POSTER SESSION P8C

ENVIRONMENTAL FATE AND EFFECTS OF PESTICIDES

Session Organiser: Dr Neil Mackay
Cambridge Environmental Assessments, Boxworth, UK

Poster Papers: P8C-1 to P8C-8

Herbicide detoxification in *Phragmites australis* – an advantage for phytoremediation of effluents from agroecosystems

P Schröder, H Maier
Institute of Soil Ecology, GSF National Research Centre for Environment and Health, D-85758 Oberschleißheim, Germany
Email: peter.schroeder@gsf.de

R Debus
Technical University Wiesbaden, Department of Chemistry, D-65428 Rüsselsheim, Germany

ABSTRACT

Unintentional loss of herbicides into drainage ditches, or other water bodies may cause large problems in farmland. Strategies for the phytoremediation of agrochemicals and especially herbicides have to be developed for agricultural areas. In order to establish effective biological pollution control, information on the detoxification capacity of riparian plants and aquatic macrophytes is important to build up effective buffer stripes. We determined the detoxification capacity of *Phragmites australis* roots and leaves for the conjugation of agrochemicals to glutathione by assaying the model substrate CDNB as well as the herbicides fenoxaprop-P, propachlor, pethoxamid and terbuthylazine. Specific GST activities were always higher in the rhizomes (6.78 ± 0.88 μkat/mg protein for CDNB) than in leaves (1.08 ± 0.21 μkat/mg protein). The detoxification capacity is distributed across an array of GST isoforms. In summary, *Phragmites australis* seems to be efficient in herbicide detoxification and a good candidate for phytoremediation of effluents from agricultural sites.

INTRODUCTION

Herbicide resistance is to a good part based on the presence of detoxification enzymes in weeds (Kreuz *et al.*, 1996). The responsible enzymes, although not physiologically connected, form a putative metabolic cascade for the detoxification of xenobiotics. This cascade or network has been compared with a "green liver", in analogy with the mammalian detoxification system (Shimabukuro, 1976; Sandermann *et al.*, 1997). Typically xenobiotic plant metabolism is subdivided into three distinct phases, i.e. (I) activation, (II) detoxification and (III) excretion of xenobiotics. In the past, xenobiotic conjugation in plants has been investigated in depth for pesticides, and several isoforms of glucosyl-transferases and glutathione S-transferases have been identified in crops (Lamoureux & Rusness, 1989). Whereas OH-, NH_2-, SH- and COOH-functions on a molecule usually trigger glycosyl-transfer mediated by glycosyltransferases (GT, E.C. 2.4.1.x, Frear, 1976), the presence of conjugated double bonds, halogen- or nitro functions determines glutathione conjugation catalyzed by glutathione S-transferases (GST, E.C.2.5.1.18, Coleman *et al.*, 1997).

Glutathione S-transferases are dimeric phase II enzymes capable of conjugating electrophilic, hydrophobic substrates to the tripeptide glutathione (GSH). They play a major role in the detoxification of a wide array of xenobiotics in plants, animals and bacteria (Lamoureux & Rusness, 1989). Schröder & Collins (2002) have recently pointed out that it is crucial to know which type of primary conjugation occurs because this will determine the final fate of the compound (Frear 1976). This might be extremely valuable information in the context of phyto-

remediation, where huge plant canopies might be exposed to pollutants of anthropogeneous origin that they are supposed to remove from the environment. The ability to detoxify herbicides will in this respect be beneficial to a plant and enhance the possibility of treating run-off water from agricultural fields.

MATERIALS AND METHODS

Plant material

Adult *Phragmites australis* plants were harvested from a pond close to Starnberg, Southern Germany, dissected into leaves, root and rhizome fractions and directly frozen in liquid nitrogen. Fresh material was incubated with herbicides for the induction studies in the lab at given concentrations. Plant parts were rinsed with water at the end of the incubation period and adherent fluid was removed with paper towels. Plant parts were then stored at -80°C.

Chemicals

Bovine serum albumin (BSA), CDNB (1-chloro-2,4-dinitrobenzene), glutathione S-transferase (rat liver), reduced glutathione (GSH), Nonidet P40, PVP K 30 (polyvinylpyrrolidone) were obtained from Sigma (Steinheim, Germany). Fenoxaprop-P (FNP-P) and pethoxamid (PA) were from Ehrenstorfer (Augsburg, Germany). Propachlor (PCl) and terbuthylazine (TBA) were from RDH (Taufkirchen, Germany). All other chemicals used were research grade commercial materials.

Enzyme extracts

Frozen leaves and roots were homogenized as previously described (Schröder *et al.,* 1990, 2005) under liquid nitrogen with mortar and pestle to yield a fine powder and extracted at 4°C in ten volumes (*w/v*) 0.1 M Tris HCl buffer pH 7.8, containing 1 % of soluble PVP (K30), 1 % Nonidet and 5 mM EDTA. Crude extracts were centrifuged at 20.000 g and 4°C for 20 min. Proteins were precipitated by stepwise addition of solid ammonium sulphate to 40 % and in a second step to 80 % saturation. After each step the extracts were centrifuged at 20.000 g and the pellets were resuspended in 1 ml of 20 mM Tris HCl buffer, pH 7.8. The extracts were desalted and further purified by gel-filtration (PD 10, Pharmacia, Freiburg, Germany).

Protein determination and spectrometric enzyme assays

Protein contents of the samples were determined by the standard method of Bradford (1976) using bovine serum albumin (BSA) as reference protein. Glutathione S-transferase (GST) activity was quantified at 25 °C in standard spectrophotometric tests following the methods of Habig *et al.*, (1974). In short, aliquots of the enzyme extract were incubated with 0.1 M Tris HCl buffer pH 7.8, 1 mM GSH with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB, $\varepsilon_{340\ nm} = 9.6$ $mM^{-1}cm^{-1}$) or 1 mM 1,2-dichloro-4-nitrobenzene (DCNB, $\varepsilon_{345\ nm} = 8.5$ $mM^{-1}cm^{-1}$) at a total assay volume of 0.6 ml. Controls lacking enzyme extracts or GSH were measured.

Kinetics/Inhibition - Measurements of steady state kinetics were performed at 25°C in 0.1 M Tris/HCl buffer. Michaelis-Menten constants for CDNB, DCNB and GSH were determined from Lineweaver-Burk plots. The measurements were performed according to standard assay

procedures (see above), with substrate concentrations varying from 0.0625 to 1 mM. The enzymatic rate of conjugation was corrected for non-enzymatic rates.

The capability to conjugate herbicides was also investigated via substrate inhibition tests, assayed with fenoxaprop-P, propachlor (PCl), pethoxamid (PA) and terbuthylazine (TBA). The concentration of the inhibitors was varied in a range from 0.0625 to 1 mM. Also in this case, GST activity for CDNB was determined at 25 °C following the standard spectrophotometric test procedures (Habig *et al.*, 1974).

HPLC - High-performance liquid chromatography (HPLC) of herbicide metabolites was performed with a Varian Model 230 HPLC System equipped with a Gynkotek SP-4 UV monitor at 254 nm. For HPLC, a RP-C_{18} Hypersil-ODS-column 5,0 µm, 250 x 4,0 mm (Bischoff) was used with acetonitrile/water/0.1 % trifluoro-acetic acid. Peaks were eluted at 1 ml/min in a gradient from 60 % acetonitrile for 5 min followed by a 15 min increase to 100 % acetonitrile.

RESULTS

Enzyme activity - Glutathione S-transferase activity for the model xenobiotic CDNB was found in leaves and rhizomes of *Phragmites*, in accordance with previous reports (Pietrini *et al.*, 2003; Schröder *et al.*, 2005). In addition, activity for the conjugation of the closely related DCNB was detected. Generally, rhizomes exhibited 10-fold higher GST activities than leaves, despite the lower protein contents (0.85 mg/g FW as compared to 9.08 mg/g FW) of the storage tissue. This suggests the importance of the enzyme in root metabolism and defence against foreign compounds. Whereas the K_M-values for CDNB are equal in both rhizomes and leaves, significant differences were measured for DCNB.

Table 1. K_M- und V_{MAX}-values for GST in *Phragmites* rhizomes and leaves. r^2 values were generally above 0.9. All measurements were done in triplicate; n.d. not determined

	leaves		rhizomes	
	K_M (mM)	V_{MAX} (µkat/mg prot)	K_M (mM)	V_{MAX} (µkat/mg prot)
CDNB	0.83	1.58	0.82	8.98
GSH/CDNB	0.19	0.86	0.16	5.15
DCNB	0.11	0.046	0.26	0.11
propachlor	0.088	0.069	2.68	17.64
terbuthylazine	n.d.	n.d.	1.34	0.70

Furthermore, the affinity of the rhizome enzyme versus the co-substrate, glutathione, was higher in rhizomes than in leaves. K_M- and v_{max}-values for the conjugation of the chloroacetamide propachlor showed a similar tendency. They were lower in leaves but extremely higher in rhizomes than for CDNB. The K_M-value for terbuthylazine was found to be 1.34 mM in the rhizomes (Table 1).

It had previously been concluded that CDNB conjugation might be competitively inhibited by herbicides (Schröder, 1997). Adding fenoxaprop-P to standard enzyme assays as an inhibitor of *Phragmites* GST resulted in a typical Dixon plot with a clear interception above the x-axis.

This is indicative of a classical competitive inhibition in leaves with a K_I of 0.55 mM and in rhizomes with a K_I of 0.09 mM (Figure 1). Contrary to this, increasing inhibitor concentrations of the herbicides pethoxamid, propachlor and terbuthylazine did not result in first order kinetics, but rather in asymptotic curves.

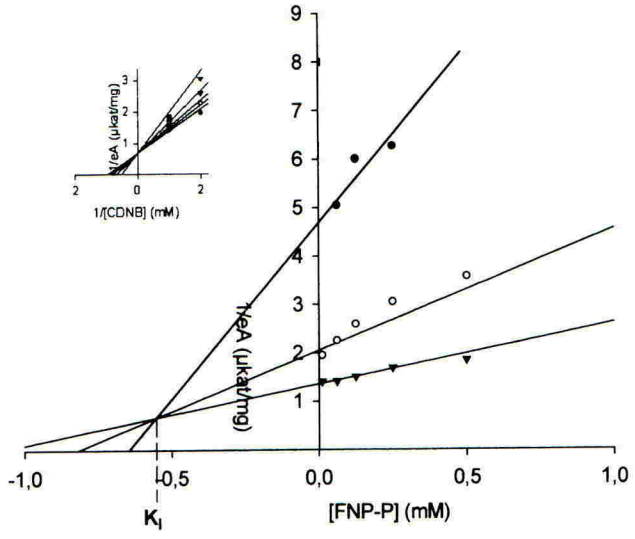

Figure 1. Inhibition of the GST activities from *Phragmites australis* leaves by fenoxaprop-P. Enzyme extraction was according to the materials and methods section and GST activity for CDNB and the herbicide fenoxaprop-P was determined. All experiments were performed in triplicate.

From these curves, at least two derivative lines can be drawn, resulting in two distinct K_I–values for the reaction under consideration. The K_I–values in leaves are 1.56 mM and 2.65 mM with pethoxamid, and 0.56 mM and 1.96 mM with propachlor. The K_I–values in rhizomes are 2.15 mM and 3.07 mM with pethoxamide and 1.12 mM with terbuthylazine. This is indicative of the presence of different sets of isoenzymes in leaves and rhizomes with different inhibitor constants. Similar results have been presented for the GSTs in tree species (Schröder & Götzberger, 1997).

The inhibition constants for other herbicidal substrates are given in Table 2. Clearly there is a huge difference between K_I values in leaves and rhizomes, with best inhibition by fenoxaprop-P. Inhibiting CDNB activity with pethoxamide yields two different K_I values, which is indicative of several isoforms of GST being involved in its detoxification. This is similar to the closely related propachlor, for which detoxification capacity seems to be only present in leaves. In contrast to this finding, terbuthylazine seems to be only conjugated by rhizomes.

Table 2. K_I-values shows the inhibition for the extracted enzymes of *Phragmites* rhizomes and leaves. The concentration of the inhibitors was varied in a range from 0.0625 to 1 mM. GST activity was determined following the standard test. R^2 values were generally above 0.9. All measurements were done in triplicate; n.d. not determined

		leaves		rhizomes	
		K_I (mM)		K_I (mM)	
fenoxaprop-P	K_I	0.55	±0,12	0.09	±0,03
pethoxamid	K_{I1}	1.56	±0,34	2.15	±0,37
	K_{I2}	2.65	±0,77	3.07	±0,98
propachlor	K_{I1}	0.56	±0,03	n.d.	
	K_{I2}	1.96	±0,14	n.d.	
terbuthylazine	K_I	n.d.		1.12	±0,32

DISCUSSION

Phragmites australis appears to be a good candidate to diminish agrochemicals in water bodies. McGonigle *et al.* (2000) reported similar catalytic properties in maize and soyabean. Their findings confirm large differences in specific activities and also higher conjugative activities for herbicides than for the standard substrate, CDNB. differences between shoot and rhizome activities suggest the existence of different sets of GST isozymes in the plant, which might be under differential developmental or environmental control. Clearly some differences exist concerning the xenobiotic binding site (Cummins, 1997). These results are of special importance, as Dixon *et al.* (2003) were able to show that plant Phi GSTs exhibit high activities for the conjugation of chloroacetanilides, whereas GSTs of the Tau family are active against aryloxyphenoxy-propionic acids. Whereas some isoforms seem to have overlapping substrate specificities, the nitrobenzylchlorides seem to be substrates to distinct GST groups (Schröder *et al.*, 2005).

Overall, reed seems to be a good candidate for phytoremediation of organic xenobiotics from water bodies. Further studies must include the role of the leaves in phytoremediation and try to elucidate the induction mechanisms as well as the GST family structure in *Phragmites*. Further studies should include the search for potent inducers of GSTs in plants for phytoremediation and the elucidation of the GST family structure.

REFERENCES

Bradford M M (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Annals of Biochemistry* **72**, 248-255.

Coleman J O D; Blake-Kalff M M A; Davies T G E (1997). Detoxification of xenobiotics by plants: Chemical modification and vacuolar compartmentation. *Trends Plant Science* **2**, 144-151.

Cummins I; Cole D J; Edwards R (1997). Purification of multiple glutathione transferases involved in herbicide detoxification from wheat *(Triticum aestivum L.)*. *Pesticide Biochemistry and Physiology* **59**, 35-49.

Dixon D P; McEwen A G; Lapthorn A J; Edwards R (2003). Forced evolution of a herbicide detoxifying glutathione transferase. *Journal of Biology and Chemistry* **278**, 23930-23935.

Frear D S (1976). Pesticide conjugation – glycosides. ACS Symposium Ser. 29, 35-54.

Habig W H; Pabst J; Jakoby W B (1974). Glutathione S-transferases, the first step in mercapturic acid formation. *Journal of Biology and Chemistry* **249**: 7130-7139.

Lamoureux G L; Rusness D G (1989). The role of glutathione and glutathione S-trans-ferase in pesticide metabolism, selectivity and mode of action in plants and insects. In: *Glutathione: Chemical, Biochemical and Medical aspects.* Series: Enzyme and Cofactors, pp. 155-195. John Wiley and Sons: New York

McGonigle B; Keeler S J; Lau S M; Koeppe M K; O´Keefe D P (2000). A genomics approach to the comprehensive analysis of the glutathione S-transferase gene family in soybean and maize. *Plant Physiology* **124**, 1105-1120.

Pietrini F; Iannelli M A; Pasqualini S; Massacci A (2003). Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. ex Steudel. *Plant Physiology* **133**, 829-837.

Sandermann H; Haas M; Messner B; Pflugmacher S; Schröder P; Wetzel A; (1997). The role of glucosyl and malonyl conjugation in herbicide selectivity. In: *Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants*, ed. K K Hatzios pp. 211-231. Kluwer Academic: Dordrecht.

Schröder P (1997). Fate of Glutathione S-conjugates in plants: Cleavage of the glutathione moiety. In: *Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants*, ed. K K Hatzios pp. 233-244. Kluwer Academic: Dordrecht.

Schröder P; Lamoureux G L; Rusness D G; Renneberg H (1990). Glutathione S-transferse activity in spruce needless. *Pesticide Biochemistry and Physiology* **37**, 211-218.

Schröder P; Götzberger C; (1997). Partial purification and characterization of glutathione S-transferse isozymes from the leaves of *Juniperus communis*, *Larix decidua* and *Taxus baccata*. *Journal of Applied Botany and Food Quality* **71**, 31-37.

Schröder P; Collins C (2002). Conjugating enzymes involved in Xenobiotic Metabolism of Organic Xenobiotics in Plants. *Int. J. Phytorem.* **4,** 247-265.

Schröder P; Maier H; Debus R (2005). Detoxification of herbicides in Phragmites australis. *Zeitschrift für Naturforsch* **60c**, 317-324.

Segel I H (1975). *Enzyme kinetics, behaviour and analysis of rapid equilibrium and steady-state enzyme systems.* Wiley-Interscience: Toronto.

Shimabukuro R H (1976). Glutathione conjugation of herbicides in plants and animals and its role in herbicidal selectivity. *Asian-Pacific Weed Science Society,* 183-186.

Significance of preferential flow as a transport mechanism for pesticides in lysimeters containing sandy soil cores

W Hein, G Fent, R Kubiak
RLP Agroscience, Institute for Agro-Ecology, Ecochemistry Group, Breitenweg 71, 67435 Neustadt, Germany
Email: Werner.hein@agroscience.rlp.de

ABSTRACT

Outdoor lysimeter studies with undisturbed soil cores have become an important tool for the identification of pesticides with a leaching potential. Besides the vertical single phase pore flow, physical non-equilibrium effects such as 'preferential flow' might also be important transport mechanisms. Sorption and degradation kinetics determine the availability of the pesticide for leaching. Soil-intrinsic properties, however, also determine the pathways that water follows. In order to describe the significance of preferential flow paths, 23 outdoor lysimeters with undisturbed soil cores were taken from a field and applied with the water tracer 2,6-difluorobenzoic acid in autumn. Two different initial soil moistures were adjusted in the soil cores. The velocity of downward percolating water in each lysimeter was determined on the basis of the tracer concentration in the leachates. Tracer findings directly after application, unlike the main breakthrough of the tracer, were used to assess the relative importance of preferential flow effects in the individual lysimeter. It was possible to identify individual lysimeters (about 25%) with the potential for preferential flow. Lysimeter leaching studies should only be carried out with lysimeters if the history of the water and tracer transport is known.

