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Toxicity assessment of pesticides to *Pseudokirchneriella subcapitata* under air-tight test environment

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Abstract

This paper presents the toxicity data of seven pesticides including atrazine, parathion, dichlorvos, malathion, fenthion, 2-methyl-4-chlorophenoxyacetic acid, and pentachlorophenol on *Pseudokirchneriella subcapitata* based on a new algal toxicity testing technique conducted under air-tight environment. The dissolved oxygen production and the cell density were adopted as the response endpoints. Median effective concentrations (EC50) range from 0.0035 to 3.40 mg/L (DO production) and from 0.0067 to 3.12 mg/L (cell density). No-observed-effect concentration (NOEC) was determined using the Dunnett's test. NOEC values are with in the range of 0.001–1.20 mg/L. In general, the two test endpoints revealed similar sensitivities. From comparisons of literature data also based on *Pseudokirchneriella subcapitata*, it is clear that conventional batch tests tend to underestimate the toxicity of pesticides due to their open test environment. Closed-system tests, i.e., microplate test, respirometer test, and our BOD-bottle test, generally provide better assessment to the effects of pesticides. Data based on our test method reveals much higher toxicity (3–100 times) than that from the conventional batch tests. Furthermore, for organophosphorus insecticides, results from the present study show that *Pseudokirchneriella subcapitata* is less sensitive than *Daphnia magna* and rainbow trout, but is more susceptible than fathead minnow. The closed-system test applied in this study provides more adequate assessment for the toxicity of pesticides than the conventional batch tests.

Keywords: Algae; EC50; Herbicides; NOEC; *Pseudokirchneriella subcapitata*; Toxicity

1. Introduction

Most pesticides are of very limited solubility and relatively stable in water [1]. However, they are prone to contaminate the surface waters or the ground waters through drift of aerial spray and/or watershed drainage. Through the above transport processes, a certain number of species in ecosystem will be eliminated, resulting in an altered community composition. Although pesticides are designed specifically to destroy unwanted target organisms, their application may cause many diverse problems to non-target organisms like fish, birds or animals, and even human being. The effects of pesticides on aquatic environment were frequently evaluated using organisms, such as fish or water flea [2–7]. Literature shows that atrazine at higher concentrations may produce harmful effects on algae and results in significant reductions on chlorophyll content and primary productivity [8]. It was also reported that atrazine may specifically inhibit the electron transport between the plastiquinones and thereby reduces the mount of transmitted energy. With respect to the photosynthesis pathway, atrazine may cause an increase in the maximum in vivo fluorescence of chlorophyll *a*, and a decrease in the maximum quantum yield [9,10]. Faust et al. [11] stated that atrazine inhibits photosystem II (PSII) by irreversible binding to D1 protein. Organophosphorus insecticides (OPs) are frequently used for the control of insects and other pests. OPs are acutely toxic to animals through inhibition of acetylcholinesterase disrupting cholinergic nerve transmission. They are bioactivated by cytochrome P-450 dependent monooxygenases, and a more potent acetylcholinesterase (AChE) inhibitor [12,13].

Batch technique is traditionally adopted by most standard algal test protocols for regulatory purposes [14–17]. These tests are basically open-system tests because the major carbon source for algal growth is from the atmospheric air. Such experimental design causes the loss of volatile organic toxicants, and consequently, underestimations of the toxicity of volatile organic chemicals. The European Centre for Ecotoxicology and Toxicology of Chemicals (1996) has concluded that current algal toxicity test protocols are unsuitable for assessing the effects

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of volatile compounds. To overcome this, we have developed a closed-system algal toxicity test technique with no headspace [18,19]. The experimental design is quite simple and the test revealed satisfactory sensitivities to both metallic and organic toxicants.

The objective of this study was to evaluate the toxicity of pesticides using an air-tight algal toxicity test. Furthermore, to compare algal responses to the toxicity of different kinds of pesticides revealed by other aquatic organisms. Four organophosphorus insecticides, two herbicides, and one fungicide were selected for this study. Two response endpoints, i.e., algal cell density and dissolved oxygen (DO) production, were used to evaluate the toxic effects of various toxicants.

