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Measuring Dermal Exposure to Pesticide Residues with Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

E. M. Doran, M. G. Yost, R. A. Fenske

Department of Environmental Health, University of Washington, Box 357234, Seattle, WA 98195-7234, USA

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Occupational pesticide exposures are of concern to workers, public health scientists, and clinical practitioners. Numerous studies have demonstrated that fieldworkers who have extensive or prolonged contact with contaminated foliage may accumulate significant concentrations of pesticides on their skin (Nigg 1980; Knaak et al. 1989; Spencer et al. 1995; Simcox et al. 1999). As a result, the primary route of exposure for these workers is via the skin. Several methods have been developed to estimate dermal exposure (WHO, 1986; Fenske 1993; Geno et al. 1996), but their ability to measure the actual concentration of residues on the skin has been questioned (Chester, 1993; Fenske et al., 1999). A clear need exists for an effective methodology to rapidly identify and quantify pesticide residues on the skin *in vivo*.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy is a surface sampling technique that allows infrared spectra to be obtained from a specimen placed in contact with an optical sensor element (optical crystal). ATR-FTIR spectroscopy is a well established technique for surface analysis of optically dense materials such as lubricants, pastes, and food products (Griffiths, 1986). Recently, ATR-FTIR has been applied to study properties of human skin (Snieder and Hansen, 1997; Pirot et al., 1997).

ATR-FTIR spectra of non-volatile compounds on the skin surface can be obtained rapidly (within a few seconds) with minimal sampling preparation. ATR-FTIR spectra provide the same characteristic infrared absorption features as other types of infrared analysis. The shape and location of absorption features indicate the structure and can be used to qualitatively identify an unknown compound. A quantitative measure of the chemical on skin can be obtained by relating the magnitude of the absorption features to spectral standards with known surface loading for the target chemicals. ATR-FTIR also can simultaneously identify and quantify multiple compounds (mixtures or formulations) on the skin surface ***in vivo*** using multivariate analysis methods such as partial least squares or classical least squares. The goal of this research was to investigate the ability of ATR-FTIR spectroscopy to identify and quantify pesticide residues in contact with or transferred to skin.

Correspondence to: R. A. Fenske

MATERIALS AND METHODS

This work consisted of four steps: 1) characterization of FTIR spectra for several pesticides; 2) instrument calibration for these compounds; 3) spectral identification of the compounds on skin; 4) measurement of pesticide concentration on skin following contact with contaminated surfaces. Three pesticides in common use were evaluated with this technique: azinphos-methyl (S-(3,4-dihydro-4-oxobenzo[d]-[1,2,3]-triazin-3-ylmethyl) O,O-dimethyl phosphordithioate; CAS 86-50-0), captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]- 1H-isoindole- 1,3(2H)-dione; CAS 133-06-2), and chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate; CAS 2921-88-2).

Analyses were performed using an out-of-compartment ATR module (Graseby Specac, Smyrna, GA), coupled to a Magna 550 FTIR (Nicolet Instrument Corp., Madison, WI). Our instrument used a liquid nitrogen cooled mercury, cadmum, telluride (MCT) type detector that offers extremely high sensitivity to IR and excellent signal to noise characteristics. In ATR-FTIR sampling, a broadband infrared light signal is generated by a ceramic source operating at approx. 1300 °K. Light from this source is modulated by a Michelson interferometer and then directed into the ATR cell. Once inside the ATR unit, the IR beam is reflected by a set of mirrors up to an internal reflecting element (IRE). The IRE is made from an infrared transparent crystal material with a uniform refractive index and defined critical angle of total reflection at the inlet and outlet surfaces. When IR light is directed into the crystal at the critical angle (or larger), the light is totally reflected by the crystal surfaces and trapped inside the IRE. This condition generates an evanescent wave which penetrates above the IRE material and into any surface in direct contact with the IRE. The depth of penetration can be estimated as,

$$\text{Penetration} = \frac{\lambda}{2\pi n_1 \sqrt{(\sin^2\theta - n_{21}^2)}}$$

Where λ represents the wavelength of infrared radiation, n, is the refractive index of the ATR crystal, n_{21} the ratio of the refractive index of the sample to the refractive index of the ATR crystal, and Θ *(theta)*, the angle of incidence. The specific configuration of our instrument has a ZnSe crystal with a 10 cm^2 (1 x 10 cm) rectangular sampling area and a refractive index of 2.4 at an incident angle of 45° that results in penetration of approximately 1 µm at a 10 micron IR wavelength. Residues to be analyzed by the instrument are placed on a 1 x 2 cm area in the center of the crystal. Some of the energy contained in the evanescent wave is absorbed by the compounds in contact with the IRE surface, leading to measurable IR spectra, which can be qualitatively and quantitatively analyzed.

Most organic compounds on or in the skin will have unique absorbance features attributable to their particular molecular structure. Typically, compounds such as

the target pesticides with large ring structures and halogen substitutions have relatively strong absorbance features at very specific wavelengths in the "fingerprint" region at wavelengths of the IR spectrum around 10 microns. These compounds on the skin in contact with the IRE interface will produce IR spectra that are characteristic of the molecular structure.

