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Response of Three Microbial Test Systems to Pesticides

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In the present day world, environmental problems are multiple and complex, especially those arising from the disposal of hazardous materials. Therefore, the most urgent need at present is to identify and assess the toxicity of such substances. Assessment of human exposure to pesticides and other toxicants through biological monitoring offers one means to evaluate the magnitude of potential health risk of these chemicals (Bhatnagar et al. 1992). Toxicity of these chemicals can also be worked out for water effluents by bioassays employing fish and macro invertebrates (Bringmann and Kuhn 1980; Crains et al. 1976). Lately, there has been an increased tendency to use microbial systems to screen toxicants as an alternative to animal tests, because these tests are rapid, inexpensive, sensitive and simple (Bitton et al. 1986; Slabbert and Grabow 1986; Walker 1988). In view of the fragmentary data on the toxicity of pesticides using Microtox™ (Chang et al. 1981), the present investigation has been carried out using three microbial tests systems namely Microtox™ (Bulich and Isenberg 1984), the Motility test (Dutka 1980) and the Growth Zone Inhibition test (Liu et al. 1989) in an attempt to assess the toxicity of selected pesticides.

MATERIALS & METHODS

A total of ten pesticides [nine technical grade pesticides - DDT, BHC, Lindane, Endrin, Sevin, Thiodan, Carbofuran, Cypermethrin, Decamethrin, and one formulation of 2,4-D (34%)] were used. Stock solution of 1 mg/mL of each pesticide was prepared and tests were carried out with original and its serial dilutions.

Microtox™ System: The experiment was carried out with a Microtox™ Model 2055 Toxicity Analyzer System (Beckman Inc, Carlsbad, USA). This system is based on monitoring changes in natural light emission of the luminescent bacteria *Photobacterium phosphoreu* when challenged with toxic compound. The toxicity end point is determined as the effective

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concentration of a test sample that causes 50% decrease in light output (EC_{50}).The instrument is equipped with a 15 well, temperature controlled incubator (10, 15 or 20°C+0.3°C) and a reaction chamber. Light output was read from the digital display. Details of the test system are described elsewhere (Bulich and Isenberg 1980; Ghosh and Doctor 1982). The toxicity endpoint was determined as the concentration of a test sample that caused a 50% decrease in light output (EC_{50}). Results were expressed in terms of gamma γ i.e. the ratio of light lost during the test time (t) to the light remaining at the time (t).

Motality Test: In this system, toxicity is determined in terms of minimum effective concentration that causes a loss of 90% motality (MEC_{90}) of *Spirillum volutans* during the test period. A strain of *S. Volutans* (ATCC 19554) obtained from Dr. B.J. Dutka, Canada Centre for Inland Waters, Canada was used for all bioassays. The standard method of test as described by Dutka (1980) was followed. Prior to testing, the cultures were transferred daily in Minimum Maintenance Medium (MMSV) and incubated at 28°C. A passage of 3 to 4 transfers was necessary at 28°C for development of optimum motality of the test organism. To perform the assay, 0.8 ml of sample was added to the sterile testing tube containing 0.1 ml Defined Test Medium (DTM) and mixed well. Sterile distilled water as well as DMSO (diluent) were added as controls. Pesticides were dissolved in a minium quantity of DMSO. About 0.1 ml of culture was added to each tube. Slides were prepared at 0, 5, 15, 30, 60, 90 and 120 min intervals and were observed under the dark field microscope (125X) to assess the motality of the organism.

Growth Zone Inhibition Test (Agar Plate Method): In this method, the growth of the test organism, *Bacillus cereus* is monitored after exposure to a toxicant (Liu et al. 1989). A locally isolated strain of *B. cereus* was used in this investigation. Three subcultures in modified nutrient broth at 20°C were done before performing the toxicity screening. Plates were seeded with the organism and the pesticide [dissolved in glycerol and DMSO (20:80)] was spotted onto the seeded agar (1O ul/spot). After 18 hr of incubation at 37°C, the zone of inhibition was measured. The minimum concentration that inhibited growth was regarded as toxic.

