that Mdr65 is an important regulator of susceptibility to nine of 14 tested insecticides in D. melanogaster. This includes all four pyrethroids, one phenylpyrazole, and four of six organophosphates. The strongest effects were seen for fipronil, malathion and malaoxon. Although the latter two insecticides are structurally very similar, fipronil is not. Furthermore, a relatively small change in structure, from malathion to phenthoate (e.g. substitution of a benzene for the C-C(O)-O-C₂H₅ group) rendered Mdr65 unable to alter toxicity. In addition, RNAi knock-down of Mdr65 was previously found to increase DDT toxicity by 12% (Gellatly et al., 2015). Thus, Mdr65 seems on one hand to have broad specificity for multiple and diverse insecticides (fipronil, malathion, malaoxon, parathion, diazinon, DDT and four pyrethroids), but on the other hand be sensitive to subtle structural modifications (malathion, but not phenthoate). Additional work will be required to clarify the structural limitations that facilitate the ability of Mdr65 to alter toxicity.

Toxicity of fipronil was enhanced 3.9-fold in flies lacking Mdr65 (Table 4) and this appears to be due to effects in multiple tissues (the nervous system, MTs and muscles). Fipronil acts on GABA gated chloride channels, so a transporter found in the nervous...
system or muscles could conceivably help protect the target site from exposure to the insecticide. Given that Mdr65 expression in the MTs also influences toxicity suggest that Mdr65 might be also working in MTs to enhance the excretion of fipronil. Thus, the protective effect of Mdr65 might be due to both altering distribution (or access) and excretion of the insecticide.

A previous study in D. melanogaster using RNAi mediated suppression of Mdr49 and Mdr50 found no effect and an increase in DDT toxicity (13%), respectively (Gellatly et al., 2015). These results for Mdr49 agree with our results in which we found no change in toxicity, except for parathion. In contrast, our results showed that suppression of Mdr50 did not change the toxicity of six insecticides, and decreased the toxicity of three others.

Given the range of endogenous compounds that ABC transporters can move it might be expected that at least some of these transporters might be required for normal development and physiology. No RNAi adults resulted from the ABCB7 (CG7955) knock-down line, indicating that it is necessary for normal development. Similarly, silencing of TeABCB-5A in Tribolium castaneum prepupae led to severe developmental defects and lethality (Broehan et al., 2013). Evaluating the role of these ABC transporters in modifying insecticide toxicity will require alternative approaches.

One surprising result is that the suppression of two ABC transporters (Mdr49 and Mdr50) decreased mortality to a few insecticides, while suppression of the other two ABC transporters (Mdr65 and ABCB6) increased mortality. This would be possible if some transporters could move insecticides out of cells (or prevent their passage through a membrane) while others move insecticides through the membrane. However, at this point ABC transporters are only known to act as exporters in insects (Merzendorfer, 2014). It is possible that this effect originates in altering the physiology of barrier tissues such as the blood–brain barrier or the excretory machinery and could be involved in reabsorption processes. It is also possible that these transporters indirectly alter the secretion of molecules that interfere or compete with these insecticides. Thus, understanding the reasons for the decreased toxicity of a few insecticides associated with the RNAi mediated suppression of Mdr49 and Mdr50 will require further study.

In conclusion, we have identified four ABC transporters that have a role in altering toxicity to one or more insecticides. Mdr65 had the largest effect and altered the toxicity of the greatest number of insecticides, although the specific relationship between insecticide structure and transport by Mdr65 is unresolved. Tissue specific RNAi suggests that the effects on insecticide toxicity mediated by Mdr65 might be to be due to both changes in distribution of the insecticide and changes in excretion.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibmb.2017.08.002.

References