INTRODUCTION

Losses of pesticides from soil by leaching may only amount to less than 1% of the application, yet the impact of the process can cause considerable environmental concern. Outdoor lysimeter studies with undisturbed soil cores have become an important tool for the identification of pesticides with a critical leaching potential regarding their use patterns and intrinsic pesticide properties. Beside the vertical single phase pore flow of water and solutes, physical non-equilibrium effects such as 'preferential flow' might also be important transport mechanisms responsible for possible groundwater contamination. Root channels, earthworm burrows, and the soil monolith sampling procedure can lead to cavities and cracks along the walls of the lysimeter, resulting in the rapid transport of water and chemicals. Because lysimeter studies are costly in terms of time and money, the definitive pesticide leaching study should be carried out only with lysimeters which are not black boxes concerning water movement and the predominant transport mechanism. In this context, a tracer experiment was carried out in order to address the following:

- Is it possible to identify individual lysimeters with the potential of preferential flow?
- Is preferential flow in lysimeters with sandy soil cores an important transport mechanism?
- Does the storage time between soil core sampling and application affect the significance of preferential flow events?

- Does the initial soil water content on the day of application affect the tracer outflow?

MATERIALS AND METHODS

Lysimeter (sampling site, collecting procedure, soil properties)

The lysimeters were collected at a field site close to the village of Birkenheide (Rhineland Palatinate) in south-western Germany. The Birkenheide field is located at 49°29' Northern Latitude: 8°15' East. The long-term average yearly air temperature at this site is 10.1°C and the mean annual natural rainfall is 634 mm. Soil cores collected from the Birkenheide site meet the requirements of the German registration authorities.

The lysimeters used in this experiment were collected between 1993 and 1999. A large excavator pressed the stainless steel cylinders into the undisturbed field soil and carefully pulled them out again. After excavation, the bottom sieve plates were attached to the base of the cores. This was to ensure the soil was kept within the container and to enable free drainage of the leachate at the lower end of the lysimeters. The lysimeters have a soil core depth of 130 cm, surface area 0.8 m^2.

According to the FAO classification of soils, the Birkenheide soil is classified as a Luvic Arenosol, developed from aeolian sand over fluvial loams. The soil is a loamy sand (> 70% sand, and < 10% clay) with a low organic carbon content (<1.5%). The water table is below 300 cm soil depth and the soil is well drained. Sorption and buffering capacity is generally low. The soil is used for viticulture and fruit growing.

A total of 23 lysimeters were used for the experiment. In the following, the different replacement series are given:

- 3 lysimeters with an initial soil moisture of 75% of the field capacity (no additional irrigation in summer 1999) (Referred to as 'Unsaturated')
- 20 lysimeters with an initial soil moisture corresponding to the field capacity (additional irrigation in summer 1999) (Referred to as 'Saturated')

Of these:

- 9 lysimeters collected in May 1995
- 7 lysimeters collected in May 1999

(7 further lysimeters were sampled at different dates and were not further considered for evaluation in this context)

Tracer (application, sampling, analytical methods)

Difluorobenzoic acid (2,6-DFBA) was used as a conservative tracer. Each lysimeter was applied with 8 g of DFBA. For this purpose, 500 g of top-soil was removed from each lysimeter and, after having thoroughly mixed the tracer into the soil, the mixture was applied onto the bare soil of the lysimeter as a thin layer of soil. The application date was September 27, 1999. Leachate was collected at least every week. After volume determination the pH, conductivity, and dissolved organic carbon were determined. For DFBA investigation, leachate samples were analysed by means of an anion-hplc coupled with conductivity detector. The limit of quantification was 1 mg/litre.

RESULTS AND DISCUSSION

Water balance

Within the experimental period (September 27, 1999 to April 19, 2000) a total amount of 348 mm natural rainfall was recorded at the lysimeter station. The additional irrigation corresponded to 98 mm, giving a total water input of 446 mm. Individual results concerning daily rainfall, daily additional irrigation, and cumulative water input are given in Figure 1. Within the first two weeks after application 28.6 mm of water reached the lysimeters, including a single irrigation of 10 mm per hour. Favourable conditions concerning the initiation of preferential flow events were therefore given.

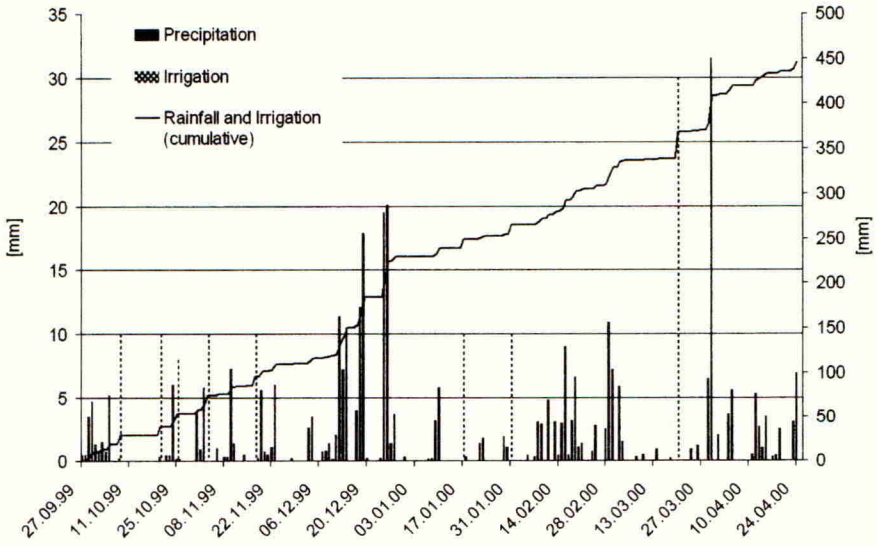

Figure 1. Daily rainfall, daily irrigation and cumulative water input

As expected, the water-saturated lysimeters revealed a significantly greater total volume of leachate than the unsaturated lysimeters. About 100 mm of leachate was collected in the saturated lysimeters before the first leachate was collected in the unsaturated lysimeters. Over the course of the experiment, a mean total of 303 mm [Coefficient of Variation (CV) 8.2 %] of leachate was collected in the saturated lysimeters, whereas a mean of only 167 mm, (CV 13.8%) was collected in the unsaturated lysimeters (Figure 2).

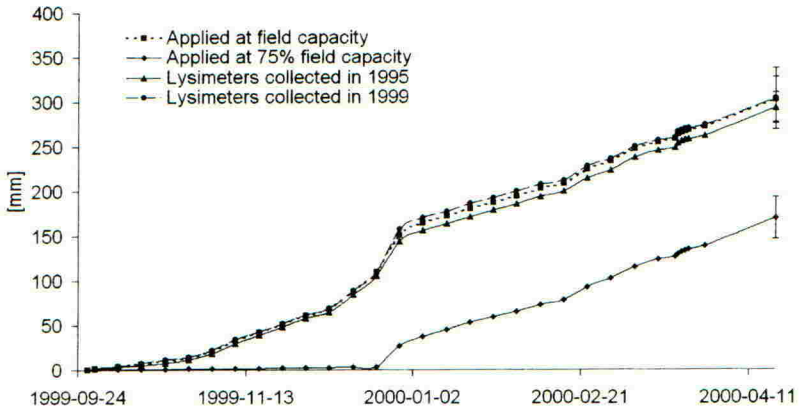

Figure 2. Comparison of cumulative leachate outflow from saturated and unsaturated lysimeters

The cumulative leachate outflow of lysimeters collected at two different sampling dates is shown in Figure 2. The comparison indicates that there was little difference between the lysimeters collected in 1999 and those collected in 1995. The dynamic of the leachate outflow and the total amount of leachate were in the same order of magnitude: mean values of 293 mm (CV 5.8%), and 303 mm (CV 10.9%), for the lysimeters collected in 1999 and 1995, respectively.

Solute movement

The DFBA breakthrough curves (BTCs) of all tested lysimeters are given in Figure 3. The BTCs clearly indicate that matrix flow was the predominant transport mechanism in the loamy sand lysimeters, although preferential flow events were determined in the first three weeks after application in some lysimeters. In most of the leachates the first tracer concentration above the limit of quantification was determined after the formation of 110 mm of leachate (87 days after application). The maximum of the BTCs was determined after 233 mm of leachate (157 days after application).

The BTCs of the lysimeters sampled in 1995 and 1999 were almost similar (Figure 4). No differences with respect to DFBA transport behaviour were observed by comparing the BTCs obtained from saturated and unsaturated lysimeters (Figure 5).

Water and solute balance

About 70% of the total water input was sampled as leachate in the water-saturated lysimeters. Since the experimental period was between autumn and spring, the evapotranspiration losses accounted for only about 30% of the total water input. In the case of the unsaturated lysimeters, about 35% of the total water input was required to increase the moisture content of the lysimeter to field capacity. About 80% of the applied DFBA was transported through the soil

cores in the course of the experiment, independent of the storage time and initial moisture of the lysimeters.

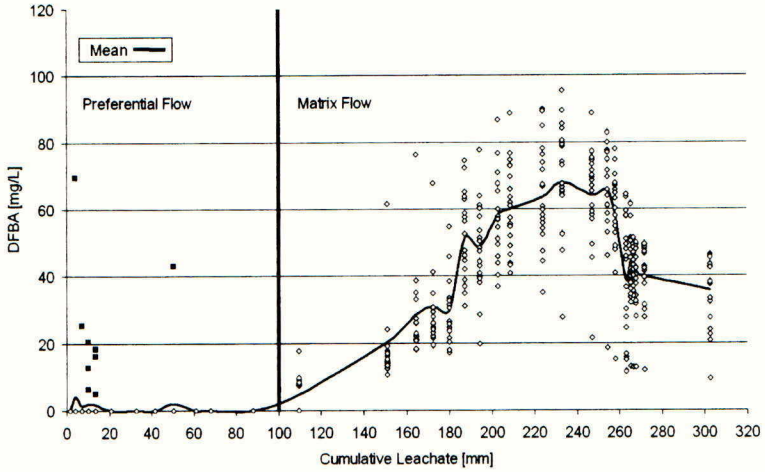

Figure 3. Breakthrough curves of DFBA (mean and individual of each lysimeter)

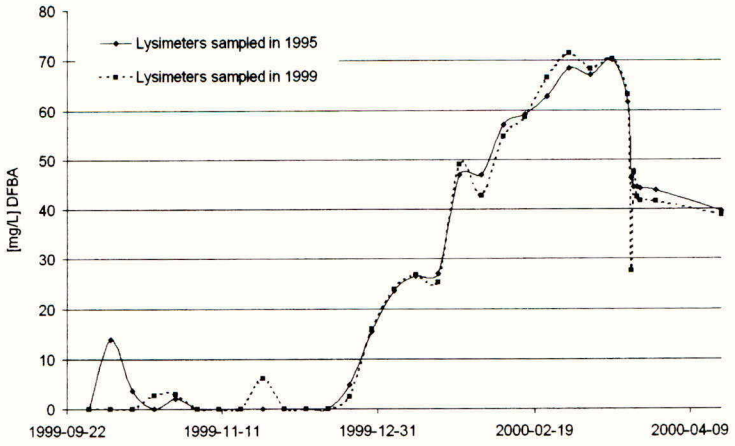

Figure 4. BTCs of the DFBA from lysimeters with different storage times

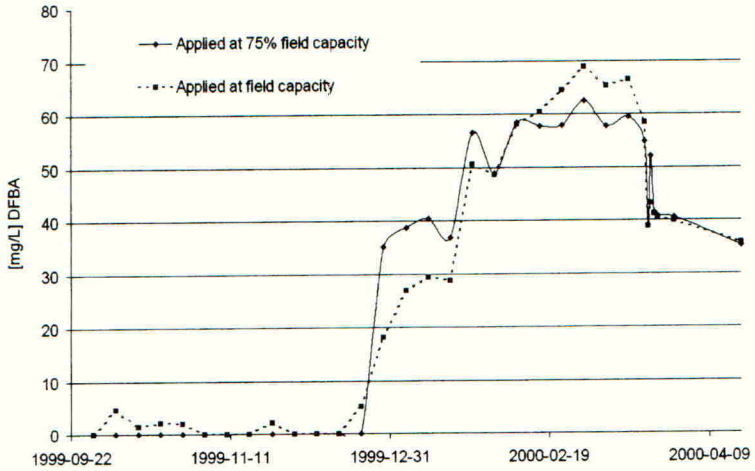

Figure 5. BTCs of the DFBA from lysimeters with different initial soil moisture

Frequency of preferential flow events

As already mentioned, the frequency of preferential flow events was not affected by the storage time between lysimeter collection and tracer application. Matrix flow was the predominant transport mechanism in about 75% of the lysimeters. Preferential flow events were detected in about a quarter of the lysimeters, resulting in solute leaching in the range of 0.7 to 7.4% of the total tracer outflow.

CONCLUSIONS

- The storage time between lysimeter collection and tracer application did not affect the frequency of preferential flow events in loamy sand lysimeters.
- It was possible to identify individual lysimeters with the potential of preferential flow. About a quarter of the lysimeters showed preferential flow events.
- Lysimeter leaching studies for registration purposes should only be carried out with lysimeters if the history of the water and tracer transport is known. This can be evaluated by the application of a conservative tracer (e.g. DFBA).
- A large number of lysimeters with a known history allows the selection of lysimeters with identical transport behaviour with respect to solute and substance transport (fewer replicates are necessary).

Fugacity concept use for prediction of carbofuran environmental behaviour in irrigated rice crops

L P M Plese, L L Foloni
Universidade Estadual de Campinas, Feagri, Campinas, 13088-970 SP, Brazil

L C Paraíba
Embrapa Meio Ambiente, CP 69, Jaguariúna, 13820-010 SP, Brazil

ABSTRACT

The objectives of this work were to use the level IV fugacity model to simulate the environmental fate of carbofuran as employed in rice cultivation. The fugacity model was used to simulate the dynamic distribution of the carbofuran in a system comprising air, water, rice plants and soil. Results indicate the preferential compartments of the pesticide, facilitating the strategies for monitoring environmental quality, and providing further knowledge of the environmental fate of carbofuran. Experiments under field conditions were carried out to verify the correspondence between simulated and measured values of carbofuran concentration in water and soil.

INTRODUCTION

Carbofuran is a systemic carbamate insecticide and a cholinesterase inhibitor that has been used in rice cultivation. It is highly toxic to vertebrates and is used to control insects in a wide variety of agricultural crops including coffee, corn, sugar cane and rice (Tomlin, 2000). In Brazil, it is usually applied directly to the soil in granular form 15 to 30 days after rice germination in order to control rice beetle larvae (*Oryzophagus oryzae*). Plese *et al.* (2005) modelled the kinetics of carbofuran hydrolysis and the subsequent degradation in an irrigated rice fields of Brazil. This study had the aim of employing a level IV fugacity model to simulate the distribution of carbofuran insecticide in different compartments of a rice field. The level IV fugacity model is presented as a system of ordinary differential equations within a fugacity framework that estimates carbofuran concentrations in different environmental compartments (Mackay, 2001).

MATERIALS AND METHODS

The relationship between fugacity and concentration is given as follows:

$$C = Zf$$

Where C is the pesticide concentration (mol/m^3), f is the pesticide fugacity (Pa) and Z is the fugacity capacity (mol/m^3 Pa^1). The compartments of the rice cultivation system modeled in this work are air (i = a), water (i = w), rice plants (i = r) and soil (i = s), i.e., $i \in I = \{a,w,r,s\}$.

