Data Evaluation Record on the leaching of clofentezine in an unaged soil column

PMRA Submission Number {.....} EPA MRID Number 47192114

Data Requirement: PMRA Data Code:
EPA DP Barcode: D342560
OECD Data Point:
EPA Guideline: 163-1

Test material:

Common name: Clofentezine.
IUPAC name: 3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS name: 3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS No.: 74115-24-5.
Synonyms: NC 21314, NC 21 314, AE B084866.
Smiles string: Clc1ccccc1c2nnc(c3ccccc3Cl)nn2 (EPI Suite, v3.12 SMILES).

Primary Reviewer: Amy Barnes
Cambridge Environmental

Signature:
Date: 12/19/07

Secondary Reviewer: Joan Harlin
Cambridge Environmental

Signature:
Date: 12/19/07

QC/QA Manager: Joan Gaidos
Cambridge Environmental

Signature:
Date: 12/19/07

Final Reviewer: Lucy Shanaman
EPA Reviewer

Signature: Lucy Shanaman
Date: 2/20/08

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 125501

CITATION: Leake, C.R. and D.J. Arnold. 1985. The leaching of (^{14}C)-clofentezine in four soil types using soil columns. Unpublished study performed by Schering Agrochemicals Limited, Essex, England; sponsored and submitted by Makhteshim Agan of North America, Inc. (location information not reported). Study number: 60J (p.4). Report number: METAB/85/10. Experiment start and completion dates were not reported. Final report issued April 23, 1985.

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Date: 12/19/07

QC/QA Manager: Joan Gaidos
Cambridge Environmental
Signature:
Date: 12/19/07

Final Reviewer: Keara Moore
EPA Reviewer
Signature:
Date:

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 125501

CITATION: Leake, C.R. and D.J. Arnold. 1985. The leaching of (^{14}C)-clofentezine in four soil types using soil columns. Unpublished study performed by Schering Agrochemicals Limited, Essex, England; sponsored and submitted by Makhteshim Agan of North America, Inc. (location information not reported). Study number: 60J (p. 4). Report number: METAB/85/10. Experiment start and completion dates were not reported. Final report issued April 23, 1985.

EXECUTIVE SUMMARY

The column leaching of unaged [tetrazine-3,6-^{14}C]-labeled 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine (clofentezine; radiochemical purity >98.1%), was investigated in four test soils from the United Kingdom: a sandy loam soil [Cottenham; pH 5.6, organic carbon 1.6%], a sand soil [Redlodge; pH 5.7, organic carbon 0.2%], a silt loam soil [Willingham; pH 7.4, organic carbon 4.7%], and a clay soil [Shelford; pH 6.0, organic carbon 5.2%]. Duplicate columns for the Redlodge sand and Shelford clay test soils, and triplicate columns for the Cottenham sandy loam and Willingham silt loam test soils were assembled using eight aluminum rings (5-cm i.d. x 5-cm) linked together with water proof adhesive tape to form a total cylinder height of 40 cm. Nylon gauze was taped across the bottom ring of each column before filling it with sand (40-100 mesh), and placing the column in a Buchner funnel in order to collect column leachate samples. Each column was packed, one segment at a time, with untreated test soil up to a total height of six segments (30 cm). The top soil segment was divided into two 2.5-cm segments. The columns were pre-conditioned by applying 0.01M $CaCl_2$ solution at *ca.* 50 mL/column per day for 2 days using a peristaltic pump, until leachate was freely draining from the soils. An eighth ring was placed on the top of the columns to hold the treated soil layer (25 g), and the treated soil layer was covered with glass wool. Radiolabelled atrazine was used as a reference standard in columns filled with two of the soil types. Each column was leached with *ca.* 34 mL of 0.01M $CaCl_2$ solution per day for 30 days. The method used to maintain a constant column head was not reported. The average total volume of $CaCl_2$ solution applied to each soil column was 1027 mL, equivalent to 34.2 mL/day over the 30-day leaching period. The method used to maintain a constant column head was not reported.

Leachate volumes were collected daily and aliquots were analyzed for total radioactivity using LSC. The soil columns were dissembled into six equal segments, each 5 cm in height (top segment divided into 2 x 2.5 cm segments) in addition to the treated soil segment at the top of each column and the sand segment at the bottom of each column. The sections were Soxhlet extracted once with dichloromethane and once with acetonitrile:water (80:20, v:v), and aliquots of the extracts were analyzed using LSC. The extracts were combined and analyzed using LSC and normal phase TLC. Following extraction, the soils were air-dried, ground to a fine powder, mixed with D-glucose, and compressed into pellets; aliquots were analyzed using LSC following combustion.