After the IR light has assumed the spectral fingerprint of the target compound by passing through the IRE surface, it was redirected and focused onto the IR detector. The detector signal was digitized and sent to a desktop computer for further analyses. The computational analyses performed in these studies used OMNIC® brand (Nicolet Instrument Corp., Madison, WI) software. The software was used to control the bench and optimize the sampling with the ATR. Subsequently this same software was used to develop and validate calibration regimes for each compound of interest.

RESULTS AND DISCUSSION

The sensitivity of the system to the characteristic reference spectra for azinphos-methyl, captan, and chlorpyrifos was determined (Table 1).

Table 1. Performance of the ATR FTIR under experimental conditions.

Compound	Range (cm^{-1})	Load ($\mu g/cm^2$)	Signal to Noise Ratio[1]	LOD[2] (ng/cm^2)
Azinphos-methyl	1495-870	5	313	48
Captan	1260-870	5	276	54
Chlorpyrifos	1400-900	5	227	66

[1]Signal to noise for 32 co-averaged spectral scans at 2 cm^{-1} resolution
[2]LOD= Limit of detection was estimated as 3 times the root mean squared nc over the range of interest.

Spectra were obtained by dissolving the technical compound at 1 µg/µl in acetone and evaporating 10 µl of liquid loaded over a 2 cm^2 region of the ATR crystal. A typical ATR-FTIR sampling regime used 32 co-averaged scans at 2 cm^{-1} resolution and could be collected in 16 seconds. These data suggested that the ATR FTIR has sufficient sensitivity to identify the pesticides of interest.

A library of reference spectra spanning a reasonable range of surface or skin loadings (Franklin et al., 1986; Fenske et al., 1990; deCock et al., 1998) for quantitative analysis was generated next. A dilution series was performed ranging from 0.5 to 5 µg/cm^2 loading of technical compound for each compound to obtain a calibration curve. The spectra were processed using a partial least squares (PLS) quantification regime and optimized with a PRESS (Predicted Residual Error Sum of Squares) protocol to create a quantification program for each compound (Table 2).

Table 2. Calibration of the optical regime with three pesticides.

Topical Loading ($\mu g/cm^2$)	Measured Load ($\mu g/cm^2$) [1]		
	Azinphos-methyl	Captan	Chlorpyrifos
0	0	0	0
0.5	0.5	0.5	0.5
1	1.0	1.0	-
1.5	1.5	1.5	1.3
2.0	2.0	2.0	-
2.5	2.5	2.5	2.5
3.0	3.0	3.0	-
3.5	3.5	3.4	-
4.0	4.0	4.0	3.8
4.5	4.5	4.5	-
5.0	5.0	5.0	5.1
Avg. Error (µg)	0.02	0.01	0.08

[1]Topical loadings were distributed over approximately 2 cm^2 area on the sampling crystal.

The results produced linear calibration curves with $R^2 > 0.99$ for each compound, and indicate that residues can be accurately quantified over a working range.

The calibration regimes developed above were tested with known concentrations of the compounds of interest *in vivo*. The participation of human volunteers in this study was monitored and approved by the University of Washington's Human Subjects Division. Known amounts (5 -10 µg) of the compound were loaded onto the IRE by evaporation as described before. Immediately afterward, the forearm of a human volunteer was placed over the residue and the spectra collected. The results, for example from an azinphos-methyl trial displayed in Figure 1, show that the target compounds can be readily identified when the skin is in contact with the residues on the IRE.

The PLS quantification regime using the above calibration data was applied to the *in vivo* spectra, and predicted the mass at the skin surface within 5% of the true values (Table 3). Our results indicate that ATR-FTIR spectra and appropriate calibration regimes can be used to measure concentrations of captan or azinphos-methyl on the skin at levels relevant to occupational exposure.

Table 3. *In vivo* exposure to pesticide residues.

Compound	Topical Load[1] ($\mu g/cm^2$)	Measured Load ($\mu g/cm^2$)	% Divergence
Azinphos-methyl	5	4.8	4
Captan	3	2.8	4

[1]Topical loadings were distributed over approximately 2 cm^2 area on the sampling crystal.

