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Scientific Paper

ACUTE TOXICITY OF VETERINARY AND AGRICULTURAL FORMULATIONS OF ORGANOPHOSPHATES DICHLORVOS AND DIAZINON IN CHICKS

Muna H. I. AL-ZUBAIDY, Yaareb J. MOUSA, Mohammad M. HASAN, and Fouad K. MOHAMMAD

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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Formulation components of organophosphate insecticidal preparations might affect their toxic action in animals. The objective of this study was to examine and compare the acute toxicity and cholinesterase inhibition in seven to 14-day-old chicks dosed orally with dichlorvos and diazinon in standard veterinary and agricultural formulations. The acute (24 h) oral median lethal doses (LD_{50}) of the formulations were determined using the up-and-down method. Respective LD_{50} of dichlorvos of the veterinary and agricultural formulations in chicks were 11.1 mg kg^{-1} and 6.51 mg kg^{-1} and those of diazinon 6.4 mg kg^{-1} and 6.7 mg kg^{-1}. Plasma and brain cholinesterase activities were measured by electrometry after *in vivo* and *in vitro* exposure to organophosphates. The chicks showed signs of cholinergic toxicosis within one hour of dosing. Dichlorvos (8 mg kg^{-1}) and diazinon (4 mg kg^{-1}) in the veterinary and agricultural formulation significantly reduced both plasma and brain cholinesterase activities in the chicks. The veterinary formulation of dichlorvos reduced plasma ChE by 60 % and agricultural by 40 % and brain ChE by 93 % and 87 %, respectively. In contrast, ChE inhibition by diazinon in the agricultural formulation of diazinon was stronger than by the veterinary formulation; 72 % *vs.* 64 % in plasma and 97 % *vs*. 80 % in the brain, respectively. The highest *in vitro* inhibitions were observed with dichlorvos in the agricultural formulation (50 %) in the brain samples and with diazinon in the agricultural formulation (52 %) in the plasma samples. While they exist, differences between formulations cannot be taken as a rule and further investigations should inventory the toxicity of standard veterinary and agricultural organophosphate formulations in addition to the known data for pure forms.

KEY WORDS: *brain, cholinergic toxicosis, cholinesterase, electrometry, inhibition, LD_{50}, plasma, poisoning*

Organophosphate (OP) insecticides are extensively used in different formulations to eradicate and control insects in veterinary practice, public health, and agriculture all over the world (1-3). These exert a variety of adverse health and environmental effects (2-7). Insecticides with similar active ingredients may contain different inactive substances such as solvents, additives, detergents, and emulsifiers (5, 8-11). Additives can increase the toxicity of the active compound in a formulation (7, 10, 12, 13) and many reports have urged that it is imperative to assess the toxicity of commercial insecticide formulations in addition to that of their active ingredients (11, 14, 15).

In mammals and birds OP insecticides inhibit cholinesterase (ChE), which leads to cholinergic overstimulation manifested through muscarinic, nicotinic, and central nervous symptoms (4, 5, 16). The toxicity of OP insecticides varies between animal species. *In vivo* avian studies usually involve chicks (17-19). The diagnostic or biomarker endpoint of OP poisoning in the avian species is the reduction of ChE activity in the plasma, serum, or brain (16, 20-22). The true and pseudo ChEs are present in the plasma of the chicks, whereas true ChE is found in the nervous tissues, but not in red blood cells (16, 20, 22) opposite to mammals (16). Reduction of blood ChE activity by 50 % or more usually confirms the diagnosis of acute poisoning with OP insecticides (5, 16, 20-23).

The aim of this study was to examine and compare acute toxicity and ChE inhibition in chicks dosed orally with OP insecticides dichlorvos and diazinon using their veterinary and agricultural formulations. Dichlorvos directly inhibits ChE activity, whereas diazinon acts via its active metabolite *in vivo* to inhibit the enzyme (23-25). Various formulations of dichlorvos and diazinon are commonly used as insecticides in Iraq and they can be obtained as over-the-counter compounds from local markets.

