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Research Article

Mixed toxicity of three different groups of Organophosphorus pesticides (Quinalphos, Malathion, Monocrotophos) on freshwater fish *Channa punctatus*

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Abstract

Assessing the cumulative toxicity of pesticides in mixtures has been an enduring challenge for environmental health research as well as ecotoxicology for the past several decades. Usage of single pesticides in agriculture belonging to one group may not be possible in nature, Group of those belonging to one class or more is after the real picture in the environment. Hence, it is not going to reveal a clear picture of assessment of toxicity singly but perhaps the mixed composition may be correct. Hence a study was under taken to evaluate the toxicity of the three different groups of organophosphate pesticides *viz* quinalphos [Organothiophosphate group - phosphorothionate], malathion [Dithiophosphate group - phosphorothiolothionate] and monocrotophos [Orthophosphate group] in mixed ratios (1:1:1, 2:1:1, 1:2:1 and 1:1:2) of commercial grade formulations to find out the combined interactions of the organophosphorus pesticides on the fish *Channa punctatus*. The nature of interactions in the mixture of the toxicants were evaluated by linear 'S' Index method and Mixture Toxicity Index method was applied to verify the nature of the combined interactions of the toxicants. More than additive nature of the combined effect observed in the mixture experiment demonstrated that all components contribute to the overall effect of the mixture.

Keywords : Organophosphorus pesticide, Quinalphos, Malathion, Monocrotophos, Mixed toxicity and *Channa punctatus.*

Introduction

Pesticides are unique chemical stressors in that they are designed to have biological activity but are intentionally placed into the environment in large quantities. Pesticide mixtures are also common in the aquatic environment, including lakes, river, streams, and other surface waters that support aquatic life (Gilliom, 2007). Concern has been expressed in recent years that exposure of non-target species to more than one pesticide in a short period may result in unpredicted toxic effects (Johnston *et al.,* 1994 a; Johnston *et al.,* 1994b; Johnston *et al.,* 1994c). Assessing the cumulative toxicity of pesticides in mixtures has therefore been an enduring challenge for environmental health research (Monosson, 2005) as well as ecotoxicology (Eggen *et al.*, 2004) for the past several decades.

The toxicity of mixtures of pesticides has been extensively investigated due to their high economic value and extensive use. Deneer (2000) reviewed the acute toxicity of 202 pesticide mixtures to aquatic organisms. He found that the toxicity of approximately 90% of the data differed from concentration addition by a factor of less than two. Unfortunately, this fact is not that useful, because the generally accepted point at which additivity stops and antagonism or synergism starts is a difference of 1.5. Thus, all that can be said without re-analysing Deneer's data is that at least 10% of the pesticide mixtures

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were either antagonistic or synergistic. Interestingly, many of the mixtures that did not exhibit concentration additivity contained chemicals with the same mode of action. This finding contradicts the model for joint action postulated by Plackett and Hewlett (1952) and the findings of Broderius and co-workers (Broderius and Kahl, 1985; Broderius, 1991; Broderius *et al*., 1995). As such, these results warrant further investigation.

In the present study combined toxicity of different groups organophosphorus pesticides mixed in different ratios has been studied and toxic effects were quantified as LC_{50} value of the mixture that is required to produce a 50% mortality response as a toxic parameter. The relative toxicities of the mixed ratios were also compared to find out which mixed ratios exhibits more toxic nature in combination.

Materials and Methods

The freshwater fish of *Channa punctatus* with length 6-8 cm, weight 6.5 to 7.5 g, irrespective of their sex, have been chosen as the test organisms in the present study. The fish were obtained from different places in and around Guntur, Andhra Pradesh, India. The fish were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28±2°C. During the period of acclimatization, the fish were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the experimentation. All the precautions laid by the Committee on toxicity tests to aquatic organisms (1975) and APHA *et al.* (1998) were followed. Quinalphos 25% EC (Ekalux EC 25) manufactured by Hikal limited, Gujarat, supplied by Syngenta India Ltd, Mumbai, Malathion 50% EC manufactured by Hyderabad chemical supplies limited Hyderabad, Monocrotophos 36% SL manufactured by Rallies India Limited Mumbai were purchased from the local market in Guntur. One percent of each of the pesticide was mixed in equal proportions (1:1:1), Two percent of quinalphos and one percent of the remaining two pesticides (2:1:1), one percent of quinalphos and monocrotophos along with two percent of the malathion (1:2:1), one percent of quinalphos and malathion along with two percent of the monocrotophos (1:1:2) were mixed and the stock solutions were prepared. Finney's probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate the LC_{50} values.

