PROTOCOL

SOIL THIN-LAYER CHROMATOGRAPHY

A. OBJECTIVE:

The objective of this study is to use thin layer chromatography to estimate the leaching potential of a test material and its degradates through various test soils. The present protocol is designed to meet environment (Canada) test requirements, in accordance with Sandoz Crop Protection Corporation's Good Laboratory Practices.

B. TEST COMPOUND:

The preferred radiolabeled test compound would have a radiochemical purity of greater than 95% and specific activity greater than 10 mCi/mmole. Information required to characterize the radiolabeled test compound includes: (1) compound name, (2) structure, (3) radiolabel position, (4) radiochemical purity, (5) source, and (6) specific activity. If it is necessary to dilute the labelled test compound with non-labeled test compound, the non-labeled compound should be analytical reference grade. All other chemicals will be reagent grade quality or better; solvents will be glass distilled.

C. GENERAL PROCEDURE AND TEST CONDITIONS:

(1) Soil Types

This test will be conducted using three types of soils. One of the soils utilized in the study will be the same as that used for the aerobic soil metabolism study. Three replicate plates will be prepared for each soil type.

(2) Soil Treatment and Aging

The aerobic aging portion of the study will be conducted for 30 days or for one half-life of the test compound in soil, whichever is shorter. The aging will be conducted at 25°C ± 1°C in darkness with the test soil at 75% of its 0.33 bar moisture level. Soil moisture will be maintained by weighing the vessel on a weekly basis and adding dionized water if weight loss is observed.

The concentration of test chemical in the soil will be equal to the maximum single application rate. This will generally be in the range of 0.1 to 10 ug/g of soil. The vessel containing the treated soil will be equipped with glass inlet and outlet tubes. The inlet tubes will be connected to an air supply; this air supply passes through a gas washing bottle containing 1.5 N KOH followed by a gas washing bottle containing distilled water, in order to provide 10 ml/min of CO_2-free moist air. The outlet tubes will be connected in series to a gas washing bottle containing ethylene glycol, followed by another gas washing bottle containing 1.5 N KOH. Volatile organic compounds are captured by the ethylene glycol, while the 1.5 N KOH traps respired CO_2. The contents of the CO_2 and organic trapping bottles will be radioassayed and replaced at weekly intervals.

An initial zero-time sample of the treated soil will be immediately frozen and 0.5 g subsample in triplicate will be radioassayed by combustion in a Packard 306 Sample Oxidizer. The remaining treated soil will be incubated until the termination of the aging period (not more than 30 days).

(3) Radiocarbon Extraction and Characterization

Radiolabeled compounds in the aged soil sample will be extracted with appropriate solvent(s) according to established procedures. Unextractable residues will be determined in the extracted sample by combustion/radioassay of triplicate 0.5 g subsamples.

A material balance will then be calculated for the soil at the end of the aging portion of the study; the balance will include $^{14}CO_2$, volatile organic fraction, extractable residues and unextractable residues. The extractable residues will then be identified by chromatographic and/or spectrometric methods. All radiocarbon fractions (degradates) greater than or equal to 10% of the applied dosage at zero time will be identified, if possible.

The collected ^{14}C-residue fraction(s) (extractable parent and degradate fractions) are now tested for the mobility within the three test soils.

(4) TLC Soil Sample Preparation

Each test soil sample will be individually air dried at 20-30°C and gently ground to reduce aggregate size. Then the soil will be sieved through a 500 µm sieve. Deionized water at pH 7.0 is added to the sieved soil until a smooth moderately fluid slurry is attained.

(5) Preparation of TLC Plates

The soil slurry is evenly and quickly across the clean glass 20 x 20 cm glass plates by using a variable thickness plate spreader. The soil layer thickness should be between 0.5 to 1.0 mm.

After achieving a uniform slurry application, the plates are air dried at 25°C for a minimum of 24 hours. After the drying period a horizontal line is scribed 12.0 cm above the glass plate bottom edge through the soil layer, exposing the plate surface. (This channel provides a consistent stopping point for solvent migration.)

(6) Chemical Mobility

The ^{14}C labeled test compound (0.1 to 1.0 ug) and a pesticide standard, whose soil mobility characteristics are already known, are spotted 2.0 cm above the bottom edge of the plate. After spotting, the soil plate is immersed with its bottom edge down, at some angle different from vertical, in a closed chromatographic chamber containing deionized water at a depth of 0.5 cm. Upon immersion, the plates are only removed after the water front reaches the scribed line, at which time they are air-dried.

