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## In Vitro Inhibition of Blood Cholinesterase Activities by Dichlorvos in Farmworkers Previously Exposed to Pesticides

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**Abstract**

Organophosphate insecticides are known to inhibit blood cholinesterase (ChE) activities *in vitro* and *in vivo* with various inhibitory kinetics outcomes. This study assessed the *in vitro* inhibition of human blood ChE activities by the organophosphate insecticide dichlorvos in farmworkers previously exposed to pesticides in comparison to non-exposed (control) participants, and the differences in time-dependent dichlorvos plasma ChE inhibition and its turnover kinetics were determined in the two groups. Dichlorvos at a 0.25 and 0.5 µM significantly inhibited *in vitro* plasma ChE activity (68%-90%), but to a lesser extent that of the erythrocytes (8%-14%) in both groups. Erythrocyte ChE activity significantly decreased (8% and 14%) in the pesticide-exposed group compared to the non-significant reductions (10% and 11%) in the control one. Dichlorvos differentially affected the rate of inhibition of plasma ChE activity between the two groups. The progress of *in vitro* inhibitory effect of dichlorvos with different incubation times on plasma ChE activity of pesticides exposed and non-exposed groups illustrated that the decline in plasma ChE activity of the non-exposed group was faster compared to that of exposed group. This differential effect in the pesticide exposed group was manifested by decreases in the inhibition rate constant (19.2%) and the overall rate of inhibition (44.5%) with concomitant increases in the half-life of inhibition (25%) and total inhibition time (24.4%) in comparison to the control non-exposed group. In conclusion, dichlorvos *in vitro* differentially inhibited plasma ChE activity compared to erythrocyte ChE activity in both groups. The calculated inhibition kinetics revealed that dichlorvos differentially also affected the progress of plasma ChE inhibition in the pesticide-exposed group. These effects might have resulted from the insensitivity of the enzyme after prior exposure to pesticides.

*Keywords:* enzyme kinetics; farmers; organophosphate insecticides; plasma cholinesterase.

## 1. Introduction

Organophosphate (OP) and carbamate (CA) insecticides are still being used widely in agriculture, at home, gardens, veterinary medicine and public health to control insects, especially in developing countries [1-4]. Over use and injudicious applications of these insecticides pose health hazards to the users, mostly the farmers [4,5] and they contaminate the environment as well [1,6]. The OP and CA insecticides poisoning is characterized by inhibiting acetylcholinesterase activity in target neuronal tissues [7]. The enzyme inhibition is irreversible with OP and reversible with CA insecticides [8,9].

Several reports have shown that exposure of agricultural workers to pesticides that also include OP and CA may suffer from reduced blood cholinesterase (ChE) activity [4,5,9-11]. Determination of plasma or serum and erythrocyte ChE activities have been widely used for biomonitoring exposure to OP and CA insecticides [7,12-16]. Evaluation of in vitro and in vivo inhibition of blood or tissue ChE activities by OP or CA insecticides has been used to assess the potential toxicity and/or ChE inhibitory action of these insecticides [14-24]. Reduced blood ChE activity in insecticide-exposed persons is considered a health risk that needs further attention [25-27]. However, as the in vitro ChE inhibition technique is used to assess the potential susceptibility of the enzyme to inhibition, a recent report found the in vitro inhibition ChE by

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carbaryl is different between pesticide-exposed and non-exposed healthy persons [11]. The turnover time-dependent kinetics of such a difference is not known. The purpose of the present study was to determine and evaluate the in vitro dichlorvos-ChE inhibition of human plasma ChE in farmers previously exposed to pesticides in comparison to none-exposed participants. The present study also reports for the first time the difference in a time-dependent manner the dichlorvos ChE inhibition and its turnover kinetics between pesticide-exposed farmerworkers in comparison to non-exposed ones. Such information could yield important insights into the susceptibility of individuals previously exposed to pesticides to further challenge by additional exposure to ChE inhibiting insecticides.

## 2. Experimental

### 2.1 Approvals

We obtained ethical approval from the Committee of Post Graduate Studies, College of Pharmacy, University of Duhok, KRG, Iraq (No. 535, October, 28, 2021) and from Research Ethics Committee of the Duhok Health Directorate, Duhok, KRG, Iraq (No. 10112021-11-2, November 10, 2021). Written consents were obtained from participants to donate venous blood samples for the purpose of the study.

