RESEARCH ARTICLE

Determination of the fate of propanil in a simulated rice field environment through electrochemical detection of 3,4-DCA

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Abstract: Electrochemical investigation of 3,4-dichlorophenylamine (3,4-dicholoroaniline or 3,4-DCA), the principal degraded product of propanil, in aqueous medium reveals that the most intense peak appears at +0.75 V *vs*. saturated calomel reference electrode (SCE). The diffusion-controlled behaviour of this peak enables direct detection of 3,4-DCA in the environment, which can be extended for investigation of the fate of propanil. Rice cultivation grown in three beds of equal dimensions prepared in a polytunnel, an effective simulated environment to which the recommended dose of propanil was applied, was used for this study. Determination of the concentrations of 3,4-DCA throughout the rice cultivation cycle indicated that the concentration of 3,4-DCA initially increases up to 4 days followed by a decrease up to 10 days, and finally reaches a plateau. The concentration-time relationships of 3,4-DCA suggest that the initial degradation of propanil and subsequent degradation of 3,4-DCA follow the mechanism of consecutive reactions, where the product of the first step becomes the reactant of the second step. Further, comparison of kinetic data with integrated rate law expressions and the mathematical model of consecutive first order reactions indicated that the degradation of both processes is of first order with relatively low rate constants, being the degradation of 3,4-DCA as the rate limiting step.

Keywords: Electrochemical detection, pesticide, propanil, rice field.

INTRODUCTION

Propanil (3',4'-dichloropropionanilide) is a much used herbicide in rice cultivation. Although propanil is moderately harzardous with a LD_{50} value ranging from 1080–2500 mg/kg, its primary degraded product 3,4-dichlorophenylamine (3,4-DCA), is more toxic with a LD_{50} value of 570 mg/kg for male rats through oral application (BAuA, 2001). The primary toxic effect of 3,4-DCA is due to methaemoglobin formation by reacting with heamoglobin in blood (McMillan *et al*., 1991). It is absorbed from the gastrointestinal tract and through the skin and lungs (Worobey & Shields, 1991; BAuA, 2001). The transformation of propanil occurs mainly due to metabolic activity, and hence the fate of propanil in agricultural fields where it is used as a herbicide is an important area of scientific investigation.

The fate of a pesticide in the environment is governed by many factors including the chemical and physical characteristics of the pesticide such as adsorption, absorption, leaching and degradation (Aydinalp & Porca, 2004; Voutsas *et al*., 2005; Brady *et al*., 2006; Isenring & Madeley, 2006), as well as environmental factors to which the pesticide is exposed, such as the composition of soil and climatic conditions (Reichman *et al*., 2000a, b; Furrer *et al*., 2001). In the case of pesticides applied as a solution, the distribution pattern between the liquid and soil phases is of utmost importance in the investigation of the fate of the pesticide (Rytwo *et al*., 2002; Tsai *et al*., 2004; Fava *et al*., 2005). Initial spreading of the pesticide from the application site is a prerequisite for its distribution. Although the interaction of a pesticide with soil particles and subsequent degradation may be relatively slow, the initial spreading mainly controlled by diffusion could be much faster in the case of an electrically neutral pesticide such as propanil.

Among many studies on the fate of propanil and 3,4-DCA, a study of a paddy-wetland ecosystem suggests that paddy soil serves as a reservoir for propanil which may be washed into drainage channels or mixed with rain water. In the wetland, propanil is either degraded rapidly or accumulated by aquatic macrophytes and retained for an extended period of time (Perera *et al*., 1999). Another study confirms that potential problems

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exist with direct aerial spraying of ponds with propanil, since propanil being a photosynthesis inhibitor would depress primary production of phytoplankton, resulting in harmful dissolved oxygen levels after its application (Perschbacher *et al*., 2002). Further, 3,4-DCA shows organic-carbon partition coefficient (K_{oc}) values typical of mobile compounds, and its ground-water ubiquity score (GUS) index is below the limit of nonleaching compounds (Fava *et al*., 2005).

