Sorption and Bound Residue Formation of Linuron, Methylparathion, and Metolachlor by Carrot Tissues: Kinetics by On-Line HPLC Microextraction

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The feasibility of determining the identities and kinetics behavior of three pesticides in carrot tissue slurries using a novel on-line HPLC microextraction was investigated. Linuron, methylparathion, and metolachlor were added to aqueous slurries of carrot tissue particles smaller than 150 μm. For each pesticide, the solution phase and the total pesticide recoverable from the whole slurry concentrations were separately monitored over a 10 day period. Kinetics curves for three categories of chemical species including solution phase pesticides, labile sorbed fractions, and unrecovered fractions were obtained. The linuron and metolachlor data are consistent with the commonly reported two-step sorption mechanism consisting of labile sorption followed by intraparticle diffusion, but the methylparathion data resemble a combination of labile surface sorption and solution phase and/or surface chemical decomposition. It is concluded that laboratory test methods of this type could be developed for assessing the risk of bound residue formation in vegetables.

Keywords: *Bound; carrots; HPLC; kinetics; linuron; methylparathion; metolachlor; microextraction; sorption; residues*

INTRODUCTION

The uptake of pesticides by tuberous vegetables such as carrots can occur by direct application or by contact with contaminated soil and water. In some cases the surface sorption of the original compound might be followed by the formation of sorbed reaction products or sorbed metabolites. In other cases, some combination of parent compound, reaction products, and metabolites might be sorbed. Once pesticide−surface contact has taken place, the subsequent phenomena can include labile surface sorption with dynamic distributions between roots and soil and diffusion into plant tissues, followed by intraparticle and/or surface bound residue formation. During the past decade Khan and various co-workers have reported many examples of pesticide-bound residues in foods and plants (Khan *et al*., 1985a, 1985b, 1987, 1990; Khan and Belanger, 1987; Khan, 1995; Dupont and Khan, 1992, 1993; Singh *et al*., 1992; Kacew *et al*., 1996). More generally, at least two categories of bound residues have been described in the literature. One category includes residues that have become covalently chemically bonded to polymeric matrices (Parris, 1980; Reiderer and Schonher, 1986). Only the chemical decomposition of the polymer matrix will release them. The other category of bound residues consists of those that have become physically trapped within the matrices of solid particles by intraparticle diffusion (Hamaker *et al*., 1996; Crank, 1975; Khan, 1982; Karickhoff *et al*., 1985; Wu *et al*., 1986; Steinberg *et al*., 1987; Brusseau, 1989; Ball *et al*., 1991; Gilchrist *et al*., 1993; Gamble *et al*., 1994; Gonzalez-Davila *et al*., 1995; Pignatello and Xing, 1996).

Muller *et al.* (1994) have proposed that each type of plant material such as waxes, cutin, carbohydrates, proteins, and air represents a different type of sorption material, described as hypothetical "compartments" within plants. The role of lipids and waxes has been emphasized in the uptake of hydrophobic organic chemicals. Tam *et al.* (1996) obtained linear correlations between distribution coefficients for chlorinated benzenes onto soybean plant tissues and the corresponding octanol/water partition coefficients. They also found correlations of the distribution coefficients with the lipid contents of the plants.

The chemical speciation of pesticides in foods, including bound residues, raises important questions about health risks. The bound residues in vegetables might be released by cooking or by the enzyme reactions of digestion. Khan and co-workers (Khan *et al*., , 1984, 1985a,b, 1987, 1990; Singh *et al*., 1992, 1993; Khan, 1995; Kacew *et al*., 1996; Khan and Belanger, 1987) have demonstrated two types of biological effects of bound residues in foods. In the examples that they examined, each type of effect was manifest in some cases but not in others. They first found that the digestive systems of animals sometimes released bound residues from foods. Following that the released contaminants were sometimes passed up to the next step in the food chain. Evidently both health protection and the prevention of false alarms require that a knowledge of chemical speciation and bound residue formation mechanisms be used for distinguishing between the cases that exhibit the hazardous effects and those that do not.

The recent work of Tam *et al.* (1996) has shown that studying the uptake of pollutants by aqueous suspen-

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S0021-8561(97)00037-X CCC: $14.00

sions of plant tissues can provide information that is useful for persistence and fate studies. Other authors have used the same strategy for obtaining data with which to predict plant uptake of pollutants from water, soil, and air (Paterson *et al*., 1994; Reiderer, 1990).

More information about the chemical speciation and binding processes is needed for the assessment of health risks. In addition, the information could be exploited by using test vegetables for monitoring soils for contamination. Supercritical fluid extraction combined with ^{14}C radiotracers could qualitatively distinguish between the two categories of bound residues once they had formed. There is, however, an additional need for an experimental method that can distinguish the free, labile sorbed and the two bound residue chemical species from each other and track the kinetics of their formation and subsequent behavior. The on-line HPLC microextraction method that was introduced by Gamble *et al.* for pesticide interactions with soils and sediments should meet much of this requirement (Gamble and Ismaily, 1991; Gamble and Khan, 1991). In this method, a solid sample is slurried in an aqueous solution of one or more pesticides. Microfiltrates obtained off-line at measured times are analyzed by HPLC to give solution concentration kinetics curves for free species. Aliquots of whole slurry are injected directly into the instrument. On-line microfilters then trap the solids ahead of the guard column, and the mobile phase becomes the extractant. This produces kinetics curves for total recoverable pesticide. Free, labile sorbed (recoverable) and lost (unrecoverable) residues kinetics curves are calculated by mass balance from the above chemical analysis curves. In at least one case, the parent compound and its hydrolysis product were monitored together in a catalytic soil (Gamble and Khan, 1991).