The environmental temperature and lighting conditions during column leaching were not reported. For all soil columns, the mean total volume of leachate collected was 968 mL (s.d. 29.6 mL).

For the **Redlodge sand soil**, the mass balance was 114.12% of the applied following 30 days of leaching. A total of 103.74% of the applied radioactivity was recovered in the treated soil layer, 9.47% in the 0-2.5 cm segment (Section 1a), 0.18% in the 2.5-5 cm segment (Section 1b), and <0.01-0.22% in the 10-30 cm layers (Sections 2-6). Extractables and non-extractables accounted for maximums of 100.99% and 2.75% of the applied, respectively. Radioactivity in the leachate volumes totaled 0.04% of the applied.

For the **Shelford clay soil**, the mass balance was 87.61% of the applied following 30 days of leaching. A total of 85.88% of the applied radioactivity was recovered in the treated soil layer, 1.29% in the 0-2.5 cm segment (Section 1a), 0.18% in the 2.5-5 cm segment (Section 1b), and <0.01-0.07% in the 10-30 cm layers. Extractables and non-extractables accounted for maximums of 71.85% and 14.03% of the applied, respectively. Radioactivity in the leachate volumes totaled <0.01% of the applied.

For the **Cottenham sandy loam soil**, the mass balance was 106.49% of the applied following 30 days of leaching. A total of 104.87% of the applied radioactivity was recovered in the treated soil layer, 1.02% in the 0-2.5 cm segment (Section 1a), 0.38% in the 2.5-5 cm segment (Section 1b), and <0.01-0.15% in the 10-30 cm layers. Extractables and non-extractables were a maximum of 95.38% and 9.49% of the applied, respectively. Radioactivity in the leachate volumes totaled <0.01% of the applied.

For the **Willingham silt loam soil**, the mass balance was 97.68% of the applied following 30 days of leaching. A total of 92.33% of the applied radioactivity was recovered in the treated soil layer, 3.91% in the 0-2.5 cm segment (Section 1a), 0.42% in the 2.5-5 cm segment (Section 1b), and 0.03-0.28% in the 10-30 cm layers. Extractables and non-extractables were a maximum of 84.59% and 7.74% of the applied, respectively. Radioactivity in the leachate volumes totaled 0.27% of the applied.

TLC analysis of the combined soil extracts and the top 2.5 cm segment showed that [^{14}C]clofentezine accounted >90% of the applied radioactivity; further details were not provided.

Study Acceptability: This study is classified as **acceptable.** No significant deviations from good scientific practices were noted. The experimental temperature and lighting conditions were not reported.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Guidelines were not reported. Significant deviations from the objectives of Subdivision N guidelines were:

The experimental temperature and lighting conditions were not reported.

COMPLIANCE: This study was not conducted in compliance with USEPA Title 40 CFR Part 160 (p.3). Signed and dated No Data Confidentiality and GLP statements were provided (pp.2-3). Quality Assurance and Authenticity statements were not provided.

A. MATERIALS:

1. Test Material [Tetrazine-3,6-^{14}C]-labeled clofentezine (p.7).

Chemical Structure: See DER Attachment 1.

Description: Technical; physical state not reported (p.7).

Purity:

Radiolabeled: Radiochemical purity: ≥98.1% via HPLC and TLC (p7).
Batch No. CFQ 2874.
Specific activity: 47.7 µCi/mg.
Locations of the label: At 3-C and 6-C of tetrazine ring (p.7).

Unlabeled: Batch No.: CDB 134522 (NC 21 314/6; p.7).
Analytical purity: 100.0%.

Storage conditions of test chemicals: Storage conditions were not reported.

Physico-chemical properties of clofentezine:

Parameter	Value	Comment
Molecular formula	Not reported.	
Molecular weight	Not reported.	
Water Solubility	Not reported.	
Vapor Pressure/Volatility	Not reported.	
UV Absorption	Not reported.	
pKa	Not reported.	
K_{ow}/log K_{ow}	Not reported.	
Stability of compound at room temperature, if provided	Not reported.	

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Description	Cottenham	Redlodge	Willingham	Shelford
Geographic location	Cottenham, Cambridgeshire, United Kingdom	Redlodge, Cambridgeshire, United Kingdom	Shippea Hill, Suffolk, United Kingdom	Little Shelford, Cambridgeshire, United Kingdom
Pesticide use history at the collection site	Not reported.			
Collection procedures	Not reported.			
Sampling depth (cm)	Not reported.			
Storage conditions	20 ± 2°C.			
Storage length	*ca.* 1 week.			
Soil preparation (eg: 2 mm sieved; air dried etc.)	Air-dried and sieved (2 mm).			

