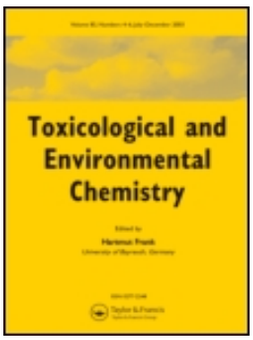

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THE DISSIPATION OF MALATHION AND DIMETHOATE FROM THE PEA PLANTS (*PISUM SATIVUM*) UNDER CONTROLLED CONDITIONS

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^{14}C-malathion, [S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate] and ^{14}C-dimethoate, [O,O-dimethyl S-(N-methylcarbamoylmethyl)phosphorodithioate] have been administered to leaves of pea (*Pisum sativum*) plants under simulated tropical conditions in the greenhouse. The results show that ^{14}C-malathion dissipated faster than ^{14}C-dimethoate from the leaf surface: After 16 days, 5.7% of the radioactive dose applied as malathion remained on the surface of the treated leaves as surface (dislodgeable) residues. The extractable residues constituted 9% while the bound (non-extractable) residues were 4.2% of the total initial radioactivity. 16.7% of the initial dose had been translocated from the treated leaves to the rest of the plant. 17.1% of the initial dose constituted ^{14}C-compounds other than $^{14}CO_2$ recovered from the transpired water. The corresponding fractions of dimethoate were 21% dislodgeable, 17.4% extractable, and 5% bound residues. Only 4.6% of ^{14}C-dimethoate residues were distributed in the plant. Although dimethoate is 170 times more soluble in water than malathion, only 10.4% of the initial pesticide dose of dimethoate constituted ^{14}C-compounds in the transpired water. Dimethoate formed dimethoxon, the oxygen analogue, while malathion did not form malaoxon in the plant, as revealed by thin layer chromatography (TLC) and confirmed by gas chromatography-mass spectrometry (GC-MS).

Keywords: ^{14}C-malathion; ^{14}C-dimethoate; dissipation; controlled conditions

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INTRODUCTION

The bioavailability of the pesticide residues in food to consumers has prompted the Food Agricultural Organisation (FAO) to initiate the pesticide programme to raise technical competence and safety in use of pesticides [1–2]. When pesticides are sprayed on plants, materials not adhering tenaciously to the plant surface slough off immediately. The portion remaining on the plant becomes an effective deposit, which then begins to be absorbed into the plant tissue [3]. The disappearance of these pesticides from plant leaf surfaces after interception is exponential and often first-order kinetics prevail [4]. The cuticle is an effective barrier for transport into and out of the leaf, holding water and solutes within the tissue and inhibiting the penetration of pathogens [5]. Apart from the thickness of the cuticle, temperature, viscosity, molecular radius, partition coefficient, and the concentration gradient of the pesticide across the cuticle are the parameters, which determine the rate of pesticide penetration into the plant [6].

In the present study ^{14}C-malathion labelled at the 2nd and 3rd carbons in the diethyl malonate moiety and ^{14}C-dimethoate labelled at the carbons of the methoxy groups, were administered to selected leaves of garden pea plants growing under simulated tropical (Kenyan) conditions in a greenhouse of the University of Bayreuth, Germany. Uptake, and distribution of ^{14}C in the plant as well as losses by respiration and transpiration have been followed over 16 days. The study was conducted between March and August 1998. The results of the study are presented in this paper.

MATERIALS AND METHODS

Chemicals were of analytical grade and solvents were pure grade. Solutions of pesticide emulsion concentrates were prepared in tap water. The pea seeds were obtained from farmers in Kenya. Malathion (2nd, 3rd carbons of the diethylmalonate moiety were ^{14}C-labelled) with specific radioactivity of 50 mCi/mmol and radiochemical purity of 99% (by HPLC) was purchased from Sigma Chemical Company, Missouri, USA. The standards of malathion and dimethoate were obtained from Chester PA, USA. ^{14}C-Methoxy-dimethoate was synthesised in the laboratory by the reaction of phosphorus pentasulphide (P_2S_5), *N*-methyl 2-chloro acetamide and labelled ^{14}C-methanol according to the method of Chen and Dauterman [7]. 250 μCi of ^{14}C-methanol with a specific radioactivity of 50 mCi/mmol, neat liquid in a sealed ampoule was purchased from ICN Pharmaceuticals, USA. 2,5-diphenyloxazole (PPO)

and 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in toluene, Carbo-Sorb and Permafluor® were from Packard BioScience B.V., Netherlands.