The fugacity capacity of air is defined as:

$$Z_a = \frac{1}{RT}$$

Where Z_a is the fugacity capacity of air, T is the air temperature (K) and R is the gas constant (i.e. 8.314 m^3 Pa/mol T). The fugacity capacity of water is defined as:

$$Z_w = \frac{1}{H} + \frac{OC_w \rho_w k_{oc}}{H}$$

Where Z_w is the fugacity capacity of water and H is Henry's constant of the pesticide (m^3 Pa/mol), OC_w is the organic carbon volumetric fraction of the water (m^3/m^3), ρ_w is the water density (kg/m^3), and k_{oc} is the organic carbon partition coefficient of the pesticide (m^3/kg). The fugacity capacity of the rice plants was estimated as:

$$Z_r = (x_w Z_w + x_l k_{ow} Z_w)\rho_r / \rho_w$$

Where Z_r is the fugacity capacity of the rice plants, ρ_r is the rice plant density (kg/m^3), k_{ow} is the pesticide octanol-water partition coefficient (m^3/m^3), x_w is the water volumetric fraction of the rice plant (m^3/m^3) and x_l is the lipids volumetric fraction of the rice plant (m^3/m^3) (Trapp & Farlane, 1995). The fugacity capacity of the soil was estimated as:

$$Z_s = \frac{\theta}{H} + \frac{OC \rho_s k_{oc}}{H}$$

Where Z_s is the fugacity capacity of the soil, θ is the water volumetric fraction of the soil (m^3/m^3), ρ_s is the density of soil (kg/m^3), OC is the organic carbon volumetric fraction of the soil (m^3/m^3), and k_{oc} is the organic carbon partition coefficient of the pesticide (m^3/kg^1). Pesticide mass flow resulting from diffusion between two contiguous compartments i and j can be calculated as:

$$N_{ij} = d_{ij}(f_i - f_j)$$

Where N_{ij} is the pesticide mass flow between compartments i and j (mol/h), and d_{ij} is the transfer coefficient (mol/Pa h). According Fick's first law, these transfer coefficients is given as:

$$d_{ij} = \frac{A_{ij} D_{pi} D_{pj} Z_i Z_j}{\delta_{ij}(D_{pi} Z_i + D_{pj} Z_j)}$$

Where A_{ij} is the contact area between compartments i and j (m^2), D_{ij} is the pesticide diffusivity in compartment i (m^2/h), D_{pj} is the pesticide diffusivity in compartment j (m^2/h), δ_{ij} is the thickness of the diffusion layer between compartments i and j (m). The water and soil contact areas between the 0.0-0.2 m depth was calculated by the following expression:

$$A_{ws} = \rho_s S_s V_s$$

Where S_s is the soil specific surface area (m^2/kg). The pesticide diffusivity in air was estimated as:

$$D_{pa} = \frac{3.6 \times 10^{-4} T^{1.75} \sqrt{M_{pa}}}{(\sqrt[3]{v_p} + \sqrt[3]{v_a})^2}$$

Where D_{pa} is the pesticide diffusivity in air (m^2/h), v_p is the molar volume of the pesticide (cm^3/mol) and v_a is the molar volume of the air (i.e. 20.0 cm^3/mol). M_{pa} is given by:

$$M_{pa} = \frac{p_m + a_m}{p_m p_a}$$

Where a_m is the molar mass of air (i.e. 28.9 g/mol). Considering that rice plants have high water volumetric fraction (>0.8 m/m), the model supposes that the diffusivity of the pesticide in rice plants is equal to the diffusivity of the pesticide in water, i.e., $D_{pr} = D_{pw}$. Pesticide disappearance or transformations in air, water, rice plants and soil can occur by physical and chemical process or biological degradations, by dilution during rice growth or by water volume variation in rice fields. These pesticide process was assumed as first-order processes and are described by:

$$\frac{dC_i}{dt} = -\lambda_i C_i$$

Where λ_i is the transformation rate (h) which were estimated by:

$$\lambda_i = \frac{\ln 2}{t_{1/2}^i}$$

Where $t_{1/2}^i$ is the pesticide half-life in compartment i (h). Thus, in the level IV fugacity model the term that describes the pesticide transformation or disappearance in a compartment i is given by:

$$V_i Z_i \frac{df_i}{dt} = -\lambda_i f_i V_i Z_i$$

Where V_i is the volume of compartment i (m^3). Pesticide advection in compartment i can be introduced in the model as a first-order process. In fact, advection can be regarded as a constant speed, defined as the algebraic sum between the entry flow $G_i C_{Bi}$ and the exit flow $G_i C_i$, or in terms of fugacity as $G_i Z_i f_i$, where G_i is the matter flow i entering compartment i (m^3/h) with concentration C_{Bi} and leaving this compartment with concentration C_i (Mackay, 2001). The mass distribution of the pesticide is given by system of ordinary differential equations:

$$\frac{df_a}{dt} = \frac{N_{wa}(f_w - f_a)}{V_a Z_a} + \frac{N_{ra}(f_r - f_a)}{V_a Z_a} + \frac{G_a C_{Ba}}{V_a Z_a} - \frac{G_a f_a}{V_a} - \lambda_a f_a$$

$$\frac{df_w}{dt} = \frac{N_{aw}(f_a - f_w)}{V_w Z_w} + \frac{N_{rw}(f_r - f_w)}{V_w Z_w} + \frac{N_{sw}(f_s - f_w)}{V_w Z_w} + \frac{G_w C_{Bw}}{V_w Z_w} - \frac{G_w f_w}{V_w} - \lambda_w f_w$$

$$\frac{df_r}{dt} = \frac{N_{wr}(f_w - f_r)}{V_r Z_r} + \frac{N_{ar}(f_a - f_r)}{V_r Z_r} - \lambda_r f_r \text{ and } \frac{df_s}{dt} = \frac{N_{ws}(f_w - f_s)}{V_s Z_s} + \frac{G_s C_{Bs}}{V_s Z_s} - \frac{G_s f_s}{V_s} - \lambda_s f_s$$

The initial condition is defined as:

$$f_a(0) = f_s(0) = 0 \text{ and } f_w(0) = (A_r P_d)/(V_w Z_w)$$

A_r is the total area of rice field (m^2) and P_d is the pesticide dose (mol m^{-2}). For $i \in I$ and $t \geq 0$, the concentrations $C_i = C_i(t)$ are obtained by $C_i = Z_i f_i(t)$.

The field experiment was carried out in a 200 ha area of irrigated rice crop located in the municipality of Bariri, State of São Paulo, Brazil (22°02'45" S and 48°43'46" W). The area was subdivided in 1.5 and 2.5 ha rice fields that were separated by irrigation and drainage channels. The entire area is managed according to usual procedures for irrigated rice crop. A soil solution sampler consisted of a porous capsule attached to a PVC tube (1.27 cm inner diameter and 30 cm length), two silicone corks (one in a plastic bottle and another in the PVC tube) and a hose. The soil solution was pumped through the hose up to the bottle using a manual pump. When the soil was dry, eight samplers were randomly installed in the experimental area at 20 cm depth, nine days before rice sowing. The paddy water, or laminar water was also collected using plastic bottles and samples were obtained by fast bottle immersion in eight randomly places in the plot. Temperature and pH were determined in all laminar water and soil solution samples using a portable pH-meter (PG1400, GENAKA). Samples were immediately placed in icebox for transportation and stored at -18°C. Laminar water and soil solution were sampled at 24, 48, 96, 192, 384 and 768 hours after carbofuran application. Carbofuran extraction from water consisted of 100 ml sample extraction of dichloromethane. Carbofuran was measured using a gas chromatography system HP-5MS capillar column (length - 30 m, diameter - 0.25 mm); (film thickness - 0.25 μm), with oven temperature programmed as follows: initial = 100°C, for 1 min; slope: 25°C up to 280°C, kept for 2 min and 30 s. Carbofuran physicochemical characteristics provided as input parameters were molar mass, molar volume, vapor pressure, aqueous solubility, octanol-water partition coefficient, and organic carbon partition coefficient in soil (Tomlin, 2000). Carbofuran was applied at rate 1.05×10^{-4} mol/m^2 (i.e. 0.23 kg/ha) that resulted in a concentration of 1.04×10^{-3} mol/m^3 in the water 24 h after application, 768 h after application the concentration had declined to 3.67×10^{-6} mol/m^3.

DISCUSSION

The carbofuran half-life in water at 29°C and pH 6.6 and soil solution in irrigated rice field capacity was estimated as 78 and 241 h, respectively (Plese *et al.*, 2005). The carbofuran half-life in air and rice plants was determined as 12 h and 36 h, respectively (Tejada & Magallona, 1985). These measured carbofuran half-life in soil, water, air and rice plants were used as

silt and sand volumetric fraction were measured using site-specific soil sample and had values of 1.54×10^{3} kg/m^3, 0.42, 0.017, 0.25, 0.09 and 0.64 m^3/m^3, respectively. The water density and organic carbon volumetric fraction of the water was 1.01×10^{3} kg/m^3 and 1.2×10^{-3} m^3/m^3, respectively. The average volumetric fraction of water in soil was 0.48 m^3/m^3 at 0-0.2 m depth. The specific surface area of the soil was estimated as 6.94×10^{4} m^2/kg. The contact areas between the compartments air and water, water and soil, air and rice plants, and water and rice plants were estimated as 2.0×10^{4}, 4.03×10^{11}, 1.5×10^{4}, and 3.0×10^{3} m^2, respectively. The density of rice plants, volumetric fraction of water and lipids in rice plants were 1.03×10^{3} kg/m^3, 0.80 and 0.02, respectively. Water density and air temperature were 1.0×10^{3} kg/m^3 and 298 K, respectively. For $i,j \in I$ the model supposes that $d_{ij} = d_{ji}$ and $d_{sr} = d_{rs}$. Volumes of air, water, rice plants and soil were 8.0×10^{4}, 2.0×10^{3}, 1.2×10^{3}, and 5.0×10^{3} m^3, respectively. The transfer coefficients between air and water, water and soil, water and rice plants, and air and rice plants was estimated as 350.16, 2.7×10^{12}, 1.03×10^{5}, 262.65 mol/Pa h. For all $i \in I$, $G_i = 0$ and $G_w = 1.89\times10^{-5}$ m^3/h. G_w was estimated considering daily precipitation, water evaporation, rice evapotranspiration and water recharge area of the rice field. The time range for numerical simulations was 1000 h. We used the algorithm proposed by Paraíba *et al.* (1999) to numerically simulate fugacity and concentration using the Matlab code. Simulations have shown that the time required for the fugacity values to stay within a range of a final equilibrium value is over 1000 hours. Fugacity decreases in a uniform way in all compartments until it reaches the equilibrium level with fugacity values around 10^{-12} Pa.

We observed that carbofuran concentrations in water decreases while it increases in air, rice plants and soil until maximas are reached (Figures 1,2). Carbofuran concentrations were highest in the following compartments: water > soil > rice plants > air. In general, carbofuran is applied only to water and then is transferred to rice plants and soil. The estimated fugacity capacities in the air, water, rice plants and soil were 4.04×10^{-4}, 2.2×10^{4}, 3.3×10^{4} and 3.8×10^{4} mol/m^3 Pa^1, respectively. The estimated carbofuran rice-water partition coefficient was 1.52 (unitless). This partition coefficient partially explains the simulated concentration levels in rice plants. Soil sorption and soil-water partition coefficient of carbofuran (k_{oc} = 0.022 m^3/kg and k_{sw} = 1.75, respectively) indicate low affinity with soil solid particles and high affinity with both laminar water and soil solution. The level IV fugacity model used in this work underestimated the water and soil solution carbofuran concentrations (Figure 2). We believe that level IV fugacity model can reasonably predict carbofuran concentration in the rice environment. As the concentrations in air and rice plants were not measured, we cannot draw conclusions concerning predictions of carbofuran concentrations in these compartments. Results suggest that the model can be used to determine which environmental compartment is more vulnerable to carbofuran.

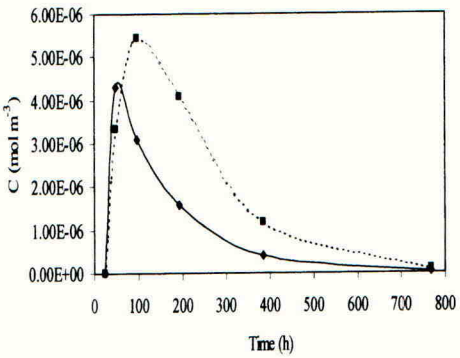

Figure 1. Carbofuran concentrations in air (◆ 1.0E+6×C_a) and rice plants (■ C_r) as simulated by level IV fugacity model.

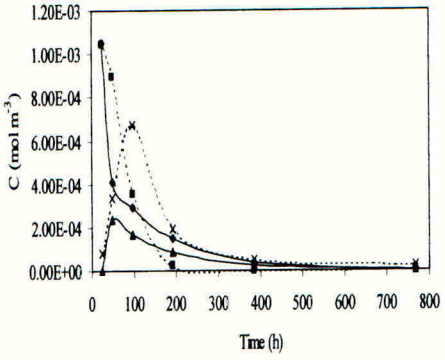

Figure 2. Measured and simulated carbofuran concentration in soil (◆ C_s - simulated) and (■ C_s - measured), and water (▲ C_w - simulated) and (× C_w - measured).

REFERENCES

Mackay D (2001). *Multimedia environmental models: the fugacity approach*, Second Edition. CRC Press: Boca Raton, FL.

Paraíba L C; Carrasco J M; Bru R (1999). Level IV fugacity model by a continuous time control system. *Chemosphere* **38,** 1763-1775.

Plese, L P M; Paraiba L C; Foloni, L L; Trevizan L R P (2005). Kinetics of carbofuran hydrolysis to carbofuran and the subsequent degradation of this last compound in irrigated rice fields. *Chemosphere* **60**, 149-156.

Tejada A W; Magallona E D (1985). Fate carbosulfan in a model ecosystem. *Philippines Entomology* **6**, 275-285.

Tomlin C D S (2000). *The pesticide manual*. British Crop Protection Council: Farnham.

Trapp S; McFarlane J C (1995). *Plant contamination: modeling and simulation of organic chemical processes.* Lewis Publishers: Chelsea.

Glutathione mediated metabolism of the safener MG-191

I Jablonkai
Institute of Biomolecular Chemistry, Chemical Research Centre, Hungarian Academy of Sciences, PO Box 17, H-1525 Budapest, Hungary.
E-mail: jabi@chemres.hu

D P Dixon, R Edwards
School of Biological and Biomedical Sciences, University of Durham, Durham DH1 3LE, UK

ABSTRACT

In vitro metabolism of the safener MG-191 was studied by incubating the safener with glutathione and various recombinant glutathione transferases such as the maize phi class *Zm*GSTF1-1 and *Zm*GSTF3-3, the maize tau class *Zm*GSTU1-1 and the *Arabidopsis* zeta class *At*GSTZ1-1 isozymes. All GSTs catalyzed the GSH-dependent dechlorination of the safener and the formation of a pyruvic aldehyde ethylene ketal was verified by GC-MS analyses. A mechanism leading through unstable glutathione conjugates for the formation of the aldehyde metabolite was suggested.

INTRODUCTION

Safeners are chemical agents that increase the tolerance of crop plants to herbicides without affecting the weed control efficacy. Studies on their mode of action account for the primary focus of the research (Hatzios & Burgos, 2004). It has been widely accepted that safeners can enhance the plant detoxifying enzymes such as glutathione S-transferases and cytochrome P-450 monooxygenases (Davies & Caseley, 1999). Unfortunately, data on safener metabolism and the biological significance of the metabolites are limited. Metabolism of dichlormid (N,N-diallyl-2,2-dichloroacetamide) in rats and maize resulted in several oxygenated metabolites and the involvement of glutathione (GSH) as well as glutathione S-transferases (GSTs) was postulated (Miaullis *et al.*, 1978). The thiazole safener flurazole (benzyl 2-chloro-4-(trifluoro-methyl)-5-thiazolecarboxylate) in addition to dechlorination by hydrolysis and deesterification underwent glutathione conjugation in maize and sorghum plants (Breaux *et al.*, 1989). Benoxacor (4-(dichloroacetyl)-3,4-dihydro-3-methyl-2*H*-1,4-benzoxazine) was metabolised in maize cell suspension cultures to formylcarboxamide and carboxycarboxamide derivatives and to a mono(GSH) conjugate by GSH-dependent and GST-catalyzed pathway (Miller *et al.*, 1996).

The herbicide safener MG-191 (1, 2-dichloromethyl-2-methyl-1,3-dioxolane, Figure 1) is a highly active molecule in safening maize against thiocarbamate and to a lesser extent chloroacetanilide herbicides (Dutka, 1991). MG-191 has been found to induce expression of maize tau class glutathione S-transferase isoform (*Zm*GSTU1-2) (Jablonkai *et al.*, 2001). A rapid metabolism of the MG-191 to water-soluble products took place in maize but the nature of metabolites has not been characterised (Jablonkai & Dutka, 1995). Nevertheless, MG-191 was found non-enzymatically stable with GSH (Jablonkai & Dutka, 1995).

Since the dichloromethyl moiety in the MG-191 structure can undergo a nucleophilic attack by GSH catalyzed by GSTs the objective of this study was to follow the *in vitro* metabolic fate of MG-191 by incubating the safener with glutathione and various recombinant glutathione S-transferase isozymes such as the maize phi class *Zm*GSTF1-1, *Zm*GSTF3-3, the maize tau class *Zm*GSTU1-1 and the *Arabidopsis* zeta class *At*GSTZ1-1.

MATERIALS AND METHODS

Chemicals

MG-191 (**1**) was prepared in our laboratory from 1,1-dichloroacetone and purified by distillation (Dutka 1991). 2-Methyl-(1,3)-dioxolane-2-carbaldehyde (**5**) was prepared in two steps from hydroxyacetone. Hydroxyacetone was condensed first with ethylene glycol in the presence of catalytic p-toluenesulfonic acid monohydrate to obtain (2-methyl-1, 3-dioxolane-2-yl)-methanol (68%) which was converted to the aldehyde **5** by a Swern oxidation (Fujisawa *et al.*, 1996). Spectral and chromatographic properties of these chemicals are shown in Table 1. Reduced glutathione was from Sigma-Aldrich. The solvents used for the extractions were HPLC grade and distilled before use.

Table 1. Spectral and chromatographic properties of the MG-191 (**1**) and its metabolite **5**

Compound	H-NMR ($CDCl_3$) * (ppm)	EI-MS m/z	R_f[a]	t_R[b] (sec)
MG-191 (**1**)	1.59 (s, 3H), 4.11 (m, 4H), 5.71 (s, 1H)	171 $(M+H)^+$ 155 $(M-CH_3)^+$ 135 $(M-Cl)^+$ 87 $(M-CHCl_2)^+$	0.54	369
2-methyl-(1,3)-dioxolane-2-carbaldehyde (**5**)	1.37 (s, 3H), 4.05 (m, 4H), 9.36 (s, 1H)	117 $(M+H)^+$, 87 $(M-CHO)^+$	0.21	162

[a] TLC retention values in hexane:ethyl acetate 1:1
[b] GC retention times, column temp.: from 50 °C to 220 °C by 10 °C /min

Enzymes

The maize glutathione transferases such as phi class *Zm*GSTF1-1, *Zm*GSTF3-3 and tau class *Zm*GSTU1-1 were recombinant proteins expressed in *Escherichia coli* and purified as described earlier (Dixon *et al.*, 1998). The *Arabidopsis* zeta GST (AtGSTZ1-1) was a recombinant protein expressed in *Escherichia coli* (Dixon *et al.*, 2000). Enzymes stored in ammonium sulfate solution were centrifuged for 5 min at 16,000 x *g* before use and the pellets were resuspended in the assay buffer.