(7) Determination of R_f Value

An AMBIS TLC radiographic imaging system is used to determine the location of the radioactive zones on the soil plate. The R_f value is calculated by dividing the migration distance of the test compound by the migration distance of the solvent front. The identify of the radioactive compounds is then established and reported.

D. CALCULATIONS

The R_f value is the ratio of the distance traveled by the test material to the distance traveled by the solvent front. The mobility of the test substance and its degradates will be reported according to the following table of mobility classes, which are based on the calculated R_f values for the respective compounds.

Table 1

R_f	Class Number	Soil Mobility Class
0.00 to 0.09	1	Immobile
0.10 to 0.34	2	Low
0.35 to 0.64	3	Intermediate
0.65 to 0.89	4	Mobile
0.90 to 1.00	5	Very Mobile

The relationship between R_f and the soil/water distribution coefficient, K_d, is given by:

$$R_f = [1 + k_d(d_s)(1/\theta^{2/3}-1)]^{-1}$$

where, d_s is bulk density of soil, and θ is the soil porosity.

K_d will be estimated using the known soil characteristics and the R_f values determined during the soil thin layer chromatography study.

E. DATA REPORTING

All raw data and calculations obtained from the soil thin-layer chromatography study will be reported. In addition, the following general information will be included with the report: (a) study initiation and termination dates; (b) test location; (c) names of principal investigators; (d) this protocol and any amendments; and (e) test chemical description. (See Section "B", Test Compound", of this protocol.) The soil characteristics used to estimate K_d will also be incorporated with the report. (See "D. Calculations" above.)

The soil thin-layer chromatography study will be conducted in accordance with Sandoz Crop Protection's Good Laboratory Practice Procedures. The completed report for this study is signed by the scientist(s) responsible for its completion and evaluation.

For Test Chemical: SAN 582H

APPROVALS:

Tsun-Min Rosa Tong — 9/14/90

Study Director — Date

J. H. Stallard — Sept. 14, 90

Director of Analytical Sciences and Formulations — Date

SANDOZ CROP PROTECTION CORPORATION
INTER-OFFICE CORRESPONDENCE

DATE: September 19, 1990 **COPIES TO:** Y. H. Atallah
D. C. Judson

TO: Project File

FROM: Tsun-Min Rosa Tong

SUBJECT: Amendment to Protocol

The Protocol "Soil Thin-Layer Chromatography" is amended as follows:

Amendment

1. In Section B. Test Compound: add information as follows:

   SAN-582H, 2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-3-^{14}C-thien-3-yl)acetamide

   CAS # 87674-68-8

   Purity: 99.3%

   Specificity: 43.2 mCi/mmole

2. Add this information: Study Sponsor

   This study will be carried out for:
   Sandoz Crop Protection Corporation
   1300 East Touhy Avenue
   Des Plaines, Illinois 60018

3. Add this information: Study Site

   This study will be carried out at:
   Sandoz Crop Protection Corporation
   1300 East Touhy Avenue
   Des Plaines, Illinois 60018

4. Add this information: Study Period

   Estimated Starting Date: September 14, 1990
   Estimated Completion Date: December 30, 1990

5. Add this information: Statistic Methods:

   A simple average will be used for this study.

Reason for Change:

The original protocol does not include Amendment 1. to 5. information.

Amendment:

6. Section C. General Procedure and Test Conditions: (2) Soil treatment and aging will not be performed.

Reason for Change:

The 30 day aged SAN-582H soil has been collected from the Aerobic Soil Metabolism of SAN-582H study.

Tsun-Min Rosa Tong
T.M. R. Tong

TMRT/rg

APPENDIX II

SAMPLE CALCULATION FOR K_d AND Koc

Kenyon Loam (SAN-582H)

Calculated according to Hamaker (1975):

$$R_f = \frac{1}{1 + \{K_{oc}(\%\text{ org. carbon}/100)(\text{bulk density})[(1/\text{vol. } H_2O)^{2/3}-1]\}}$$

where: bulk density is g/cm^3 and vol. H_2O content is cm^3/cm^3

$$k_d = K_{oc} \frac{(\%\text{ organic carbon})}{100}$$

$$0.21 = \frac{1}{1 + \{K_{oc}(2.2/100)(1.2)[(1/0.451)^{2/3}-1]\}}$$

$K_{oc} = 83.80$

$k_d = 83.80 \times \frac{(2.2)}{100}$

$= 1.84$

$R_f = 0.21$ for SAN-582H in Kenyon Loam

% org. Carbon = 2.2 in Kenyon Loam

bulk density = 1.2 g/cm^3 in Kenyon Loam

Vol. H_2O Content = 0.451 cm^3/cm^3 for loam soil