MATERIAL AND METHODS

We performed our experiment on mix-breed seven to 14-day-old broiler chicks of both sexes (55 g to 95 g body weight). They were receiving water and feed *ad libitum* and were kept in batches of 20 to 30 at room temperature of 30 °C to 34 °C with constant lighting. Floor litter consisted of wood shavings. Commercial insecticidal concentrate solutions of the OPs used were as follows: dichlorvos for veterinary use (55 % 2,2-dichlorovinyl dimethyl phosphate, DDVP, Fertil Kimya San. Ic., Turkey), dichlorvos for agricultural use (50 %, Super Nogos 50 EC, Pacific Agriscience, Australia), diazinon for veterinary use (60 % Diazinon-60EC, VAPCO, Jordan), and diazinon for agricultural use (60 %, Zell Chemie Internacional, S.L., Spain). They were further diluted in distilled water to obtain desired concentrations for dosing by gavage in a volume of 5 mL kg^{-1} body weight. The solutions were freshly prepared before use, and all insecticide doses were based on active ingredients.

Acute toxicity of OP insecticides

We first determined the acute, 24-hour oral lethal dose (LD_{50}) for each OP insecticide/formulation using the up-and-down method (26). The chicks were individually observed for the signs of cholinergic toxicosis for one hour and then we recorded 24-hour lethality. For the LD_{50} experiments we used only 5 to 6 chicks for each formulation group.

In vivo *effects of OP insecticides on plasma and brain ChE activities*

Forty chicks were randomly divided into five groups of eight birds each. Through gavage the chicks received the oral doses of distilled water at 5 mL kg^{-1} b. w. (control), dichlorvos at 8 mg kg^{-1} or diazinon at 4 mg kg^{-1} b.w. in either the veterinary or agricultural formulation. The doses of the OP were chosen so that they did not cause acute signs of cholinergic toxicosis or death in chicks within one hour after the dosing. One hour after each OP dosing, chicks were euthanised to obtain the plasma and whole brain for determination of ChE activity by an electrometric method described earlier (18, 19, 27). All samples were kept at -20 °C, pending ChE analysis within one week.

Whole brain was homogenised in a pH 8.1 barbital-phosphate buffer solution as described earlier (18, 19, 27). Plasma and brain ChE activity was determined using the electrometric method as described in our earlier study (18): the reaction mixture in a 10-mL vial contained 3 mL distilled water, 0.2 mL plasma or whole brain homogenate and 3 mL of pH 8.1 buffer described above. The initial pH of the mixture (pH_1) was measured with a glass electrode using a pH meter (Hanna, Romania), and then 0.10 mL of the substrate 7.5 % acetylthiocholine iodide was added to the mixture and incubated at 37 °C for 30 min. At the end of the incubation period, we measured the pH of the reaction mixture (pH_2). Enzyme activity (expressed as ΔpH/30 min) was calculated as follows:

Activity (ΔpH/30 min) = (pH_1-pH_2) - ΔpH of blank

The blank was without the plasma or brain homogenate sample.

The percentage of inhibition was calculated as follows:

% Inhibition = (Activity - $Activity_{OP}$ / Activity) x 100

In vitro *effects of OP insecticides on plasma and brain ChE activities*

To determine *in vitro* ChE inhibition we used the plasma and whole brains of chicks that had not been exposed to the oral doses of OP insecticides (three per control and each OP concentration). The OP insecticides

were prepared in distilled water and individually added in a volume of 0.1 mL to the plasma or whole-brain homogenate, and the final reaction volume was 6.3 mL (28). Control reaction mixtures did not contain any insecticide and served as baseline. In treated samples, dichlorvos and diazinon concentrations were 0.5 μmol L^{-1}, 1.0 μmol L^{-1}, and 2.0 μmol L^{-1}. The reaction mixtures were incubated at 37 °C for 10 minutes, and the residual ChE activity was measured as described above. All measurements were done in duplicate.

We used the analysis of variance, followed by the least significant difference test (29) with significance set at $p<0.05$.