Metabolism assays

Assays at pH 9.5. In glycine buffer (10 ml, 0.1 M, pH 9.5) deareated by bubbling nitrogen was dissolved reduced GSH (30 mg, 0.1 mM) and MG-191 (1.71 mg, 0.01 mM). To this solution enzymes (*Zm*GSTF1-1, 0.375 mg; *Zm*GSTF3-3, 0.350 mg; and *Zm*GSTU1-1, 0.446 mg) were added and the solutions were incubated at 37 °C in water bath. Aliquots (0.5 ml) from

incubation mixtures were taken at various time intervals and extracted with ethyl acetate (0.5 ml). Ethyl acetate extracts were analysed by GC and GS-MS. The aqueous solutions were freeze-dried and the residual solid material agitated with 80% methanol (0.5 ml). The solvents separated from the insolubles were analysed by FAB/ES-MS. Experiments were carried out three replicates.

Assays at pH 7.5. To a deareated potassium phosphate buffer (2.77 ml, pH 7.5) ethanol solution of MG-191 (0.03 ml, 0.51 mg, 1mM) and GSH (9.2 mg, 30 mM) and the respective GSTs (*Zm*GSTF1-1, 0.45 mg; *Zm*GSTU1-1, 1.34 mg; and *At*GSTZ1-1, 0.3 mg) in buffer (0.20 ml) were added and the mixture incubated at 37 °C up to 4 hours. Sample preparation from the aliquots taken were identical, as described above, and the analyses performed by GC, GC-MS and FAB/ES-MS. Experiments were carried out in three replicates.

Analyses

Gas chromatography. Analyses of ethyl acetate extracts were carried out using a Perkin-Elmer F-22 gas chromatograph fitted with flame ionisation detector (Perkin-Elmer, Norwalk, CT, USA). The glass column (1m x 2.7 mm I.D.) was packed with 80-100 mesh Gaschrom Q coated with 3% OV-17 stationary phase. Column temperature was programmed from 50 °C to 220 °C at 10 °C min^{-1}. Injector and detector temperatures were 220 and 240 °C, respectively. Nitrogen carrier gas was supplied at 30 ml/min.

Mass spectrometric analyses. Mass spectrometric analyses of ethyl acetate extracts from incubations of enzymes with MG-191 were performed on a VG-ZAB-SEQ type mass spectrometer (Manchester, United Kingdom). For gas chromatography/mass spectrometry (GC-MS) GC experimental conditions were as follows; HP-5890 GC instrument equipped with a capillary column (25m x 0.25 mm x 0.25 μm Cp-Sil 8) and the carrier gas (2 ml/min) was helium. In these experiments electron impact ionization (EI) in positive mode was performed at 70 eV electron energy and at 200°C source temperature. Analyses of 80% MeOH soluble metabolites from the incubations were run in fast atom bombardment (FAB) and electrospray (ES) ionization mode.

^{1}H-NMR analyses. NMR spectra were recorded with Varian Unity Inova (400 MHz for ^{1}H and 100 MHz for ^{13}C) and Varian Gemini-2000 (200 MHz for ^{1}H) spectrometer (Varian Inc., Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Glutathione mediated *in vitro* metabolism of the safener MG-191 was studied by using recombinant GST isoforms such as phi class maize *Zm*GSTF1-1 and *Zm*GSTF3-3, tau class maize *Zm*GSTU1-1 as well as zeta class *Arabidopsis At*GSTZ1-1. *Zm*GSTF1-1 is the major constitutive GST in maize roots and shoots, has broad-ranging specificity, relatively little towards herbicides and is only modestly increased by safeners (Dixon *et al.*, 1997). *Zm*GSTF3-3 is expressed constitutively in both root and shoots and found inducible by safeners and highly active in detoxifying chloroacetanilide herbicides (Dixon *et al.*, 1997). Recombinant *Zm*GSTU1-1 showed high GST activity towards diphenyl ether herbicide fluorodifen and was inducible by dichlormid (Dixon *et al.*, 1998). *At*GSTZ1-1 differs from other GSTs in showing

no glutathione conjugating activity towards xenobiotics but catalyzes the GSH-dependent dehalogenation of dichloroacetic acid to glyoxylic acid (Dixon *et al.*, 2000).

Non-enzymatically no reaction took place between MG-191 and glutathione at both pH 7.5 and 9.5 (Table1). Despite at elevated pH the great majority of GSH exists in the form of the more reactive GS^- anion the safener remained unchanged. GC analysis of the organic soluble fraction revealed that in presence of *Zm*GSTF1-1 during 2 hours at pH 9.5 about 20% while after 4 hours at both pH more than 30% of the parent compound was metabolized. Slower metabolism of the safener was detected with *Zm*GSTF3-3. The catalytic effect of *Zm*GSTU1-1 was hardly detectable and 95% of the safener found unreacted after 4 hours. *At*GSTZ1-1 was the most active enzyme, which catalyzed the metabolism of MG-191. At the lower pH only 22% of the parent molecule was detected proving that this glutathione transferase was found active in dechlorination of dichloroacetic acid.

GC-EI/MS analyses of the ethyl acetate extracts of the assay mixtures revealed the formation of the metabolite **5** with M=116 assigned as the ethylene ketal of the pyruvic aldehyde (Table 1, Figure1). The formation of this metabolite was confirmed with all glutathione transferases used in our experiments. The molecule is a dechlorinated derivative of the MG-191 and had a low intensity molecular ion detected at *m/z* 117 ($[M+H]^+$). The high intensity fragment ion at *m/z* 87 corresponds to the loss of the CHO fragment from the parent molecule. The spectrum of this metabolite was identical with that of synthetic standard prepared from hydroxyacetone (Fujisawa *et al.*, 1996). The formation of other metabolites with M=118, 134 and 162 was also observed but their structure has not been confirmed. Dechlorination and aldehyde formation took place during the GSH-dependent metabolism of dichloroacetamides such as dichlormid and benoxacor safeners (Miaullis *et al.*, 1978; Miller *et al.*, 1996) as well as the antibiotic chloramphenicol (Martin *et al.*, 1980). Also, metabolism of dihalomethanes by rat liver cytosol fractions yielded formaldehyde (Ahmed & Anders, 1976).

Table 1. GSH and GST mediated metabolism of MG-191

GSTs	Parent MG-191 in the assay mixture (%)[a]				Metabolite detected by GC-MS
	pH 9.5		pH 7.5		
	2HAT	4HAT	2HAT	4HAT	
no enzyme	100.0	100.0	100.0	100	-
*Zm*GSTF1-1	82.7	68.6	86.4	66.4	5
*Zm*GSTF3-3	89.3	79.3	-	-	5
*Zm*GSTU1-1	98.0	95.2	100	95.8	5
*At*GSTZ1-1	-	-	43.9	22.0	5

[a] Calculated from GC chromatograms

FAB- and ES-MS studies showed no formation of glutathione conjugates in the 80% methanol extracts of the assay mixtures. The major peak of the MS spectra was from the reduced glutathione (*m/z* 308.5). Except benoxacor metabolism, which yielded glutathione conjugates (Miller *et al.*, 1996) no stable GSH conjugates were shown during the GSH-dependent metabolism of molecules having dichloroacetyl or dihalo moieties (Miaullis *et al.*, 1978;

Martin *et al.*, 1980; Ahmed & Anders, 1976). Formation of initial unstable glutathione conjugate, which undergoes rapid hydrolytic cleavage, was postulated.

Based on our results and on literature findings of metabolic transformation of dihalogenated substrates we can also presume that maize and *Arabidopsis* GSTs metabolise MG-191 by conjugation with glutathione displacing one or both chlorine atoms from the MG-191 (Figure 1). The unstable intermediate ∀-halothioether (**2**) or diglutathione conjugate (**3**) then undergoes nonenzymatic hydrolysis to yield the corresponding hemimercaptal **4**. The elimination of the glutathione from the hemimercaptal leads to the formation of the final pyruvic aldehyde derivative **5**.

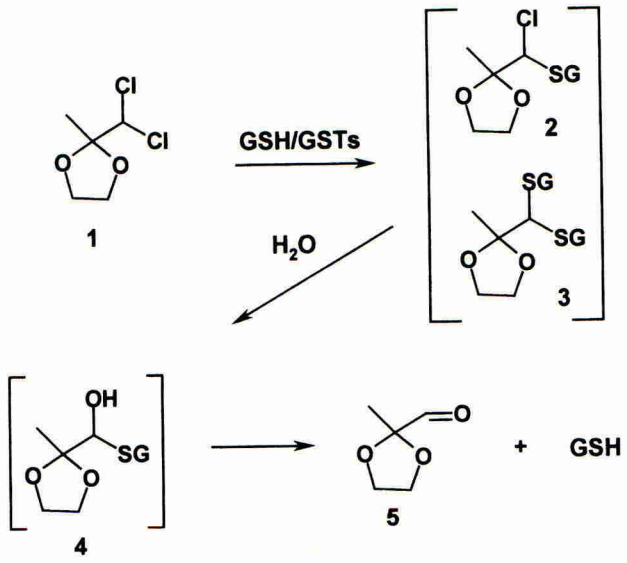

Figure 1. Proposed pathway of the glutathione-dependent metabolism of MG-191 safener catalyzed by maize and Arabidopsis glutathione transferases

Our results demonstrate that dechlorination of the safener MG-191 is a glutathione-dependent metabolism catalyzed by various GST isozymes. It appears that the safener, which can elevate the activity of GSTs, can also be a substrate for the GST isoforms. Nevertheless, the biological significance of the observed dechlorination in the mode of safener action remains to be elucidated since the aldehyde **5** formed was found having no safening activity against the herbicide acetochlor (data not shown).

REFERENCES

Ahmed A E; Anders M W (1976). Metabolism of dihalomethanes to formaldehyde and inorganic halide. *Drug Metabolism and Disposition* **4**, 357-361.

Breaux E J; Hoobler M A; Patanella J E; Leyes G A (1989). Mechanism of action of thiazole safeners. In: *Crop safeners for herbicides,* eds. K K Hatzios, R E Hoagland, pp. 163-175, Academic Press: San Diego, USA.

Davies J; Caseley J C (1999). Herbicide safeners: a review. *Pesticide Science* **55**, 1043-1058.

Dixon D P; Cole D J; Edwards R (1997). Characterisation of multiple glutathione transferases containing the GST I subunit with activities toward herbicide substrates in maize (*Zea mays*). *Pesticide Science* **50**, 72-82.

Dixon D P; Cole D J; Edwards R (1998). Purification, regulation and cloning of a glutathione transferase (GST) from maize resembling the auxin-inducible type-III GSTs. *Plant Molecular Biology* **36**, 75-87.

Dixon D P; Cole D J; Edwards R (2000). Characterisation of a zeta class glutathione transferase from *Arabidopsis thaliana* with putative role in tyrosine catabolism. *Archives of Biochemistry and Biophysics* **384**, 407-412.

Dutka F (1991). Bioactive chemical bond systems in safeners and prosafeners. *Zeitschrift für Naturforschung* **46c**, 805-809.

Fujisawa T; Kooriyama Y; Shimizu M (1996). Switchover of diastereofacial selectivity in the condensation reaction of optically active *N*-sulfinimine with ester enolate. *Tetrahedron Letters* **37**, 3881-3884.

Hatzios K K; Burgos N (2004). Metabolism-based herbicide resistance: regulation by safeners. *Weed Science* **52**, 454-467.

Jablonkai I; Dutka F (1995). Uptake, translocation and metabolism of MG-191 safener in corn (*Zea mays* L.). *Weed Science* **43**, 169-174.

Jablonkai I; Hulesch A; Cummins I; Dixon D P; Edwards R (2001) The herbicide safener MG-191 enhances the expression of specific glutathione S-tarnsferases in maize. *Proceedings of the BCPC Conference – Weeds 2001,* **2**, 527-532.

Martin J L; George J W; Pohl L R (1980). Glutathione-dependent dechlorination of chloramphenicol by cytosol of rat liver. *Drug Metabolism and Disposition* **8**, 93-97.

Miaullis J B; Thomas V M; Gray R A; Murphy J J; Hollingworth R M (1978) Metabolism of R-25788 (N,N-diallyl-2,2-dichloroacetamide) in corn plants, rats, and soil. In: *Chemistry and action of herbicide antidotes,* eds F M Pallos & J E Casida, pp. 109-131. Academic Press: London.

Miller K D; Irzyk G P; Fuerst E P; McFarland J E; Barringer M; Cruz S; Eberle W J, Fory W (1996). Time course of benoxacor metabolism and identification of benoxacor metabolites isolated from suspension-cultured *Zea mays* cells 1h after treatment. *Journal of Agriculture and Food Chemistry* **44**, 3326-3334.

Multiresidue analysis of pesticides for evaluation of surface water quality of the river Ganga in West Bengal

R K Kole, S Alam, P Mukherjee, D Sardar, S Kole, T Saha
Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Kalyani – 741235, West Bengal, India
Email: rkkole@rediffmail.com

M Sengupta
National River Conservation Directorate, Govt. of India, Lodhi Road, New Delhi – 110 003, India

ABSTRACT

Surface water samples from the river Ganga were collected every month from two sites (middle and a discharge point) at four locations (a 300 km stretch) in West Bengal and analyzed for multiple residues of pesticides using glc. About 87.8% of 278 samples were found to contain combinations of organochlorine (OC) pesticide residues such as HCH, DDT and endosulfan in the range of 0.004 to 18.78 µg/L and 62.8% were detected for organophosphorus (OP) pesticides such as dimethoate, malathion or methyl parathion in the range of 0.008 to 5.53 µg/L. In terms of total (OC+OP) pesticides, about 95% of the samples were found to be contaminated in the range of 0.01-18.78 µg/L of which 57.3% were found to exceed the MRL in drinking water (0.5 µg/L). Total OC pesticide reached maxima during summer (mean = 1.96 µg/L) and for total OP pesticides maxima occurred during winter (0.69 µg/L). There was no significant variation observed in pesticide residues due to change in sampling locations nor were there any significant adverse impacts attributable to the residual level due to raw discharges in the river. Principle Component Analysis identified some pesticides (among other quality parameters) to form the Minimum Data Set for prediction of Water Quality Index.

INTRODUCTION

Among the various classes of pesticides, herbicides dominate the world market while insecticides lead in the Indian market (Adityachaudhury *et al.*, 1997). About 50% of the insecticides in India belong to the chemical group organophosphate (OP). Although the use of organochlorine (OC) insecticides has been restricted by most countries because of their persistence in the environment, their illegal use in agriculture is not uncommon (Tuncer *et al.*, 1998) and many countries still employ these chemicals in public health programmes. Consequently, the toxic residues of pesticides occurring in food commodities and drinking water have created concern among the environmentalists due to their possible health hazards through bioaccumulation. Pesticidal chemicals are introduced into water systems from agricultural run off (Scott *et al.*, 1999), sewage treatment plants (Honnen *et al.*, 2001), industrial effluents or accidental spills (Lambert, 1997). The presence of OC pesticides even at very low concentration in water is undesirable for long time consumption by wild life (Bindra, 1972) and may be toxic to fish (Brown, 1979; Tilak *et al.*, 1991). The hazard to aquatic life is further increased by possible biomagnification of the pesticides from water by aquatic organisms (Kurunthchalam *et al.*, 1999; Murti, 1986). Furthermore, the presence of pesticides in rainfall and in the atmosphere indicates the possibility of agricultural pesticides playing a role in forest decline (Trevisan *et al.*, 1993). The presence and accumulation of OC pesticides like DDT, HCH, etc. in various aquatic resources is well documented including river water in

India (Agnihotri *et al.*, 1994; Halder *et.al.* 1989; 1990; Kole & Bagchi, 1995; Mohapatra *et al.*, 1995) and elsewhere (Badaway, 1998; Eichelberger & Litchenberg, 1971; Honnen *et al.*, 2001; Rovedatti *et al.*, 2001; Schulz, 2001). Current levels of OC and OP residues in water samples of the most important and scared river Ganga in West Bengal have not been assessed systematically. Therefore, as a part of our research programme on the assessment of water quality, we monitored the occurrence of some OC and OP pesticide residues in surface water of the river Ganga in West Bengal, India.

MATERIALS AND METHODS

Sampling: Surface water samples from the river Ganga were collected once in every month (from October 2001 to September 2004) from two sites: the middle of the river and from a mixing point of municipal drain/ agricultural canal/ bathing ghat near the river bank (termed as discharge points) at four locations of West Bengal: Berhampore (District: Murshidabad), Palta (N-24 Pgs), Dakshineswar (Kolkata) and Uluberia (Howrah) along the 300 km stretch of the river (Figure 1) in India. Nansen Type samplers were used for collection of water from a depth of 0.3 m from the surface. Chloroform (10 ml/litre) was added as preservative in the samples taken in amber-coloured glass bottles and immediately transported to the laboratory under ice-cold condition for analysis.

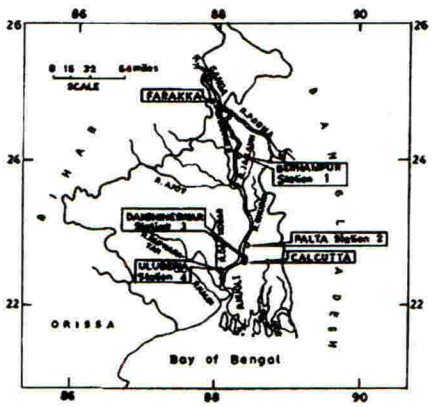

Figure 1. Location of permanent monitoring stations of the river Ganga in West Bengal

Extraction and Clean up: Water samples (1.0 litre) were extracted by liquid-liquid partitioning in a separatory funnel after adding 10 g of solid NaCl. The mixture was then extracted thrice with 100, 75 and 75 ml of n-hexane:dichloromethane (85:15, *v/v*) by vigorous shaking. The separated layers were collected over anhydrous Na_2SO_4 and evaporated to dryness by a rotary vacuum evaporator. The residue thus obtained was taken in 5 ml volumetric flask using *n*-hexane for glc analysis.