Carrots are an ideal choice of vegetable for this kind of investigation because they are in direct contact with soils and soil pore water. The pesticides selected for the research have conveniently high water solubilities and also are expected to be sorbed by such vegetables as potatoes (Miliadis *et al*., 1990) and carrots (D'Amato *et al*., 1993). Methylparathion has been found in carrots and is used as a general insecticide in various crops (Youngman *et al*., 1989). Metolachlor is a herbicide frequently used in soybean, corn, and tomato (Gaynor *et al*., 1992) production and has the potential to contaminate ground water due to its high mobility in soils (Muller *et al*., 1995).

The objective of this work was to adapt and apply the on-line HPLC microextraction method to the identification and kinetics measurements of pesticide chemical species in slurried carrot tissues. The chemical species of particular interest are any bound residues that might be formed.

MATERIALS AND METHODS

All pesticides used were purchased from Chem Service, West Chester, PA, and were at least 98% pure. Pesticide grade acetonitrile was purchased from BDH, Vancouver, BC. A Supelco C_{18} guard column and a Supelco C_{18} reversed phase (5 μm particle size, 25 cm length) from Supelco, Oakville, ON, were used for HPLC determinations. Cameo 3N (0.45 μm) nylon filters from MSI Co., Westboro, MA, were used for off-line filtering of sample slurries. Five micrometer stainless steel on-line filters (Alltech Associates Inc., Deerfield, IL) were used for on-line filtration of slurries. HPLC analyses were carried out using a Perkin-Elmer Series 410 pump and a Perkin-Elmer Model LC 90 UV−visible detector. The injector was equipped with a 20 μL sample loop. Data acquisition and management were done using Chromperfect software from Justice Innovations, Palo Alto, CA.

Individual stock solutions of linuron, methylparathion, and metolachlor were prepared by dissolution of the pure compounds in acetonitrile giving final concentrations of 4.29×10^{-1}, 8.01×10^{-1}, and 8.01×10^{-1} M, respectively. A mixed stock solution containing linuron, methylparathion, and metolachlor was prepared by combining appropriate volumes of each individual stock in a 100 mL volumetric flask. The solvent was evaporated by gently blowing pure nitrogen, and the flask was brought to volume with Milli-Q water. The solution was sonicated for 15 min to ensure total dissolution of the compounds. The concentrations of each component in the mixed stock solution were found to be 8.54×10^{-5}, 3.20×10^{-4}, and 6.48×10^{-5} M for linuron, methylparathion, and metolachlor, respectively.

Carrots purchased from a local market were peeled, grated, and homogenized in a blender, followed by further homogenization using a Polytron. The resulting pulp was spread into a thin layer on a clean aluminum foil sheet and dried by pressing with an absorbent tissue paper several times until no more moisture was removed. The pulp was allowed to dry overnight. Alternatively, the pulp was freeze-dried. The dried pulp was then ground and sieved through a 150 μm metal sieve. The fraction <150 μm was used in the experiments. This fraction was stored in aluminum foil pouches until needed for experiments.

Preparation of Carrot Tissue Slurries. Experimental samples were prepared as slurries. Portions (30 mg) of carrot tissue were weighed into 15 mL glass vials, followed by the addition of 9 mL of distilled water. Slurries were mixed in a vortex shaker for approximately 10 min. The measured pH was 5.4. One milliliter of the mixed stock solution was next added to each slurry. The initial concentrations of linuron, methylparathion, and metolachlor were 8.54×10^{-6}, 3.20×10^{-5}, and 6.48×10^{-6} M, respectively. This concentration level was selected to ensure pseudo-first-order sorption kinetic conditions based on the ratio of pesticide to total organic carbon present in the slurries estimated from the concentration of carrot tissue and, at the same time, to be within the analytical range for each pesticide. The vials were shaken for 10 min and kept in the dark at an ambient temperature of about 22 °C. Control solutions containing the same concentrations of pesticides were prepared in water at the same pH as the slurries. Standard solutions in acetonitrile at the same concentration as in the control solutions were also prepared. All slurries and control solutions were bubbled with pure nitrogen and sealed with Teflon-lined septa until analysis aliquots were taken.

The concentrations used for methylparathion were 3−5 times higher than those for linuron and metolachlor, because the lower molar extinction coefficients of its UV−visible spectrum gave a lower analytical chemical sensitivity. Because of possible decomposition of the carrot tissues even after oxygen had been removed by purging with pure nitrogen, the experiments were run for only 10−13 days. It was observed that the slurries began to flocculate after this length of time. This imposes an experimental limitation that is not encountered with soils. The stability in aqueous solutions of the pesticides under study was monitored to check for possible losses due to hydrolysis and other side reactions. The data indicate that linuron and metolachlor are stable in aqueous solutions of the same pH as the slurries for the duration of the experiments. Their detector responses were stable throughout that period of time. Percent losses of each pesticides were 12.7% and 9%, respectively, for methylparathion and linuron and <1% for metolachlor. These values are all within the uncertainty of the measurements, which was estimated at near 15% (coefficient of variation).