### 2.2 Study design and participants

#### 2.2.1 Farmworkers

The design of the study was a case-control one in which heparinized venous blood samples (5 mL) were obtained from ten pesticide exposed farmworkers (case) compared with their non-exposed counterparts (control, n=10). The details of the exposure and blood sampling procedure for examining the plasma and erythrocyte ChE activities have been described in a recent report [4]. Briefly, male participants were farmworkers who were exposed to pesticides within the past five years in Duhok, Iraq. The control group consisted of age-matched males not exposed previously to pesticides. The blood samples were centrifuged at 3000 rpm for 15 min to separate the plasma from erythrocytes, and they were kept in deep freeze at –20 ºC to be analyzed within one month.

### 2.3 *In vitro* inhibition of blood ChE activities by dichlorvos

Plasma and erythrocyte samples of 10 pesticide-exposed and 10 non-exposed participants were pooled separately. We used the 10-min *in vitro* ChE-OP incubation method for measuring plasma or erythrocyte ChE inhibition in the 0.2 mL blood aliquot as described in details previously [4,28,29]. The aqueous solution of the insecticide dichlorvos (Dichlorvos 50% EC, Nicoz, China) was freshly prepared before the experiment, and it was used to obtain final concentrations (0.25 and 0.5 μM) of dichlorvos in the enzymatic reaction mixture (6.3 mL). The use of *in vitro* concentrations of dichlorvos in the present study depended on previous reports [28,29]. The enzyme-inhibitor reaction mixtures were incubated in a water bath at 37 ºC for 10 min to initiate ChE inhibition; the residual ChE activity in the blood sample was then measured by an electrometric method using 7.1% acetylcholine iodide as a substrate as described in details elsewhere [4,11,24,30]. All assays were done in triplicate in the control (0 μM) and each of the dichlorvos concentrations. The percent of inhibition of ChE activity in the plasma or erythrocytes was calculated as follows [4]:

% ChE inhibition = [ChE activity in ΔpH/20 min (base-line control) – ChE activity (with dichlorvos)/ ChE activity (base-line control)] × 100

### 2.4 Determination of *in vitro* plasma ChE inhibition kinetics by dichlorvos

The pooled plasma samples of 10 pesticide-exposed and 10 non-exposed participants were used. According to a preliminary experiment, we determined the concentration of dichlorvos in the reaction mixture to be 0.25 μM. The incubation times after dichlorvos addition to plasma samples were 5, 10, 15, 30 and 60 minutes; with the value of 0-time representing the plasma ChE activity without the addition of dichlorvos, and it was considered 100% baseline activity. Assays at each time point were done in duplicate. A proper blank was included in each time frame of incubation. Enzyme activity (Δ pH/20 min) and ChE inhibition were calculated as described above.

The steady state kinetic relationship was applied on the rate of decline of ChE activity vs. time [0 - 60 min], and the equations used for calculating *in vitro* inhibition kinetics were according to previous studies [31-33]:

$\text{Log (ChE activity)} = \text{Log (ChE activity)}_0 - 0.434kt$

$\text{Slope} = 0.434k$

$-k = \text{slope}/0.434$

$\text{Total inhibition time} = 1/k$

$\text{Inhibition rate} = (\text{ChE activity})_0 \times k$

where, $(\text{ChE activity})_0$ is the steady state plasma ChE activity at time 0, (ChE activity) is the plasma ChE activity at t time (60 min), and k is the inhibition rate constant which is multiplied by

t (60 min). These calculations were also statistically verified by the linear regression using the statistical software program Past4.11 (https://www.nhm.uio.no/english/research/resources/past/) as well as using the online program Omni Calculator (Chemistry Calculators, https://www.omnicalculator.com/chemistry).

## 3. Results

### 3.1 *In vitro* ChE inhibition

*In vitro* addition of dichlorvos, significantly and in a concentration dependent manner, inhibited plasma and erythrocyte ChE activities in both pesticide exposed and non-exposed controls. In the pesticide exposed group, dichlorvos at 0.25 and 0.5 µM significantly inhibited plasma ChE activity by 68% and 85%, respectively and significantly inhibited erythrocyte ChE activity by 8% and 14%, respectively, when compared with the respective baseline values (Table 1). In the non-exposed control group, dichlorvos at 0.25 and 0.5 µM significantly inhibited plasma ChE activity by 72% and 90%, respectively, with no significant inhibition of erythrocyte ChE activity when compared with the respective baseline values (Table 1). The baseline plasma ChE activity but not that of the erythrocytes in the pesticide exposed group was significantly below that of the control (non-exposed) group by 37% (Table 1).