As the fate of a pesticide is a complex issue governed by many factors, it needs to be understood to safeguard the quality of the environment. A satisfactory approach is to apply a suitable model for a set of conditions/parameters defined for the system of investigation. Models on pesticidal fate have aided satisfactory extrapolation to predict where and how pesticides enter the environment (Carsel *et al*., 1984; USEPA, 2004; McCarthy *et al*., 2007). Nevertheless, application of available models for local environmental conditions has become doubtful, because models designed using different soil types and for different meteorological conditions would not provide satisfactory predictions for a new set of local environmental conditions. Limited data available on environmental fate studies of 3,4-DCA promote detailed investigation on this issue (McMillan *et al*., 1991; Dups *et al*., 2003).

The goal of this research is to study the fate of propanil in a simulated rice field by means of a kinetic model for the determination and quantification of its primary degraded product, 3,4-DCA. As propanil is not electroactive at bare electrode surfaces, its direct electrochemical determination is not possible and consequently, electrodes modified with metalloporphyrin catalysts have been reported as a reliable means for its detection (Priyantha & Weerabahu, 1996; Priyantha *et al*., 2004). On the other hand, electroactivity of 3,4-DCA makes it possible to investigate the fate of propanil through the detection of 3,4-DCA. For this investigation, the application of propanil followed by regular sampling of floating and leaching water from the soil bed, where rice plants are grown under simulated conditions by applying actual farmer practices, is important. Further, detection of 3,4-DCA, in replicate, is neccssary to improve the precision of measurements. Detection of 3,4-DCA by steady-state amperometry, an economical and simple analytical method, is a feature of this study.

METHODS AND MATERIALS

Materials: A standard sample of 3,4-DCA (98 %) was purchased from Fluka (Germany). The electrolyte, NaCl, and stearic acid for electrode modification were of analytical grade purchased from BDH (England). Acetonitrile, used for the preparation of 3,4-DCA standards and of electrolyte solutions, was distilled prior to use.

Instrumentation: A CV-1B cyclic voltammograph and an X-Y recorder, both from Bioanalytical Systems, USA, were used for cyclic voltammetric and amperometric experiments. Cyclic voltammetric analyses and amperometric determinations were performed in a three-electrode, single-compartment cell, which was constructed using a glassy carbon working electrode (GCE), a platinum wire counter electrode, and a saturated calomel reference electrode (SCE). All potentials were measured with respect to SCE, and all the experiments were conducted under N_2 saturated conditions.

Research design: Based on the findings of a previously reported research work (Navaratne *et al*., 2009), stearic acid-coated GCEs were used for the detection of 3,4-DCA. Cyclic voltammetric experiments of 160 ppm 3,4-DCA in 0.1 mol dm^{-3} NaCl [prepared in $H_2O:CH_3CN$ (3:1 V/V) mixed solvent system] were carried out by changing experimental conditions in order to optimize the potential operated for amperometric detection.

Experiments on simulated rice field environment: Three rice beds of similar dimensions (1.0 m in length × 1.0 m in width × 0.5 m in depth) were prepared in a polytunnel and filled up to 0.25 m with paddy soil obtained from the Rice Research and Development Institute (RRDI), Batalagoda. Weeds grown in normal paddy fields were introduced to these soil beds and allowed to grow under water saturated conditions.

After the weeds grew up to about 0.3 m in height, the fields were filled with water up to 3 cm above the soil level, and this level was kept constant by addition of water to counteract the effect of evaporation on the final results. Water samples from the rice field were analysed for propanil and 3,4-DCA residues for a period of 3 d, before application of any pesticide, because pesticides had been introduced to the soil during previous rice cultivations at the RRDI. For this purpose, water samples were withdrawn, in triplicate, from each bed from the top layer as well as from the bottom (after passing through the soil bed).

