Toxicokinetic Analysis of Injected Diazinon and Diazoxon in Resistant and Susceptible Houseflies, 
*Musca domestica* L.

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The internal dynamics of diazinon and diazoxon when administered by injection were studied in a susceptible and resistant strain of housefly. Flies which received diazinon by injection showed only a small interstrain difference in the internal amount of diazinon. In contrast, large interstrain difference was observed in the internal amount of diazoxon when the flies received diazinon or diazoxon by injection. Several kinetic models were postulated and examined to describe the internal dynamics of diazinon. Interstrain comparison of the kinetic parameter values estimated by computer simulation revealed that high degradation of diazoxon played an important role. It is also suggested that high degradation of diazinon and less activation are responsible for the decreased accumulation of diazoxon.

*Key words*: Toxicokinetics, diazinon, housefly, resistance, mathematical model

INTRODUCTION

A housefly strain originally collected in 1984 from sanitary landfill in Yachiyo City, Japan, had an extremely high *LD*$_{50}$ for diazinon, i.e. 50 µg per fly by topical application after several selections with the insecticide in the laboratory. In vitro studies identified three enzyme systems responsible for resistance, i.e. cytochrome P-450 dependent monooxigenases (MFO), glutathione transferase (GST), and phosphorothriester hydrolase (Ot et al., 1990). Insensitivity at the target enzyme, AChE was also a resistance factor in this strain (Ot et al., 1990). A subsequent study (Ot et al., 1992) on the in vivo dynamics of dermally applied diazinon demonstrated reduced cuticular penetration as an additional factor for the resistance. A comparison of the kinetic parameter values estimated by computer simulation suggested a remarkable interstrain difference in the ratio of diazoxon degradation compared to the activation of diazinon. This resulted in little accumulation of diazoxon in the resistant strain.

To determine the significance of these pathways responsible for diazinon resistance more accurately, this paper deals with the internal dynamics of diazinon applied by injection. Several kinetic models are postulated and examined for toxicokinetic analy-

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sis of activation and degradation of diazinon and degradation of diazoxon in resistant and susceptible houseflies.

MATERIALS AND METHODS

Houseflies. The diazinon resistant Yachiyo strain and susceptible CSMA strain of housefly *Musca domestica* L., were the same as those described previously (Ot et al., 1990, 1991 and 1992). LD$_{50}$ values determined by topical application were 50 $\mu$g and 0.035 $\mu$g per fly in Yachiyo and CSMA strains, respectively (Ot et al., 1990). Three- to six-d-old female flies were used throughout the study.

Chemicals. [Ethoxy-$^{14}$C]diazinon, O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate (sp. act. 67 kBq/mg (>99% radiochemical purity) was the same as described previously (Ot et al., 1990). Nonradioactive diazinon was obtained from Nihonkayaku Co., Ltd., and purified before use. Bovine serum albumin fraction V (BSA) was purchased from Wako Pure Chemical Industries, Ltd. Sorpol 3005XH® surfactant was purchased from Toho Chemical Co. All other chemicals used were of the highest quality and commercially available.

Toxicity test. Diazinon solution (0.1 $\mu$l) in saline was injected into the thoracic scutum of flies using a glass capillary attached to an Arnold hand micro applicator (Kiya Manufacturing Co., Ltd.). The saline contained BSA (5%, w/v) and a surfactant, Sorpol 3005XH®. The concentration of the surfactant was adjusted to 1% (w/v) when the diazinon concentration was 1% (w/v) or below, or equal to the diazinon concentration when it was above 1%.