**Table 1:** *In vitro* inhibition of plasma and erythrocyte cholinesterase (ChE) activities (ΔpH/20 min) by dichlorvos in control participants not exposed to pesticide and in farmworkers exposed to pesticides.

| **Dichlorvos (µM)** | Plasma ChE activity | % inhibition | Erythrocyte ChE activity | % inhibition |
|---|---|---|---|---|
| **Not exposed to pesticides (control)** | | | | |
| **0** | 1.17 ± 0.057 | - | 1.43 ± 0.040 | - |
| **0.25** | 0.33 ± 0.035$^{*}$ | 72 | 1.28 ± 0.052 | 10 |
| **0.5** | 0.12 ± 0.026$^{*a}$ | 90 | 1.27 ± 0.015 | 11 |
| **Exposed to pesticides** | | | | |
| **0** | 0.74 ± 0.032$^{\dagger}$ | - | 1.27 ± 0.023 | - |
| **0.25** | 0.24 ± 0.027$^{*}$ | 68 | 1.17 ± 0.026$^{*}$ | 8 |
| **0.5** | 0.11 ± 0.026$^{*a}$ | 85 | 1.09 ± 0.01$^{*}$ | 14 |

Values are mean ± SE of 3 measurements/concentration.
% ChE inhibition = [(ChE activity without dichlorvos – ChE activity with dichlorvos)/ ChE activity without dichlorvos] x 100
$^{*}$Significantly different from the baseline (0 µM concentration), $p<0.05$.
$^{a}$Significantly different from the 0.25 µM concentration of dichlorvos, $p<0.05$.
$^{\dagger}$Significantly different from the non-exposed group, $p<0.05$.

### 3.2 *In vitro* plasma ChE inhibition kinetics

The progress of *in vitro* inhibitory effect of dichlorvos (0.25 µM) with different incubation times on plasma ChE activity of pesticides exposed and non-exposed groups illustrated that the decline in plasma ChE activity of the non-exposed group was faster compared to that of exposed group (Table 2, Figure 1). Kinetic values shown in table 3 were derived from the steady state equation, Log (plasma ChE activity) = Log (plasma ChE activity)$_0$ – 0.434 kt, using the results of table 1 and by applying the linear regression analysis with confirmation using the software Omni Calculator as mentioned above.

As shown in table 3, the kinetic response of pesticide-exposed participants to the progress of plasma ChE inhibition was affected differently compared to the control non-exposed group. This differential effect in the pesticide exposed group was manifested by decreases in the inhibition rate constant (19.2%) and the overall rate of inhibition (44.5%) with concomitant increases in the half-life of inhibition (25%) and total inhibition time (24.4%) in comparison to the control non-exposed group. These inhibitory kinetics parameters coincided with the above-mentioned differential inhibition of plasma ChE activity between the exposed and the non-exposed control (Table 1).

## 4. Discussion

Organophosphate insecticides are generally used against insects in agriculture, public health and veterinary clinical practice with potential environmental impacts and toxic effects of their residues which can be readily absorbed and become bioavailable in man or animals [2-5,34,35]. *In vitro* ChE inhibition is a useful method to evaluate the potential antiChE activity of chemicals and drugs as well as organophosphate and carbamate insecticides in humans and animals, and the technique is

supportive of in vivo poisoning studies induced by these chemicals [11,18,20,29,36,37].

**Table 2:** The progress of *in vitro* inhibitory effect of dichlorvos (0.25 µM) with different incubation times on plasma cholinesterase (ChE) activity (ΔpH/20 min) of pesticide-exposed farmworkers and non-exposed control group

| **Time (min)** | **Plasma ChE activity-Control** | **Plasma ChE activity-Exposed** |
|---|---|---|
| **0** (no dichlorvos) | 1.02 | 0.70 |
| **5** | 0.68 | 0.52 |
| **10** | 0.50 | 0.38 |
| **15** | 0.38 | 0.28 |
| **30** | 0.19 | 0.20 |
| **60** | 0.12 | 0.12 |

Values are the mean of duplicate measurements of 10 pooled samples per group.