After 10–14 d of planting rice, the recommended dose of propanil (400 cm^3 of 670 ppm per bed of 1 m^2 area) was introduced to the field. Water samples were collected daily up to 10 d, followed by every 8th day thereafter to determine the concentration of 3,4-DCA.

RESULTS AND DISCUSSION

Parameter optimization for the detection of 3,4-DCA was conducted using cyclic voltammetric studies at bare GCE in 0.1 mol dm^{-3} NaCl (3:1 H$_2$O/CH$_3$CN). The voltammograms recorded between +1.0 V and –1.0 V showed two reduction peaks and two oxidation peaks at the potentials of +0.10 V, –0.10 V (broad), +0.35 V and +0.75 V, respectively, which are in agreement with literature (Navaratne *et al.*, 2008). The optimum operational potential for the amperometric detection of 3,4-DCA was subsequently determined to be +0.70V at bare GCE at which the highest amperometric response was observed. Noisy amperometric responses at this potential were improved using stearic acid coatings. Analytical characteristics for the detection of 3,4-DCA, such as sensitivity of detection, linear dynamic range, minimum detection limit and lifetime were determined to be 1.23 µA ppm^{-1}, 0.08 – 0.80 ppm, 0.03 ppm and 5 weeks, respectively.

Levels of 3,4-DCA, as determined by steady-state amperometry at stearic acid coated GCE in three beds of two cultivation cycles over a period of 52 days after application of propanil are shown in Table 1. The average concentration of 3,4-DCA over the three beds at each sampling day has also been reported for comparison. Levels of 3,4-DCA were not detected after 52 days of propanil application, indicating that almost all propanil and 3,4-DCA have degraded into other products. The differences in the levels of 3,4-DCA among the three

rice beds, as observed by high standard deviations in Table 2, can be explained considering many variations in the preparation of the rice beds such as the composition and particle size of soil, which cannot be fully controlled in any field trial although the soil for the preparation of the beds was obtained from a single location of a rice field used for research purposes. The pooled standard deviation (σ_p) was calculated using equation 1,

$$\sigma_p = \left\{ \frac{\sum (x_i - \bar{x}_1)^2 + \sum (x_i - \bar{x}_2)^2}{(N-k)} \right\}^{1/2} \qquad ...(1)$$

where x_i is any individual measurement of a particular cultivation cycle on a given sampling day, $\bar{x}_i$ is the average concentration of that cycle, N is the total number of measurements of both cycles ($N = 6$), and k is the number of cultivation cycles ($k = 2$).

It should be stressed that residual levels of neither propanil nor 3,4-DCA were present, according to the background measurements, when the rice cultivation cycle began under simulated conditions. Further, levels of 3,4-DCA in the leachate were found to be below the detection limits, which is characteristic of a nonleaching pesticide such as 3,4-DCA, as already reported (Reichman *et al.*, 2000a).

Although there were differences among the three beds, the average concentrations of 3,4-DCA at each sampling time between the two cultivation cycles

Table 1: Concentration of 3,4-DCA, in ppm, in surface water of three beds of two cultivation cycles measured over a period of 52 days using steady state amperometry. UD ≡ undetectable

Day	Cycle 1				Cycle 2			
	Bed 1	Bed 2	Bed 3	Average	Bed 1	Bed 2	Bed 3	Average
1	UD	UD	UD	--	UD	UD	UD	--
2	2.15	2.28	2.69	2.38	1.94	2.37	2.62	2.31
3	2.69	2.90	2.53	2.71	2.79	2.75	2.43	2.66
4	3.87	4.24	3.84	3.99	3.40	3.81	3.73	3.65
5	3.11	3.5	3.05	3.22	3.11	3.56	3.05	3.24
6	2.40	2.38	1.49	2.09	2.22	2.33	1.70	2.08
7	1.88	2.30	1.90	2.03	1.62	2.33	1.90	1.95
8	1.44	1.99	1.59	1.67	1.49	1.91	1.65	1.68
9	1.25	1.60	1.20	1.35	1.25	1.62	1.20	1.36
10	1.20	1.34	0.94	1.16	1.30	1.26	1.12	1.23
20	2.20	2.11	1.70	2.00	2.20	2.14	1.62	1.99
28	2.11	1.86	1.46	1.81	2.03	1.86	1.46	1.78
36	2.56	1.88	1.51	1.98	2.19	1.90	1.44	1.84
44	2.15	2.28	1.88	2.11	1.94	2.19	1.78	1.97
52	0.78	0.87	0.39	0.68	0.91	1.05	0.62	0.86