Fate of diazinon injected. Groups of ten flies with three replications were injected with various doses of $^{14}$C-diazinon as mentioned above. After the injection, the flies were kept in a scintillation vial (20 ml) at 25°C. At various time intervals, the flies were homogenized in a mixture of chloroform (4 ml) and water (2 ml) using an ultra-dispenser (Yamato Scientific Instruments Co., Model LK-21). The homogenate was stirred vigorously and centrifuged at 1,300 $\times$ g for 10 min. Radioactivity of the chloroform-soluble and water-soluble fractions was quantified by LSC (Aloka LSC 650) using a liquid scintillator (Patterson and Green, 1965). Unextracted radioactivity from the housefly residue should be below the detectable level as shown by the previous paper (Ot et al., 1992). Excreta in the holding vial was suspended in 1 ml of water for the determination of radioactivity. A preliminary study showed no chloroform-soluble radioactivity in the holding vial.

Fate of diazoxon following the injection of diazinon or diazoxon. After the injection of nonradioactive diazinon or diazoxon, the internal diazoxon was extracted with chloroform as described above at various time intervals. The amount of internal diazoxon was determined biochemically by inhibiting head AChE from the CSMA strain with a portion of the chloroform according to the method of Ot et al. (1992).

Estimation of kinetic parameters by computer simulation. Kinetic parameters from various proposed mathematical models were estimated by computer simulation through a commercially available program (Nankoudou, Inc., Tokyo) which was made on the basis of the paper of Yamaoka et al. (1981). Details of the simulation were described in the previous paper (Ot et al., 1992). When a time-course obtained with radioactive diazinon was simulated, the weight value 1 was used so that larger values could fit (smaller values were easily subjected to experimental error). On the other hand, when
a time-course obtained biochemically was simulated, the weight value \( 1/C_i^2 \) was used so that all values could fit since accuracy of smaller values were the same as larger values, where \( C_i \) is measured value at time \( t_i \).

**Selection of kinetic models.** Kinetic models were examined based upon AIC (An Information Criterion), which is incorporated in the above program. The AIC can be determined using the following equation:

\[
AIC = n \ln SS + 2m
\]

where \( n \), \( SS \) and \( m \) denote number of data, sum of squares and number of parameters, respectively, in the kinetic model. A model which gives minimum AIC is judged as the most suitable model.

**RESULTS**

**Toxicity test**

The LC_{50} (95% C.L.) of the CSMA and the Yachiyo strain by injection of diazinon were 0.012 (0.009–0.016) and 0.76 (0.67–0.87) \( \mu \)g/female, respectively.

**Dynamics of organo-soluble radioactivity after the injection of radioactive diazinon**

Five doses, i.e., 0.02, 0.05, 0.2, 1 and 5 \( \mu \)g/female were used. Results are shown in Fig. 1. The radioactivity recovery was greater than 80% throughout the experiments. A decrease in organo-soluble radioactivity, consisting mostly of diazinon and a small amount of diazoxon, was more rapid with the Yachiyo strain than with the CSMA strain at all the doses examined.

Dose dependency was suggested because the relative rate of decrease at the higher two doses were obviously slower than at the lower three doses.

**Fate of diazoxon after the injection of diazinon**

Since no diazoxon was detected in the Yachiyo strain at the lower three doses used in the previous experiment, two higher doses were used. As shown in Fig. 2, there was a remarkable interstrain difference. In the CSMA strain, a large amount of diazoxon was found and the concentration was constant or gradually decreased with time, while in the Yachiyo strain a smaller amount of diazoxon was found and decreased rapidly.

**Fate of diazoxon after the injection of diazoxon**

To investigate the interstrain difference in the degradation of diazoxon, the amount of diazoxon was determined at various time intervals after the injection of diazoxon (Fig. 3). Two doses, i.e. 0.01 and 0.1 \( \mu \)g/female were used. The result again showed the interstrain difference and dose dependency of this process.