Radioactivity was measured with a Liquid Scintillation Counter (Canberra Packard 2500 TR). Biological material was combusted in a Packard 307 Oxidiser in which the produced $^{14}CO_2$ was trapped in Carbo-Sorb and counted in the liquid scintillation counter. On chromatograms radioactivity was located with a Phosphor Imager (Fuji film BAS-2500) or a TLC Scanner (Berthold LB 2760). For GC-MS a Carlo Erba HRGC FractoVap 4160 equipped with a FID and coupled to a Double Focused Finnigan MAT 95 Spectrometer was used.

Application of the Pesticides and Sampling of the Plants

Green pea seeds were grown in half-litre PVC pots on benches in the greenhouse, which was illuminated by Hg-lamps of 5 × 250 W for 11 hours per day. The temperatures in the greenhouse ranged from 10 °C (night) to 26 °C (day). The average relative humidity was 70%. When the plantlets were 21 days old, they were treated with the pesticide. Each leaf received 100µl of the pesticide solution in tap water on a spot with a diameter of 15 mm. Only the first three foliage leaves on each plant were treated with pesticides as emulsifiable concentrate with initial radioactivity of 0.4 µCi for both malathion and dimethoate. A sample taken for analysis consisted of all treated leaves and the rest of the plant in each pot. The samples in triplicates were analysed for surface (dislodgeable) residues, extractable and bound (non-extractable) radioactivity. The rest of the plant was analysed for the translocated fraction of the pesticide. Samples awaiting analysis were frozen at −20 °C.

Analytical Methods

The dislodgeable residue on the malathion-treated leaves was removed by repeated washing with double distilled water. One ml aliquot of the combined washing was mixed with 4 ml of the counting cocktail (Lumasafe™ Plus) in a vial and counted in the Liquid Scintillation Counter. The dimethoate treated-leaf samples were rinsed with both double distilled water and pure acetone. The acetone fractions were combined and stripped of the acetone, and the residue was dissolved in the water fraction. One ml of the combined rinsing was mixed with the cocktail and radioassayed. The rinsed leaves were blotted carefully with filter paper and then frozen at −20 °C prior to extraction and analysis.The extractable residues in the rinsed leaves were extracted according to the method of Hugh *et al.* [8]. The resulting extract was highly

coloured and was cleaned according to the method of Leon *et al.* [9]. The column for clearing of the extracts was restored by the method of Hopper [10]. The eluted extracts were concentrated to 5 ml and an aliquot was radioassayed. The bound residue in air-dried extracted leaf samples was determined by combusting a sub-sample of 500 mg in a folded filter paper in a biological materials oxidiser. The released $^{14}CO_2$ was trapped by 7 ml of Carbo-sorb® and mixed with 7 ml of Permafluor E cocktail and counted in the Liquid scintillation counter. The recovery of the oxidiser as checked with a labelled standard of 1-pentanol and 1,3-butanediol was 94%. The translocated fraction in the rest of the plant was determined by combusting a sub-sample of 500 mg in a folded filter paper. The $^{14}CO_2$ released was radioassayed. Sample extracts, after clean up, were analysed by GC-MS.

The ^{14}C-compounds in the transpired water were determined by enclosing the potted pea plants in glass bell jars placed over troughs as shown in