2. Materials and methods

Algal inoculum (*Pseudokirchneriella subcapitata*) taken from a chemostat at steady state was transferred into 300-mL BOD-bottle, together with growth medium (US EPA bottle medium, [16]) and toxicants. The initial cell density was 15,000 cells/mL. The BOD-bottles were completely filled up with no head-space left. Water seal was provided to ensure a closed test environment. The bottles were then placed on an orbital shaker operated at 100 rpm for 48 h (test duration). Temperature and light intensity were kept at $24 \pm 1\,^{\circ}C$ and $65\,\mu E/m^2\,s \pm 10\%$ (4300 lx), respectively. The initial dissolved oxygen level was between 1 and 3 mg/L and the initial pH for the growth medium was 7.5. Two response endpoints were used to evaluate the toxicity of toxicants: dissolved oxygen production (ΔDO) and the 48 h final yield based on cell density. The median effective concentration (EC50) was defined as the toxicant concentration that resulted in 50% reduction with respect to the response endpoints (i.e., ΔDO and cell density). Probit analysis was used to obtain EC50 values. A detailed description regarding the test technique can be found from the author's previous work [19].

Seven pesticides, including parathion, dichlorvos, malathion, fenthion (organophosphorus insecticides), atrazine, 2-methyl-4-chlorophenoxyacetic acid (herbicides) and pentachlorophenol (fungicide), were tested in this study. Their physical and chemical characteristics are given in Table 1. All chemicals used were of reagent grade and all tests were performed in triplicate. HPLC-grade (99% purity) acetonitrile were used for analytical standards. Stock solutions of toxicants were prepared in acetonitrile and were allowed to reach equilibrium at room temperature in foil-wrapped glass containers. Before commencing the experiment, stock solution was freshly prepared and its concentration was analyzed using a HPLC analyzer. Both median effective concentration (EC50) and the no-observed-effect concentration (NOEC) were calculated, based on the aforementioned two response endpoints. EC50 values were estimated using the probit model. Solvent controls were conducted to ensure that the amounts of solvent used were adequate.

One-tail Dunnett's procedure was applied for the estimation of NOEC values at 5% level of significance. The author's previous work [20] introduced the cut-off value as an expression of the variability as well as data quality for NOEC values: the studentized range (SI) can be calculated according to Eq. (1) as shown below:

$$\mathrm{SI} = \frac{\mathrm{Xc} - \mathrm{Xi}}{\mathrm{Sw}\sqrt{(1/\mathrm{nc}) + (1/\mathrm{ni})}} \tag{1}$$

where Xc and Xi are mean observations from controls and treatments, respectively. Sw is the square root of the within-group-variance, and nc and ni are the numbers of replicates for the control and treatment, respectively. A specific treatment is considered to be significantly different from the controls if the corresponding SI value is greater than the critical value (T). Therefore, T serves as a cut-off point for the Dunnett's test. We may hence calculate the cut-off value (in terms of the % reduction of the inhibitory effects) by transforming Eq. (1) into the following expression:

$$\%\,\mathrm{Reduction} = \frac{\mathrm{Xc} - \mathrm{Xi}}{\mathrm{Xc}} \times 100 = \frac{T}{\mathrm{Xc}} \times \mathrm{Sw}\sqrt{\frac{1}{\mathrm{nc}} + \frac{1}{\mathrm{ni}}} \times 100 \tag{2}$$

3. Results and discussion

Table 2 displays a typical set of algal responses with respect to the toxicity of malathion. For the test control, the dissolved oxygen concentration increased from 2.70 mg/L at the beginning to a final DO concentration of 8.85 mg/L. The increase in dissolved oxygen content was a production from algal photosynthesis reactions. The cell density increased from an initial value of 15,000 cells/mL to a final yield of 294,467 cells/mL. Generally speaking, at a specific malathion concentration, the inhibition rate based on DO production is greater than that based on Δcell density. Concentration response curves for the