RESULTS AND DISCUSSION

The Microtox response (EC_{50}) to these compounds in the decreasing order is given in Table 1. It is seen from the table that the synthetic pyrethroids (cypermethrin and decamethrin) and carbofuran are less toxic, while lindane, endrin and sevin are toxic at higher concentrations (EC_{50} values >=48 mg/L). On the other hand, the EC_{50} values of DDT, BHC, thiodan and 2,4-D are extremely low indicating they are very toxic even at low concentrations. It is also observed that temperature (Table 2) and incubation period (Table 3) have a profound effect on the toxicity of each

compound. Analysis of variance indicated that the degree of toxicity differed significantly ($p<0.001$). Grouping by time and temperature also gave significant results ($p<0.001$). Turkey's post hoc tests were utilized to conduct pairwise comparisons. Carbofuran was significantly less toxic ($p<0.01$) than all other pesticides, as its EC_{50} value was highest (203 mg/L)

Table 1. EC_{50}* values of various pesticides using Microtox™ ($T_{15°C}$; t_{15min}) according to decreasing toxicity

Pesticide	EC_{50} (mg/L)
2,4-D	22±10
Thiodan	28±10
BHC	31±8
DDT	39±7
Lindane	48±10
Endrin	55±16
Sevin	63±14
Decamethrin	186±22
Cypermethrin	192±22
Carbofuran	203±43

*Results are expressed as Mean±S.D.

Table 2. Effect of temperature on EC_{50}*values (mg/L) of various pesticides as determined by Microtox™ (t=15 min)

Pesticide	Temperature		
	10°C	15°C	20°C
DDT	36±5	38±7	44±6
BHC	26±7	31±7	36±7
Lindane	42±7	47±9	54±12
Endrin	46±10	53±19	64±12
Sevin	57±9	62±18	70±10
Thiodan	18±4	29±11	36±8
Carbofuran	190±20	184±54	234±33
Cypermethrin	176±16	194±17	207±23
Decamethrin	170±16	186±12	201±25
2,4-D	14±4	23±8	30±11

*Results are expressed as Mean±S.D.

On the other hand, 2,4-D was the significantly most toxic compound ($p<0.05$) and its EC_{50} value was the lowest (22 mg/L). However, the toxicity of 2,4-D was not significantly different from thiodan (28 mg/L). In general, the lowest EC_{50} values were observed when incubation was done at 10°C, while the highest EC_{50} value was encountered during the 20°C incubating temperature. The difference in the EC_{50} values at these two temperatures was significant ($p<0.001$). A similar trend was observed with

incubation time. The lowest EC_{50} values were always encountered with 5 min exposure periods, while the EC_{50} became significantly higher as incubation time increased to 30 min ($p<0.01$).

Results from the Motality test (MEC_{90}) are given in Table 4. Some of the pesticides like DDT, BHC, thiodan and 2,4-D required a 60 min exposure time in order to calculate MEC_{90} values, while lindane, endrin and sevin

Table 3. Effect of time on EC_{50}* values (mg/L) of pesticides as determined by Microtox™ (T=15°C)

Pesticide	Time (min)		
	5	15	30
DDT	33±4	40±5	45±5
BHC	23±5	32±6	37±6
Lindane	38±4	50±7	56±8
Endrin	39±10	56±9	69±10
Sevin	49±9	64±6	77±9
Thiodan	19±6	28±9	36±10
Carbofuran	181±14	193±14	234±27
Cypermethrin	172±12	192±14	213±19
Decamethrin	166±12	186±13	205±20
2,4-D	13±3	23±7	31±11

*Results are expressed as Mean ± S.D.

Table 4. Mean MEC_{90} values of pesticides using the Motility test

Pesticide	MEC_{90}	
	Exposure time (min)	Concentration (mg/L)
2,4-D	60	30
Thiodan	60	32
BHC	60	54
DDT	60	58
Lindane	90	61
Endrin	90	67
Sevin	90	89
Decamethrin	120	N.D.
Cypermethrin	120	N.D.
Carbofuran	120	N.D.

N.D. = Not detected

required 90 min. At the higher concentrations tested, cells became immediately lysed. MEC_{90} values for carbofuran, cypermethrin and decamethrin were not possible to detect in this system even after allowing

120 min exposure (the maximum recommended time). The MEC_{90} values of 2,4-D, thiodan, BHC and DDT were 30, 32, 54 and 58 (mg/L) respectively, while lindane, endrin and sevin possessed MEC_{90} values of 61, 67 and 89 mg/L respectively.