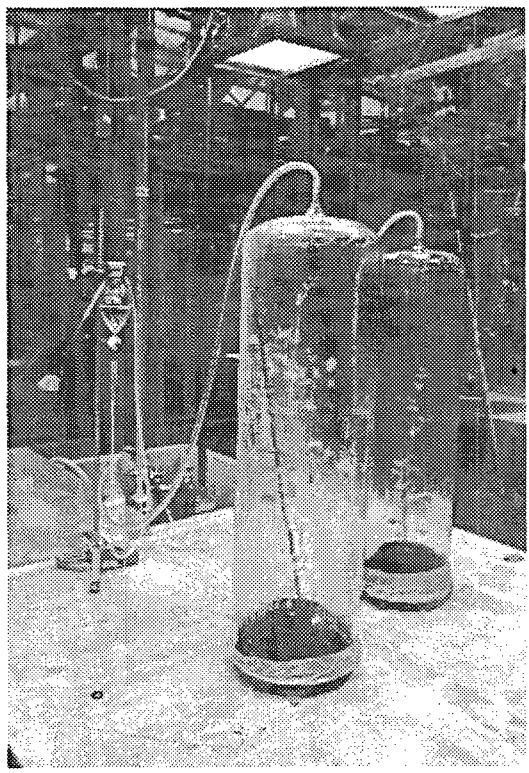

FIGURE 1 Experimental set-up for the collection of ^{14}C-compounds in the transpired water from pea plants.

Figure 1. The transpired water, which collected in the trough, was withdrawn with a hypodermic syringe at intervals of 12 hours and the radioactivity was measured in aliquots thereof. Two identical experiments were performed, one acting as a control in which no pesticide was applied. The recovery of the radioactivity from the plants spiked at a concentration of 10 mg/sample with malathion and 2.3 mg/sample with dimethoate were 89% and 83.7%, respectively and results were corrected according to these recovery rates. The data were subjected to statistical analysis according to the method of Mactaggart and Farewell [11].

RESULTS AND DISCUSSION

Figures 2 and 3 show the disappearance of 2nd, 3rd ^{14}C-labelled malathion and methoxy-^{14}C-dimethoate, respectively from the pea plants in the greenhouse over a period of 384 hours (16 days). The dislodgeable residues of the pesticides on the leaves initially decrease very fast and later slowly. The decrease in concentration of the dislodgeable residues of the pesticides on the foliar surface of the treated leaves is due to volatilisation into the surrounding air, penetration of the pesticide into the cells of the leaves, binding of the pesticide residues to the plant tissues and losses by transpiration [12]. The radioactivity of the extractable residues of both pesticides initially increased, reached a maximum and then decreased slowly. Initially the pesticide residues had penetrated into the cells and accumulated there

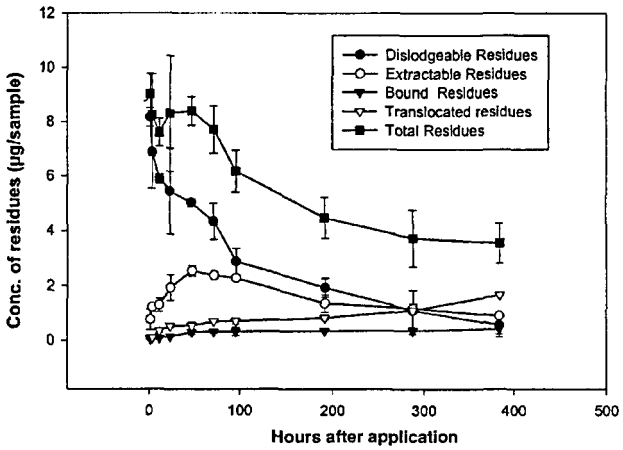

FIGURE 2 Foliar dissipation of malathion from the pea plant.

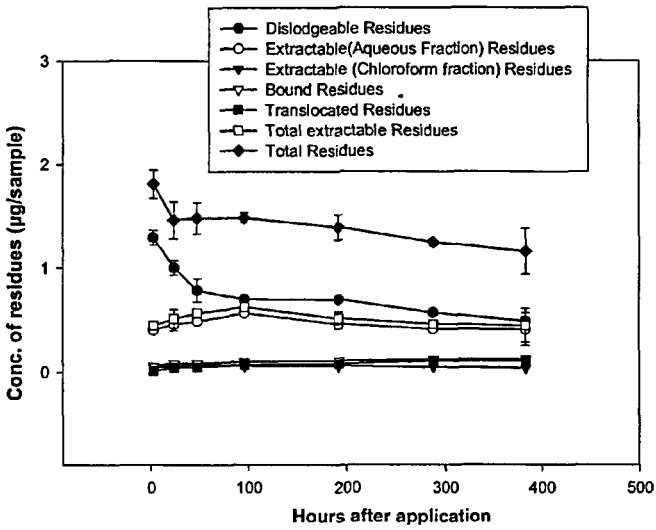

FIGURE 3 Foliar dissipation of dimethoate from pea plants.