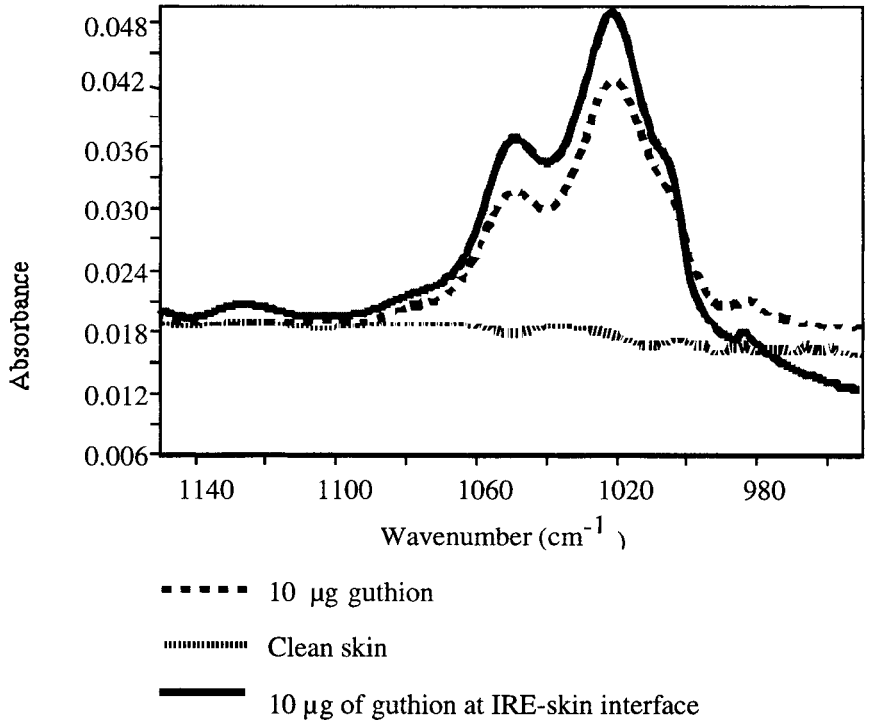

Figure 1. Azinphos-methyl *in vivo* exposure trial.

A final set of trials was performed to test the ability of the ATR-FTIR instrument to measure the transfer of pesticide residues onto skin after contact with a pesticide-contaminated surface. In this trial the subjects (n=2) stroked their index fingers across a clean glass slide, then placed it on the IRE for analysis. This produced the spectrum for the baseline for uncontaminated skin. Next, the subjects lightly stroked the same finger across a glass slide that had been loaded with either captan or chlorpyrifos residue using the evaporation method described previously. The finger was then placed on the IRE and the spectrum collected for a period of 10 seconds. The example spectra shown in Figure 2 clearly show the presence of captan residue.

The spectra collected after contact with a contaminated surface were then analyzed with a PLS quantification regime (Table 4).

Table 4. *In vivo* transfer of pesticide residues.

Compound	Surface Load ($\mu g/cm^2$)	Dermal Load[1] ($\mu g/cm^2$)	% Transfer
Captan	3.3	0.5	15
Chlorpyrifos	4	3.1	77

[1]Dermal loadings are based on an estimated area of exposed skin.

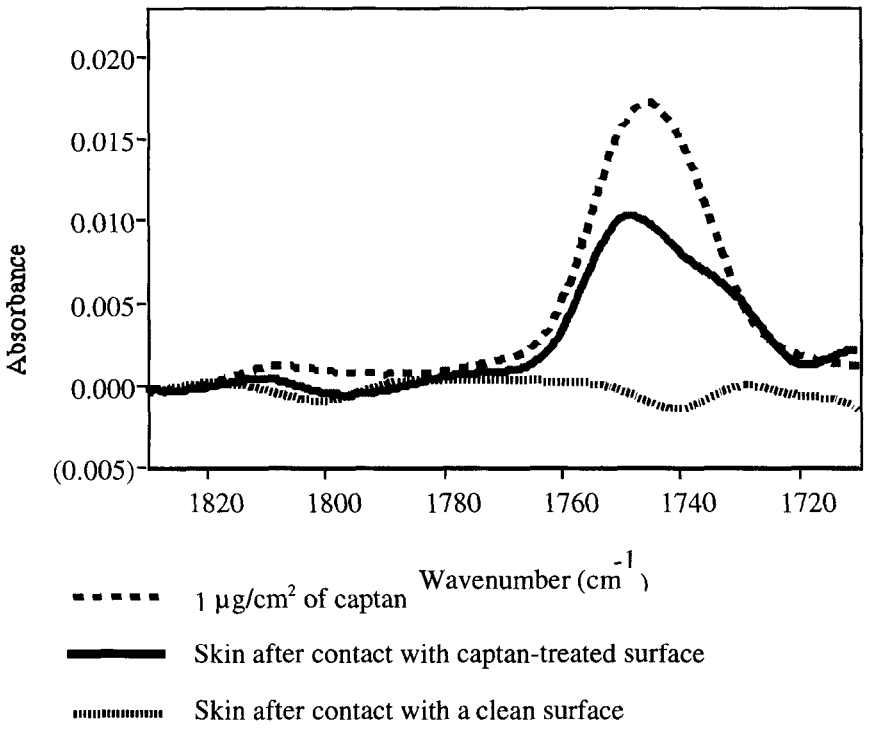

Figure 2. *In vivo* measurement of skin after surface contact.

These tests demonstrated the ability of the ATR-FTIR method to rapidly identify and quantify pesticide residues on the skin surface following contact with a pesticide-contaminated surface, and to distinguish this from contact with a clean surface. Experiments are currently being performed to identify the effects of individual compound, contact pressure, and surface characteristics on the efficiency of transfer.

These studies demonstrate that ATR-FTIR spectroscopy can provide rapid, quantitative data on dermal pesticide exposures for skin loading ranges that are relevant to occupational or residential settings. They also indicate that this technique can quantify pesticides in contact with the skin and residues transferred by contact with a contaminated surface.

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