Data were obtained from p.8 of the study report.

Table 2: Properties of the soil.

Property	Cottenham	Redlodge	Willingham	Shelford
Soil texture (USDA)[1]	Sandy loam	Sand	Silt loam	Clay
% Sand (63 µm-2 mm)	64.94	91.03	14.69	35.73
% Silt (2 µm-63 µm)	20.93	5.19	68.56	16.31
% Clay (<2 µm)	14.13	3.78	16.75	47.96
pH (1:2.5 in 0.01M $CaCl_2$)	5.6	5.7	7.4	6.0
Organic carbon (%)[2]	1.6	0.2	4.7	5.2
Organic matter (%)	2.7	0.4	8.0	9.0
CEC (meq/100 g)	12.8	3.3	12.7	44.2
Moisture at 1/3 atm (%)	Not reported.			
Moisture content (%)	10.3	4.7	55.7	35.3
Bulk density (g/cm^3)	1.14	1.23	0.92	0.86
Biomass (mg microbial C/100 g or CFU or other)	Not reported.			
Soil taxonomic classification	Not reported.			
Soil mapping unit (for EPA)	Not reported.			

Data were obtained from Table 2, p.9 of the study report.

1 Textural classifications were confirmed using the NRCS soil texture calculator http://soils.usda.gov/technical/aids/investigations/texture/ which calculates soil texture based on the percent sand and clay.

2 Ccalculated as % organic matter ÷ 1.72.

C. STUDY DESIGN:

1. Preliminary study: No preliminary studies were reported.

2. Definitive study experimental conditions: The mobility of [tetrazine-3,6-^{14}C]-labeled 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine (radiochemical purity >98.1%; specific activity 47.7 µCi/mg) was investigated using sandy loam, sand, silt loam, and clay soils (pp.7-8).

The test soils were air-dried and sieved (2 mm) prior to use in the study (p.8). Soil biomass determinations for the test soils were not conducted.

Aliquots (2 x 25 g) of each test soil were placed into glass beakers and treated with 0.2 mg of [^{14}C]clofentezine, dissolved in dichloromethane, at a field application rate of 1.0 kg a.i./ha (p.11). For all soils, the solvent was allowed to evaporate prior to application of the treated soils to the top of the appropriate soil columns. Two single samples of Cottenham loamy sand and Willingham silt loam test soils were also treated with [^{14}C]atrazine, dissolved in ethanol, at 0.1983 mg/25 g dry soil, equivalent to a field application rate of 1.0 kg a.i./ha. The solvent was allowed to dry in a similar manner prior to application to the appropriate soil columns.

For the column leaching study, duplicate columns for the Redlodge sand and Shelford clay test soils, and triplicate columns for the Cottenham sandy loam and Willingham silt loam test soils were assembled using eight aluminum rings (5-cm i.d. x 5-cm) linked together with water proof adhesive tape to form a total cylinder height of 40 cm (p.10). Nylon gauze was taped across the bottom ring of each column before filling it with sand (40-100 mesh), and placing the column in a Buchner funnel in order to collect column leachate samples. Each column was packed, one segment at a time, with untreated test soil up to a total height of six segments (30 cm). The top soil segment was divided into two 2.5-cm segments. The columns were pre-conditioned by applying 0.01M $CaCl_2$ solution at *ca.* 50 mL/column per day for 2 days using a peristaltic pump, until leachate was freely draining from the soils. An eighth ring was placed on the top of the columns to hold the treated soil layer (25 g) and the treated soil layer was covered with glass wool. Each column was leached with *ca.* 34 mL of 0.01M $CaCl_2$ solution per day for 30 days (p.1). The method used to maintain a constant column head was not reported. The average total volume of $CaCl_2$ solution applied to each soil column was 1027 mL, equivalent to 34.2 mL/day over the 30-day leaching period (p.13). The method used to maintain a constant column head was not reported.

Column leachate volumes were collected daily (p.11). Following 30 days of leaching, columns were drained for 3 days. One soil column for each test soil was dissembled into six equal segments, each 5 cm in height (top segment divided into two 2.5-cm segments) in addition to a treated soil segment and a sand segment. The columns were segmented by removing the water proof tape and cutting the soil with a knife.

3. Description of analytical procedures:

Extraction/clean up/concentration methods: Each column segment of soil, including the originally treated soil and sand layers, was Soxhlet extracted with dichloromethane for 18 hours followed by acetonitrile:water (80:20, v:v) for 18 hours (p.11). The [^{14}C]atrazine columns were sectioned and air-dried without solvent extraction.