**Kinetic analysis of the injected diazinon**

Two mathematical models were assumed to describe the overall detoxification of organo-soluble diazinon and diazoxon (Fig. 4). The following equations were derived from the Type I model:

\[
\frac{dX_c}{dt} = -k_{cw}X_c \tag{1}
\]

\[
\frac{dX_w}{dt} = k_{cw}X_c \tag{2}
\]

Fig. 5 presents the experimental data in the CSMA strain (Fig. 1, 0.02 \( \mu \)g/female) and
Fig. 1 Degradation of organo-soluble internal radioactivity following injection of various doses of diazinon in CSMA (●) and Yachie (○) strains. The curves were obtained by computer simulation, based on the rate constants listed in Table 2. The ratio of radioactivity was expressed based upon the total radioactivity recovered.

A corresponding curve predicted by computer simulation using equation 1. The $k_{ow}$ values are listed in Table 1. There is a considerable deviation between the experimental and predicted results.

Therefore, the Type II model in Fig. 4 was proposed, which includes a distribution factor such as partitioning of diazinon/diaxoxon between the hemolymph and internal tissues. The following equations were derived from the model:
Toxicokinetics of Injected Diazinon and Diazoxon

Fig. 2. Formation of diazoxon following injection of diazinon with time in CSMA (S) and Yachiyo (R) strains. Applied doses are 1 µg/♀ (●) and 5 µg/♀ (○). The curves were obtained by computer simulation, based on the rate constants listed in Table 4.

Fig. 3. Percent diazoxon remaining following injection of diazoxon in CSMA (S) and Yachiyo (R) strains. Applied doses are 0.01 µg/♀ (●, ○), and 0.1 µg/♀ (▲, △). The lines were obtained graphically from solid points, based on the rate constants listed in Table 5.

\[
\begin{align*}
\frac{dX_H}{dt} &= - (k_{H\text{W}} + k_{H\text{T}})X_H + k_{TH}X_T \\
\frac{dX_T}{dt} &= k_{HT}X_H - k_{TH}X_T \\
X_0 &= X_H + X_T
\end{align*}
\]

The parameter values were estimated by computer simulation using the experimental
data shown in Fig. 1 and equations 3-5. With regard to $k_{HT}$ and $k_{TH}$, 1.75 and 0.806 h⁻¹, respectively, were assumed in both strains obtained by preliminary stimulation for the estimation of the $k_{HW}$ values, which are listed in Table 2. Judging from the smaller AIC values as compared to the values obtained for the Type I model (Table 1), the Type II model appears more appropriate. Predicted curves obtained by the Type II model shows good agreement with the experimental data (Fig. 1). The Type II model assumes that diazinon and diazoxon partitioned into the internal tissues and thus was not available for detoxification reactions. Lack of this process in the Type I model will explain the deviation in the experimental data from the predicted data (Fig. 5).
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![Graph showing the percentage of diazinon and diazoxon over time](image)

Fig. 5. Comparison between predicted time course of organo-soluble internal radioactivity determined by computer simulation for the Type I model using the rate constants listed in Table 1 and the experimental data. The CSMA strain was subjected to injection of 0.02 μg diazinon at zero time.

$$\begin{align*}
X_4 & \xrightarrow{k_{14}} X_5 \\
& \xleftarrow{k_{15}} X_6 \\
& \xrightarrow{k_{67}} X_7
\end{align*}$$

Type III

$$\begin{align*}
X_4 + E_0 & \xrightarrow{k_{16}} X_5 \\
& \xleftarrow{k_{67}} X_6 \\
& \xrightarrow{k_{67}} X_7
\end{align*}$$

Type IV

Fig. 6. Kinetic models describing the dynamics of injected diazinon. The type III model consists of first-order processes. The type IV model consists of diazinon activation under bimolecular reaction, and apparent inactivation of the activation enzyme and degradation of diazoxon under first-order reactions. $X_4$: internal diazinon; $X_5$: metabolites of diazinon; $X_6$: internal diazoxon; $X_7$: metabolites of diazoxon; $E_0$: residual P-450 activity.