Table 1
Physical and chemical characteristics of pesticides

Pesticides	CAS no.	MW[a]	Formula	Chemical class
Atrazine	1912-24-9	215.69	$C_8H_{14}ClN_5$	Triazine herbicide
2-methyl-4-chlorophenoxyacetic acid (MCPA)	94-74-6	200.62	$C_9H_9ClO_3$	Phenoxy herbicide
Dichlorvos	62-73-7	220.98	$C_4H_7C1_2O_4P$	Phosphorate insecticide
Malathion	121-75-5	330.36	$C_{10}H_{19}O_6PS_2$	Phosphorodithioate insecticide
Parathion	56-38-2	291.27	$C_{10}H_{14}NO_5PS$	Phosphorothioate insecticide
Fenthion	55-38-9	278.33	$C_{10}H_{15}O_3PS_2$	Phosphorothioate insecticide
Pentachlorophenol (PCP)	87-86-5	266.35	C_6HCl_5O	Chlorophenol fugicide

[a] MW: molecular weight.

Table 2
Toxicity data of malathion

Concentration (Mg/L)	Initial DO (mg/L)	Final DO (mg/L)	Final cells (cells/mL)	ΔDO (mg/L)	IR (DO)	ΔCell density (cells/mL)	IR (cell density)
Control	2.7000	8.850	294467	6.150	0	279467	0
6	3.3833	3.503	32333[a]	0.120[a]	0.980	17333	0.938
4	2.8500	3.823	43967[a]	0.973[a]	0.842	28967	0.896
2	2.4500	5.790	185467[a]	3.340[a]	0.457	170467	0.390
1.2	2.2933	7.050	283233	4.757[a]	0.227	268233	0.040
0.5	2.6167	8.407	300333	5.790	0.059	285333	−0.021
EC50					2.04		2.32
NOEC					0.5		1.2

IR: inhibition rate.
[a] Statistically different from the control.

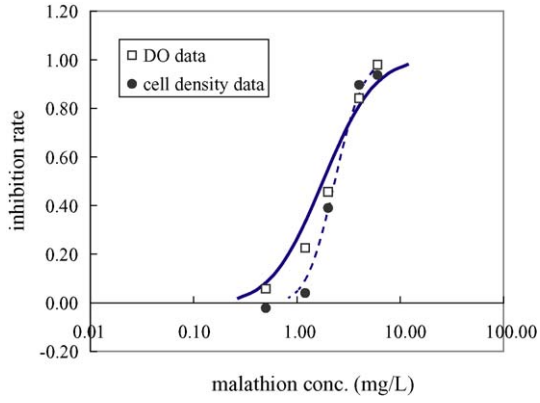

Fig. 1. The dose–response relationships of malathion on *Pseudokirchneriella subcapitata*.

aforementioned response endpoints are shown in Fig. 1. These curves were obtained through linear regression assuming a log-normal distribution (probit model) of the tolerances. Based on the probit analyses, EC50 values were found to be equal to 2.32 (Δcell density) and 2.04 mg/L (ΔDO), respectively. According to the dose–response curves in Fig. 1, we may also conclude that endpoint based on DO production is slightly more sensitive than cell density. The no-observed-effect concentration (NOEC) is determined using the one-tail Dunnett's test at $p = 0.05$. Individual treatments that were statistically different from the control are marked with an asterisk. Therefore, for malathion, the NOEC value based on DO production is also smaller than that obtained based on cell density.

Table 3 lists the EC50 values and the 95% confidence intervals for all the test compounds listed in Table 1. EC50 values range from 0.0035 to 3.397 mg/L (based on ΔDO) and from 0.0067 to 3.115 mg/L (based on Δcell density). For the endpoint of DO production, the toxicity order is: PCP > atrazine > dichlorvos > parathion > fenthion > malathion > MCPA. On the other hand, a slightly different toxicity order can be found based on cell density as: PCP > atrazine > parathion > fenthion > dichlorvos > malathion > MCPA. The insecticide dichlorvos has an apparent tendency to interfere algal photosynthesis reaction, which results in a much smaller EC50 value compared to that based on cell density. For the other compounds tested in this study, however, the two test endpoints revealed similar sensitivities.