Off- and On-Line HPLC Analysis. Filtration of samples prior to injection into the HPLC (off-line filtrates) was done using a 1 mL glass syringe and the 0.45 μm nylon filters. For injection of unfiltered samples (whole slurry), a custom syringe with a wide-bore needle was used (Hamilton) for sampling the slurry. Vigorous stirring was maintained to ensure representative sampling in both cases. For the analysis of off-line

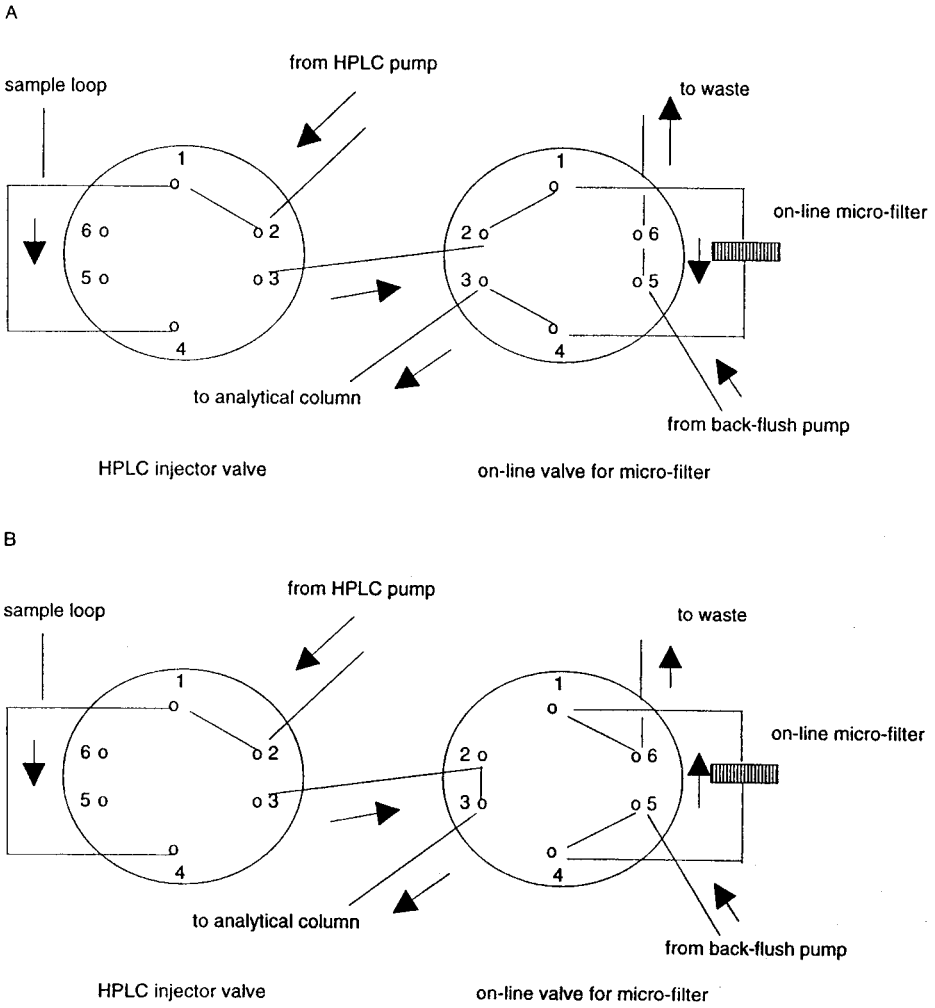

Figure 1. Switching valve connections for on-line HPLC microextraction, with a bypass for solids removal by back-flush: (A) running mode; (B) back-flushing mode to remove particles remaining on the microfilter.

filtrates for free species, a 20 μL sample of the filtered slurry was injected into the HPLC. Filtration times were recorded for analyses done during a 10 day period. Unfiltered slurry aliquots were taken with the Hamilton microsyringe. On-line microextraction was then achieved by direct injection of the 20 μL slurry aliquots into the HPLC, with the microfilter set on-line with the switching valve. The microfilter was kept on-line for 30 s during each slurry injection. After each slurry injection, the microfilter was taken off-line using the switching valve (Figure 1). It was then back-flushed with mobile phase, to wash out the trapped solids after extraction. During slurry injections the system pressure did not change significantly from the normal operating value. The HPLC was operated under isocratic conditions using a 60% acetonitrile and 40% water mobile phase. The flow rate was set at 1 mL/min, and the detector wavelength was set at 220 nm.

Triplicate experiments were run for the multiresidue cases. Injections of mixed aqueous pesticide standards at the same pH as the slurries were used as controls throughout the experiments.