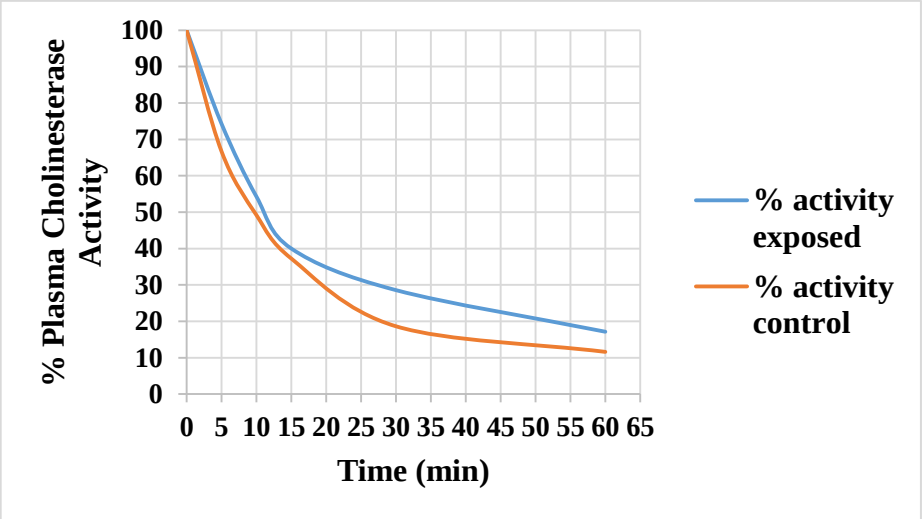


**Figure 1:** The decline in the percentage of plasma cholinesterase activity against time by *in vitro* incubation of plasma samples with dichlorvos (0.25 µM) at 37 ℃ for 5, 10, 15, 30 and 60 min in pesticides- exposed and non-exposed control groups. The values are duplicate readings at each time point from 10 pooled plasma samples in each group.

**Table 3**: Plasma cholinesterase inhibitory kinetic parameters of control and pesticide exposed participants following *in vitro* incubation of plasma samples with dichlorvos at a concentration of 0.25 µM

| Parameters | Control | Pesticide exposed | % Change from control |
|---|---|---|---|
| Inhibition rate constant (k), $min^{-1}$ | 0.0343 | 0.02773 | –19.2 |
| Half-life of inhibition ($t_{1/2}$), min. | 20 | 25 | +25 |
| Inhibition rate, activity ΔpH/ min. | 0.035 | 0.01942 | –44.5 |
| Total inhibition time, min | 29 | 36.07 | +24.4 |

Additionally, the metabolites of OP insecticides in mammals or plants might have deleterious toxic impacts [7,8,38,39,]. Dichlorvos, a synthetic OP insecticide was found in the present study to inhibit plasma and erythrocyte ChE activities *in vitro* in a concentration-dependent manner in both the pesticide-exposed and the non-exposed participants. This ChE inhibitory effect is in agreement with the known *in vitro* inhibitory effects of dichlorvos on blood ChE activities in various species [18,22,28,29,36,37]. Observing the data of table 1, a differential *in vitro* ChE inhibitory effect of dichlorvos was seen in both groups, as the plasma ChE activity was susceptible to dichlorvos inhibition more than that of the erythrocytes (68%-90% vs. 8%-14%). This effect is agreement with the previously reported differential action of dichlorvos on plasma ChE activity [38,40-42]. Furthermore, it was reported that the susceptibility of plasma ChE activity to *in vitro* inhibition by dichlorvos variably increased in diabetic patients [43,44]. Within this context, it is reasonable to suggest that the differences seen in blood ChE inhibition *in vitro* by dichlorvos between the pesticide-exposed and non-exposed subjects could be associated with the previous exposure to pesticides. This phenomenon, which is confirmed by the present study, has been reported earlier by using carbaryl for *in vitro* blood ChE inhibition in farmers previously exposed to pesticides [11]. The reason for such an effect is not clear at present; it could, however, results from the insensitivity of the enzyme after prior inhibition which confers some protection from further inhibition compared to the non-exposed individuals [45]. Such a protective effect has been demonstrated using metoclopramide (a weak ChE inhibitor) in rats treated with the OP compound paraoxon [45] and in young chicks dosed with the OP diazinon [46].