with time, prior to export and degradation. Other processes, such as binding of the pesticides to the plant tissues and transpiration likewise reduced the amount of the extractable residues. At some stage the rate of these processes exceeded the rate of uptake of the pesticides from the foliar surface. Both the bound and translocated residues of the pesticides increased slowly with time. After 384 hours (16 days), there was 5.7% of the initially applied malathion remaining as dislodgeable residues on the surface of the treated leaves. In contrast, a considerable fraction (21.2%) of the initially applied dimethoate remained on the surface of the treated leaves as dislodgeable residues. The extractable residue of malathion was 9.1% while extractable residue of dimethoate was 19.4% in the treated leaves. The bound residues were similar with 4.2% and 5% for malathion and dimethoate, respectively. Translocation of labelled compounds from malathion were considerably higher (16.7%) than those from dimethoate (4.6%).

Overall, total malathion residues disappeared faster than the total residues of dimethoate from the pea plants. The data showed a good correlation between the log of concentration of the residues with time (Figures 4 and 5) and the first order kinetics of the data give half-life values of 4.4 days for the dislodgeable residues of malathion on the surface of the treated leaves, and 11 days for the total residues of malathion in the plant. The corresponding values for the dislodgeable residues and total residues of dimethoate were 13 and 29 days, respectively. The half-life periods for dimethoate residues were

higher than for malathion residues. The behaviour of the residues of the pesticides on the foliar surface and in the plant is well correlated with their physical properties. The solubility of malathion in water is 143 mg/l and its log KO/w (partition coefficient between octanol and water) is 2.36 while the values for dimethoate are 25,000 mg/l and 0.78, respectively [13]. It is known that compounds of intermediate lipophilicity (log P = 1–2) and log S (molar water solubility) of −1.5 to −3.5 show high rates of uptake through the cuticle of leaves [14]. Malathion can be regarded as a pesticide of intermediate lipophilicity and is therefore expected to penetrate the cuticle faster than dimethoate.

The results agree with the above statement: 16 days after pesticide application, only 5.7% of the malathion residues was remaining as dislodgeable residues on the surface of the treated leaves. Dimethoate, being of lower lipophilicity could not be taken up as fast through the cuticle. This explains why still 19.4% of dimethoate residues could be dislodged from the surface of the treated leaves after 16 days. The higher lipophilicity of malathion is

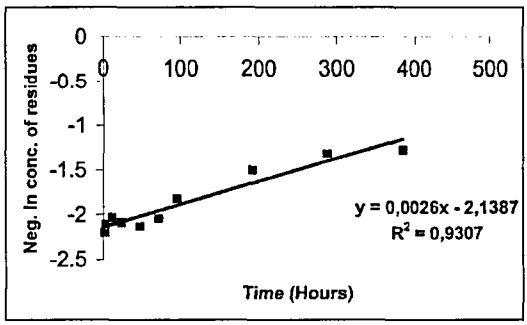

FIGURE 4 Regression curve for foliar disappearance of malathion from pea plants.

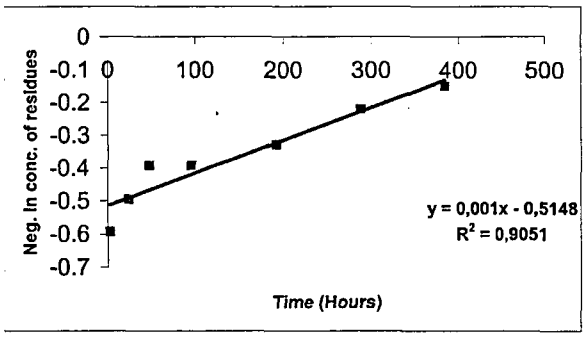

FIGURE 5 Regression curve for foliar disappearance of dimethoate from pea plants.