Calculations. Replicate measurements were averaged, and then each of the slurry and filtrate curves was fitted by least squares. The kinetics curves for free, labile sorbed and unrecovered species were calculated from the fitted analysis curves. A model assuming two consecutive kinetic steps involving reversible labile sorption followed by an irreversible (unrecoverable) loss of pesticides was tested. The *a priori* selection of this model is based on past experiences with pesticide sorption kinetics in soils (Li *et al.*, 1996a,b). Alternative models may include two independent steps leading to labile sorption and lost species directly from solution. Pseudo-first-order labile sorption rate constants were estimated using the ordinary first-order integral equation for methylparathion, while for metolachlor and linuron the initial rate method was used. Pseudo-first-order rate constants for the irreversible kinetic step were estimated by iterative exponential fitting using initial estimates from Guggenheim's plots. The "irreversibility" of the second kinetic step justified this approach. In accordance with diffusion theory (Crank, 1975; Gamble *et al.*, 1994), the diagnostic test plots for intraparticle diffusion were done with sections of the curves for which the labile sorption was under approximately steady state conditions.

RESULTS AND DISCUSSION

Figure 2 shows chromatograms of standard solutions of linuron, methylparathion, and metolachlor and of slurry and filtrate samples using the on-line HPLC microextraction system. When filtrates and slurries were analyzed, the chromatograms were similar except that compounds or polymeric materials extracted from the carrots gave a mixture of overlapping peaks at very early retention times. They were well separated from the following pesticide peaks. Small shifts in the retention times of the pesticides were noted on consecutive days, during a series of sample extractions. This was caused by the retention of tissue components on the guard column. The replacement of the guard column after every 10 slurry injections effectively controlled the problem.

Sorption Kinetics. For each of the three pesticides, Figure 3 shows evidence of labile sorption and for the formation of unrecoverable pesticide fractions. This is especially clear for methylparathion. The species kinet-

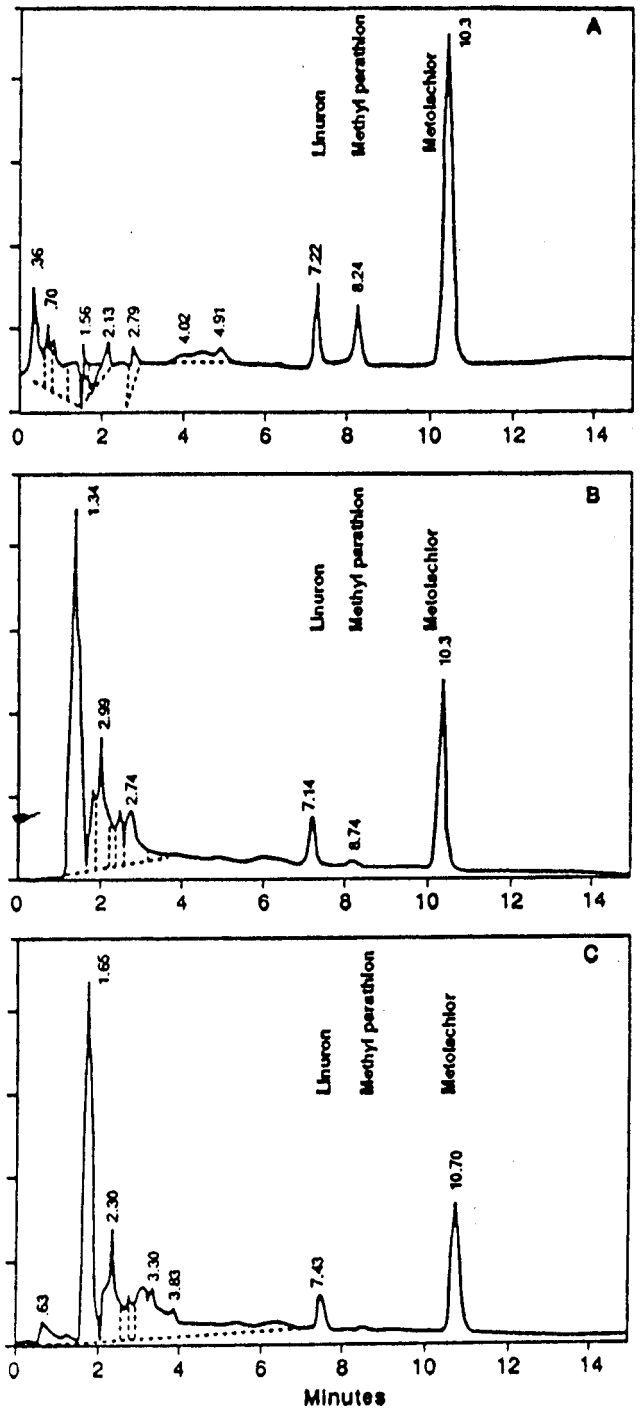

Figure 2. Chromatogram of the mixed standard (A), slurry (B), and filtrate (C). Pesticide concentrations in the standard are 8.5×10^{-6} M linuron, 3.5×10^{-5} M methylparathion, and 6.5×10^{-6} M metolachlor. Filtrate and slurry measurements are at 9 days.