To further elaborate on the *in vitro* inhibitory differences in plasma ChE activity between the pesticide-exposed and non-exposed individuals, we attempted in the present study to examine the progress of *in vitro* inhibitory effect of dichlorvos (0.25 μM) with different incubation times on plasma ChE activity to determine the turnover parameters of the enzyme inhibition in both groups. It is known that dichlorvos inhibits plasma and erythrocyte ChE activities at different rates [38,40,44]. The parameters of inhibition kinetics of dichlorvos which were reported by other investigators included 50% plasma ChE activity inhibition (IC50, 0.03 μM) with a Michaelis-Menton constant ($K_m$) of 7.1mM and a maximum velocity ($V_{max}$) of 143 nmol/min/mL [41,47,48]. However, in the present study a different approach was attempted by examining the inhibition kinetics (percentage activity versus time) because of the initial finding that dichlorvos inhibits plasma ChE activity in pesticide exposed farmworkers less than that of the non-exposed controls (Table 1). The calculated inhibition kinetics revealed that dichlorvos differentially affected the progress of plasma ChE inhibition according to the incubation time, where, the inhibition rate constant was decreased by 19.2% and the rate of inhibition was decreased by 44.5% in the pesticide exposed group with concomitant increases in the half-life of inhibition by 25% and the total inhibition time by 24.4% in comparison to the control plasma samples. These parameters of the inhibitory kinetics, being a unique finding in the present study, coincided with the above-mentioned differential inhibition of plasma ChE activity comparing the pesticide-exposed and the non-exposed individuals. Depending on the steady state equations [31-33], these findings confirm the sensitivity of plasma ChE in the none-exposed group to dichlorvos inhibition more than that of the pesticide-exposed farmworkers. Furthermore, within this context, it would be interesting to apply the present experimental protocol on additional pesticides that pose a health hazard to the public occupationally [49], or in monitoring exposure to residues of OP insecticides [50].

## 5. Conclusions

In conclusion, dichlorvos *in vitro* differentially inhibited plasma ChE activity compared to erythrocyte ChE activity in both groups. The calculated inhibition kinetics revealed that dichlorvos differentially also affected the progress of plasma ChE inhibition in the pesticide-exposed group. These effects might have resulted from the insensitivity of the enzyme after prior exposure to pesticides. The clinical toxicological implication of this finding needs additional studies.