**Kinetic analysis of diazinon activation and diazoxon degradation**

Two models are postulated to describe the activation of injected diazinon and the subsequent degradation of diazoxon (Fig. 6). The following equations are derived from the Type III model:
Fig. 7. Disappearance of injected diazinon in CSMA (●) and Yachiyo (▲) strains. Applied doses were 1 µg/l and 5 µg/l. The curves were obtained by computer simulation using the rate constants listed in Table 3.

Table 3. Kinetic parameters for diazinon disappearance determined by the method of residuals

\[ [X_4 = Ae^{-at} + Be^{-bt}] \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 µg/l</th>
<th>5 µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>A (^a)</td>
<td>74.4</td>
<td>59.3</td>
</tr>
<tr>
<td>a (^b)</td>
<td>0.262</td>
<td>0.361</td>
</tr>
<tr>
<td>B (^a)</td>
<td>25.6</td>
<td>40.8</td>
</tr>
<tr>
<td>b (^b)</td>
<td>5.02</td>
<td>5.77</td>
</tr>
</tbody>
</table>

\(^a\) A and B: % diazinon.
\(^b\) a and b: rate constant (h\(^{-1}\)).

\[ X_4 = Ae^{-at} + Be^{-bt} \]
\[ dX_4/dt = k_46X_4 - k_67X_6 \]

For the estimation of \(k_{46}\) and \(k_{67}\), Eq. 6 was employed to describe a disappearance of diazinon caused by degradation of diazinon and activation to diazoxon. A comparison of predicted data and the experimental data on diazinon disappearance shows good agreement (Fig. 7). The parameter values for the process are listed in Table 3.

However, the experimental data on internal amount of diazoxon \(X_6\) and the predicted data did not show good agreement, especially with the Yachiyo strain (Fig. 8). The AIC values obtained from these computations were -17.9 and 15.0 for the CSMA strain and 35.7 and 52.4 for the Yachiyo strain at doses of 1 and 5 µg/female, respectively. Two reasons are possible for this disagreement: a decrease in activation over time or an increase in degradation of diazoxon over time. The former appears more reasonable since little increase in degradation potential of diazoxon was actually observed following the injection of diazoxon (Fig. 3).

Activation of a phosphorothioate to a phosphate is catalyzed by MFO system where cytochrome P-450 plays an important role (Nakatsugawa and Morelli, 1976). It is unlikely that the cytochrome P-450 involved in the activation of diazinon may be competitively inhibited by diazoxon, because in vitro activation of diazinon was not inhibited by the addition of diazoxon (Ot et al., 1990).
Toxicokinetics of Injected Diazinon and Diazoxon

Fig. 8. Time-course of diazoxon present in CSMA (S) and Yachiyo (R) strains. Applied doses were 1 μg/♀ (●) and 5 μg/♀ (○). The curves, showing deviation from the experimental data (particularly in the Yachiyo strain), were obtained by computer simulation using the Type III model.

Table 4. Kinetic parameters for diazinon activation, diazoxon detoxification, and apparent inactivation for the Type IV model given in Fig. 6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose (μg/♀)</th>
<th>Parameter value (h⁻¹) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>( k_{46} ) (activation)</td>
<td>1</td>
<td>0.319±0.024</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.115±0.010</td>
</tr>
<tr>
<td>( k_{67} ) (detoxification)</td>
<td>1</td>
<td>5.62±0.63</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.06±0.52</td>
</tr>
<tr>
<td>( k_P ) (apparent inactivation)</td>
<td>1</td>
<td>-0.0942±0.0259</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-0.137±0.016</td>
</tr>
<tr>
<td>AIC</td>
<td>1</td>
<td>-25.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-10.3</td>
</tr>
</tbody>
</table>