ics curves in Figure 4 quantitatively show a range of pesticide sorption kinetics characteristics but have some important features in common. Each pesticide had at least one fast sorption process during the first 10 min. The labile sorption rate constants (k_{s1}) for metolachlor and linuron were of the order of 10^{-6} s^{-1}, which is the same order of magnitude as the result for the methylparathion rate constant estimated from Figure 5 and by the initial rate method to be $\approx(2.2 \pm 1.1) \times 10^{-6}$ and $(3 \pm 1) \times 10^{-6}$ s^{-1}, respectively. Comparable values have been reported for atrazine in soils (Gamble *et al.*, 1994; Gonzalez-Davila *et al.*, 1995; Gamble and Ismaily, 1991; Gamble and Khan, 1991). For atrazine in a soil,

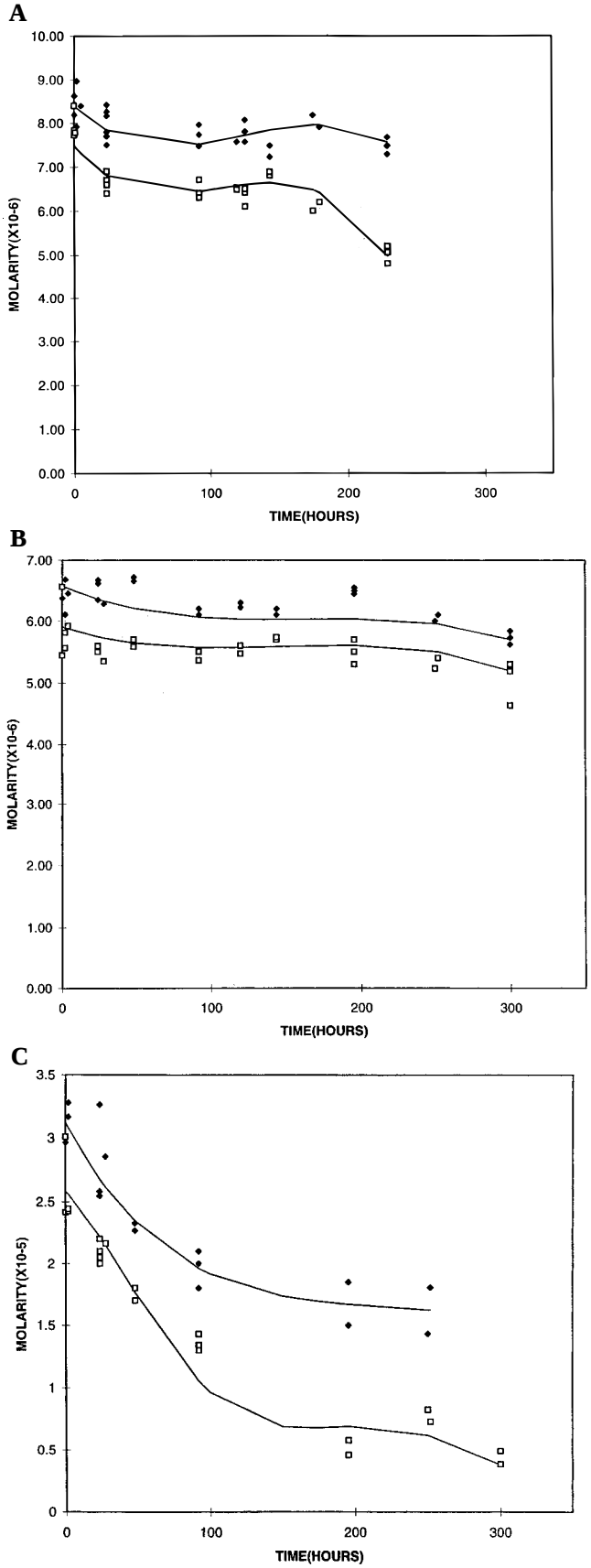

Figure 3. Chemical analysis kinetic curves: (A) linuron; (B) metolachlor; (C) methylparathion; (◆) whole carrot slurries; (□) microfiltrates.

k_{s1} was previously found to be approximately 1.0×10^{-6} s^{-1} (Gamble and Khan, 1991; Li *et al.*, 1996a,b). Gilchrist *et al.* (1993) found a value about 2 orders of