RESULTS

Table 1 shows the acute oral LD_{50} values of the OP insecticides in both formulations. Signs of OP poisoning appeared within two to five minutes in chicks dosed with dichlorvos formulations, and the birds died within 18 minutes. Signs of poisoning and death were delayed in chicks dosed with diazinon.

Regardless of the insecticide, the signs of cholinergic toxicosis appeared within one hour and included salivation, lacrimation, gasping, frequent defecation, droopy wings, tremors, convulsions, and recumbency.

Oral dosing with dichlorvos (8 mg kg^{-1}) in both significantly reduced plasma ChE activity by 60 % and 48 % and that of the brain by 93 % and 87 %, respectively when compared to control values (Table 2). Similarly, oral dosing with diazinon (4 mg kg^{-1}) in both formulations significantly reduced plasma ChE activity by 64 % and 72 % and that of the brain by 80 % and 97 %, respectively in comparison with control values (Table 2). The veterinary formulation of dichlorvos was more effective in reducing plasma

Table 1 *24-hour oral median lethal dose (LD_{50}) of dichlorvos and diazinon in veterinary and agricultural formulations in chicks*

	Dichlorvos		Diazinon	
	veterinary formulation	agricultural formulation	veterinary formulation	agricultural formulation
LD_{50} / mg kg^{-1}	11.10	6.51	6.40	6.66
Range of the doses / mg kg^{-1}	9 to 12	3 to 9	5 to 7	5 to 9
Initial dose / mg kg^{-1}	9	9	5	5
Last dose / mg kg^{-1}	9	6	5	7
Number of chicks	5 (OXOXO)*	6 (XXOOXO)*	5 (OXOXO)*	6 (OOXXOX)*
Increase or decrease in the dose / mg kg^{-1}	3	3	2	2
Range of latency to the onset of poisoning / min	2 to 4	3 to 5	10 to 25	9 to 33
Range of latency to the onset of death / min	12 to 18	9 to 17	43 to 46	33 to 64

**X = died; O = survived.*

Table 2 In vivo *inhibition of plasma and whole-brain cholinesterase (ChE) activity in chicks dosed orally with dichlorvos (8 mg kg^{-1}) and diazinon (4 mg kg^{-1}) in veterinary and agricultural formulations*

Treatment	Plasma ChE activity		Whole-brain ChE activity	
	ΔpH/30 min	Inhibition / %	ΔpH/30 min	Inhibition / %
Control	0.25±0.011		0.30±0.010	
Dichlorvos (veterinary)	0.10±0.011*	60	0.02±0.003*	93
Dichlorvos (agricultural)	0.13±0.013*	48	0.04±0.005*,†	87
Diazinon (veterinary)	0.09±0.007*	64	0.06±0.008*	80
Diazinon (agricultural)	0.07±0.006*	72	0.009±0.005*,†	97

Cholinesterase activity values are means ± SE, n = 8 chicks per group. ChE activity was determined one hour after OP dosing.
**Significantly different from the respective control ($p<0.05$).*
†Significantly different from the respective veterinary dose group ($p<0.05$).

and brain ChE activities than the agricultural formulation by 12 % and 6 %, respectively. In contrast, diazinon in the agricultural formulation was 8 % and 17 % more potent ChE inhibitor in plasma and brain, respectively, than diazinon in the veterinary formulation.

Table 3 shows that *in vitro* ChE inhibition by dichlorvos and diazinon was concentration-dependent. The highest inhibitions were observed with dichlorvos in the agricultural formulation (50 %) in the brain samples and with diazinon in the agricultural formulation (52 %) in the plasma samples.

DISCUSSION

Acute (24-hour) LD_{50} of dichlorvos and diazinon in the veterinary and agricultural formulations determined in our study are generally in agreement with other reports (18, 19, 23-25, 30). In our study, the agricultural formulation of dichlorvos appeared to be more lethal than the veterinary one (6.51 *vs.* 11.1 mg kg^{-1}, respectively). This could be attributed to the additive effect of organic solvents, detergents and emulsifiers used to formulate insecticides (7, 10-13). However, this is not a rule, as the LD_{50} values of diazinon did not differ much between the veterinary and agricultural formulation (6.4 mg kg^{-1} *vs*. 6.7 mg kg^{-1}, respectively).