The toxicity endpoint of these pesticides was also assayed using the Growth Zone Inhibition test and the results are given in Table 5. It is seen from this table that the concentration required to produce a 'halo' was very low for thiodan and 2,4-D (10 ug each), slightly higher for DDT and BHC (15 ug each) and gradually increased for endrin (25 ug), lindane (50 ug)

Table 5. Toxicity screening of pesticides using Growth Zone Inhibition Test ($T_{28°C}$;$t_{18\ hr}$)

Pesticide	Mean minimum concentration required to produce Clear Zone (ug)	Mean diameter of 'Halo' (mm)	Final concentration (mg/L)
2,4-D	10	8	25
Thiodan	10	12	30
BHC	15	7	35
DDT	15	8	48
Lindane	50	10	52
Endrin	25	11	55
Sevin	75	8	70
Decamethrin	200	-	N.D.
Cypermethrin	200	6	200
Carbofuran	200	5	200

N.D. = Not detected

and sevin (75 ug). However, the toxicity of cypermethrin and carbofuran only be detected at the maximum concentration tested (200 ug/spot). Decamethrin was not toxic even at this concentration. The mean diameter of the 'halo' observable for thiodan (12 mm) was the largest observed, while it gradually decreased with the other compounds, [endrin (11 mm), lindane (10 mm) and DDT (8 mm)]. The toxicity endpoints indicated that 2,4-D, thiodan, BHC and DDT were more toxic than lindane, endrin and sevin, while carbofuran and synthetic pyrethroids were less toxic.

Table 6 displays the results obtained from the three microbial test systems. This table shows that the sensitivity of Microtox system is always greater than the other two systems. MEC_{90} values in Motality Test System are always higher than EC_{50} values and Growth Zone Inhibition results. Among the three systems, the Motility Test appears to be least sensitive. Decamethrin, cypermethrin and carbofuran failed to give any response in

this system, while only decamethrin did not give a response in the Growth Zone Inhibition system. These three give results in the Microtox™ system, but their EC_{50} values were quite high. Although the response of each compound varied with the test system, the order of toxicity remained same in each system. It is generally assumed that the determination of the responce to a sample by any single organism can not completely predict the response of the other organisms to the same sample. Therefore, a battery of short tests should be used for a more comprehensive and meaningful toxicity assessment.

Table 6. Toxicity screening of pesticides using three microbial test systems

Assay	System		
	Microtox™	Motility Test	Growth Zone Inhibition Test
Organism used	*Photobacterium phosphoreum*	*Sprillum volutans*	*Bacillus cereus*
Optimum conditions	$T_{15°C};t_{15\ min}$	$T_{28°C};t_{60/90/120\ min}$	$T_{28°C};t_{18\ hr}$
Toxicity endpoint (Concentration mg/L)	EC_{50}	MEC_{90}	'Halo' (Clear Zone)
2,4-D	22	30	25
Thiodan	28	32	30
BHC	31	54	35
DDT	39	58	48
Lindane	48	61	52
Endrin	55	67	55
Sevin	63	89	70
Decamethrin	186	N.D.	N.D.
Cypermethrin	192	N.D.	200
Carbofuran	203	N.D.	200

N.D. = Not detected

All the tests employed in this investigation met the criterion for rapid toxicity screening. They are simple, reproducible and inexpensive, which is the reason why there is an increased awareness of using these tests to screen toxicity of these chemicals (Elnabarawy et al. 1988). Toxicity screening using Microtox™ has been conducted and compared with more conventional bioassays for monitoring the toxicity of a wide variety of pure chemicals (Dutka and Kwan 1981; Qureshi et al. 1962), complex effluents (Bulich et al. 1981), distilled and tap water, pesticides (Chang et al. 1981) and mycotoxins (Yates and Porter 1982). The present investigation gives a comprehensive account on pesticide toxicity in three microbial test

systems indicating a battery of short term tests would be more efficient and meaningful for toxicity assessment for the compounds which respond differently in different systems.

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