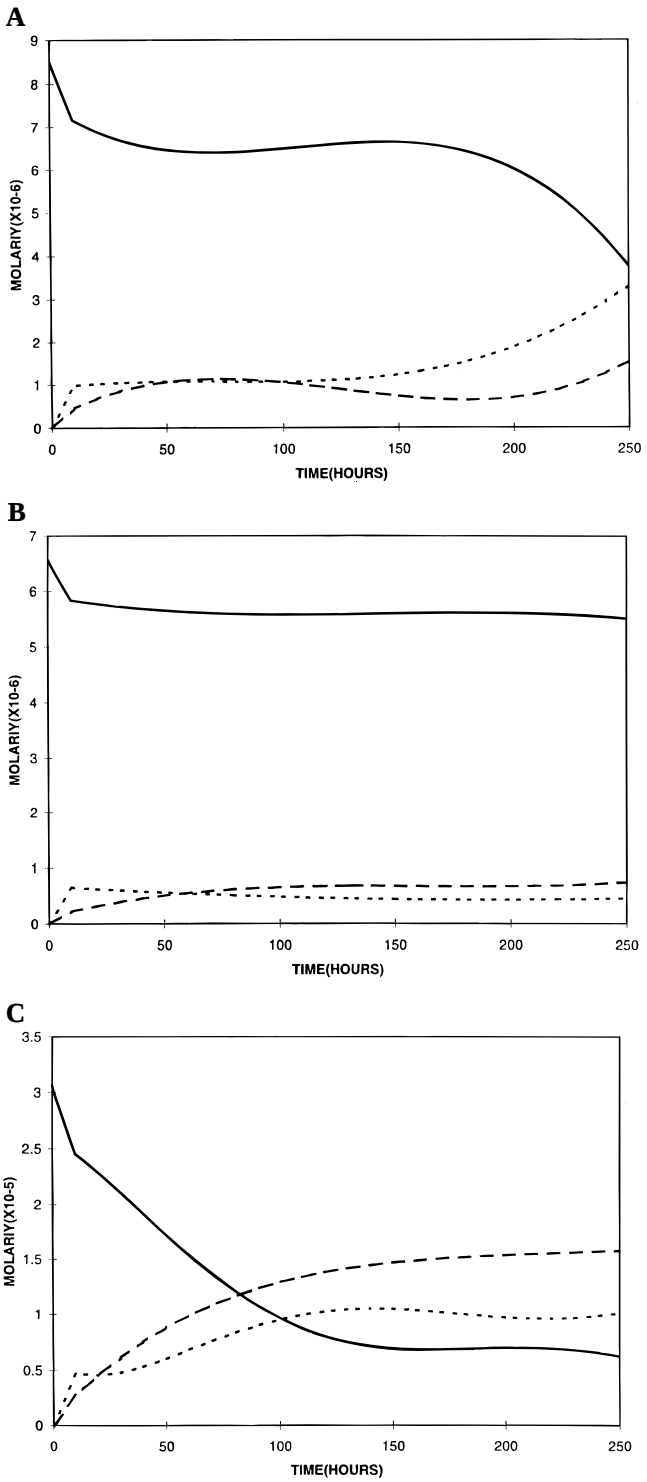

Figure 4. Chemical species in carrot slurries: (A) linuron; (B) metolachlor; (C) methylparathion; (—) in solution; (- - -) labile sorbed; (— —) unrecovered.

magnitude greater, however, for atrazine sorption by the Na^+ form of montmorillonite.

The concentrations of recoverable (labile) species of linuron and metolachlor reached a maximum at about 24 h, after which time, labile linuron slowly increased and labile metolachlor remained more or less constant. This unusual behavior of linuron may be due to biological degradation of the labile fraction, but this remains unconfirmed. The methylparathion loss was monitored for about 10 days, and it reached a maximum after 6 days. During the first 4 days, its first-order rate constant for labile sorption was about $(2.2 \pm 1.1) \times 10^{-6}$ s^{-1}, using the integral rate law equation and $(3 \pm 1) \times 10^{-6}$ s^{-1} using the initial rate law method.

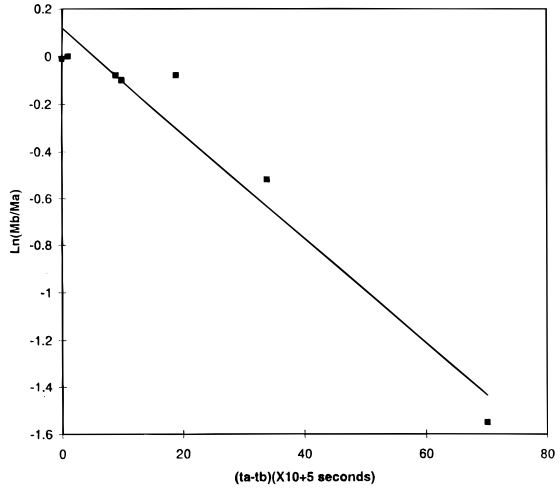

Figure 5. Estimate of the pseudo-first-order rate constant k_{s1}, for methylparathion sorption between 10 min and 195 h. $k_{s1} = (2.2 \pm 0.2) \times 10^{-6}$ s^{-1}, $r^2 = 0.950484$.

The rate constants for the formation of unrecoverable species, k_{s2}, were estimated to be $\approx(6 \pm 3) \times 10^{-6}$, $(8 \pm 4) \times 10^{-6}$, and $(1 \pm 0.5) \times 10^{-5}$ s^{-1} for linuron, metolachlor, and methylparathion, respectively.

Nature of Unrecoverable Residues. To investigate the nature of the lost residues, the possibility of degradation reactions was considered. For example, degradation of linuron to 3-(3,4-dichlorophenyl)-1-methylurea (DCPMU), 3-(3,4-dichlorophenyl)urea (DCPU), and 3,4-dichloroaniline (DCA) (Miliadis *et al.*, 1990; D'Amato *et al.*, 1993) can be a source of losses in these experiments. These compounds may be sorbed onto tissue particles or remain free in solution. They are also expected to have shorter chromatographic retention times than the parent compound under the present chromatographic conditions of analysis (DiCorcia *et al.*, 1991). None of these compounds were detected by our HPLC-UV system, although the system was not optimized for them. DiCorcia *et al.* (1991) showed that DCA has a shorter retention time than linuron in water/methanol gradients using C_{18} columns. The use of an isocratic solvent system in the present experiments may have caused DCA, and other products, if present, to elute with the tissue components. Attempts to gain further information by using off-line extraction followed by GC/MS were not successful. The absence of decomposition products in the GC/MS scans does not necessarily mean that these compounds were not formed. The concentration of lost species or bound residues of linuron toward the end of the experiments was of the order of 1×10^{-6} M, which is quite close to the GC/MS detection level for linuron in vegetables samples (Fillion *et al.*, 1995). Less than 10% of the total linuron was converted into bound or lost residues by the end of the experiments, while approximately 23% was still extractable (labile species) and the rest was free in the aqueous phase.