The predicted toxicity of mixtures was estimated by the equation described by Preston *et al*. (2000). The nature of the combined interactions of the organophosphorus pesticides in the mixture of the toxicants is evaluated by linear 'S' Index method (Konemann, 1980). For interpretation of toxicity data, the results obtained as 50% effect point values were converted into a Mixture Toxicity Index (MTI) for comparing quantitatively the results of the mixture toxicity, with the formula of Konemann (1981).

Results and Discussion

In the present study, the relative toxicity of mixed ratios of commercial grade formulations in static and continuous flow through systems to the fish *Channa punctatus* were in the order 1:1:2 > 1:1:1 > 1:2:1 > 2:1:1.

The nature of the combined interactions of the organophosphorus pesticides in the mixture of the toxicants was evaluated by linear 'S' Index method. The index expressed the toxicity quantitatively in which the action of mixture was found to be more than additive (Synergistic) since the "S" value was found to be <1. After assigning zero as a reference point and establishing linearity for formula S<1.0 Additive index (AI) = (1/S)-1.0, An AI value greater than 0, conformed the synergistic toxicity given in the Table - 1. The Mixture Toxicity Index method was applied to verify the nature of the combined interactions of the toxicants and was found to be concentration additive since

Table - 1. Calculated Linear 'S 'value and Additive index (AI) for mixed ratios exposed to fish *Channa punctatus*

Mixed ratios	Type of test	method	LC_{50} value	S value	AI value	Type of Joint action
1:1:1	24	Static	1.1845	0.4549	1.1981	synergistic
		C.F	1.1540	0.4778	1.0926	
	48	Static	1.1688	0.4657	1.1469	
		C.F	1.1439	0.5378	0.8594	
	72	Static	1.1420	0.4987	1.0050	
		C.F	1.1268	0.6242	0.6019	
	96	Static	1.1102	0.5879	0.7007	
		C.F	1.0968	0.7500	0.3332	
2:1:1	24	Static	1.4621	0.5615	0.7808	
		C.F	1.4468	0.5991	0.6691	
	48	Static	1.4397	0.5737	0.7429	
		C.F	1.4221	0.6686	0.4956	
	72	Static	1.4195	0.6199	0.6130	
		C.F	1.4089	0.7805	0.2811	
	96	Static	1.3971	0.7399	0.3515	
		C.F	1.3797	0.9435	0.0598	
1:2:1	24	Static	1.3447	0.5164	0.9362	
		C.F	1.3268	0.5494	0.8201	
	48	Static	1.3130	0.5232	0.9111	
		C.F	1.3020	0.6121	0.6336	
	72	Static	1.2995	0.5675	0.7619	
		C.F	1.2847	0.7117	0.4050	
	96	Static	1.2795	0.6776	0.4757	
		C.F	1.2544	0.8578	0.1657	
1:1:2	24	Static	0.9667	0.3712	1.6933	
		C.F	0.9356	0.3874	1.5811	
	48	Static	0.9394	0.3743	1.6712	
		C.F	0.9262	0.4354	1.2964	
	72	Static	0.9143	0.3993	1.5043	
		C.F	0.9044	0.5010	0.9958	
	96	Static	0.9067	0.4801	1.0825	
		C.F	0.8819	0.6030	0.6581	

* 'S' value is smaller than 1.0 and 'AI' value greater than 0, so it is synergistic.