were comparable having much smaller changes in the average results (column 5, Table 2). This provides valuable information indicating the repeatability of the amperometric method employed for the detection of 3,4-DCA. The statistical parameter, the F index, [$F = (\sigma_1/\sigma_2)^2$ where σ_1 and σ_2 are the variances of each set and $\sigma_1 > \sigma_2$] calculated for the standard deviations of the two beds for each day was close to unity for many sampling days (column 6, Table 2). The F-test thus indicates that there is no statistical difference between the average results of the two cultivation cycles at a high confidence level, which is also supported by having low concentration differences shown in Table 2. Consequently, the cumulative average of the three beds of the two cultivation cycles can be used for investigation of the fate of propanil in the simulated rice field environment.

Table 2: Standard deviations (individual and pooled), difference in the average concentrations (ppm) and the *F* value associated with concentration measurements of 3,4-DCA shown in Table 1

Day	σ_{cycle1}	σ_{cycle2}	σ_p	$\bar{x}_{(cycle\ 1)} - \bar{x}_{(cycle\ 2)}$	*F* value
1	--	--	--	--	--
2	0.28	0.34	0.31	0.07	1.49
3	0.19	0.20	0.19	0.05	1.13
4	0.22	0.22	0.22	0.34	1.05
5	0.24	0.28	0.26	-0.02	1.30
6	0.52	0.34	0.44	0.01	2.39
7	0.24	0.36	0.30	0.08	2.28
8	0.28	0.21	0.25	-0.01	1.80
9	0.22	0.23	0.22	-0.01	1.11
10	0.20	0.09	0.16	-0.07	4.61
20	0.27	0.32	0.29	0.01	1.43
28	0.33	0.29	0.31	0.03	1.26
36	0.53	0.38	0.46	0.14	1.98
44	0.20	0.21	0.21	0.14	1.03
52	0.26	0.22	0.48	-0.18	1.35

Considering the variation of the average concentration of 3,4-DCA over the entire sampling period, the initial increase up to 4 days (Figure 1) is a clear indication of the degradation of propanil to form 3,4-DCA. This is well in agreement with earlier observations as it has been reported that 3,4-DCA reaches the maximum levels within 1-5 days of propanil application, and remain high for 1-2 weeks (McMillan *et al.*, 1991).

Assuming that the degradation is quantitative, and that 3,4-DCA does not undergo significant degradation within the initial period of 4 days, the concentration of propanil at each detection point of 3,4-DCA can be determined. This assumption is justifiable because it has already been reported that propanil is metabolized rapidly in aerobic soil with a half-life of 0.5 days (McMillan *et al.*, 1991).

The variation of the propanil concentration calculated with the aid of the concentration of 3,4-DCA at different sample points within the initial period is shown in Figure 2. The concentration of 3,4-DCA at day 1 was not detectable, and hence it was assumed to be negligible.

This investigation mainly focused on the rate of degradation processes in order to understand the fate of propanil. Kinetics analysis performed for initial degradation of propanil, assuming that the degradation of propanil forming 3,4-DCA is the major conversion step, indicated that the degradation process is of first order according to the integrated first order rate law as shown in equation 2,

$$\ln [P] = \ln [P]_0 - k_1 t \qquad ...(2)$$

where $[P]_0$ is the initial concentration of propanil and $[P]$ is the concentration of propanil at time *t*, and k_1 is the first order rate constant (Figure 3). It should be noted that there are only four measurements as the concentration of 3,4-DCA started decreasing after the 4th day of sampling according to Figure 1. The linear fit of the integrated rate equation for first order kinetics shows a regression coefficient close to one (0.9523), as compared to more deviated values with the rate laws of other orders (0.9044 for zeroth order and 0.9134 for second order). The rate constant for degradation of propanil (k_1) estimated from Figure 3 is 4.51×10^{-6} s^{-1}.