Table 4 lists the NOEC, the square root of the within-group-variance (Sw), and the cut-off values for the pesticides tested in this study. Using malathion as an example (Table 2), one can see that an individual treatment will be considered as statistically different from the controls if its inhibitory effect (DO endpoint) is greater than 16%. For the case of cell density, treatments with their effects greater than 7.98% will be considered as significantly different from the controls at $p = 5\%$. The means for the cut-off values are 11.77 and 9.9% with respect to the DO and cell-density endpoints. Hence, on an average basis, our NOEC values are generally equivalent to EC10. As shown in Table 4, NOEC ranges from 0.001 (PCP) to 1.20 mg/L (malathion) with respect to cell density. For each specific pesticide, NOEC is approximately equal to one-third to one-tenth of the EC50 value. For the DO endpoint, most tests did not cover the range of NOEC values. However, we may also find that, at low concentration levels, more significant inhibition based on DO production was observed than that based on the final yield of cell density. Hence, for MCPA, dichlorvos, malathion and fenthion, NOEC values

Table 3
EC50 values and 95% confidence intervals of pesticides

Toxicants	EC50 (DO)	95% C.I.			EC50 (cell density)	95% C.I.		
Atrazine	0.0899	0.086	–	0.095	0.0784	0.075	–	0.082
MCPA	3.397	2.13	–	8.88	3.115	2.68	–	3.69
Dichlorvos	0.737	0.698	–	0.77	1.616	1.54	–	1.69
Parathion	1.162	1.10	–	1.22	0.927	0.88	–	0.97
Malathion	2.04	1.24	–	4.86	2.32	1.47	–	1.94
Fenthion	1.288	1.02	–	2.15	1.049	0.64	–	1.53
PCP	0.0035	3.03E-3	–	3.79E-3	0.0067	0.003	–	0.012

Unit: mg/L. C.I. = confidence interval.

Table 4
NOEC, Sw, and cut-off values for seven pesticides

Toxicants	NOEC, mg/L (DO)	Sw[a]	Cut-off value (%)	NOEC, mg/L (cell density)	Sw	Cut-off value (%)
Atrazine	0.02	0.26	11.08	0.02	9635.93	11.83
MCPA	<0.5	0.44	10.85	1.00	11400.34	7.27
Dichlorvos	0.106	0.44	15.47	0.212	19116.46	15.71
Parathion	0.35	0.55	15.41	0.35	13880.02	14.32
Malathion	0.5	0.47	16.01	1.20	11159.67	7.98
Fenthion	<0.3	0.25	7.43	0.3	10758.54	7.74
PCP	0.001	0.25	6.19	0.001	7533.40	4.43
Mean			11.77			9.90

[a] Sw: square root of the within-group-variance.

based on the DO endpoint are smaller than that based on cell density.

In Table 5, literature data (US EPA ECOTOX database, [21]) based on *Pseudokirchneriella subcapitata* were compared with our test data. To avoid listing pages of references, the original reference numbers assigned by the ECOTOX database will be shown in the table and will not be included in our references. To explore the differences between the conventional batch tests and our closed-system technique, for the case of atrazine, 14 EC50 values with 72 or 96 h test duration (standard exposure times for OECD or US EPA protocols [16,17]) are summarized with the mean equal to 0.292 mg/L. Therefore, the closed-system test is about three to four times more sensitive than the conventional batch tests. On the other hand, results from microplate technique revealed much better sensitivity than the batch tests. The reason is that microplate tests were conducted with sealed test vessels using PVC or parafilm covers to minimize evaporation [22,23]. On average, EC50 values from the microplate tests are approximately 30–40% greater than that from our test. Versteeg (reference no. 17639, [24]) has reported a remarkably low atrazine's EC50 value (0.05 mg/L, 96 h) using the bottle technique. In Versteeg's article [24], however, the detail of algal toxicity test was not given. The only information available is that a chronic toxicity testing procedure was adopted. Such a good sensitivity from Versteeg's work is apparently deviated from the general performance of the conventional batch tests. Since it has been recognized that the amount of algal inoculum has a significant influence on test sensitivity [25], it is likely that a very low initial cell density was applyed in Versteeg's tests. Furthermore, from atrazine's data, one may find that good sensitivity can be achieved when inhibition on algal photosynthesis reactions was used as an endpoint. These data were mainly derived using respirometer or DO meter conducted under air-tight environment. Hence, based on atrazine's data, it is obvious that tests