Table - 2. Sum of the toxic units (M) and the verified Mixed Toxicity Index (MTI) to interpret the type of interaction of the compound

Mixed ratios	Type of test	method	LC_{50} value	Sum of the toxic units (M)	MTI	Type of joint action
1:1:1	24	Static	1.1845	0.1516	1	
		C.F	1.1540	0.1552	1	
	48	Static	1.1688	0.1662	1	
		C.F	1.1439	0.1959	1	

	72	Static	1.1420	0.1592	1	concentration addition
		C.F	1.1268	0.1792	1	
	96	Static	1.1102	0.2080	1	
		C.F	1.0968	0.2500	1	
2:1:1	24	Static	1.4621	0.2390	1	
		C.F	1.4468	0.2447	1	
	48	Static	1.4397	0.2675	1	
		C.F	1.4221	0.3271	1	
	72	Static	1.4195	0.2573	1	
		C.F	1.4089	0.2919	1	
	96	Static	1.3971	0.3475	1	
		C.F	1.3797	0.4284	1	
1:2:1	24	Static	1.3447	0.1663	1	
		C.F	1.3268	0.1681	1	
	48	Static	1.3130	0.1796	1	
		C.F	1.3020	0.2075	1	
	72	Static	1.2995	0.1749	1	
		C.F	1.2847	0.1906	1	
	96	Static	1.2795	0.2157	1	
		C.F	1.2544	0.2527	1	
1:1:2	24	Static	0.9667	0.0935	1	
		C.F	0.9356	0.0943	1	
	48	Static	0.9394	0.1005	1	
		C.F	0.9262	0.1208	1	
	72	Static	0.9143	0.0976	1	
		C.F	0.9044	0.1096	1	
	96	Static	0.9067	0.1260	1	
		C.F	0.8819	0.1515	1	

*If MTI=1 possible types of joint action is concentration addition

Table - 3. Experimental LC_{50} values of Individual compounds and observed LC_{50} mix *versus* Predicted LC_{50}mix values

Mixed ratios	Method	Duration	Individual Toxicity			Predicted LC_{50}mix	Observed LC_{50}mix
			Quinalphos LC_{50}	Malathion LC_{50}	Monocrotophos LC_{50}		
1:1:1	Static	24	3.7031	9.0196	316.8623	1.3361	1.1845
		48	3.5509	8.7954	312.1074	1.3240	1.1688
		72	3.1538	8.5937	303.2597	1.3082	1.142
		96	2.4569	8.3952	288.2431	1.3061	1.1102
	CF	24	3.3615	8.8195	309.677	1.3132	1.154
		48	2.8484	8.6439	294.9933	1.3231	1.1439
		72	2.3108	8.4874	290.9933	1.3348	1.1268
		96	1.7912	8.1951	282.0739	1.3468	1.0968

Mixed ratios	Method	Duration	Individual Toxicity			Predicted LC_{50}mix	Observed LC_{50}mix
			Quinalphos LC_{50}	Malathion LC_{50}	Monocrotophos LC_{50}		
2:1:1	Static	24	3.7031	9.0196	316.8623	1.7011	1.4621
		48	3.5509	8.7954	312.1074	1.6844	1.4397
		72	3.1538	8.5937	303.2597	1.6870	1.4195
		96	2.4569	8.3952	288.2431	1.7242	1.3971
	CF	24	3.3615	8.8195	309.677	1.7041	1.4468
		48	2.8484	8.6439	294.9933	1.7140	1.4221
		72	2.3108	8.4874	290.9933	1.7564	1.4089
		96	1.7912	8.1951	282.0739	1.8081	1.3797
1:2:1	Static	24	3.7031	9.0196	316.8623	1.5110	1.3447
		48	3.5509	8.7954	312.1074	1.4811	1.313
		72	3.1538	8.5937	303.2597	1.4791	1.2995
		96	2.4569	8.3952	288.2431	1.4870	1.2795
	CF	24	3.3615	8.8195	309.677	1.5017	1.3268
		48	2.8484	8.6439	294.9933	1.4926	1.302
		72	2.3108	8.4874	290.9933	1.5004	1.2847
		96	1.7912	8.1951	282.0739	1.5071	1.2544
1:1:2	Static	24	3.7031	9.0196	316.8623	1.0602	0.9667
		48	3.5509	8.7954	312.1074	1.0337	0.9394
		72	3.1538	8.5937	303.2597	1.0148	0.9143
		96	2.4569	8.3952	288.2431	1.0275	0.9067
	CF	24	3.3615	8.8195	309.677	1.0332	0.9356
		48	2.8484	8.6439	294.9933	1.0358	0.9262
		72	2.3108	8.4874	290.9933	1.0304	0.9044
		96	1.7912	8.1951	282.0739	1.0334	0.8819