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## 7. References


1. Mali, H., Shah, C., Raghunandan, B. H., Prajapati, A. S., Patel, D. H., Trivedi, U., & Subramanian, R. B. (2023). Organophosphate pesticides an emerging environmental contaminant: Pollution, toxicity, bioremediation progress, and remaining challenges. *Journal of environmental sciences (China)*, *127*, 234–250. https://doi.org/10.1016/j.jes.2022.04.023
2. Pandya, I. Y. (2018). Pesticides and their applications in agriculture. *Asian Journal of Applied Science and Technology*, *2(2)*:894–900.
3. Rashid, S., Rashid, W., Tulcan, R. X. S., & Huang, H. (2022). Use, exposure, and environmental impacts of pesticides in Pakistan: a critical review. *Environmental science and pollution research international*, *29*(29), 43675–43689. https://doi.org/10.1007/s11356-022-20164-7
4. Odisho, S. K. ., & Mohammad, F. K. (2022). Blood cholinesterase activities and oxidative stress status among farmworkers using pesticides in Duhok, KRG, Iraq. *Journal of ideas in health*, *5(4)*: 786-793. https://doi.org/10.47108/jidhealth.Vol5.Iss4.264
5. Samareh, A., Asadikaram, G., MojtabaAbbasi-Jorjandi, Abdollahdokht, D., Abolhassani, M., Khanjani, N., & Nematollahi, M. H. (2022). Occupational exposure to pesticides in farmworkers and the oxidative markers. *Toxicology and industrial health*, *38*(8), 455–469. https://doi.org/10.1177/07482337221106754
6. Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (2021). Agriculture Development, Pesticide Application and Its Impact on the Environment. *International journal of environmental research and public health*, *18*(3), 1112. https://doi.org/10.3390/ijerph18031112
7. Wilson, B. W. (2014). Cholinesterase inhibition. In: Wexler P (Ed). Encyclopedia of Toxicology. 3rd ed. Elsevier, Amsterdam. pp. 942-951.
8. Vale, A., & Lotti, M. (2015). Organophosphorus and carbamate insecticide poisoning. *Handbook of clinical neurology*, *131*, 149–168. https://doi.org/10.1016/B978-0-444-62627-1.00010-X
9. Aguera, R. G., Freires, C. D. S., Oliveira, L. O., Monteiro, L. R., Lini, R. S., Romoli, J. C. Z., Freire, B. M., Nerilo, S. B., Machinski Junior, M., Batista, B. L., & Mossini, S. A. G. (2022). Risk evaluation of occupational exposure of southern Brazilian flower farmers to pesticides potentially leading to cholinesterase inhibition and metals exposure. *Environmental toxicology and pharmacology*, *93*, 103874. https://doi.org/10.1016/j.etap.2022.103874
10. Sine, H., Achbani, A., & Filali, K. (2022). Measuring butyrylcholinesterase activity and hematological parameters in farmers exposed to pesticides: a case and control study from Morocco. *Archives of environmental & occupational health*, *77*(4), 345–350. https://doi.org/10.1080/19338244.2021.1886034
11. Mohammed, A. A. & Mohammad, F. K. (2022). Monitoring blood cholinesterase activity of farmworkers: *In vitro* inhibition by diphenhydramine and carbaryl. *Malaysian applied biology*, *51(2)*,23–32. Available from: https://jms.mabjournal.com/index.php/mab/article/view/2204.
12. Assis, C. R. D., Linhares, A. G., Cabrera, M. P., Oliveira, V. M., Silva, K. C. C., Marcuschi, M., Maciel Carvalho, E. V. M., Bezerra, R. S., & Carvalho, L. B., Jr (2018). Erythrocyte acetylcholinesterase as biomarker of pesticide exposure: new and forgotten insights. *Environmental science and pollution research international*, *25*(19), 18364–18376. https://doi.org/10.1007/s11356-018-2303-9
13. Kapka-Skrzypczak, L., Cyranka, M., Skrzypczak, M., & Kruszewski, M. (2011). Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art. *Annals of agricultural and environmental medicine : AAEM*, *18*(2), 294–303.
14. Mohammad, F. K., Garmavy, H. M. S., Mohammed, A. A., & Rashid, H. M. (2023). First meta-analysis study of cholinesterase inhibition in experimental animals by organophosphate or carbamate insecticides under the influence of diphenhydramine. *Veterinary world*, *16*(1), 118–125. https://doi.org/10.14202/vetworld.2023.118-125
15. Wilson, B. W., Arrieta, D. E., & Henderson, J. D. (2005). Monitoring cholinesterases to detect pesticide exposure. *Chemico-biological interactions*, *157-158*, 253–256. https://doi.org/10.1016/j.cbi.2005.10.043
16. Nigg, H. N., & Knaak, J. B. (2000). Blood cholinesterases as human biomarkers of organophosphorus pesticide exposure. *Reviews of environmental contamination and*