A similar argument is valid for metolachlor. Metolachlor has been reported to degrade in soils to a sulfonic acid metabolite and can also decompose to 2-[2-ethyl-6-methylphenyl)amino]-1-propanol (Aga *et al.*, 1996). As with linuron, these degradation products, if present, were not detected in our experiments. After 10 days of equilibration, approximately 12% of the initial concentration of metolachlor was bound or lost, while about

7% was in the labile form. The remaining percentage was free in the aqueous phase.

In contrast to linuron and metolachlor, methylparathion showed a greater interaction with the carrot tissue. Methylparathion can decompose into *p*-nitrophenol, paraoxon, and dimethyllphosphoric and oxalic acids (Heim *et al*., 1995). This decomposition does occur in aqueous solutions, and it is certain to have contributed to the losses of the dissolved species seen in our experiments, even though we did not detect any of these compounds either in the HPLC runs or in the GC/MS scans of extracted slurries. Our control solutions, in distilled water, showed losses between 12% and 15% after a 6−10 day period, although *p*-nitrophenol was not detected in soil slurry extracts or in soil filtrates (these losses were compensated for when the losses due to reactions of the pesticides with the carrot tissues were calculated). As before, this may have been due to unsuitable mobile phase and detection conditions. This may also suggested that *p*-nitrophenol may further degrade as shown by others (Ou and Sharma, 1989). At the end of the experiment approximately 40% of the total methylparathion was converted into bound or lost species, 31% formed labile species, and the remaining fraction (29%) remained free in solution.

The lost species may be the result of (1) surface sorption followed by diffusion of the parent pesticide into the interior of the particles, (2) decomposition reactions at the surface of the carrot tissues followed by diffusion of decomposition products into the interior of the gel-like structure of the tissues, (3) diffusion of the pesticide to the interior of the tissue particles followed by decomposition, or (4) solution phase decomposition processes due to interactions with dissolved carrot tissue components or any combination of these. Although it is difficult to differentiate each of these possible contributions to the formation of lost species with the present technique, certain approximations are valid. Contributions of solution phase decomposition processes are likely to be very small for linuron and metolachlor, on the basis of our control experiments, but may be of significance for methylparathion, especially if dissolved tissue components interact strongly with this pesticide. Nevertheless, this implicates tissue surface reactions as the main cause of lost residue formation. The nature of these surface reactions is unknown at present, but models explaining the origin of lost species can be postulated.

Proposed Sorption Model. A sorption model based on consecutive reversible sorption and intraparticle diffusion of the parent pesticides can be tested to find out whether the lost species are the result of retarded intraparticle diffusion. Tests for intraparticle diffusion have been previously used (Gamble *et al.*, 1994; Li *et al*., 1996a,b) in the study of atrazine sorption to soils. A plot of $\ln(\theta)_b$ versus $\ln(t)$, where $(\theta)_b$ is the amount of pesticide lost (or diffused into the particles) in moles per grams of tissue and t is the time, should result in a straight line with slope equal to 0.5. Figure 6 shows the results of applying such test to our data. The Z values are 0.42 ± 0.04, 0.68 ± 0.01, and 0.48 ± 0.01 for linuron, methylparathion, and metolachlor, respectively. The values for both linuron and metolachlor are consistent with steady state surface sorption and intraparticle diffusion kinetics, while methylparathion deviates significantly from the expected value of 0.5. This deviation may be due to a second mechanism of removal from solution, possibly solution phase hydrolysis caused by dissolved tissue components. Considering the available evidence, a subsequent search for definitive proof such as through the use of radiolabeled pesticides and supercritical fluid extraction would therefore be justified. Also, methods in which the carrot tissues are selectively decomposed without the destruction of any trapped compounds would be of interest.

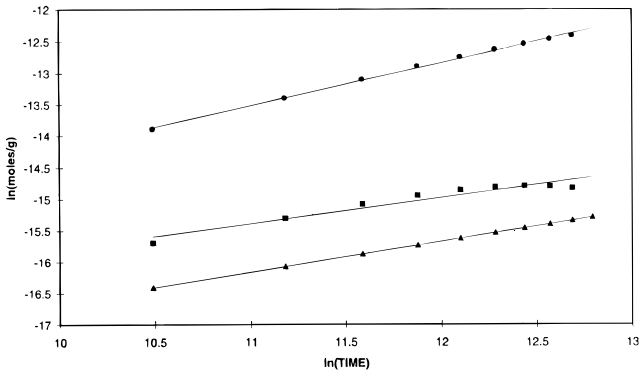

Figure 6. Diagnostic tests for intraparticle diffusion: (◆) linuron, $Z = 0.42 \pm 0.04$, $r^2 = 0.931503$; (■) metolachlor, $Z = 0.48 \pm 0.01$, $r^2 = 0.997379$; (●) methylparathion, $Z = 0.68 \pm 0.01$, $r^2 = 0.997460$.