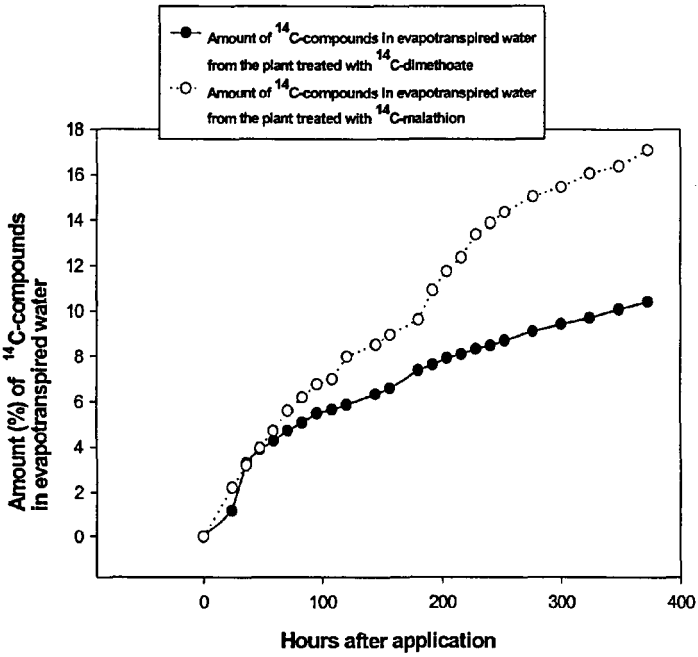

FIGURE 6 Amount of ^{14}C-compounds in evapotranspired water from the plant to which the pesticides were applied on the foliar surface.

also reflected in the higher amount of the pesticide translocated from the treated leaves to the rest of the plant. Whereas 16.7% of malathion residues were found with the rest of the plant, only 4.6% of dimethoate residues were exported from the leaves in the course of 16 days.

In a parallel experiment the amounts of ^{14}C-compounds in the transpired water were determined. Figure 6 shows that a higher proportion of ^{14}C was found in transpired water from the plant treated with ^{14}C-labelled malathion. After 15.5 days, the amount of ^{14}C-compounds in the transpired water from the plant to which malathion was applied was 17.1% while only 10.4% was recorded in the transpired water from the plant to which methoxy-^{14}C-dimethoate had been applied. Since the solubility of dimethoate in water is 25,000 mg/l while the solubility of malathion is 143 mg/l, one could expect higher radioactivity in transpired water from dimethoate. The results show that solubility of the pesticides in water did not determine the amounts of ^{14}C-compounds, which were lost by transpiration. Rather, only ^{14}C taken up by the plant could be transpired and obviously there was no evaporation of the pesticides from the leaf surface. The higher rate (16.7%) of transloca-

tion of malathion than dimethoate (4.6%) explains why there is higher radioactivity in the transpired water from the plant to which malathion was applied than from the plant to which dimethoate was applied.

Metabolites in the samples were separated and tentatively identified by thin layer chromatography (TLC), and further analysed by GC-MS. The samples from the plant to which malathion was applied contained O,O-dimethyl phosphorothioate, diethyl mercaptosuccinate and the parent compound, malathion. Malaoxon, an oxygen analogue of malathion, was not detected. The samples from the plant treated with dimethoate yielded dimethoxon, O,O-dimethyl phosphorothioate, dimethoate acid, dimethoxonic acid and the parent compound, dimethoate. Acids of dimethoate and dimethoxon were detected by mass spectrometry as trimethylsilyl (TMS) derivatives. The formation of the acids of dimethoate and dimethoxon could be due to the action of a carboxyamidase on the amide of dimethoate and dimethoxon [15]. The TMS derivative of dimethoate acid gave peaks at m/z 273 and 199. The peak at m/z 273 is due to the M-15 ion that results from the loss of a silylated methyl group. The peak at m/z 199 is due to M-89, which results from the loss of three silylated methyl groups and further loss of a CO_2 molecule. The TMS derivative of dimethoxonic acid gave peaks at m/z 272, 257, 213, and 183. The peak at m/z 272 is due to the molecular ion, M. The peak at m/z 257 is due to M-15 resulting from the loss of one silylated methyl group. The peak at m/z 213 is due to M-59 resulting from the loss of one silylated methyl group and further loss of a CO_2 molecule. The peak at m/z 183 is due to M-89 resulting from the loss of three methyl groups and further loss of a CO_2 molecule. The same fragments have been observed in TMS derivatives of alcohols and carboxylic acids upon electron impact ionisation [16]. The results show that dimethoate is readily activated to the oxygen analogue, dimethoxon, on the surface and in the pea plant probably by UV-radiation on the surface and a monooxygenase in the plant.