The variation of the relative magnitude of the concentration of 3,4-DCA over a period of 52 days is indicative of degradation of propanil followed by subsequent degradation of 3,4-DCA qualifying the overall process for a consecutive first order reaction, which can be represented as follows (Levine, 2002).

where k_2 represents the rate constant of degradation of 3,4-DCA. Additionally, the magnitude of k_2 should be
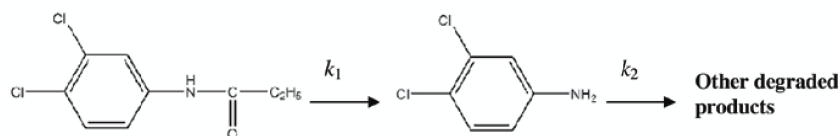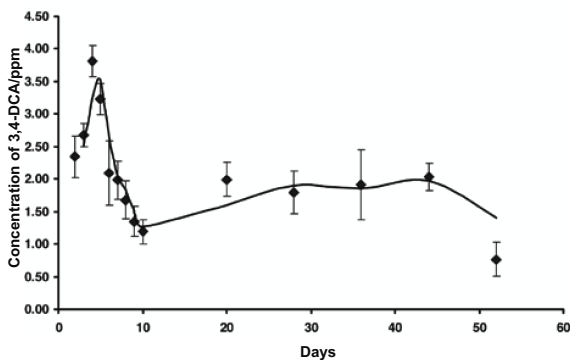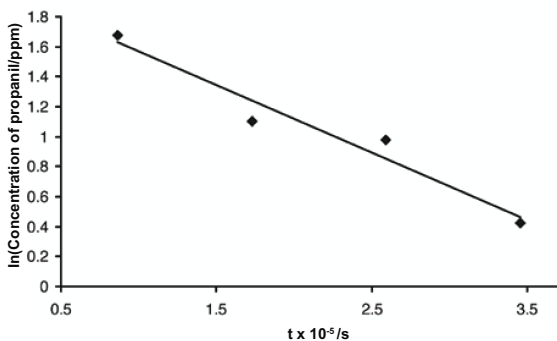
Figure 1: Average concentration of 3,4-DCA in surface water of simulated rice fields over two cultivation cycles of three beads, together with associated standard deviation
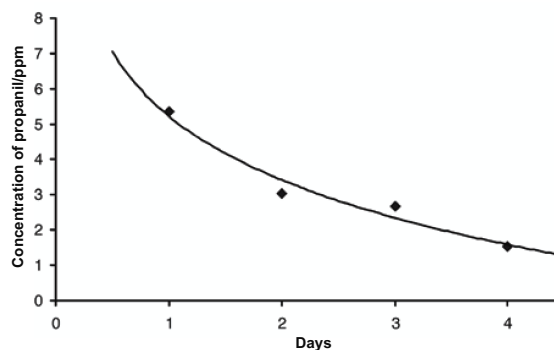
Figure 2: The variation of the propanil concentration in simulated rice fields within the first 4 days

Figure 3: Illustration of the integrated rate law for first order kinetics for degradation of propanil in the simulated rice field environment
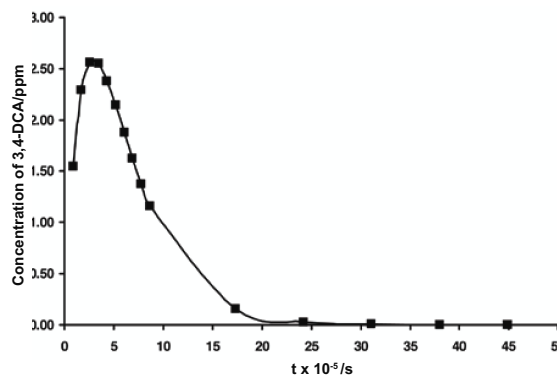
Figure 4: Concentration of 3,4-DCA as calculated with the aid of equation 3 for $k_2 = (0.55)k_1$

smaller than that of k_1 to observe a curve similar to that is shown in Figure 1.