Table 1. Partition Coefficient Estimates

pesticides	log K_D[a]	log K_D[b]	log K_{OW}[c]
linuron	1.74	2.04	2.76
methylparathion	2.52	2.88	3.04
metolachlor	1.46	1.83	3.28

[a] Determined after 100 h on the basis of the concentration of labile bound species. [b] Determined after 100 h on the basis of the total bound species. [c] From Noble (1993).

Although not experimentally addressed in the present study, it is important to identify the nature of the surfaces with which the pesticides interact. Cell wall materials including possibly phospholipids, polysaccharides, pectins, starches, carotenes, and fatty acids present in the carrot tissues are likely to interact with pesticides. Tam *et al.* (1996) noted that the lipid content of soybean tissues could be considered as the main component responsible for the sorption of chlorinated hydrocarbons. They were also able to correlate the equilibrium partition coefficients (K_D) of tested compounds between water and the tissue with their respective octanol−water partition coefficients (K_{OW}) and the tissue lipid contents. They did not report the formation of bound or diffused residues. The nature of the sorption sites on the carrot tissue is likely to be heterogeneous. We estimated partition coefficients (Table 1) using the traditional method, which assumes equilibrium between total bound (total lost as measured by mass balance) and solution phase concentrations, and using the labile bound fraction as the fraction involved in any equilibrium with the solution phase. The fact that the total bound concentration of the pesticides is made up of two fractions, one labile and the other unrecoverable and therefore not engaged in the equilibrium, indicates that using total bound concentration values overestimates partition coefficients in these systems, although they follow the same trend as those determined using the labile sorbed fraction concentrations. Another important observation is that according to the log K_{OW} values, the partition coefficients for the tissue/water systems should increase as linuron < methylparathion < metolachlor. The experimental values follow the trend metolachlor < linuron < methylparathion. This suggests that a simple partition

model based on hydrophobic forces cannot explain the sorption of these pesticides to carrots tissues.

The present study provides some evidence for a sorption mechanism of linuron and metolachlor to carrot tissues based on two consecutive kinetics steps: labile surface sorption followed by intratissue diffusion. The possibility of surface reactions as the source of lost species still needs to be investigated. In the case of methylparathion, the situation is more complex, involving surface labile sorption and possibly surface and solution phase promoted decomposition and or intratissue particle diffusion.

A model using retarded intraparticle diffusion has been proposed by Gonzalez-Davila *et al.* (1995) to explain desorption hysteresis of chlorinated pesticides from chitin slurries. Their system has some resemblance to our carrot tissue slurries in that both contain significant concentrations of polysaccharides. This model can be coupled with one in which the formation of bound residues due to biotic and abiotic reactions at the cell wall level occurs. Pogany *et al.* (1990) demonstrated that compounds such as 4-chloroaniline and 2,4-dichlorophenol can distribute among the components of the cell wall material including pectin, lignin, starch, protein, and hemicellulose. The result of this distribution is the formation of β-D-glucosides (conjugates), mainly in the starch fraction of the cells cultures. The release of the parent compounds from these conjugates requires enzymatic hydrolysis. It is possible that the lost or bound residues seen in our experiments, in particular for the case of methylparathion, are the result of such processes.

In the present study, it has been demonstrated that the on-line HPLC microextraction method for determining reaction kinetics and chemical speciation can be adapted to vegetables such as carrots. The adaptation of the method to vegetables should be further refined to improve the quality of the data produced. The available evidence suggests that the method might have been monitoring the formation of metolachlor, linuron, and methylparathion labile sorbed and bound residues. The nature of the bound residues is still under question. For linuron and metolachlor there is evidence of intratissue diffusion as the cause of bound residues. For methylparathion a tissue surface and solution phase degradation, possibly coupled with intratissue diffusion, is likely to be the origin of the lost fraction. Further studies are needed to investigate the nature of the bound residues.

The kinetics of the lost species in Figure 4 have important implications for the use and interpretations of sample spiking in the pesticide analyses of vegetables. Samples must be extracted immediately after spiking if the results of their analysis are to be used in method validation exercises. Conventional sorption studies only monitor the disappearance from solution and the total concentration on the tissue particles (Tam *et al.*, 1996; Paterson *et al.*, 1994). We believe that it is essential to monitor the species formation to be able to derive valid physicochemical interaction parameters.

Some issues for future research include study of epithelial cells as well as tissues from other plant parts of a vegetable. In addition, the effects of plant growth on sorption and bound residue formation should be accounted for if possible.

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Received for review January 13, 1997. Revised manuscript received May 28, 1997. Accepted June 20, 1997.[⊗] This work was partly funded by the Matching Investment Initiatives Program of the Research Branch of Agri-Food and Agriculture Canada.

JF970037B

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1997.