Dichlorvos and diazinon are highly toxic compounds for chicks (2, 4, 16, 23). Although direct extrapolation is not intended here from the avian species to mammals, several studies have reported similar sensitivity of the chicken and mammals to acute OP poisoning (2, 23-25).

The signs of cholinergic toxicity seen in our chicks correspond to those reported elsewhere for chickens acutely poisoned with OP insecticides (17, 19, 27). In both avian and mammalian species OP inhibits the true ChE in the nervous tissues, which results in the appearance of nicotinic, muscarinic and central nervous symptoms (5, 16, 20, 21). Dichlorvos directly inhibits the target ChE, whereas and diazinon acts through its bioactive oxon metabolite (2, 23-25). Hence the delay in the onset of the signs of poisoning and death in chicks dosed with diazinon compared to those dosed with dichlorvos (Table 1). It is not known

Table 3 In vitro *inhibition of plasma and whole brain cholinesterase (ChE) activity in chicks by dichlorvos and diazinon in veterinary and agricultural formulations*

Organophosphate	Plasma ChE activity		Whole-brain ChE activity	
concentration / µmol L^{-1}	ΔpH/30 min	Inhibition / %	ΔpH/30 min	Inhibition / %
Dichlorvos (veterinary)				
0	0.210		0.296	
0.5	0.153	27	0.213	28
1	0.150	29	0.200	32
2	0.127	40	0.170	43
Dichlorvos (agricultural)				
0	0.237		0.300	
0.5	0.153	35	0.206	31
1	0.143	40	0.190	37
2	0.140	41	0.150	50
Diazinon (veterinary)				
0	0.210		0.303	
0.5	0.147	30	0.213	30
1	0.140	33	0.180	41
2	0.130	38	0.163	46
Diazinon (agricultural)				
0	0.223		0.300	
0.5	0.153	31	0.203	32
1	0.110	51	0.186	38
2	0.107	52	0.180	40

Cholinesterase activities are expressed as means of duplicate measurements in three chicks per concentration.

whether additives in insecticidal formulations affect the bioactivation of diazinon or not.

In chicken, the extent of OP poisoning is measured by the drop in plasma, serum or brain ChE activities (17-20, 27, 31). Other studies have also shown concurrent inhibition of plasma and brain ChE activities in chickens acutely poisoned with dichlorvos and diazinon (19, 27, 31). Many factors such as the type of the OP, dose and formulation, route and duration of exposure, toxicokinetic aspects of the insecticide, type of tissue, and sampling time might contribute to the extent of ChE inhibition by an OP compound (2, 4, 5, 16, 20, 23). However, the most important factor is ChE inhibition in the nervous tissues (17, 20-25).

The *in vitro* inhibition of plasma and brain ChE activities by dichlorvos and diazinon confirms the *in vivo* findings and indicates that both insecticides inhibit the enzyme almost indiscriminately, regardless of the formulation. The assessment of ChE inhibition *in vitro*, can also serve to evaluate the anticholinesterase activity of OPs in pure forms or in pharmaceutical and agricultural formulations (28, 32).

The limitation of our study is that we could not verify the formulations used in the veterinary and agricultural preparations, as they are industrial secrets.

In conclusion, acute exposure of chicks to OP insecticides in veterinary and agricultural formulations is associated with concurrent reductions in brain and plasma ChE activities. While they exist, differences in toxicity between formulations cannot be taken as a rule and further investigations should inventory the toxicity of standard veterinary and agricultural OP formulations in addition to the known data for pure forms.