CONCLUSIONS

Malathion does not persist long on the foliar surface of the pea plant because it is readily taken up into the cells of the leaves and translocated to various parts of the plant where it should continue to exert its insecticidal effects especially to sucking insects. However, the high amount of ^{14}C-compounds in the transpired water could become an environmental problem. Dimethoate persists longer on the foliar surface of the plant and thus is likely to contaminate those who can come into contact with the foliar

surface of the treated plants. Furthermore, dimethoate is converted on the foliar surface to the oxygen analogue, dimethoxon, which is more toxic than dimethoate itself.

Acknowledgements

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References

[1] D.L. Suett, Organophosphorus pesticides: organic and biological chemistry, C.R.C. Press, Cleveland: 16–30 (1980).
[2] Anonymous, Proceedings of the FAO conference on ecology in relation to plant pest control, Rome, Italy, 11–15 December (1972).
[3] Anonymous, Degradation of synthetic organic chemicals in the atmosphere. Proceedings of a conference held in San Francisco, California, June 12–13, 1971, National Academy of Sciences, Washington D.C. (1972).
[4] G.H. Willis and L.L. McDowell, Pesticide persistence on foliage. *Rev. Environ. Contam. Toxicol.*, Springer-Verlag, New York, **100**: 24–29 (1987).
[5] G. Kerstiens, Plant cuticles, Bio Scientific Publishers, Harcourt: 33 (1996).
[6] D.F. Cutie, K.L. Alvin and C.E. Price, A review of the factors influencing the penetration of pesticides through plant leaves. The plant cuticle, papers presented at an international symposium organised by the Lennean Society of London, held at Burlington House, London, 8–11 September 1980, Academic Press, London, 238 (1982).
[7] P.R.S. Chen and W.C. Dauterman, Studies on the toxicity of dimethoate analogs and their hydrolysis by sheep liver amidase. *Pestic. Biochem. Physiol.*: 340–348 (1971).
[8] M.B. Hugh, B.B. Lena, E.L. David and J.H. Pamela, Metabolic pathways and residue levels of thifensulfuron methyl in soybeans. *J. Agric. Food Chem.*, **41**: 1724–1730 (1993).
[9] V. Leoni, A.M. Caricchia, S. Chiavarini, Multiresidue method for the quantitation of organophosphorus pesticides in vegetables and animal feeds. *J. AOAC internat.*, **75**: 511–518 (1992).
[10] M.L. Hopper, Improved method for partition of organophosphate pesticide residues on a solid phase partition column. *J. AOAC internat.*, **71**: 731–734 (1988).
[11] D.L. Mactaggart, S.O. Farwell, Analytical use of linear regression. Part I: Regression procedures for calibration and quantitation. *J. AOAC internat.*, **75**: 594–606 (1992).
[12] W.C. Dauterman, G.B. Viado, J.E. Casida and R.D. O'brien, Persistence of dimethoate and metabolites following foliar application. *J. Agric. Food Chem.*, **8**: 115–119 (1960).
[13] P.H. Howard, Handbook of environmental fate and exposure data for organic chemicals, Lewis Publishers, New York, **3**: 4–6 (1991).
[14] P.D. Berger, B. Deviselty and F.R. Hall, Pesticide formulations and application systems. AST Publication, Philadelphia, **13**: 243–254 (1993).
[15] G.S. Gruzdyer, The chemical protection of plants, M.P. Publisher, Moscow, 207–215 (1983).
[16] S. Safe and S. Hutzinger, Organophosphorus pesticides. Mass spectrometry of pesticides and pollutants, CRC Press, Cleveland, 52–56 (1976).