Table 5
Comparisons of EC50 values for *Pseudokirchneriella subcapitata* with literature data

Pesticides	EC50 (mg/L)		Reference EC50 (mg/L)			
	DO	$Yield_{final}$	*P. subcapitata* (literature)	Test conditions	Effects	Sources (ECOTOX reference number)
Atrazine	0.089	0.078	Mean = 0.292 (0.103–0.96, $n = 14$)[a]	Batch, 72–96 h	PGRT, ABND, BMAS, CHLO	([a])
			0.164, 0.093	Semistatic microplate, 72 h	PGRT	17613
			0.187 (Static)	Microplate, 72 h	GPOP	13728
			0.118 (Semistatic)	Microplate, 72 h	GPOP	13728
			0.019–0.026	Microplate, 96 h, renewal	ENZ, GPOP–	17098
			0.05	Batch, 96 h	–	17639
			0.07	Sealed vessel, 24 h	PSYN	11780
			0.034–0.053	24 h	PSYN	11777
MCPA	3.39	3.12	17.8	Microplate, 96 h	PHY, PSYN	17098
			18.4	Microplate, 96 h	POP, ABND	17098
			2.13	Microplate, 96 h	LC50, cytometry	17098
			1.94	Microplate, 96 h	IC50, cytometry	17098
Dichlorvos	0.74	1.62	–			
Malathion	2.04	2.32	0.01	Microcosms, 96 h	POP, GPOP	10181
Parathion	1.16	0.93	–			
Fenthion	1.29	1.05	1.0–1.2	96 h	POP, ABND	344
PCP	3.54E−3	0.0067	0.42	Batch, 96 h	PGRT	13171

Effect: PHY = physiology; POP = population; GRO = growth; HIS = histology; ENZ = enzymes; PSYN = photosynthesis; GPOP = population changes, general; PSYN = photosynthesis; ABND = abundance; GGRO = growth, general; CHLO = chlorophyll; PGRT = population growth rate; PRLF = proliferation; BMAS = biomass.

[a] Sources: 16010, 19285, 18440, 19852, 17613, 18093, 18933, 19461, 61707.

Table 6
Comparisons of EC50 values from algae, fish, and water flea

Pesticides	EC50 (mg/L)		Reference		
	DO	$Yield_{final}$	Rainbow trout	Fathead minnow	*Daphnia magna*
Atrazine	0.089	0.078	Mean = 14.92 (4.5–24), n = 6 (96 h, LC50, MOR)[18]	11–20 (96 h, LC50, MOR)[5]	>50 (24 h, EC50, BEH, EQUL)[1]
					>39 (48 h, EC50, ITX, IMBL)[2]
					5.2–8.1 (48 h, LC50, MOR)[3]
MCPA	3.39	3.12	0.53–1.51 (48 h, LC50, MOR)[4]	–	>180 (48 h, EC50, ITX, IMBL)[5]
					>11 (48 h, LC50, MOR)[6]
Dichlorvos	0.74	1.62	0.5 (24 h, LC50, MOR)[7]	2.5–3.7 (96 h, LC50, MOR)[8]	1–3.5 (24 h, EC50, ITX, IMBL)[9]
			0.6–0.9 (96 h, LC50, MOR)[5]		0.8–1.4 (48 h, EC50, ITX, IMBL)[5]
Malathion	2.04	2.32	0.15–0.36 (24 h, LC50, MOR)[7]	10.3–16 (24 h, LC50, MOR)[7]	0.00033–0.0006 (21 days, EC50, ITX, IMBL)[11]
			0.16–0.24 (96 h, LC50, MOR)[10]	6.45–11.5 (96 h, LC50, MOR)[7]	
Parathion	1.16	0.93	1.39–2.98 (24 h, LC50, MOR)[7]	1.77–4.87 (24 h, LC50, MOR)[13]	0.00072–0.0014 (48 h, EC50, ITX, IMBL)[11]
			1.2–1.6 (96 h, LC50, MOR)[12]		
Fenthion	1.29	1.05	1.19–2.05 (24 h, LC50, MOR)[7]	2.48–4.87 (24 h, LC50, MOR)[7]	0.0045–0.006 (48 h, EC50, ITX, IMBL)[5]
				1.37–3.8 (96 h, LC50, MOR)[7]	
PCP	3.54E-3	0.0067	0.164–0.235 (48 h, LC50, MOR)[14]	0.193–0.274 (24 h, LC50, MOR)[7]	0.3–0.42 (48 h, EC50, ITX, IMBL)[17]
			0.35–0.66 (96 h, LC50, MOR)[12]	0.21–0.286 (48 h, MOR)[15]	
				0.42–0.55 (96 h, EC50, MOR, HTCH)[16]	