Total ^{14}C measurement: Aliquots (*ca.* 1 mL) of the leachate volumes and extracts were analyzed for total radioactivity using LSC (p. 11). Samples were added to 12 mL of scintillant and allowed to equilibrate at 16°C in ultraviolet-filtered light prior to counting.

Non-extractable residues, if any: Following extraction, the soils were air-dried and ground with a Copley mill to a fine powder (p. 12). Aliquots (3 x 0.2-0.3 g) were mixed with *ca.* 0.2-0.3 g of D-glucose, compressed into pellets using a Parr pellet press, and combusted. The $^{14}CO_2$ released was absorbed into 'Carbosorb' and analyzed by LSC. The soil segments from the [^{14}C]atrazine treated soils were dried and combusted in a similar manner. Combustion efficiency was not reported.

Derivatization method, if used: A derivatization method was not employed in this study.

Identification and quantification of parent compound: Aliquots of the soil extracts were analyzed for clofentezine using normal phase TLC (p.12). Normal phase two-dimensional TLC was conducted in paper-lined chromatography tanks at room temperature using Machery-Nagel F_{254} plates (20 cm x 20 cm, 0.25 mm thickness) developed in toluene:ethanol:ethyl acetate:glacial acetic acid (80:10:5:0.5, v:v:v:v; ratio by volume; Solvent System A) and chloroform:glacial acetic acid (95:5, v:v; ratio by volume; Solvent System B). Aliquots of the soil extracts were applied to the plates as two narrow bands. One band contained the extract while the other was mixed with aliquots of solutions of appropriate authentic potential transformation products. Unlabeled reference compounds were visualized under UV light (254 nm). Images of the developed plates were obtained using an Isomess IM 3000 linear analyzer and autoradiography (Agfa M3 X-ray film). [^{14}C]Clofentenzine was identified by comparison to the retention time of an unlabeled reference standard of clofentenzine (p. 7).

Identification and quantification of transformation products, if appropriate: As described for parent compound.

Detection limits (LOD, LOQ) for the parent compound: Detection limits for LSC and TLC analyses were not reported.

Detection limits (LOD, LOQ) for the transformation products, if appropriate: Detection limits for LSC and TLC analyses were not reported.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: Details of the test conditions were not reported. The experimental temperature and lighting conditions were not reported. The method used to maintain a constant column head during leaching was not reported.

For all soil columns, the mean total volume of leachate collected was 968 mL (S.D. 29.6 mL; p.13).

B. MASS BALANCE: Following 30 days of leaching, mass balances were 114.12%, 87.61%, 106.49%, and 97.68% of the applied for the Redlodge sand, Shelford clay, Cottenham sandy loam, and Willingham silt loam soils, respectively (Appendix III, p.17).

C. LEACHING: For all test soils, the majority of the applied radioactivity remained in the topmost (treated) section of the soil columns following 30 days of leaching (p.13; Appendix III, p.17).

For the **Redlodge sand soil**, a total of 103.74% of the applied radioactivity was recovered in the treated soil layer, 9.47% in the 0-2.5 cm segment (Section 1a), 0.18% in the 2.5-5 cm segment (Section 1b), and <0.01-0.22% in the 10-30 cm layers (Sections 2-6; p. 13; Appendix III, p.17). The sand layer accounted for <0.01% of the applied radioactivity. Extractables accounted for 100.99% of the applied in the treated soil layer, 7.48% in the top 2.5 cm layer, and was a maximum of 0.19% in the bottom 2.5-30 cm and sand layers. Non-extractables accounted for 2.75% of the applied in the treated soil layer, 1.99% in the top 2.5 cm layer, and was a maximum of 0.14% of the applied in the bottom 2.5-30 cm and sand layers. Radioactivity in the leachate volumes totaled 0.04% of the applied.

For the **Shelford clay soil**, a total of 85.88% of the applied radioactivity was recovered in the treated soil layer, 1.29% in the 0-2.5 cm segment (Section 1a), 0.18% in the 2.5-5 cm segment (Section 1b), and <0.01-0.07% in the 10-30 cm layers (Sections 2-6; p. 13; Appendix III, p.17). The sand layer accounted for 0.08% of the applied radioactivity. Extractables accounted for 71.85% of the applied in the treated soil layer, 0.11% in the top 2.5 cm layer, and was a maximum of 0.08% in the bottom 2.5-30 cm and sand layers. Non-extractables accounted for 14.03% of the applied in the treated soil layer, 1.18% in the top 2.5 cm layer, and was a maximum of 0.15% of the applied in the bottom 2.5-30 cm and sand layers. Radioactivity in the leachate volumes totaled <0.01% of the applied.