The variation of the concentration of 3,4-DCA, when modelled according to the integrated rate law for first order consecutive reactions is shown in equation 3

$$[D] = \frac{k_1[P]_0}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \qquad ...(3)$$

where [D] is the concentration of 3,4-DCA at time *t*. The concentration of 3,4-DCA, [D], calculated from equation 3 shows the best fit for $k_2 = 0.55\, k_1 = 2.48 \times 10^{-6}\, s^{-1}$ when compared with the experimentally obtained values

(Figure 1). The calculated concentration values of 3,4-DCA according to the proposed kinetics model is shown in Figure 4. The deviation between the modelled and experimental values after 10 days of the application of propanil is probably due to its release into water after the death of plants that had adsorbed propanil during initial introduction.

The decrease in the concentration of 3,4-DCA down to undetectable levels beyond 45 days is indicative of the absence of 3,4-DCA at appreciable levels. This is either due to the degradation of 3,4-DCA leading to simpler molecules which cannot be detected electrochemically and/or leaching and other sorption effects becoming significant.

CONCLUSION

The degradation of propanil forming 3,4-DCA and its subsequent degradation can be modelled through a consecutive first order reaction. The rate constants of the two degradation processes were 4.51×10^{-6} s^{-1} and 2.48×10^{-6} s^{-1}, respectively, suggesting that the degradation of 3,4-DCA forming various degraded products is the rate limiting step. The deviation of the calculated curve derived for the concentration of 3,4-DCA from the experimental curve may be due to the release of residual propanil or its degraded products from dead plants that had been present after initial application. Such kinetics models provide valuable information on the levels of degraded products and residual pesticides over reasonably lengthy time periods. Further refinement of this kinetics model can be achieved by considering the quantity of propanil lost during spraying, and due to volatilization and plant absorption.

Acknowledgement

REFERENCES

1. Aydinalp C. & Porca M.M. (2004). The effects of pesticides in water resources. *Journal of Central European Agriculture* **5**(1): 5–12.

2. Brady J.A., Wallender W.W., Werner I., Fard B.M., Zalom F.G., Oliver M.N., Wilson B.W., Mata M.M., Henderson J.D., Deanovic L.A. & Upadhaya S. (2006). Pesticide runoff from orchard floors in Davis, California, USA: a comparative analysis of diazinon and esfenvalerate. *Agriculture, Ecosystems and Environment* **115**(1–4): 56–68.

3. Carsel R.F., Smith C.N., Mulkey L.A., Dean J.D. & Jowise P. (1984). *Users Manual for Pesticide Root Zone Model (PRZM)*. United States Environmental Protection Agency, Athens, Ohio, USA.

4. Dups I.G., Brown C.D. & Beulke S. (2003). Sources of uncertainty in pesticide fate modeling. *Science of the Total Environment* **317**(1–3): 53–72.

5. Fava L., Orru` M.A., Crobe A., Caracciolo A.B., Bottoni P. & Funari E. (2005). Pesticide metabolites as contaminants of groundwater resources: assessment of the leaching potential of endosulfan sulfate, 2,6-dichlorobenzoic acid, 3,4-dichloroaniline, 2,4-dichlorophenol and 4-chloro-2-methylphenol. *Microchemical Journal* **79**(1–2): 207–211.