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REFERENCES

1. Coggon D. Work with pesticides and organophosphate sheep dips. Occup Med 2002;52:467-70.
2. International Program on Chemical Safety (IPCS). Organophosphate Pesticides [displayed 14 June 2011]; Available at http://www.inchem.org/d Cuments/pims/chemical/pimg001.htm
3. Kachaiyaphum P, Howteerakul N, Sujirat D, Siri S, Swannapong N. Serum cholinesterase levels of Thai-chilli farm workers exposed to chemical pesticides: prevalence estimates and ass Ciated factors. J Occup Health 2010;52:89-98.
4. Jaga K, Dharmani C. Sources of exposure to and public health implications of organophosphate pesticides. Rev Panam Salud Publica 2003;14:171-85.
5. Kwong TC. Organophosphate pesticides: biochemistry and clinical toxicology. Ther Drug Monit 2002;24:144-9.
6. Eddleston M, Eyer P, Worek F, Mohamed F, Senarathna L, von Meyer L, Juszczak L, Hittarage A, Azhar S, Dissanayake W, Rezvi Sheriff MH, Szinicz L, Dawson AH, Buckley NA. Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. Lancet 2005;366:1452-9.
7. Peter JV, Jerobin J, Nair A, Bennett A. Is there a relationship between the WHO hazard classification of organophosphate pesticide and outcomes in suicidal human poisoning with commercial organophosphate formulations? Regul Toxicol Pharmacol 2010;57:99-102.
8. Hill EF, Camardese MB. Toxicity of anticholinesterase insecticides to birds: technical grade versus granular formulations. Ecotoxicol Environ Saf 1984;8:551-63.
9. Sidhu KS, Collisi MB. Case of an accidental exposure to a veterinary insecticide product formulation. Vet Hum Toxicol 1989;31:63-4.
10. Kanikkannan N, Kandimalla K, Lamba SS, Singh M. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Cur Med Chem 2000;7:593-608.
11. Karallieddea LD, Edwards P, Marrs TC. Variables influencing the toxic response to organophosphates in humans. Food Chem Toxicol 2003;41:1-13.
12. Guhathakurta S, Bhattacharya S. *In vitro* inhibition of goat brain acetylcholinesterase by pure and commercial anticholinesterase pesticides. Ecotoxicol Environ Saf 1989;17:16-20.
13. Axelrad JC, Howard CV, McLean WG. Interactions between pesticides and components of pesticide formulations in an *in vitro* neurotoxicity test. Toxicology 2002;173:259-68.
14. Sartorelli P, Aprea C, Bussani R, Novelli MT, Orsi D, Sciarra G. *In vitro* percutaneous penetration of methyl-parathion from a commercial formulation through the human skin. Occup Environ Med 1997;54:524-5.
15. Griffin P, Payne M, Maso H, Freedlander E, Curran AD, Cocker J. The *in vitro* percutaneous penetration of chlorpyrifos. Hum Exp Toxicol 2000;19:104-7.
16. Wilson BW. Cholinesterase inhibition. In: Wexler P, editor. Encyclopedia of toxicology. 2nd ed. Vol. 1. New York (NY): Elsevier; 2005. p. 588-99.
17. Farage-Elawar M, Francis MB. Effect of multiple dosing of fenthion, fenitrothion and desbromoleptophos in young chicks. J Toxicol Environ Health 1988;23:217-28.
18. Al-Badrany YMA, Mohammad FK. Effects of acute and repeated oral exposure to the organophosphate insecticide chlorpyrifos on open-field activity in chicks. Toxicol Lett 2007;174:110-6.
19. Mohammad FK, Al-Badrany YM, Al-Jobory MM. Acute toxicity and cholinesterase inhibition in chicks dosed orally with organophosphate insecticides. Arh Hig Rada Toksikol 2008;59:145-51.