Effect: MOR = mortality; BEH = behavior; ITX = intoxication; HTCH = hatch; DFRM = deformation; EQUL = equilibrium; IMBL = immobile. Source: (1) 56301; (2) 13154; (3) 344; (4) 35; (5) 344; (6) 6270; (7) 6797; (8) 17138; (9) 17456; (10) 10656; (11) 6449; (12) 11519; (13) 983; (14) 3386; (15) 10574; (16) 11958; (17) 17289; (18) 344, 546, 6797, 18806.

conducted under a closed environment appeared to be more sensitive than those under open environment.

Literature data for other pesticides were quite limited as compared to atrazine. For MCPA, EC50s (cell counts and photosynthesis) based on the microplate technique are approximately 18 mg/L and are six times greater than our values. On the other hand, LC50 and IC50 values derived by a flow-cytometry technique were found to be 2.13 and 1.94 mg/L, respectively (reference no. 17098, [26]). The above LC50 and IC50 values are even smaller than EC50s from our BOD-bottle technique. According to the author, the flow-cytometry technique is able to distinguish between viable and non-viable cells [26]. For

Table 7
Comparisons of NOEC values with literature data

Toxicants	NOEC (mg/L)		Reference NOEC (mg/L)			
	DO	$Yield_{final}$	*P. subcapitata*[a]	Ciliate[b]	*Daphnia magna*	Fish
Atrazine	0.02	0.02	0.075 (24–96 h, POP, BMAS)[1]	14.5 (48 h, GPOP)[2]	–	2.0 (96 h, MOR, MORT)[c3]
				21.5 (96 h, GPOP)[2]	–	–
MCPA	<0.50	1.00	–	–	–	–
Dichlorvos	0.106	0.212	–	–	0.000109–0.000206 (21 days, GRO, GGRO)[4]	0.07–0.19 (28 days, GRO, GGRO)[d4]
Parathion	0.35	0.35		–	0.000002 (21 days, BEH, EQUL)[5]	<0.2 (48 h)[e]
Malathion	0.5	1.20	–	–	0.00015 (21 days, ITX, IMBL)[6]	<15.6 (2 h, MOR, MORT)[f7]
					0.0003 (21 days, REP, GREP)[6]	
Fenthion	<0.30	0.30	–	–	0.6 (14 days, REP, GREP)[8]	–
PCP	0.001	0.001	0.005–0.04 (96 h, GRO)[9]	0.08 (48 h, PGRT)[2]	50 (14 days, ITX, IMBL)[10]	0.056–0.11 (28 days, GRO, GGRO)[d11]
			0.1	0.1 (96 h, PGRT)[2]		

Effect: POP = population; BMAS = biomass; GRO = growth; GGRO = growth, general; GPOP = population changes, general; BEH = behavior; EQUL = equilibrium; ITX = intoxication; IMBL = immobile; REP = reproduction; GREP = reproduction, general; MOR = mortality. Source: (1) 18093; (2) 4008; (3) 18805; (4) 17138; (5) 6628; (6) 6449; (7) 15030; (8) 18530; (9) 12735; (10) 8764; (11) 14078.