6. Federal Institute for Occupational Safety and Health Notification Unit (BAuA) (2001). *Risk Assessment: 3,4-dichloroaniline*. Institute for Health and Consumer Protection, European Chemical Bureau, Berlin, Germany.

7. Furrer G., Schulin R., Wei J. & Kaufmann S. (2001). Influence of clay minerals on the hydrolysis of carbamate pesticides. *Environmental Science and Technology* **35**(11): 2226–2232.

8. Isenring R. & Madeley J. (2006). *Paraquat: Unacceptable Health Risks for Users*, 3rd edition. Pesticide Action Network UK, London, UK.

9. Levine I.N. (2002). *Physical Chemistry*, 5th edition, pp. 537–538. Tata McGraw-Hill Publishing Company Limited, New Delhi, India.

10. McCarthy A.M., Baled J.D., Cope W.G. & Shea D. (2007). Modelling pesticide fate in a small tidal estuary. *Ecological Modelling* **200**(1–2): 149–159.

11. McMillan D.C., Bradshaw T.P., Hinson J.A. & Jollow D.J. (1991). Role of metabolites in propanil-induced hemolytic anemia. *Toxicology and Applied Pharmacology* **110**(1): 70–78.

12. Navaratne A.N., Hafil A.M. & Ekanayake C.B. (2009). Cost effective electroanalytical methodology for the detection of 3,4-dichloroaniline (3,4-DCA), the primary residue of propanil. *Ceylon Journal of Science: Physical Sciences* **15**: 57–64.

13. Perera A., Burleigh J.R. & Davis C.B. (1999). Movement and retention of propanil N-(3,4-dichlorophenyl) propanamide in a paddy-riverine wetland system in Sri Lanka. *Agriculture, Ecosystems and Environment* **72**(3): 255–263.

14. Perschbacher P.W., Ludwig G.M. & Slaton N. (2002). Effects of common aerially applied rice herbicides on the plankton communities of aquaculture ponds. *Aquaculture* **214**(1–4): 241–246.

15. Priyantha N. & Weerabahu D. (1996). An amperometric sensor for detection of propanil. *Analytica Chimica Acta* **320**: 263–268.

16. Priyantha N., Navaratne A., Jayawickrama D. & Weliwegamage U.S.K. (2004). Amperometric method for the determination of propanil in simulated rice field environment. *Journal of the National Science Foundation of Sri Lanka* **32**(3 & 4): 115–125.

17. Reichman R., Wallache R. & Mahrer Y. (2000a). A combined soil-atmosphere model for evaluating the fate of surface-applied pesticides. 1. model development and verification. *Environmental Science and Technology* **34**(7): 1313–1320.

18. Reichman R., Wallache R. & Mahrer Y. (2000b). A combined soil-atmosphere model for evaluating the fate of surface-applied pesticides. 2. the effect of varying environmental conditions. *Environmental Science and Technology* **34**(7): 1321–1330.

19. Rytwo G., Tropp D. & Serban C. (2002). Adsorption of diquat, paraquat and methyl green on sepiolite: experimental results and model calculations. *Applied Clay Science* **20**(6): 273–282.

20. Tsai W.T., Lai C.W. & Hsien K.J. (2004). Adsorption kinetics of herbicide paraquat from aqueous solution onto activated bleaching earth. *Chemosphere* **55**(6): 829–837.

21. United States Environmental Protection Agency (USEPA) (2004). *Exposure Analysis Modeling System (EXAMS): User Manual and System Documentation* (ed. L.A. Burns). Athens, Ohio, USA.

22. Voutsas E., Vavva C., Magoulas K. & Tassios D. (2005).

Estimation of the volatilization of organic compounds from soil surfaces. *Chemosphere* **58**(6): 751–758.

23. Worobey B.L. & Shields J.B. (1991). Preliminary studies on the bioavailability and disposition of bioincurred carrot residues of [^{14}C] linuron and [^{14}C] 3,4-dichloroaniline in rats. *Food Additives and Contaminants* **8**(2): 193–200.