Estimation by glc: A gas chromatograph (Hewlett Packard Model 5890 A) coupled with an Electron Capture Detector (Source: ^{63}Ni) and a Chemito 1000 data processor was used for estimation of the OC and OP pesticide residues. The carrier gas (N_2) flow rate was maintained at 30 ml/min. A megabore column (DB – 1701 of 30 m length with 0.35 mm id and film thickness 1.5 µm) was programmed initially at 160°C (for 1 min) with an increasing rate of 3°C/min, to a final temperature of 250°C to stay for 5 min for satisfactory resolution of the

pesticides. The injector and detector temperatures were maintained at 275°C and 340°C respectively during the analysis.

Analytical grade pesticides HCH (α, β, γ, and δ), DDT (*o,p*-DDT, *p,p*-DDT, *o,p*-DDD, *p,p*-DDE), endosulfan (α, β and endosulfan sulfate), dimethoate, malathion and methyl parathion (purity 97-99.5%) were used to prepare a mixed solution to serve as external standards. The retention times (RT) for various pesticides were found in the range of 5.39 to 20.70 min. The cleaned up extract of samples (3–7 µL) was injected to the glc. Various pesticides in the sample were detected by comparing the RT with those obtained for the standard and quantified. The recovery of different pesticides was obtained in the range of 75-98% and the method was adopted for analysis.

Statistical analysis: To understand the effect of various factors like collection site, location and season on the residual content of individual or total pesticides in river water, the residual data were subjected to Analysis of Variance (ANOVA) using SPSS statistical package. The two years residual (October 2001 – September 2003) data were also subjected to Principal Component Analysis (PCA) along with other physico-chemical and biological quality parameters to form the minimum data set (MDS) to derive the water quality index (WQI).

RESULTS AND DISCUSSION

A total of 278 water samples of the river were collected during the three years study period and analysed for OC pesticide residues. The OC insecticides HCH, DDT and endosulfan were detected in 83.4, 49.3 and 32.7% of the samples (Table 1). Among the carcinogenic HCH isomers (Reuber, 1980), the level of β-HCH (0.005-18.65 µg/L) was higher in comparison to α, γ and δ- isomers of HCH. Frequency of occurrence of the metabolites of DDT (i.e. *o,p*-DDD, *p,p*-DDE) was much higher as compared to its isomers (*o,p*-DDT, *p,p*-DDT). The occurrence of endosulfan was significantly lower in comparison to HCH and DDT. The results are comparable with earlier findings in Ganga water (Agnihotri *et al.*, 1994; Halder *et al.*, 1989; 1990; Kole & Bagchi, 1995; Samanta *et al.*, 1994).

Among the 278 samples, 239 were also analysed for OP pesticides collected during the period April'02 to Sept'04. Out of these 239 samples analysed for both OC and OP pesticides about 95% were found to be contaminated either with OC or OP pesticides or both of these pesticides in the range of 0.01-18.78 µg/L (Table 1). Although OP pesticides do not move freely in soils with water (Vasu, 1996) and their presence is usually not detected in river water (Rovedatti *et al.*, 2001) but their occurrence in river water is not unusual (Mohapatra *et al.*, 1995). Among the OP pesticides analysed in 239 samples of Ganga water, the residue level of Malathion was found to be highest (0.008 - 4.83 µg/L) followed by methyl parathion, 0.008-3.05 µg/litre. Dimethoate, malathion and methyl parathion detected in 11.7, 48.1 and 16.3% samples respectively (Table 1).

Statistical analysis of the residual data using ANOVA indicated no significant difference in the residual levels either due to variation between collection sites (middle of the river or discharge points) or due to changes in sampling locations. No spatial trend in pesticide residues was also observed in surface water under the flowing condition of the river Reconquista in Argentina (Rovedatti *et al.*, 2001). However, the seasonal effect was evident when considering the residual level of some OC and OP pesticides. The average seasonal concentrations of total HCH and total OC pesticides were found to reach maxima during the summer. But for total OP maximas were observed during winter. The total (OC+OP) pesticides content in Ganga water

also exhibited wide temporal changes the maximum seasonal mean (2.73 µg/litre) being observed during summer (Figure 2).

Table 1. Residues of organochlorine (OC) and organophosphorus (OP) pesticides detected in Ganga water

Pesticide	No. of samples detected*	Range of residues (µg/litre)		No. of samples above 0.1 µg/litre
		Minimum	Maximum	
α-HCH	75	0.001	1.38	15
β-HCH	202	0.005	18.65	160
γ-HCH	96	0.001	1.86	26
δ-HCH	72	0.009	1.60	34
Total HCH	*232*	*0.014*	*18.65*	*191*
p, p-DDE	76	0.002	2.09	28
o, p-DDD	80	0.005	3.69	22
o, p-DDT	19	0.010	3.94	4
p, p-DDT	35	0.001	2.06	6
Total DDT	*137*	*0.001*	*6.00*	*46*
α-Endosulfan	53	0.002	2.23	9
β-Endosulfan	43	0.001	1.52	9
Endo Sulphate	27	0.002	3.11	4
Total Endo	*91*	*0.001*	*3.62*	*24*
Total OC	*244*	*0.004*	*18.78*	*202*
Dimethoate	28	0.002	1.94	15
Malathion	115	0.008	4.83	79
Methyl parathion	39	0.010	3.05	33
Total OP	*150*	*0.008*	*5.53*	*117*
Total Pesticides	*227*	*0.010*	*18.78*	*137***

*Out of 278 for OC and 239 for OP; Total OC = HCH + DDT + Endosulfan; Total Pesticides = OC+OP; ** No. of samples above 0.5 µg/L for total pesticides

Further statistical analysis using PCA revealed the MDS of 25 quality parameters including DDT, HCH, endosulfan and malathion which are responsible for the variation of the river water quality. The MDS may be combined to derive WQI. The process will lead to formulate suitable water quality indexing system for assessment over time.

Global contamination due to OC pesticides probably occurs as these chemicals volatilize into the atmosphere and deposit with rains (Kole & Bagchi, 1995). Water solubility of OC pesticides is typically extremely low and they have the capability to accumulate in lipid tissues due to their lipid solubility. Therefore, OC pesticides have the potential to persist for a longer period than many other groups of pesticides. DDT is toxic to aquatic life at concentration well below 1 mg/litre (Joshi, 1992). Many OC pesticides in water have the potential for significant bioconcentration and biomagnification. It has been reported that dieldrin and DDT at a level of 10^{-5} mg/litre in water may result in long-term impacts for wildlife (Bindra, 1972). DDT and endosulfan are considered toxic to fresh water fish at 16 and 1µg/litre respectively (Brown, 1979). In our present observation it has been found that the residue levels for DDT were well within the safety limit for fresh water fish and for endosulfan the limit has been exceeded only in limited number of cases (7 out of 278 samples).

By comparison with the permissible threshold for any individual pesticides (0.1 µg/litre) in drinking water in the European Union, about 72.7% and 48.9% of the samples breached the drinking water threshold as a consequence of OC and OP pesticide contamination respectively.

In terms of total pesticides (OC + OP), 57.3% of the samples were found to breach the EU permissible level (0.5 µg/litre) for total pesticides in drinking water (Table 1).

Therefore, monitoring programmes for the pesticide residues such as this would enable us to develop a pesticide-monitoring database for the river Ganga throughout the year. This is considered necessary for determining the quality of potable water for different uses including aquaculture, source water for drinking, recreational purpose, etc. An important conclusion from this monitoring assessment is that care should be taken for the use of the river Ganga, as a source of drinking water. Further it is recommended that the waterworks engaged in water treatment should implement a means of removing these pesticide residues before supply for community use.

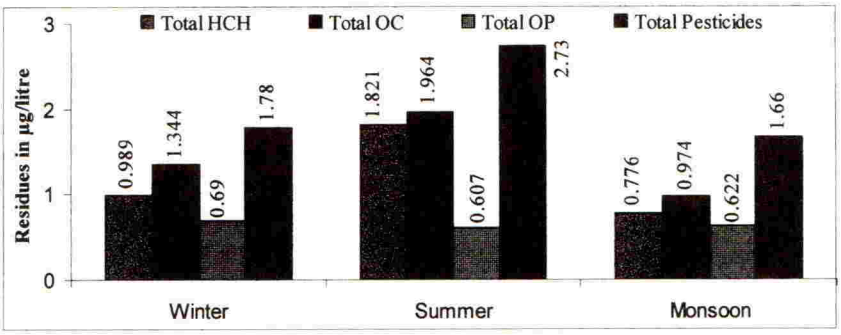

Figure 2. Seasonal change in the occurrence of pesticides in river water

ACKNOWLEDGEMENTS

Financial support by the National River Conservation Directorate, Mins. of Env. & Forests, Govt. of India and infrastructural facilities provided by BCKV is gratefully acknowledged.

REFERENCES

Adityachaudhury N; Banerjee H; Kole R K (1997). An appraisal of pesticides use in Indian agriculture with special reference to their consumption in West Bengal. *Science and Culture* **63**, 223-228.

Agnihotri N P; Gajbhiye V T; Mohapatra S P; Mukesh K; Kumar M (1994). Organochlorine insecticide residues in Ganga river water near Farrukhabad, India. *Environmental Monitoring and Assessment* **30**, 105-112.

Badawy M I (1998). Use and impact of pesticides in Egypt. *International Journal of Environmental Health Research* **8**, 223-229.

Bindra O S (1972). Pesticidal pollution of water. *Pesticides* **6**, 77-82.

Brown AWA (1979). *Ecology of pesticides,* pp.174.Wiley Interscience Publication: New York.

Eichelberger J W; Lichtenberg J J (1971). Persistence of pesticides in river water. *Environmental Science and Technology* **5**, 541-544.

Halder P; Raha P; Bhattacharyya P; Chowdhury A; Adityachaudhury N (1989). Studies on the residues of DDT and Endosulfan occurring in Ganga water. *Indian Journal of Environmental Health* **31**, 156-161.

Halder P; Kole R K; Bhattacharya A; Chowdhury A; Adityachaudhury N (1990). Studies on the residues of the BHC isomers (α, β, γ and δ) occurring in Ganga waters. *Pollution Research* **9**, 51-56.

Honnen W; Rath K; Schlegel T; Schwinger A; Frahne D (2001). Chemical analyses of water, sediment and biota in two small streams in southwest Germany. *Journal of Aquatic Ecosystem Stress and Recovery* **8**, 195-213.

Joshi H C (1992). Environmental mediated stress on flood plain lakes. II. Pesticides and agricultural run-off. In: *Compendium of FAO sponsored Foodplain Fish Management,* eds Y S Yadav & V V Suguman. Central Inland Capture Fisheries Research Institute: Barrackpore, West Bengal, India.

Kole R K; Bagchi M M (1995). Pesticide residues in the aquatic environment and their possible ecological hazards. *Journal of Inland Fisheries Society of India* **27**, 79-89.

Kurunthachalam S; Kurunthachalam K; Sinha R K; Tanabe S; Giesy J P; Senthilkumar K; Kannan K (1999). Bioaccumulation profiles of polychlorinated biphenyl congeners and organochlorine pesticides in Ganges river dolphins. *Environmental Toxicology and Chemistry* **18**, 1511-1520.

Lambert M R K (1997). Environmental effects of heavy spillage from a destroyed pesticide store near Hargeisa (Somaliland) assessed during dry season, using reptiles and amphibians as bioindicators. *Archives of Environmental Contamination and Toxicology* **32**, 80-93.

Mohapatra S P; Gajbhiye V T; Agnihotri N P; Manju R; Raina M (1995). Insecticide pollution in Indian rivers. *Environmentalist* **15**, 41-44.

Murty A S (1986). *Toxicity of pesticides to fish.* **Vol.1**. CRC Press: Boca Raton, Florida.

Reuber M D (1980). Carcinogenicity of benzene hexachloride and its isomers. *Journal of Environmental Pathology and Toxicology* **4,** 355-372.

Rovedatti M G; Castane P M; Topalian M L; Salibian A (2001). Monitoring of organochlorine and organophosphorus pesticides in water of the Reconquista River (Buenos Aires, Argentina). *Water Research* **35**, 3457-3461.

Samanta S; Jana K; Kole R K; Das A K; Chowdhury A (1994). Studies on pesticide residues occurring in various environmental substrates with special reference to West Bengal condition. *Journal of Inland Fisheries Society of India* **26**, 43-48.

Schulz R (2001). Rainfall induced sediment and pesticide input from orchards into the Lourens river, Western Cape, South Africa: Importance of a single event. *Water Research* **35**, 1869-1876.

Scott G I; Fulton M H; Moore D W; Wirth E F; Chandler G T; Key P B; Daugomah J W; Strozier E D; Devane J; Clark J R; Lewis M A; Finley D B; Ellenberg W; Karnaky K J Jr. (1999). Assessment of risk reduction strategies for the management of agricultural nonpoint source pesticide runoff in estuarine ecosystem. *Toxicology and Industrial Health* **15**, 200-213.

Tilak K S; Rao N H J J; Lakshmi J (1991). Effect of pesticides mixed in different ratios on freshwater Labeo rohita. *Journal Ecotoxicology and Environmental Monitoring* **1**, 49-52.

Trevisan M, Montepiani C, Ragozza L, Bartoletti C, Ioannilli E and Del Re AAM (1993). Pesticides in rainfall and air in Italy. *Environmental Pollution* **80**, 31-39.

Tuncer G; Karakas T; Balkas T I; Gökcay C F; Aygnn S; Yurteri C; Tuncel G (1998). Land-based sources of pollution along the Black Sea coast of Turkey: Concentrations and annual loads to the Black Sea. *Marine Pollution Bulletin* **36**, 409-423.

Vasu K (1996). Pollution of ground water and methods to minimise from environmental angle. In: *Agrochemicals and Sustainable Agriculture* (ed. N K Roy), pp.238. APC Publications: New Delhi.

Catalytic photodegradation of the insecticide imidacloprid in aqueous media exposed to sunlight

S Roy, R K Kole, M A Sarkar and A Chowdhury
Department of Agricultural Chemicals, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur – 741252, West Bengal, India
Email: rkkole@yahoo.com

ABSTRACT

A laboratory experiment was conducted using hplc to understand the kinetics of photodegradation of analytical grade imidacloprid [1- (6-chloro-3-pyridylmethyl)-N-nitroimidazolidin -2-ylideneamine, **I**] in double distilled water (DDW, 10 µg/ml) under sunlight as compared to its commercial formulation (200 SL) in irrigation water (10 µg a.i./ml) in presence or absence of TiO_2 (0.01 M). Half-life values indicated accelerated action of TiO_2 on degradation of **I** in DDW (DT_{50} = 27.4 h and 75.3 h with and without TiO_2). However, the reverse effect of TiO_2 was recorded in the formulation (DT_{50} = 60.2 h and 37.6 h respectively with and without TiO_2) in irrigation water attributable to the turbidity produced in the solution preventing the absorption of light of appropriate wavelength. Column chromatography of the bulk irradiation mixture of **I** (2.0 g in DDW exposed to sunlight for 60 h) yielded four photoproducts (**II** – **V**) purified by repeated crystallization. Based on their physical and spectral (ir, ^{1}H-nmr and ms) evidence the structure of the metabolites were assigned as 1-(6-chloro-3-pyridylmethyl)-N-nitro-4-imidazolidene-2-ylideneamine (**II**), 6-chloronicotinic acid (**III**), 1-[(6-chloro-3-pyridyl) methyl] imidazolidin-2-one (**IV**) and 6-hydroxy nicotinic acid (**V**). The photolytic process affected the imidazolidine moiety while the 6-chloropyridine moiety remained unchanged. The proposed phototransformation pathway involved hydrolysis, oxidation and dehydration.

INTRODUCTION

Imidacloprid [1-(6-chloro-3-pyridylmethyl) – N – nitroimidazolidin – 2 –ylideneamine, **I**] is a nitromethylene insecticide developed by Bayer (Leverkusen, Germany). It has a novel mode of action, acting as an agonist of the nicotinyl acetylcholine receptor (Bai *et al.*, 1991; Liu *et al.*, 1995). It is the first highly effective insecticide for which the mode of action has been found to deviate from the almost complete and irreversible blocking of the postsynaptic nicotinergic acetylcholine receptors (Yamamoto *et al.*, 1995). It belongs to a class called "Chloronicotinoids" and shows a high activity, especially against a great number of sucking pests such as aphids, leaf and plant hoppers, thrips, white flies and other pest species including strains resistant to other insecticides. Exposure to the active ingredient (a.i.) can be through contact as well as through ingestion. Nevertheless, it shows excellent systemic properties, which makes it suitable for seeds, soil and foliar treatment. Due to its high insecticidal activity at very low application rates (0.02 to 0.07 kg a.i./ha), imidacloprid is regarded as a promising insecticide alternative (Kagabu *et al.*, 1992).

The metabolism of ^{14}C imidacloprid has been investigated in plant cell suspension cultures (Koester, 1992). Soil biodegradation studies with imidacloprid have demonstrated that the major metabolite is the 6-chloronicotinic acid leaving no soil residue three months after sowing

(Rouchaud *et al.*, 1994). ^{14}C carbon dioxide was found to be the main product of ^{14}C imidacloprid soil biodegradation when incubated under laboratory conditions (Scholz & Spiteller, 1992). The persistence and metabolism of imidacloprid in different soils have also been studied (Sarkar *et al.*, 2001). The main imidacloprid metabolites identified in soil include imidacloprid-urea, 6-chloronicotinic acid and 6-hydroxy nicotinic acid (Rouchaud *et al.*, 1996). The sorption-desorption of imidacloprid and its metabolites has been studied in soil (Cox *et al.*, 1997) and the persistence of imidacloprid as affected by pH and type of formulation has also been reported (Sarkar *et al.*, 1999).