Therefore, the Type IV model which includes an apparent inactivation process of the cytochrome P-450 responsible for activation of diazinon was postulated (Fig. 6). By assuming a linear equation for the apparent decrease in the P-450 activity and a bimolecular reaction of the P-450 and diazinon, the following equations were derived from the model:

\[
\begin{align*}
\dot{X}_6 &= k_{46} X_4 E_P - k_{67} X_6 \\
\dot{E}_P &= -k_P E_P \text{ (initial condition of } E_P \text{ is 1)}
\end{align*}
\]
Table 5. Kinetic parameters for diazoxon detoxication obtained graphically from solid points in Fig. 3

<table>
<thead>
<tr>
<th>Dose (μg/♀)</th>
<th>Parameter value (h⁻¹) ± S.D.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>5.60 ± 0.19</td>
<td>S</td>
</tr>
<tr>
<td>0.1</td>
<td>4.57 ± 0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.5 ± 4.2a</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>9.75 ± 0.46</td>
<td></td>
</tr>
</tbody>
</table>

* The weighted value of \( X⁻¹ \) was used for the computation.

Parameter values estimated by computer simulation with Eqs. 6, 8 and 9, time-course of diazoxon formation (Fig. 2) and the parameter values listed in Table 3 are listed in Table 4. The AIC values for the Type IV model are smaller than those for the Type III model. Predicted data also showed good agreement with the experimental data (Fig. 2), indicating that the Type IV model describes the internal event of diazinon more accurately. An interstrain comparison shows that the \( k_{46}, k_{67} \) and \( k_{46} \), all of which are determinants of the internal amount of diazoxon, are in favor of affording resistance to the Yachiyo strain.

DISCUSSION

A remarkable interstrain difference was observed in the amount of diazoxon found following the injection of diazinon (Fig. 2). This result is similar to those obtained previously when diazinon was applied dermally by topical application. This difference was quantified by \( k_{RW} \) in Table 2, and \( k_{46}, k_{67} \) and \( k_{46} \) in Table 4. Values for \( k_{46} \) and \( k_{67} \) were not obtained correctly when diazinon was applied dermally (Or et al., 1992). In the present study, the values were obtained more accurately, as indicated by standard deviations of the parameters.

The \( k_{RW} \) value should be regarded, approximately, as the sum of \( k_{46} \) and \( k_{46} \) values which indicate disappearance of diazinon, because organo-soluble radioactivity was almost all diazinon. Therefore \( k_{46} \) is calculated by subtracting \( k_{46} \) (Table 4) from \( k_{RW} \) (Table 2). The \( k_{46} \) values thus obtained were 0.668 and 1.82 at 1 μg/♀ in the CSMA and the Yachiyo strain, respectively, and similarly 0.333 and 0.514 at 5 μg/♀, indicating that the interstrain difference of diazinon degradation was smaller than that of diazoxon degradation (Table 4).

The greater degradation of diazoxon was confirmed by the injection of diazoxon (Fig. 3) and the parameter values for diazoxon degradation were estimated graphically from the slope of the regression lines obtained from the data illustrated with the solid symbols (Table 5). The increased degradation of diazoxon indicated by the higher \( k_{67} \) values (Tables 4 and 5) is probably due to phosphorotriester hydrolyase activity which was present in all the subcellular fractions of the Yachiyo strain (Or et al., 1990), and this factor was supported by the synergistic effect of DEF, a phosphorotriesterase inhibitor (Or and Motoyama, 1991).

Kinetic analysis of the fate of injected diazinon suggested less activation of diazinon in the Yachiyo strain (Table 4), although this factor was not shown through in vitro study (Or et al., 1990). The presence of a cytochrome P-450 isozyme which is responsible for less activation of phosphorothionate to phosphate is not surprising. A cytochrome P-450, which has less specificity for activation, was recently reported for methyl
parathion resistance in the tobacco budworm (Konno et al., 1989).

In conclusion, the presence of an extremely low level of diazoxon in the Yachiyo strain (Fig. 2) should be the result of three factors: greater degradation of diazoxon (the main factor), greater degradation of diazinon, and less activation of diazinon.

REFERENCES