20. Wilson BW. Clinical enzymology. In: Loeb WF, Quimby FW, editors. The clinical chemistry of laboratory animals. Philadelphia (PA): Taylor and Francis; 1999. p. 399-454.
21. Cocker J, Mason HJ, Garfitt SJ, Jones K. Biological monitoring of exposure to organophosphate pesticides. Toxicol Lett 2002;134: 97-103.
22. Wilson BW, Arrieta DE, Henderson JD. Monitoring cholinesterases to detect pesticide exposure. Chemico Biol Interact 2005;157-158:253-6.
23. World Health Organization (WHO). Organophosphorus Insecticides: A General Introduction. Environmental Health Criteria No. 63. Geneva: WHO; 1986.
24. World Health Organization (WHO). Dichlorvos. Environmental Health Criteria No. 79. Geneva: WHO; 1989.
25. World Health Organization (WHO). Diazinon. Environmental Health Criteria No. 198. Geneva: WHO; 1998.
26. Dixon WJ. Efficient analysis of experimental observations. Ann Rev Pharmacol Toxicol 1980;20:441-62.
27. Al-Zubaidy MIH, Mohammad FK. Met Clopramide protection of diazinon-induced toxicosis in chickens. J Vet Sci 2007;8:249-54.
28. Mohammad FK, Alias AS, Ahmed OAH. Electrometric measurement of plasma, erythrocyte and whole blood cholinesterase activities in healthy human volunteers. J Med Toxicol 2007;3:25-30.
29. Petrie A, Watson P. Statistics for Veterinary and Animal Science. Oxford: Blackwell Science Ltd; 1999.
30. Naidu NV, Reddy KS, Janadhan A, Murthy MK. Toxicological investigation of dichlorvos in chicks. Indian J Pharmacol 1978;10:323-6.
31. Abdelsalam EB. Neurotoxic potential of six organophosphorus compounds in adult hens. Vet Hum Toxicol 1999;41:290-2.
32. Karanth S, Pope C. *In vitro* inhibition of blood cholinesterase activities from horse, cow and rat by tetrachorvinphos. Int J Toxicol 2003;22:429-33.

Sažetak

AKUTNA TOKSIČNOST VETERINARSKIH I POLJOPRIVREDNIH FORMULACIJA ORGANOFOSFATA DIKLORVOSA I DIAZINONA U PILIĆA

Sastojci formulacija mogu djelovati na toksičnost organofosfatnih insekticidnih pripravaka u životinja. Cilj je ovog istraživanja bio ispitati i usporediti akutnu inhibiciju kolinesteraza u pilića starih 7 do 14 dana koji su na usta primili organofosfatne insekticide diklorvos i diazinon u odgovarajućim formulacijama za veterinarsku odnosno poljoprivrednu primjenu. Dvadesetčetverosatna akutna letalna doza (LD_{50}) diklorvosa bila je 11,1 mg kg^{-1} u veterinarskim odnosno 6,51 mg kg^{-1} u poljoprivrednim formulacijama, a diazinona 6,4 mg kg^{-1} odnosno 6,7 mg kg^{-1}. Do kolinergičke toksikoze u pilića došlo je jedan sat nakon primjene. Nakon izlaganja organofosfatima *in vivo* i *in vitro* izmjerena je aktivnost kolinesteraza u plazmi i mozgu s pomoću elektrometrije. Oralne doze diklorvosa (8 mg kg^{-1}) odnosno diazinona (4 mg kg^{-1}) putem veterinarskih odnosno poljoprivrednih formulacija značajno su smanjile aktivnost kolinesteraza u plazmi i mozgu pilića. *In vitro* su također oba organofosfata inhibirala aktivnost kolinesteraza, bez obzira na formulaciju. Poljoprivredna formulacija diklorvosa izazvala je najjaču inhibiciju (50 %) u mozgu pilića, dok je poljoprivredna formulacija diazinona najjače inhibirala (52 %) aktivnost u plazmi. Ovo istraživanje pokazuje da različite formulacije organofosfatnih insekticida mogu dovesti do različita otrovanja i različito djelovati na kolinesteraznu aktivnost u mozgu i plazmi.

KEY WORDS: *elektrometrija, inhibicija, kolinergička toksikoza, kolinesteraza, LD_{50}, mozak, otrovanje, plazma*

CORRESPONDING AUTHOR:

Fouad K. Mohammad
Department of Physiology, Biochemistry and Pharmacology
College of Veterinary Medicine, University of Mosul,
Mosul, Iraq
E-mail: *fouadmohammad@yahoo.com*