Under field conditions only a small portion of the applied pesticide reaches the biological target. The majority is released into the ecosystem where it undergoes biological and abiotic degradation reducing potential for accumulation. The degradation process usually leads to the formation of less harmful breakdown products, but in some instances more toxic products can also be produced, which may be of risk to the environment (Aharonson, 1987). A further possibility is that the pesticide may be resistant to degradation by any means and thus remain unchanged in the environment for prolonged time (Edwards, 1973). In aqueous media and soil, pesticides may react chemically where they may be subject to hydrolysis or oxidation. In surface water and in the upper soil cover the degradation of substances can be acclerated *via.* photolysis. Xenobiotics can undergo either direct phototransformation through an excited state or indirect phototransformation by photosensitized processes assisted by the presence of sensitizers, for example, TiO_2 and quenchers. Our objective was to study the photochemical transformation of imidacloprid in aqueous system under sunlight. An attempt has also been made to characterise the photoproducts thus formed and to determine their probable mechanism of formation.

MATERIALS AND METHODS

Chemicals: Analytical grade imidacloprid (**I**) along with its commercial formulation 'Confidor' 200 SL were provided by Bayer AG, Germany. **I** was isolated in bulk from the formulation by column chromatography and subsequently purified by repeated crystallization from dichloromethane:hexane (8:2 *v/v*), m.p. 143.6°C (99.0% pure). All solvents used were AR grade.

Apparatus and chromatography: The purity of **I** and quantification of results were carried out with hplc (Hewlett-Packard, HP – Model 1050) equipped with an HP 1050 UV/VIS detector set at λ_{max} = 270 mm and coupled to an HP (Model 3392A) integrator. The column used was reversed-phase hypersil C_{18} (ODS) of Shadon HPLC, UK (15 cm length, 4.6 mm id). The mobile phase used was methanol:water (6:4 *v/v*) at a flow rate of 0.5 ml/min. The retention time (RT) was 5.51 min. Mass spectra (electron impact, ionization potential 70 eV, direct insertion) were recorded on a JOEL JMS 600 mass spectrometer. ^1H-nmr spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz) using CD_3OD as solvent and TMS as an internal standard. IR spectra were taken on KBr pellets by using a Perkin-Elmer (Model 1310) spectrophotometer.

Silica gel G was used for TLC on 20 x 20 cm glass plates (0.5 mm) using iodine as chromogenic reagent. Column chromatography was conducted using glass columns packed with a slurry of silica gel (60-120 mesh) in hexane.

Irradiation experiment: The solution of analytical grade imidacloprid (**I**, 10 µg/ml) in double distilled water (DDW, pH 6.5) and its formulated product 200 SL (10 µg a.i./ml) in irrigation

water (after filtration using Whatman No. 42 filter paper, pH 7.6) were exposed to natural sunlight (at Kalyani, 22°57′ N latitude, 7.8 m altitude, India) with and without addition of TiO_2 (0.01 M). The temperature of the solution did not exceed 28°C throughout the experiment. Samples were withdrawn at an interval of 5 h and extracted with dichloromethane. The extracts were concentrated by rotary vacuum evaporator (RVE) and finally diluted with methanol for analysis using hplc.

The kinetics of photodegradation of imidacloprid and its SL formulation was determined using the first order reaction i.e. $[C_t] = \ln [C_0] - K_t$ and the dissipation half-lives ($DT_{50} = \ln 2/b$) were calculated (Timme *et al.*, 1986; Walter *et al.*, 1993).

To obtain enough photoproducts for structure elucidation and confirmation, eight batches each containing 250 mg of **I** (a.i.) in 1.0 litre DDW were irradiated for 60 hours. The irradiated solutions were extracted with dichloromethane and concentrated by RVE (~ 40°C). The combined extract was subjected to column chromatography over silica gel for isolation of photoproducts which were purified by repeated crystallization.

RESULTS AND DISCUSSION

The results of photokinetic assessments of imidacloprid and its formulation are summarised in Table 1. The kinetics study showed that 50% of analytical grade imidacloprid was degraded in 27.37 h and 75.26 h respectively with and without TiO_2. But in case of the formulation (200 SL) the corresponding half-lives (DT_{50}) were 60.21 h and 37.63 h with and without TiO_2 respectively (Figure 1). TiO_2 was observed to be an effective photocatalyst for the degradation of analytical grade imidacloprid in DDW. Titania in the form of anatase and in presence of water generates $OH^{\bullet}$ radicals, known as strongly oxidative and poorly selective agent according to the following mechanism:

$$TiO_2 + h\nu \rightarrow e\_ + p^+ \quad ; \quad O_2 + e\_ \rightarrow O_2\text{-}$$
$$H_2O \rightarrow OH\_ + H^+ \quad ; \quad 2HOO^{\bullet} \rightarrow H_2O_2 + O_2$$
$$H_2O_2 + e\_ \rightarrow OH^{\bullet} + OH\_ \quad ; \quad O_2\text{-} + H^+ \rightarrow HOO^{\bullet}$$
$$OH\_ + p^+ \rightarrow {}^{\bullet}OH \quad ; \quad R\text{-}H + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O \rightarrow \text{Intermediate} \rightarrow CO_2$$

In some cases photoproduced holes (p^+) created in the valence band (Oliver & Carey, 1986) of TiO_2 (with light of energy higher than the bandgap) can react directly with the adsorbed reactants such as carboxylic acids or anions: $R\text{-}COO\_ + p^+ \longrightarrow R^{\bullet} + CO_2$

Table 1. Calculated values of rate constant, K and the DT_{50} values

Sl. No.	Exposure condition	Regression equation	K (10^{-3} s^{-1})	DT_{50} (h)
1.	**I** in DDW + TiO_2	Y = 2.11- 0.011 X	11	27.37
2.	**I** in DDW only	Y = 2.01- 0.004 X	4	75.26
3.	200 SL in irrigation water + TiO_2	Y = 2.03 - 0.005 X	5	37.63
4.	200 SL in irrigation water only	Y = 2.03 - 0.008 X	8	60.21

In the case of the SL formulation the rate of degradation was decreased in presence of photocatalyst TiO_2. The described deceleration is probably an order of formulation, the effect of which is reinforced by the supplement of TiO_2. In addition, it may be due to the fact that the irradiated solutions differ in colour. Irradiated imidacloprid delivered a bright yellow solution,

whereas the formulation without TiO_2 produced a yellowish brown solution and in presence of TiO_2 a light brown solution. It can be assumed that the entire wavelength range is no longer available due to the increasing brown dyeing of the solution. A low solution turbidity observed also prevents the entire range of light intensity from being used (Warnhoff & Schneider, 1999).

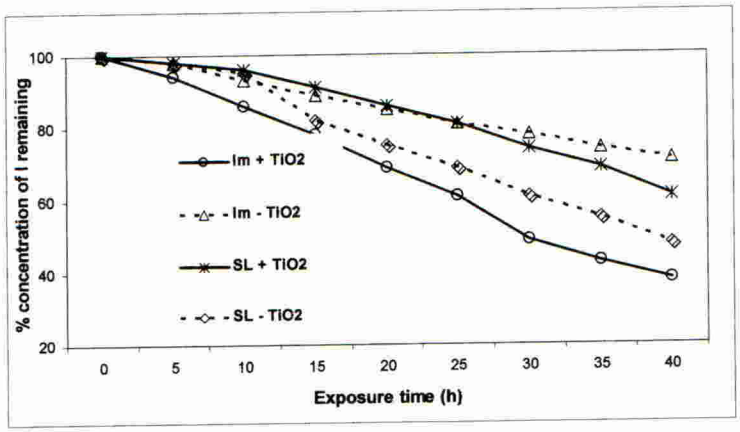

Figure 1. Photokinetics of imidacloprid, I (10 µg/ml) in aqueous solution undersunlight. Im = Analytical grade; SL = Formulation 200 SL

Identification of photoproducts: Hexane elution of the column containing the concentrated extract of sunlight irradiated aqueous solution of **I** (2g) yielded compound **II**, crystallized from hot-cold hexane as brownish white needle, (m.p. 132-133°C). The i.r. spectrum of **II** indicated the presence of aromatic-CH group at 3346 cm^{-1}, pyridine at 3000 cm^{-1} in addition to 1580 cm^{-1} (- NO_2, >C = N-NO_2), 1535 cm^{-1} (>C = C <). The ^1H nmr spectrum of **II** was as follows: $\delta_{TMS}^{CD_3OD}$ 5.21 [s, 2H, olifinic protons (H_d)]; 7.078, 7.070 [d, 1H, J = 2.4 Hz, olefinic double bond protons present (H_e , H_f)]; 7.496, 7.468 [d, 1H, J = 8.4 Hz, ortho, Ar-(H_C)]; 7.876, 7.868, 7.849, 7.841 [dd, 1H, J = 2.4, 8.1, meta, ortho, Ar-H (H_b)]; 8.446, 8.439 [d, 1H, J = 2.1, meta, Ar-H (Ha)]. Upon mass analysis the compound gave a molecular ion peak at m/e 253 (Table 2). On the basis of ir, nmr and ms data, the structure of the photoproduct **II** could be assigned as 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidene-2-ylideneamine.

Further elution of the column with hexane-benzene (1:1, *v/v*) produced compound **III**, crystallized from methanol as white crystals (m.p. 190°C). The i.r. spectrum of **III** indicated the presence of aromatic –CH group at 3057 cm^{-1}, pyridine (>N) at 2893, 2689 and 2565/ cm^{-1} and >C =O at 1683 cm^{-1}. The nmr spectrum of **III** was as follows: 7.639, 7.611 [d, 1H, J = 8.4 Hz, ortho, Ar-H (H_c)]; 8.388, 8.381, 8.360, 8.353 [dd, 1H, J = 2.1, 8.4 meta, ortho, Ar-H (H_b)]; 8.927, 8.920 [d, 1H, J = 2.1, meta, Ar-H (Ha)]. The ms of the compound gave a molecular ion peak at m/e 157 (Table 2). From the spectral record the structure of the photoproduct **III** was assigned as 6-chloronicotinic acid.

The product **IV** was obtained from benzene:chloroform (9:1 *v/v*) elution, crystallized from hexane as white needles, (m.p. 138-140°C). IR spectrum of **IV** indicated the presence of aromatic – CH at 3278 cm^{-1}, aromatic – NH at 3147 cm^{-1}, > C = O, -N-CO-NH at 1670 cm^{-1} and >C=O at 1582 cm^{-1}. The nmr spectrum of the compound was as follows: 3.67 [s, 2 (-CH_2-), (Ar-CH_2-N)]; 4.545 [s, 4(-CH_2-CH_2-), (-N-CH_2-CH_2-N)], 4.846 [solvent (H_2O)]; 7.584, 7.557 [d, 1H, J = 8.4 Hz, Ortho, Ar-H (H_c)]; 7.884, 7.876, 7.857, 7.845 [dd, 1H, J = 8.4, 2.4; ortho,

meta Ar-H (H_b)]; 8.383, 8.376 [d, 1H, J = 2.4, meta, Ar-H (H_a)]. The ms of the compound gave a molecular ion peak at m/e 211 (Table 2). From the spectral evidence the structure of **IV** was determined as 1-[(6-chloro- 3- pyridyl) methyl] imidazolidin- 2- one.

Table 2. Mass spectral data of photometabolites of imidacloprid

Product	Mass fragmentation pattern
II	253 [M^+], 255 [M^++ 2] (3 : 1); 236 [M^+-NH_3]; 208 [M^+-{(N-NO_2)+O}]; 193 [M^+-{(N-NO_2)+O}-$NH^•$]; 171 [M^+-$C_3N_2H_2O$]; 126 [M^+-$C_3N_4H_3O_2$]; 111 [M^+-$C_3N_4H_3O_2$-CH_3]; 91 [C_6NH_5]; 82 [$C_3N_2OH_2$]
III	157 [M^+]; 159 [M^+ + 2]; 139 [M^+ + H_2O]; 122 [M^+ - Cl]; 110 [M^+ - CO_2H_3]; 81 [$(C_5NH_7)^+$]; 75 [M^+ - CO_2H_3 – Cl^+]; 65 [$C_5H_6^+$]
IV	211 [M^+]; 212 [M^+ + 1]; 209 [M^+ - H_2]; 175 [M^+ - HCl]; 147 [M^+ - HCl – CO]; 126 [M^+ - (CO-NH-CH_2-CH_2-N)]; 111 [M^+ - (CO-NH-CH_2-CH_2-N)- CH_3]; 91 [C_5H_4N-CH_2-1]; 80 [$C_3H_2ON_2$ – 2]
V	139 [M^+]; 122 [(M – OH)$^+$]; 111 [(M-CO)$^+$]; 94 [(M-COOH)$^+$]; 67 [$(C_5H_7)^+$]

Further elution of the column with benzene:chloroform (1:1 *v/v*) yielded compound **V**, crystallized from ethanol as white crystal (m.p. > 300°C). IR spectrum of **V** indicated the presence of –OH and aromatic –CH at 3231 and 3120 cm^{-1}, aromatic –CH and pyridine at 3080 cm^{-1}, pyridine (-NH) at 2992, 2923, 2602 and 2494 cm^{-1}, >C = O at 1708, 1640 and 1608 cm^{-1}. The nmr spectrum of the compound was as follows: 6.550, 6.518 [d, 1H, J = 9.6 Hz, ortho, Ar-H (H_c)]; 8.049, 8.041, 8.017, 8.009 [dd, 1H, J = 2.4, 9.6 Hz, meta, ortho, Ar-H (H_b)]; 8.166, 8.158 [d, 1H, J = 2.4 meta, Ar – H (H_a)]. The molecular ion peak appeared at m/e 139 (Table 2). Based on these spectral data the structure of V was assigned as 6-hydroxy nicotinic acid.

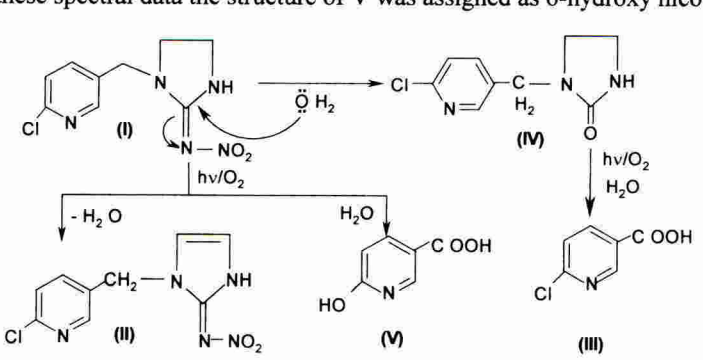

Figure 2. Hypothetical photodegradation pathway of imidacloprid (I) in aqueous media

The photolytic pathway of imidacloprid is presented in Figure 2. During irradiation of **I** the photoreaction affects only the imidazolidine moiety of the molecule while the 6-chloropyridine moiety remains unchanged. This photodegradation pathway is supported by some postulated metabolic pathways of imidacloprid in plants after spray and granular application (Koester, 1992; Nauen *et al.*, 1998). Thus it seems that a potential exists for photolysis in the removal of traces of chloronicotinoid insecticides. Moreover, the present study indicates that the main phototransformation pathway of **I** involves hydrolysis, oxidation and dehydration.

ACKNOWLEDGEMENTS

Thanks to Bayer India Ltd. for supplying analytical grade imidacloprid and financial assistance. The infrastructural facility provided by B.C.K.V. is also thankfully acknowledged.

REFERENCES

Aharonson N (1987). Potential contamination of groundwater by pesticides. *Pure and Applied Chemistry* **59**, 1419-1446.

Bai D; Lummis S C R; Leicht W; Beer H; Sattelle B D (1991). Action of imidacloprid and a related nitromethylene on chlolinergic receptors of an identified insect motor neurone. *Pesticide Science* **33**, 197-204.

Cox L; Koskinen W C; Yen P Y (1997). Sorption-desorption of imidacloprid and its metabolites in soils. *Journal of Agricultural and Food Chemistry* **45**, 1468-1472.

Edwards C A (1973). *Persistent pesticides in the environment.* (2nd edn), CRC Press: Cleveland.

Kagabu S; Moriya K; Shibuya K; Hattori Y; Tsuboi S; Shiokawa K (1992). 1-(6-Halonicotinyl) –2 – nitromethylene – imidazolidines as potential new insecticides. *Bioscience Biotechnology and Biochemistry* **56**, 362-363.

Koester J (1992). Comparative metabolism of (pyridinyl – ^{14}C-methyl) – imidacloprid in plant cell suspension cultures. *Proceedings 1992 Brighton Crop Protection Conference – Pests & Diseases*, 901-906.

Liu M; Latli B; Casida J E (1995). Imidacloprid affinity binding site in Musca nicotinic acetylcholine receptor: interactions with physostigmine and a variety of nicotinic agonists with chloropyridyl and chlorothiazolyl substituents. *Pesticide Biochemistry and Physiology* **52**, 170-181.

Nauen R; Tietjen K; Wagner K; Klbert A (1998). Efficacy of plant degradates of imidacloprid against *Myzus persicae* and *Aphis gossypii*. *Pesticide Science* **52**, 53-57.

Oliver B G; Carey J H (1986). In: *Homogeneous and heterogeneous photocatalysis.* eds E Pelizzetti & N Serpone, p 629. Reidel: Dordrecht, The Netherlands.

Rouchaud J; Gustin F; Wauters A (1994). Soil biodegradation and leaf transfer of insecticide imidacloprid applied in seed dressing in sugar beet crops. *Bulletin of Environmental Contamination and Toxicology* **53**, 344-350.

Rouchaud J; Gustin F; Wauters A (1996). Imidacloprid insecticide soil metabolism in sugar beet field crops. *Bulletin of Environmental Contamination and Toxicology* **56**, 29-36.

Sarkar M A; Biswas P K; Roy S; Kole R K; Chowdhury A (1999). Effect of pH and type of formulation on the persistence of imidacloprid in water. *Bulletin of Environmental Contamination and Toxicology* **63**, 604-609.