*toxicology*, *163*, 29–111. https://doi.org/10.1007/978-1-4757-6429-1_2

17. Badawy S. M. (2020). Validation and kinetic of enzymatic method for the detection of organophosphate insecticides based on cholinesterase inhibition. *Toxicology mechanisms and methods*, *30*(2), 134–138. https://doi.org/10.1080/15376516.2019.1669248
18. Ellinger C. (1985). Enzyme activities after in vitro and in vivo application of dichlorvos. *Biomedica biochimica acta*, *44*(2), 311–316.
19. Zdrazilová, P., Stěpánková, S., Komersová, A., Vránová, M., Komers, K., & Cegan, A. (2006). Kinetics of 13 new cholinesterase inhibitors. *Zeitschrift fur Naturforschung. C, Journal of biosciences*, *61*(7-8), 611–617. https://doi.org/10.1515/znc-2006-7-823
20. Mohammed, A. A & Mohammad, F. K. (2022). Recognition and assessment of antidotal effects of diphenhydramine against acute carbaryl insecticide poisoning in a chick model. *Toxicology international*, *29:(3)*,339-352. https://doi.org/10.18311/ti/2022/v29i3/29732
21. Wilson, B.W. (2010). Cholinesterases. In: Robert Krieger, editor. Hayes' Handbook of Pesticide Toxicology. 3rd edition.: Academic Press, Amsterdam. pp.1457-147.
22. Alias, A. S., Al-Zubaidy, M. H., Mousa, Y. J., & Mohammad, F. K. (2011). Plasma and whole brain cholinesterase activities in three wild bird species in Mosul, IRAQ: In vitro inhibition by insecticides. *Interdisciplinary toxicology*, *4*(3), 144–148. https://doi.org/10.2478/v10102-011-0022-x
23. Mohammad, F. K., Al-Badrany, Y. M., & Al-Jobory, M. M. (2008). Acute toxicity and cholinesterase inhibition in chicks dosed orally with organophosphate insecticides. *Arhiv za higijenu rada i toksikologiju*, *59*(3), 145–151. https://doi.org/10.2478/10004-1254-59-2008-1873
24. Garmavy, H. M. S., Mohammed, A. A., Rashid, H. M., & Mohammad, F. K. (2023). A meta-analysis of normal human blood cholinesterase activities determined by a modified electrometric method. *Journal of medicine and life*, *16*(1), 22–34. https://doi.org/10.25122/jml-2022-0215
25. Ciesielski, S., Loomis, D. P., Mims, S. R., & Auer, A. (1994). Pesticide exposures, cholinesterase depression, and symptoms among North Carolina migrant farmworkers. *American journal of public health*, *84*(3), 446–451. https://doi.org/10.2105/ajph.84.3.446
26. Environmental Protection Agency. (2015). How to Comply with the 2015 Revised Worker Protection Standard for Agricultural Pesticides: What Owners and Employers Need to Know. EPA, Washington DC, USA. Available from: https://www.epa.gov/pesticide-worker-safety/pesticide-worker-protection-standard-how-comply-manual
27. Lessenger J. E. (2005). Fifteen years of experience in cholinesterase monitoring of insecticide applicators. *Journal of agromedicine*, *10*(3), 49–56. https://doi.org/10.1300/j096v10n03_06
28. Mohammad, F. K., Al-Baggou, B. K., Naser, A. S. & Fadel, M. A. (2014). In vitro inhibition of plasma and brain cholinesterases of growing chicks by chlorpyrifos and dichlorvos. *Journal of applied animal science, 42(4)*, 423-8. https://doi.org/10.1080/09712119.2013.875912
29. Mohammad, F. K., Al-Baggou, B. K. , Alias, A. S. & Faris GA-M. (2006). Application of an electrometric method for measurement of in vitro inhibition of blood cholinesterases from sheep, goats and cattle by dichlorvos and carbaryl. *Veterinarni Medicina, 51(2)*, 45-50. https://doi.org/10.17221/5516-VETMED.
30. Mohammad, F. K. (2022). Clarifying an electrometric method for determining blood cholinesterase activity: a scientific letter. *Asia pacific journal of medical toxicology*, *11(1)*, 30-32. doi: 10.22038/apjmt.2022.19924
31. Brodie, B. B., Costa, E., Dlabac, A., Neff, N. H., & Smookler, H. H. (1966). Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. *The Journal of pharmacology and experimental therapeutics*, *154*(3), 493–498. PMID: 5928249.
32. Reid, W. D., Volicer, L., Smookler, H., Beaven, M. A., & Brodie, B. B. (1968). Brain amines and temperature regulation. *Pharmacology*, *1*(6), 329–344. https://doi.org/10.1159/000135983
33. Al-Baggou, B. K. & Mohammad, F. K. (2022). The use of medetomidine to determine glutathione turnover rate in cadmium-treated mice. Proceeding of the Ahi Evran 2nd International Conference on Scientific Research, Kırsehir Ahi Evran University, Turkey, October 21-23, 2022; Vol. 3: 100-104. (ISBN - 978-625-8246-64-3).
34. Abdel-Gawad, H., Abdel-Hameed R.M., Affi L. M. & Hegazi B. (2008). Distribution and

degradation of 14C-ethyl prothiofos in potato plant and the effect of processing. *Phosphorous, sulfur, and silicon and related elements, 183 (11),* 2734-2751. https://doi.org/10.1080/10426500801977970