the MTI value is equal to 1. Calculated values of sum of the toxic units (M) and verified type of interaction was given in Table-2. The predicted LC_{50} value of the mixture and the experimentally observed LC_{50} value of the mixture were compared and the experimentally observe values were found to be less than the predicted values which are given in the Table - 3.

The results of the mixture experiment demonstrate that mixtures of organophosphorus pesticides have the capacity to act in combination and that their effects can be predicted based on the concentration–response curves of the individual mixture components according to the principles of concentration addition. Thus, we can conclude that the combined effect of the mixture does not deviate from additivity. This is consistent with the *a priori* assumption of this concept, which is dependent upon the components of the mixture acting via a common mechanism to contribute to the overall mixture effect. Toxicity studies involving pesticide mixtures have resulted in a full spectrum of responses in which the complexity of the interactions depends on differences in the chemical properties and modes of toxic action of the pesticides. Studies that examine the effects of pesticides from the same class are usually the easiest to interpret, because the observed effects are often additive in nature. Additive effects can be expected for compounds with a similar mode of action, while synergistic effects trigger a more than additive effect in the exposed organism due to changes in chemical biotransformation (Belden and Lydy, 2000).

Zhi-Yong Zhang *et al*. (2009) reported acute toxicities to *Brachydanio rerio* zebrafish (Hamilton and Buchanan) determined for two organophosphorus insecticides dichlorvos and phoxim. The organophosphate dichlorvos showed low toxicity, but phoxim showed high or intermediate toxicities to zebrafish, and the toxicities of binary mixtures of permethrin and dichlorvos or phoxim, bifenthrin and dichlorvos or phoxim and etofenprox and phoxim (48, 72 and 96 hr exposure) reported to be very high. The toxicities of binary mixtures of tetramethrin and dichlorvos or phoxim, etofenprox and dichlorvos and etofenprox and phoxim (24 hr exposure) were high.

Although the importance of multiple stressors is widely recognized in aquatic eco-toxicology (Eggen *et al.,* 2004), pesticide mixtures continue to pose major challenges for natural resource agencies (Gilliom, 2007). These challenges include the data gaps that exist in many individual chemicals, experimental design difficulties (e.g., near-insurmountable factorial complexity for large numbers of chemicals), poorly understood path- ways for chemical interaction, potential differences in response among species, and the need for more sophisticated statistical tools for analyzing complex data.

The effects observed provide strong evidence of the capacity for mixtures of similarly acting chemicals to behave in an additive manner according to the principles of concentration addition.

The more than additive nature of the combined effects observed in the mixture experiment demonstrates that all components contribute to the overall effect of a mixture. This implies that the overall effects will always exceed the highest individual effect of the mixture components. By this line of reasoning, low-effect concentrations of the individual components may give rise to considerable mixture effects. This phenomenon is of particular importance for the environmental hazard assessment of chemicals because it indicates that concentrations of chemicals that show no effect when applied singly may provoke substantial effects when acting in combination. When mixtures contain chemicals with the same mode of action, it is assumed that the toxicity is concentration additive. The vast majority of the literature shows that mixtures of chemicals with the same mode of action have concentration additive toxicity irrespective of the number of components in the mixture. These findings indicate that concentration addition may be a valuable tool for predicting the hazards posed by these types of mixture.

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