Sarkar M A; Roy S; Kole R K; Chowdhury A (2001). Persistence and metabalism of imidacloprid in different soils of West Bengal. *Pest Managment Science* **57**, 598-602.

Scholz K; Spiteller M (1992). Influence of ground cover on the degradation of ^{14}C-imidacloprid in soil. *Proceedings 1992 Brighton Crop Protection Conference – Pests & Diseases*, 883-888.

Timme G; Frehse H; Laska V (1986). Zur Statistischen Interpretation und graphischen Darstellung des Abbauverhaltens von Pflanzenschutzmittel – Rückständen. *Pflanzenschutz – Nachr* **39**, 188-204.

Walter H F; Freshe H; Timme G (1993). Zur Statistischen Interpretation und graphischen Darstellung des Abbauverhaltens von Pflanzenschutzmittel – Rückständen. *Pflanzenschutz – Nachr* **46**, 265-690.

Warnhoff H; Schneider V (1999). Photodegradation of Imidacloprid. *Journal of Agricultural and Food Chemistry* **47**, 1730-1734.

Yamamoto I; Yabuta G; Tomizawa M (1995). Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. *Journal of Pesticide Science* **20**, 33-40.

Pencycuron application to soils: degradation and effect on microbiological parameters

A Chowdhury, R Pal, K Chakrabarti
Department of Agricultural Chemistry & Soil Science, Calcutta University, 35 Ballygunge Circular Road, Calcutta, Pin-700 019, India

A Chakraborty
Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, Pin- 741 252, India

ABSTRACT

Clay loam soil from agricultural fields of alluvial soil (Typic udifluvent) and coastal saline soil (Typic endoaquept) were investigated for the degradation and effect of pencycuron applied at normal, 2-times, and 10-times field dose rate with and without decomposed cow manure, on soil microbial variables under laboratory conditions. Pencycuron spiking at normal and 2-times the field dose rate resulted in a short-lived (in case of 10-times slightly longer) and transitory toxic effect on soil microbial biomass-C ergosterol content. Amendment of decomposed cow manure did not seem to have any counteractive effect of the toxicity of pencycuron on the microbial variables.

INTRODUCTION

The intensive use of pesticidal chemicals may be a matter of environmental concern, because of the potential for adverse effect of these chemicals on soil microorganisms (Araújo *et al.*, 2003), which may impair soil fertility (Schuster & Schröder, 1990). In order to use the new generation pesticides in a flexible, but environmentally sensitive manner, the degradation of pesticides in soil and their effect on microorganisms should be studied.

Pencycuron [1-(4-chlorobenzyl)-1-cyclopentyl-3-phenylurea] is a relatively new non-systemic protective phenylurea fungicide for the control of diseases caused by *Rhizoctonia solani* and *Pellicularia* spp. of various crops and can be used as foliar spray and dust application or by soil incorporation (Tomlin, 1997).

The use of pesticides may alter the structure and function soil ecology. Many toxicity tests have been developed to assess the risks of pesticide application to soils. The total soil microbial biomass is an important parameter of ecological tests (Beelen & Doelman, 1997). Ergosterol, predominantly occurring in the phospholipid bilayer of the fungal cell membrane, may be a useful index of fungal presence (Hart & Brookes, 1996).

The majority of the degradation and ecotoxicological studies of pesticide applications have been carried out in non-problematic soils. The present study deals with the degradation of pencycuron and its side effects on certain microbiological variables, in two different soil types under laboratory conditions with and without decomposed cow manure (DCM). This is considered critical since microorganisms play a vital role in ensuring maintenance of soil fertility and the degradation of organic matter and pollutants in soils.

MATERIALS AND METHODS

Soils

Two different soils were used in this study: an alluvial (AL; udifluvent) soil and a coastal saline (CS; endoaquept) soil . Samples of AL soils were collected from the surface layer (0-15 cm) at the Experimental Farm of Bidhan Chandra Krishi Viswavidyalaya at Mohanpur. CS soils were collected from the surface layer (0-15 cm) at the Agricultural Experimental Farm of Central Soil Salinity Research Institute, Canning Town, West Bengal, India. The soils had no history of receiving any pesticide treatment 6 months prior to this study. All the physicochemical parameters of the two soils have been analysed following standard procedures (Jackson, 1973; Black, 1965).

Experimental plan

Decomposed cow manure was used as an organic amendment @ 10 t /ha. The DCM contained 14.0% organic C and 1.36% total N on dry weight basis with pH 7.4. Pencycuron (99.6% pure) obtained from Bayer CropScience India Ltd., India, was applied to the soil at normal field dose rate (FR) (187.5 g a.i. /ha), 2-times (2FR) and 10-times (10FR) the dose rate, with and without DCM amendment. The conversion of the field application to mg of pencycuron per kg of soil was calculated assuming an even distribution of pencycuron and/or DCM in the 0-15 cm layer (bulk density AL-2.6 and CS-2.3). The pencycuron transformation study was carried out under nonsterile and sterile conditions under dark. Conical flasks fitted with nonabsorbent cotton plug containing 50 g soil were sterilized by autoclaving on three consecutive days at 121°C for 20 min. and the sterile condition was maintained throughout the study period. From the whole set of sterilized flasks, three units of each were taken for analyses. The other units were retained for subsequent periodic analyses. The incubation study was carried at 60% of maximum water holding capacity of soil for 90 days (AL soil) and 60 days (CS soil). The moisture content of the soils was maintained with the aseptic addition of sterile distilled water.

To minimize the influence of soil processes and highlight the side effects of pencycuron on non-target soil micro-organisms two further application rates were employed: 2FR and 10FR field dose. The 10FR dose is recommended in laboratory tests to assess the side effects of pesticides on soil microflora (as per Sommerville, 1987).

Soil sample preparation

Moist soils were collected and brought to the laboratory in labeled and sealed polythene bags. The sieved field soil samples (< 2 mm) were homogenized and kept in polyethylene boxes and at *c.* 60% Water Holding Capacity (WHC). The boxes containing soil were stabilized at 25°C in the dark for one week. The stabilized soil samples were divided into three portions. Each portion was used separately for the sterile and nonsterile transformations of pencycuron and the microbiological studies. The incubation studies were carried out at ca 60% WHC at 30°C. It is noted that other temperature and moisture conditions prevail for agriculture in India.

Pencycuron treatment

FR corresponds to a weighed amount of 0.084 mg pencycuron per kg soil (dry weight), A solution of pencycuron in acetone was applied to 50 g soil in individual amber coloured Erlenmeyer flasks (250 mL) plugged with cotton pad. After complete removal of acetone by

evaporation at room temperature, the flasks were incubated at 30°C for the periodic incubation study. The control samples received only acetone and underwent the same procedure.

Determination of pencycuron residues

Pencycuron was quantified using high performance liquid chromatography (HPLC 1050 Hewlett Packard equipped with UV detector and 3392A integrator. The soil samples were immediately extracted with acetone three times (100ml each) with shaker (1 h), each followed by ultrasonicator for 5 min. After centrifugation at 3000 rpm for 10 min., the extracts were combined and pencycuron was partitioned in $CHCl_3$ (100+50+50 ml). The $CHCl_3$ layer was evaporated to dryness, rinsed with HPLC grade methanol and filtered (0.2μm) for direct HPLC analysis. Pencycuron was separated on an Intersil 150 x 4.6 mm ODS 2, 5μ (RP C_{18}) column using a mobile phase of methanol and water (90:10) at a flow rate of 1 ml/min and column temperature at 40°C. Quantification was performed against pencycuron standard at a wavelength of 240 nm. Under this condition the retention time of pencycuron was 3.2 min., the limit of detection was 0.01 mg and the sensitivity of the method was 0.005 mg/ kg. The average recovery was found to be 92.0 – 94.1%.

Microbial assay

The MBC was determined by fumigation extraction method (Joergensen, 1995) followed by determination of K_2SO_4 extractable C (Vance *et al.*, 1987). Biomass C was estimated as: Biomass C = 2.64 Ec, where Ec is the difference between K_2SO_4 extractable C from the fumigated and unfumigated soils. Ergosterol content of the soil samples was determined by the method described by Djajakirana *et al.* (1996).

Soil samplings were done at 1, 7, 15, 30, 45, 60, 75 and 90 days and 1, 7, 15, 30, 45 and 60 days for AL soil and CS soil respectively following pencycuron application. One flask of each treatment for each sample was destructively sampled.

Statistical method

Treatments were replicated three times in a completely randomized design. Statistical analysis was conducted using the IRRISTAT statistical package developed by International Rice Research Institute, Philippines. For comparison of averages Duncan's Multiple Range Test was used at the 5% probability level.

RESULTS AND DISCUSSION

Pencycuron degradation

Higher rates of degradation in nonsterilized soil than in sterilized soil confirmed the role of microbes in its degradation. Percent degradation of pencycuron by abiotic and biotic factors is presented in Table 1. Pencycuron degradation also took place in sterilized soils, indicating the role of chemical and abiotic factors other than photodegradation as the soils were kept in the dark during the experimental period. However, there might have been the possibility of bonding to humic substances or entrapment due to sequestration reactions of the compound not recovered by the extraction method. Generally, reversible or irreversible sorption and/or

sequestration mechanisms in combination with the microbially driven turnover of soil organic matter strongly influence pesticide metabolism although the key processes involved are still not understood precisely (Burauel & Führ, 2001). To further characterise this behaviour it would be necessary to conduct assessments with radiolabelled compound in order to quantify the mineralized component as CO_2.

After 30 days incubation, between 29.4 – 38.7 % (AL soil) and 83.3 – 87.2% (CS soil) of initial pencycuron had undergone degradation (and/or was bound to organic matter) under sterile conditions. On the other hand, 32.0 – 46.7 % (AL soil) and 4.9 – 12.8% (CS soil) of initial pencycuron had undergone degradation (and/or was bound to organic matter) following amendment with DCM. The high level of abiotic degradation and low level of microbial degradation would suggest that binding may be the major dynamic in these systems rather than degradation.

Microbial biomass-C

Evidence of the depressive effect of pencycuron treatment on the content of soil MBC at FR, 2FR and 10FR was prominent (Table 2). The MBC decreased significantly ($p< 0.05$) in AL soil up to day 15 at FR and 2FR and day 30 at 10FR compared to the control treatments, irrespective of DCM amendment. In CS soil, however, MBC decreased significantly ($p< 0.05$) up to day 15 irrespective of pencycuron application rates. The decrease was linked to the toxic effect of pencycuron on soil microorganisms, which were not adapted to this fungicide. The microbial biomass in pencycuron treated AL soil returned almost to its original values on day 90 in contrast to day 60 in CS soil.

The initial decrease of MBC in AL soil upto day 15 at FR and 2FR pencycuron spiking corresponded to their half-lives. The MBC decreased upto day 30 at 10FR, which did not correspond to the half-life. In CS soil the depressive effect on MBC persisted beyond the half-life period. The persistence of the detrimental effect on MBC exceeding the half-life period might be due either to the inhibition of degrading microorganisms at higher concentration in AL soil and all the concentrations in CS soil (Gan *et al.*, 1995; Gevao *et al.*, 2000) or the build up of higher level of toxic metabolites. Amendment of DCM did not lessen the toxic effect of pencycuron, but in general, increased the MBC. Organic matter is an important source of nutrients and the activity and size of soil microbial populations are enhanced with greater organic C contents (Anderson & Domsch, 1989).

Table 1. Comparative role of biotic and abiotic degradation processes for pencycuron in alluvial and coastal saline soils following 30 day incubation

Treatment	Percent Dissipation		
	Nonsterilized	Sterilized	DCM Amendment
Alluvial soil			
Pencycuron FR	71.7d	38.7a	33.0c
Pencycuron 2FR	71.3e	38.2b	33.1c
Pencycuron 10FR	69.5f	37.5c	32.0d
Pencycuron FR + DCM	77.7a	33.3d	44.4b
Pencycuron 2FR + DCM	77.3b	32.8e	44.5b
Pencycuron 10FR + DCM	76.1c	29.4f	46.7a
Coastal saline soil			
Pencycuron FR	91.8d	85.6b	6.20e
Pencycuron 2FR	92.1c	87.2a	4.90f
Pencycuron 10FR	91.3e	84.2e	7.10d
Pencycuron FR + DCM	95.0b	84.4d	10.6b
Pencycuron 2FR + DCM	95.1b	85.2c	9.80c
Pencycuron 10FR +DCM	96.0a	83.3f	12.8a

FR Field rate; DCM Decomposed cow manure Values in the same column followed by different letters are statistically different at $p< 0.05$* by DMRT

Ergosterol

Ergosterol is a fungal specific biomarker and has been used as a sensitive measure of pesticidal side effects (Hart & Brookes, 1996). At all rates of pencycuron application the ergosterol content decreased (Table 2) to a greater extent in the amended and unamended controls during the initial stages of incubation. The application of pencycuron to AL soil at FR and 2FR decreased the ergosterol content upto day 30 and day 45 at 10FR, irrespective of DCM applied or not. At all rates of pencycuron application to CS soil, with and without DCM, ergosterol content decreased upto day 15. At the end of incubation of both the soils at FR and 2FR, the ergosterol content remained similar to the respective controls in contrast to 10FR, which remained lower.

The duration of the depressive action of pencycuron was shorter on MBC compared to ergosterol. This indicated that pencycuron affected mostly the fungal population of the MBC. The depressive effect of pencycuron on ergosterol was enhanced in DCM amended soil, compared to the unamended soil. Hart & Brookes (1996) in a study with the fungicides triadimefon and epoxiconazole in straw amended soil also reported similar results. The greater depressive effect of pencycuron on the ergosterol content of soil indicated that newly generated fungal species (following organic matter addition) were more sensitive than the original population.

Degradation of pencycuron was soil dependent. Microbial mediated degradation was more prominent in AL soil than in CS soil. Organic matter amendment to soil resulted in more rapid degradation of pencycuron than the unamended soil. Pencycuron application had a toxic effect

on the microbial variables studied, although the duration was short-lived. The duration of the toxic effect remained unchanged with addition of DCM. The toxicity of pencycuron to the newly generated soil microflora appeared to be stimulated by the addition of DCM. Pencycuron application at recommended rates had no detrimental effect on the soil microbial, biochemical and ecophysiological parameters. Results of the controlled laboratory studies cannot be reliably extrapolated to field conditions. Field studies represent a more realistic approach for conclusive evidence of the ecological impact of pencycuron.

ACKNOWLEDGEMENTS

The authors would like to thank Department of Agricultural Chemistry & Soil Science, University of Calcutta for the delightful collaboration in the scientific activities of the work. The author is grateful to Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, India, for excellent technical assistance and Bayer CropScience Ltd, India for sponsoring the research project.

REFERENCES

Alexander M (1977). *Introduction to soil microbiology*. Wiley Eastern Limited: New York.

Anderson T H; Domsch K H (1985b). Determination of eco-physiological maintenance requirements of soil microorganisms in a dormant state. *Biol. Fertil. Soils* **1**, 81-89.

Araújo A S F; Monteiro R T R; Abarkeli R B (2003). Effect of glyphosate on the microbial activity of two Brazilian soils. *Chemosphere* **52**, 799-804.

Beelen P V; Doelman P (1997). Significance and application of microbial toxicity tests in assessing ecotoxicological risks of contaminants in soil and sediment. *Chemosphere* **34**, 455-499.

Black C A (1965). Methods of soil analysis (parts 1 & 2). *American Society of Agronomy*, Madison, WI.

Burauel P; Führ F (2001). Long-term fate of organic chemicals in lysimeter experiments and the role of the bound pesticide fraction. In: *Humic Substances and Chemical Contaminants*. eds C E Clapp, M H B Hayes, N Senesi, P R Bloom & P M Jardine, pp. 289-301. Soil Science Society of America: Madison.

Djajakirana G; Joergensen R G; Meyer B (1996). Ergosterol and microbial biomass relationship in soil. *Biol. Fertil. Soils* **22**, 299-304.

Gerber H R; Anderson J P E; Bugel-Mongensen B; Castle D; Domsch K H; MalKomes H P; Arnold D J; Werf H V D; Verbeken R; Vonk J W (1989). Revision of recommended laboratory tests for assessing side effects of pesticides on soil microflora. In: *Proceedings of the 4th International Workshop, Leverkusen*.

Gevao B; Semple K T; Jones K C (2000). Bound pesticide residues in soils: a review. *Environmental Pollution* **108**, 3-14.

Hart M R; Brookes P C (1996). Effects of two ergosterol-inhibiting fungicides on soil ergosterol and microbial biomass. *Soil Biol. Biochem.* 28, 885-892.

Jackson M L(1967). *Soil chemical analysis*. Prentice Hall of India: New Delhi, India.

Joergensen R G (1995). Microbial biomass. In: *Methods in applied soil microbiology and biochemistry*, eds K Alef & P Nannipieri, pp. 382-386. Academic Press: London.

Martin J P (1963). Influence of pesticide residues on soil microbiological and chemical properties. *Res. Rev.* **4**, 96-129.

Nelson D.W; Sommers L E (1982). Total carbon, organic carbon and organic matter. In: *Methods of Soil Analysis. Part 2. 2nd edition,* eds D L Sparks, A L Page, P A Helmke, R H Loeppert, P N Soltanpour, M A Tabatabai, C T Johnson & M E Sumner, pp. 539-579. ASA and SSSA: Madison, WI

Perucci P; Dumontet S; Bufo S A; Mazzatura A; Casucci C (2000). Effects of organic amendment and herbicide treatment on soil microbial biomass. *Biol. Fertil. Soils* **32**, 17-23.

Reddy B R; Sethunathan N (1985). Salinity and the persistence of parathion in flooded soil. *Soil Biol. Biochem.* **17**, 235-239.

Sannino F; Filazzola M T; Violante A; Gianfreda L (1999). Fate of herbicides influenced by biotic and abiotic interactions. *Chemosphere* **39**, 333-341.