[a] *Pseudokirchneriella subcapitata* = *Selenastrum capricornutum*.
[b] *T. pyriformis* = *Tetrahymena pyriformis*.
[c] Rainbow trout = *Oncorhynchus mykiss*.
[d] Fathead minnow = *Pimephales promelas*.
[e] Gold fish = *Poecilia reticulate*.
[f] Rainbow trout = *Oncorhynchus mykiss*.

the case of malathion, a very sensitive result (0.01 mg/L) was obtained based on microcosm study. The continuous-flow test condition, which is normally applied in microcosm study, could be the reason for such good sensitivity. Previous test result for fenthion is almost identical with our data. Finally, a mark difference between the batch tests and the closed-system tests is observed from the case of PCP. EC50 values derived from the above two test methods differ by a factor of greater than 100.

Table 6 compares the toxicity of pesticides between algae, fish, and water flea. Toxicity data from acute tests with an exposure time of 96 h or less were selected for comparison. *Pseudokirchneriella subcapitata* seems to be very sensitive to herbicides and fungicide (PCP), compared to water flea and fish. Secondly, rainbow trout revealed better sensitivity than our closed-system test for MCPA, dichlorvos, and malathion. On the other hand, *Daphnia magna* were found to be the most sensitive species of organism to malathion, parathion, and fenthion. EC50 values based on *Daphnia magna* for the above three toxicants are 2–3 orders smaller in magnitude compared to other organisms in Table 6. Most of the aforementioned toxicants, except MCPA, are organophosphorus insecticides that are expected to be more harmful to animals considering their toxicity mechanisms [12,13]. Furthermore, endpoints such as hatchability and behavior do not seem to be more sensitive than lethality. However, in Table 6, data with longer exposure times (>96 h) were not considered. It is also quite interesting to find that, although algae are expected to be less sensitive to organophosphorus insecticides due to lacking of a nervous system, *Pseudokirchneriella subcapitata* still seems to be more sensitive than fathead minnow.

Literature NOEC values for algae, ciliate, water flea, and fish are listed in Table 7. Previous data (atrazine and PCP) on *Pseudokirchneriella subcapitata* are at least three to five times greater than our BOD-bottle test results. Ciliate also seems to be relatively insensitive to atrazine and PCP. For *Daphnia magna*, results from 21-day tests indicate that dichlorvos, parathion, and malathion are extremely toxic. However, for the case of fenthion, NOEC value from a 14-day test did not reveal better sensitivity than our 48-h test's results. Finally, NOEC values show that fishes are more susceptible to dichlorvos and parathion than algae. Generally speaking, chronic toxicity data are more reliable than acute toxicity data because chronic tests were normally conducted using flow-through systems. Overall, Table 7 has revealed a consistent conclusion as from Table 6 that, organophosphorus insecticides exert stronger harmful effects on water flea and fish. Herbicides and fungicides, on the other hand, are more toxic to algae.

4. Conclusions

This paper presents the toxicity data of seven pesticides on *Pseudokirchneriella subcapitata* derived based on a new algal toxicity testing technique conducted under air-tight environment. The dissolved oxygen production and the cell density were adopted as the response endpoints. Median effective concentrations (EC50) and no-observed-effect concentrations (NOEC) were derived using the probit analysis and the Dunnett's test, respectively. In general, the two test endpoints revealed similar sensitivities. From comparisons of literature data also based on *Pseudokirchneriella subcapitata*, it is clear that conventional batch tests tend to underestimate the toxicity of pesticides due to their open test environment. Closed-system tests, i.e., microplate test, respirometer test, and our BOD-bottle test, generally provide better assessment to the effects of pesticides. Data based on our test method reveals much higher toxicity (3–100 times) than that from the conventional batch tests. Furthermore, for organophosphorus insecticides, results from the present study show that *Pseudokirchneriella subcapitata* is less sensitive than *Daphnia magna* and rainbow trout, but is more susceptible than fathead minnow. The closed-system test applied in this study provides more adequate assessment for the toxicity of pesticides than the conventional batch tests.

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