35. Abdel-Gawad, H., Abdel-Hameed, R. M., & Witczak, A. (2013). Residues of $^{14}$C-ethion along the extraction and refining process of maize oil, and the bioavailability of bound residues in the cake for experimental animals. *Bulletin of environmental contamination and toxicology*, *91(2),* 240–245. https://doi.org/10.1007/s00128-013-1027-2
36. Ranjan, A., Chauhan, A., & Jindal, T. (2018). In-silico and in-vitro evaluation of human acetylcholinesterase inhibition by organophosphates. *Environmental toxicology and pharmacology*, *57*, 131–140. https://doi.org/10.1016/j.etap.2017.12.014
37. Mohammadi, H., Jalilian, J., Karimi, M. Y., & Shetab-Boushehri, S. V. (2017). *In Vitro* Cysteine Reactivates Organophosphate Insecticide Dichlorvos-Inhibited Human Cholinesterases. *Sultan Qaboos University medical journal*, *17*(3), e293–e300. https://doi.org/10.18295/squmj.2017.17.03.006
38. WHO (1986). Environmental Health Criteria 63: Organophosphorus Insecticides: A General Introduction. Geneva, Switzerland. https://apps.who.int/iris/handle/10665/40198
39. Abdel-Gawad, H. & Hegazi, B. (2010). Fate of 14C-ethyl prothiofos insecticide in canola seeds and oils. *Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes, 45(2),* 116–122. https://doi.org/10.1080/03601230903471878
40. WHO (1989). Environmental Health Criteria 79. Dichlorvos. Geneva, Switzerland. https://apps.who.int/iris/handle/10665/39329?search-result=true&query=environmental+health+criteria+79&scope=%2F&rpp=10&sort_by=score&order=desc
41. Richter, P. & Corcoran, J. (1997). Toxicological Profile for Dichlorvos. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, Georgia USA.
42. Moon, J., Chun, B., & Lee, S. (2015). Variable response of cholinesterase activities following human exposure to different types of organophosphates. *Human & experimental toxicology*, *34*(7), 698–706. https://doi.org/10.1177/0960327114558890
43. Rashid, H. M., Mohammad, F. K. & Persike, D. S. (2021). Blood cholinesterase activity and oxidative stress in type 2 diabetic patients. *Duhok Medical Journal*, *15(1),* 48-57. https://doi.org/10.31386/dmj.2021.15.1.4
44. Rashid, H. M., Mohammad, F. K. & Persike, D. S. (2021). *In vitro* inhibition of blood cholinesterases by the organophosphate dichlorvos in typ-2 diabetic patients. *Indian journal of forensic medicine and toxicology, 15(3),* 3110-3124. https://doi.org/10.37506/ijfmt.v15i3.15780
45. Petroianu, G. A., Hasan, M. Y., Kosanovic, M., Vijayasarathy, C., & Saleh, A. M. (2003). Metoclopramide protection of cholinesterase from paraoxon inhibition. *Veterinary and human toxicology*, *45*(5), 251–253. PMID: 14513894.
46. Al-Zubaidy, M. H., & Mohammad, F. K. (2007). Metoclopramide protection of diazinon-induced toxicosis in chickens. *Journal of veterinary science*, *8(3),* 249–254. https://doi.org/10.4142/jvs.2007.8.3.249
47. Assis, C. R., Amaral, I. P., Castro, P. F., Carvalho, L. B., & Bezerra, R. S. (2007). Effect of dichlorvos on the acetylcholinesterase from tambaqui (Colossoma macropomum) brain. *Environmental toxicology and chemistry*, *26*(7), 1451–1453. https://doi.org/10.1897/06-488r1.1
48. Carter, M. K. & Maddux, B. (1974). Interaction of dichlorvos and anticholinesterases on the in vitro inhibition of human blood cholinesterases. *Toxicology and applied pharmacology*, *27*(2), 456–463. https://doi.org/10.1016/0041-008x(74)90217-8

49. Shalaby, S., Elmetwally, I., Abou-elella, G. & Abdou, G. (2022). Occupational workers understanding of pesticide labels and safety practices in Egypt. *Egyptian Journal of Chemistry*, *65*(6), 367–380. doi: 10.21608/ejchem.2021.94069.4430
50. Elsayed, A., Eissa, F., Ghanem, K. & Mahmoud, H. (2023). Monitoring and risk assessment of organophosphorus pesticide residues in surface and drinking water in some Egyptian governorates. *Egyptian Journal of Chemistry*, (in press), -. doi: 10.21608/ejchem.2023.210533.7967