Tomlin C D S (ed.) (1997). Pencycuron. In: *The Pesticide Manual, Eleventh edition*, pp. 935-937. British Crop Protection Council: Farnham.

Table 2. Microbial biomass carbon and ergosterol content of alluvial and coastal saline soils treated with pencycuron both with and without decomposed cow manure

Treatment	Day of incubation															
	1	7	15	30	45	60	75	90	1	7	15	30	45	60	75	90
	Soil microbial biomass-C (lg g/1 oven dry soil)								Soil ergosterol content (lg g/1 oven dry soil)							
Alluvial soil																
Control	306b	282c	241c	211e	180g	165g	132g	122h	4.62b	4.14b	3.83b	3.62c	3.28cd	3.96c	3.57d	4.12f
Pencycuron FR*	286c	270d	232d	254c	289b	305b	328b	332d	4.07c	3.76c	3.32c	3.02d	3.38c	3.98c	4.08c	4.54d
Pencycuron 2FR	252e	238f	212f	237d	265d	293c	317c	326e	3.62e	3.15d	2.55e	2.10f	3.30cd	3.82c	4.02c	4.36e
Pencycuron 10FR	185g	137h	114h	98g	173h	245e	289e	312f	1.98g	0.88e	0.64f	0.45h	0.32e	1.35f	2.86e	3.37h
Control + DCM**	317a	305a	323a	264b	247e	212f	187f	163g	5.53a	6.54a	7.36a	6.98a	6.20a	5.87a	5.44a	5.36b
Pencycuron FR + DCM	316a	287b	254b	277a	306a	314a	340a	361b	4.78b	4.12b	3.67b	4.25b	4.96b	5.61b	5.52a	5.61a
Pencycuron 2FR + DCM	281d	242e	223e	256c	283c	310a	329b	372a	3.82d	3.27d	2.80d	2.43e	3.18d	3.64d	4.75b	5.14c
Pencycuron 10FR + DCM	213f	172g	149g	122f	187f	266d	306d	343c	2.16f	1.03e	0.78f	0.63g	0.37e	1.62e	3.00e	3.76g
Coastal saline soil																
Control	239b	224b	198b	166e	136f	107f			3.61b	3.33b	2.98b	2.63d	2.39e	2.11e		
Pencycuron FR	220d	200c	187c	204b	216b	242b			3.19cd	2.96cd	2.62bc	3.00c	3.42cd	3.63c		
Pencycuron 2FR	199e	175d	154d	173d	208c	235c			2.88d	2.29e	1.78d	2.62d	3.15d	3.57c		
Pencycuron 10FR	121g	75g	46f	99g	141f	201d			1.76f	0.63f	0.35e	0.78f	1.53f	2.47d		
Control + DCM	252a	238a	223a	188c	163e	138e			4.49a	5.53a	6.28a	6.01a	5.43a	4.82a		
Pencycuron FR + DCM	249a	205c	192c	216a	227a	256a			3.59b	3.27bc	2.91b	3.62b	4.12b	4.39b		
Pencycuron 2FR + DCM	232c	165e	152d	187c	219b	252a			3.28bc	2.89d	2.39c	3.19c	3.69c	4.10b		
Pencycuron 10FR + DCM	151f	101f	77e	121f	189d	237bc			2.17e	0.91f	0.53e	1.31e	2.37e	3.43c		

Values in the same column followed by different letters are statistically different at $p < 0.05$ by DMRT.

* Field rate; ** Decomposed cow manure

Studies on the fate of imidacloprid-a neonicotinoid insecticide in a rice ecosystem

A Bhattacharyya, J Bhattacharyya, H Banerjee
Pesticide Residue Laboratory, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India
Email: anjan_84@rediffmail.com

ABSTRACT

Imidacloprid [1-(6-chloro-3-pyridinylmethyl) – N- nitro imidazolidine-2-ylideneamine], a neonicotinoid broad-spectrum systemic insecticide, was recently introduced in India. It has a novel mode of action acting as an agonist of the nicotinyl acetylcholine receptor. This chloronicotinyl compound is very effective in controlling sucking insect, soil insects, termites and some species of chewing insects as well as seed dressing, soil treatments and foliar treatments in different crops. It is very popular in rice cultivation to control brown plant hopper (BPH). In order to determine the fate of the imidacloprid molecule in rice ecosystem, a field study was conducted at Bidhan Chandra Krishi Viswavidyalaya Research Farm, for two consecutive seasons (2002 kharif and 2003 rabi). Imidacloprid was applied in a rice field at the normal dose-rate of 17.8 g a.i. /ha, and at 35.6 g a.i. /ha on three occasions at intervals of 10 d and an untreated control was included. Soil samples were taken at an interval of 0, 5, 10 and 30 d, and rice water samples were drawn 0, 3, 7 and 10 d after the last application along with straw and grain at harvest. All the samples were extracted, cleaned and characterised by hplc coupled with UV/VIS detector. The study revealed that the dissipation of imidacloprid in soil followed 1st order kinetics and DT_{50} values ranged from 3.16-4.04 d. The estimated water phase dissipation half-life was found to be 1.91 d irrespective of treatment and season. No residue could be detected in rice straw and grain at harvest.

INTRODUCTION

Rice (*Oryza sativa*) is the most important cereal crop of India grown in about 25% of agricultural land, measuring about 43 million ha with total production of about 80 million tonnes. In every year, there are reductions in yield due to the impact of various insect pests like stem borers, leaf folders, brown plant hoppers (BPH) and plant hoppers. There are various insecticides recommended for their control, amongst them imidacloprid [1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine], a new generation, highly active neonicotinoid broad-spectrum systemic insecticide, that was recently introduced in India. It has a novel mode of action, acting as an agonist of the nicotinyl acetylcholine receptor (Bai *et al.*, 1991; Mullins *et al.*, 1993). It shows high activity, especially against a great number of sucking pests such as aphids, leaf- and plant hoppers, thrips, whiteflies, soil insects, termites and some species of chewing insects, as well as seed dressing, soil and foliar treatments in different crops (Jarande *et al.*, 1994; Kumar & Santharam, 1999; Kumar *et al.*, 2001; Mote *et al.*, 1994; Sarkar *et al.*, 2001; Tomlin, 2000). Recently it has been observed that imidacloprid is very popular in rice cultivation to control brown plant hopper (BPH). So far, no studies have been conducted to investigate the fate of imidacloprid molecule in the rice ecosystem. This investigation was

therefore initiated to determine the dissipation pattern as well as residue levels of imidacloprid present in soil, water, grain and straw samples.

MATERIALS AND METHODS

A field study was conducted at Bidhan Chandra Krishi Viswavidyalaya Research Farm for consecutive two seasons: 2002 kharif (rainy season) and 2003 rabi (dry) in rice (cv. Khitish). The commercial formulation of imidacloprid ('Confidor' 200 g a.i./litre, SL from Bayer CropScience) was applied in a rice paddy field three times at intervals of 10 d at 17.8 g a.i. /ha (recommended dose, I_1) and 35.6 g a.i. /ha (double the recommended dose, I_2), including an untreated control I_3. Sprays were applied at high volume (750 litres/ha) with a knapsack sprayer. Each treatment, including control was replicated three times in a randomised block design. For the dissipation study, soil samples were taken at 0, 5, 10 and 30 d intervals after the first application and soil samples were also collected at harvest. Field water samples were collected at 0, 3, 7 and 10 d after each application. Grain and straw samples were collected at harvest.

Soil samples were collected at a depth of 0-15 cm with a soil auger and representative amounts of sample (100 g) were dissolved in a mixture of methanol:water (8:2 *v/v*) overnight. This was shaken for 1 h in a mechanical shaker and filtered through Buchner funnel followed by washing with 100 ml of same solvent mixture. The extract was concentrated in a rotary vacuum evaporator at 40°C. The concentrated extract was partitioned with 100 ml mixture of hexane:ethylacetate (8:2 *v/v*) and the organic layer was discarded. The aqueous layer was again partitioned three times with (100+50+50) ml dichloromethane. The organic layer was passed through anhydrous sodium sulphate and evaporated to dryness using rotary vacuum evaporator at 40°C. The concentrated extract was chromatographed over florisil (10 g) in a glass column with 1 cm layer of anhydrous sodium sulphate at the top as adsorbent. The column was eluted first with 100 ml mixture of hexane:ethylacetate (8:2 *v/v*) and the fraction was discarded. It was further eluted with 100 ml of a mixture of acetonitrile:methanol (9:1 *v/v*). The eluate was then concentrated and reconstituted with acetonitrile for hplc-analysis.

Representative amounts (50 g) of each sample (grain and straw) were blended with 150 ml of mixture of methanol:water (8:2 *v/v*) in a Remi automix blender for 2 min and filtered through a Buchner funnel. The residue was re-extracted twice (2x50 ml) with the same solvent mixture. Similar steps were then followed as described above.

Field water samples (500 ml) were taken in a separating funnel and partitioned three times with (100+50+50) ml dichloromethane by adding 50 ml of saturated aqueous sodium chloride solution. The dichloromethane layer was collected through anhydrous sodium sulphate. The organic layer was then concentrated in a rotary vacuum evaporator at 40°C and finally its volume was made up with acetonitrile for hplc analysis.

Final analysis of imidacloprid residue in soil, water, grain and straw samples were done by hplc (Hewlett Packard series 1050) with UV/VIS detector coupled with 3392A integrator. The spherisorb reverse phase C18 (cartridge column), 15 cm x 4.6 mm i.d. column was used. The mixture of acetonitrile and water (8:2 *v/v*) was used as mobile phase for the detection of imidacloprid residue. The other parameters like flow, wavelength (λ), retention time, limit of

detection and limit of quantification (LOQ) were 0.33 ml/min, 270 nm, 3.38 min, 0.001 ppm and 0.05 ppm respectively.

In order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up steps employed for the present study, soil, water, grain and straw samples were spiked with 1, 2 and 5 ppm analytical standard of imidacloprid. Average recoveries were 91.47, 87.93, 90.67 and 88.40% respectively.

DISCUSSION

The persistence of imidacloprid residues in soil and water has been characterized with results (regression equation, dissipation half-lives for consecutive two seasons) (Tables 1-4). The initial residues of imidacloprid were found to be in the range of 0.36-0.51 ppm for soil and 0.10-0.13 ppm for water for the recommended dose (17.8 g a.i. /ha I_1) and 0.84-0.89 ppm for soil and 0.25-0.27 ppm for water at double the recommended dose (35.6 g a.i. /ha I_2). No residue was detected in the untreated control (I_3). It has been observed from the result that the residues of imidacloprid in soil and water declined progressively with dissipation of imidacloprid in soil and water following first order kinetics in both the dosage regimes irrespective of any season. The results demonstrated that dissipation DT_{50} values in soil ranged from 3.16 - 4.50 d. The dissipation half-lives for rice field water were found to be in the range of 1.86 - 1.91 d. No residue was detected in soil, grain and straw samples at harvest which is also substantiated from the recent study reported by Zhu & Xu (2000) that, at harvest, grain samples have no measurable imidacloprid residues. From these results it may be concluded that the imidacloprid will not pose residual problems in rice production ecosystems.

Table 1. Persistence of imidacloprid in soil cropped with paddy (1st season)

DAT	I (dose rate)	Residue in ppm				Dissipation (%)
		R_1	R_2	R_3	Mean±SE	
I_1 (17.8g.a.i. /ha)						
0		0.42	0.36	0.29	0.36±0.065	-
5		0.19	0.15	0.10	0.15±0.045	58.33
10		0.06	0.04	0.02	0.04±0.020	88.89
30		BDL	BDL	BDL	-	-
I_2 (35.6g.a.i. /ha)						
0		0.84	0.69	0.98	0.84±0.15	-
5		0.30	0.49	0.62	0.56±0.09	33.33
10		0.10	0.19	0.24	0.18±0.07	78.97
30		BDL	BDL	BDL	-	-
Regression equation:		I_1; $y = 2.5886-0.0954x$			I_2 ; $y = 2.9770-0.0669x$	
Half-life ($T_{1/2}$):		$I_1 = 3.16$ d			$I_2 = 4.50$ d	

BDL= Below detectable limit ≤ 0.05 ppm; R replicates 1, 2, 3

Table 2. Persistence of imidacloprid in soil cropped with paddy (2nd season)

DAT I (dose rate)	Residue in ppm				Dissipation (%)
	R_1	R_2	R_3	Mean±SE	
I_1 (17.8g.a.i. /ha)					
0	0.32	0.69	0.52	0.51±0.185	-
5	0.10	0.19	0.13	0.14±0.046	72.55
10	0.05	0.09	0.06	0.07±0.021	86.27
30	BDL	BDL	BDL	-	-
I_2 (35.6g.a.i. /ha)					
0	0.75	1.12	0.80	0.89±0.200	-
5	0.32	0.59	0.50	0.47±0.140	47.19
10	0.11	0.20	0.16	0.16±0.045	82.02
30	BDL	BDL	BDL	-	-

Regression equation: I_1; $y = 2.6642-0.0863x$ I_2 ; $y = 2.9812-0.0745x$

Half-life ($T_{1/2}$): $I_1 = 3.49$ d, $I_2 = 4.04$ d

BDL= Below detectable limit ≤ 0.05 ppm; R replicates 1, 2, 3

Table 3. Persistence of imidacloprid in water of paddy (1st season)

DAT I (dose rate)	Residue in ppm				Dissipation (%)
	R_1	R_2	R_3	Mean±SE	
I_1 (17.8g.a.i. /ha)					
0	0.11	0.19	0.08	0.13±0.057	-
3	0.04	0.03	0.01	0.03±0.015	76.92
7	BDL	BDL	BDL	-	-
10	BDL	BDL	BDL	-	-
I_2 (35.6g.a.i. /ha)					
0	0.28	0.20	0.34	0.27±0.070	-
3	0.09	0.07	0.12	0.09±0.025	66.67
7	0.02	0.01	0.03	0.02±0.01	92.59
10	BDL	BDL	BDL	-	-

Regression equation: I_2 ; $y = 2.4341-01616x$

Half-life ($T_{1/2}$): $I_2 = 1.86$ d

BDL= Below detectable limit ≤ 0.05 ppm; R replicates 1, 2, 3

Table 4. Persistence of imidacloprid in water of paddy (2nd season)

DAT I (dose rate)	Residue in ppm				Dissipation (%)
	R_1	R_2	R_3	Mean±SE	
I_1 (17.8g.a.i. /ha)					
0	0.12	0.18	0.10	0.13±0.055	-
3	0.03	0.05	0.02	0.03±0.015	76.92
7	BDL	BDL	BDL	-	-
10	BDL	BDL	BDL	-	-
I_2 (35.6g.a.i. /ha)					
0	0.25	0.18	0.32	0.25±0.070	-
3	0.10	0.08	0.11	0.10±0.015	66.67
7	0.02	0.01	0.03	0.02±0.010	92.59
10	BDL	BDL	BDL	-	-

Regression equation: I_2 ; $y = 2.4252-0.1577x$

Half-life ($T_{1/2}$): $I_2 = 1.91$ d

BDL= Below detectable limit ≤ 0.05 ppm; R replicates 1, 2, 3

Table 5. Harvest time residues of imidacloprid in grain, straw of paddy and soil (1st season)

Sample	Residue in ppm		
	R_1	R_2	R_3
I_1 (17.8g.a.i. /ha)			
Grain	BDL	BDL	BDL
Straw	BDL	BDL	BDL
Soil	BDL	BDL	BDL
I_2 (35.6g.a.i. /ha)			
Grain	BDL	BDL	BDL
Straw	BDL	BDL	BDL
Soil	BDL	BDL	BDL

BDL= Below detectable limit ≤ 0.05 ppm; R replicates 1, 2, 3

Table 6. Harvest time residues of imidacloprid in grain, straw of paddy and soil (2nd season)

Sample	Residue in ppm		
	R_1	R_2	R_3
I_1 (17.8g.a.i. /ha)			
Grain	BDL	BDL	BDL
Straw	BDL	BDL	BDL
Soil	BDL	BDL	BDL
I_2 (35.6g.a.i. /ha)			
Grain	BDL	BDL	BDL
Straw	BDL	BDL	BDL
Soil	BDL	BDL	BDL

BDL= Below detectable limit ≤ 0.05 ppm; R replicates 1, 2, 3

ACKNOWLEDGEMENT

We are grateful to Bayer CropScience India Ltd., Mumbai, for financial assistance.

REFERENCES

Bai D; Lummis S C R; Leicht W; Beer H; Sattele B D (1991). Action of imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neurone. *Pesticide Science* **33**,197-204.

Jarande N T; Dethe M D (1994). Imidacloprid for effective control of sucking pests of chilli. *Pestology* **18** (12), 17-18.

Kumar K; Santharam G (1999). Effect of imidacloprid against aphids and leaf hoppers on cotton. *Annals of Plant Protection Science* **7**(2), 212-254.

Kumar R; Dikshit A K; Prasad S K (2001). Bioefficacy and residues of imidacloprid in mustard. *Pesticide Research* **13** (2), 213-217.

Mote V N; Datkhile R V; Pawar S A (1994). Imidacloprid as a seed dressing against sucking pests of okra. *Pestology* **18** (3), 5-9.

Mullins J W (1993). Imidacloprid: a new nitroguanidine insecticide, in pest control with enhanced environmental safety. *American Chemical Society*, pp. 183-198. ACS Symposium Series 524: Washington DC

Sankar S A; Roy S; Kole R K; Chowdhury A (2001). Persistence and metabolism of imidacloprid in different soils of West Bengal. *Pest Management Science* **57**, 598-602.

Tomlin C (ed.) (2001). *The Pesticide Manual*, 12th edn, pp 537-538. British Crop Protection Council: Farnham, UK.

Zhu Y; Xu H (2000). Degradation dynamics and residues of imidacloprid in rice and soil. *Acta Agriculturae Zhejiangensis* **12** (6), 400-403.