For the **Cottenham sandy loam soil**, a total of 104.87% of the applied radioactivity was recovered in the treated soil layer, 1.02% in the 0-2.5 cm segment (Section 1a), 0.38% in the 2.5-5 cm segment (Section 1b), and <0.01-0.15% in the 10-30 cm layers (Sections 2-6; p.13; Appendix III, p.17). The sand layer accounted for <0.01% of the applied radioactivity. Extractables accounted for 95.38% of the applied in the treated soil layer, 0.40% in the top 2.5 cm layer, and was a maximum of 0.06% in the bottom 2.5-30 cm and sand layers. Non-extractables accounted for 9.49% of the applied in the treated soil layer, 0.62% in the top 2.5 cm

layer, and was a maximum of 0.32% of the applied in the bottom 2.5-30 cm and sand layers. Radioactivity in the leachate volumes totaled <0.01% of the applied.

For the **Willingham silt loam soil**, a total of 92.33% of the applied radioactivity was recovered in the treated soil layer, 3.91% in the 0-2.5 cm segment (Section 1a), 0.42% in the 2.5-5 cm segment (Section 1b), and 0.03-0.28% in the 10-30 cm layers (Sections 2-6; p.13; Appendix III, p.17). The sand layer accounted for 0.02% of the applied radioactivity. Extractables accounted for 84.59% of the applied in the treated soil layer, 2.24% in the top 2.5 cm layer, and was a maximum of 0.11% in the bottom 2.5-30 cm and sand layers. Non-extractables accounted for 7.74% of the applied in the treated soil layer, 1.67% in the top 2.5 cm layer, and was a maximum of 0.31% of the applied in the bottom 2.5-30 cm and sand layers. Radioactivity in the leachate volumes totaled 0.27% of the applied.

TLC analysis of the combined soil extracts and the top 2.5 cm segment showed that [^{14}C]clofentezine accounted >90% of the applied; further details not provided (p.13).

III. STUDY DEFICIENCIES

Complete details of the experimental design employed for the leaching portion of the study were not provided. The experimental temperature was not reported. An incubation temperature within the range of normal environmental parameters (18-30°C) should have been used in the study. It was not stated whether leaching was conducted in the dark to minimize photodegradation. Also, the method used to maintain a constant column head during leaching of the soil column was not reported.

IV. REVIEWER'S COMMENTS

1. The study authors report that TLC analysis of combined solvent extracts from the originally treated soils and the top 2.5 cm segment showed that the majority of the radioactivity (>90%) was clofentezine (p.13). However, TLC chromatograms were not provided.

2. Clofentezine was determined to be immobile in the four test soils (p.14).

3. Combustion of the soil segments from the [^{14}C]atrazine-treated columns showed that there was movement in the Cottenham sandy loam and Willingham silt loam soil columns, with only a small amount radioactivity (1.26-8.04% of the applied) remaining in the treated soil layer. The majority of the radioactivity moved into segments 1 and 2 (22.64-25.92% of the applied) in the Cottenham sandy loam soil column, and segments 4 and 5 (15.60-19.21% of the applied in the Willingham silt loam soil column (p.13; Appendix IV, p.18).

4. For all soil columns, an average of 1027 mL of total volume of $CaCl_2$ solution was applied to each soil column; however, the mean total volume of leachate collected was 968 mL (s.d. 29.6 mL; p.13). The study authors suggested that the loss may be due to evaporation.

5. It was stated that samples were analyzed for transformation products; however the transformation products were not identified (p.12).

V. REFERENCES

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 163-1. Mobility studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738.

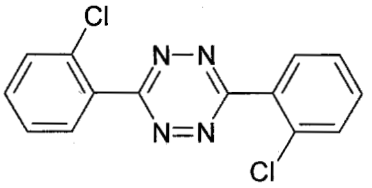
4. U.S. Environmental Protection Agency. 2003. Guidance for Calculating Sorption Coefficients in Batch Equilibrium Studies.

Attachment 1: Structure of Test Material

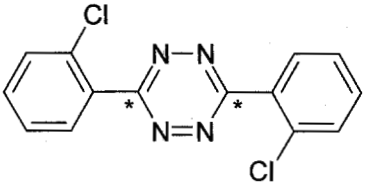
Clofentezine [NC 21314, NC 21 314, AE B084866]

IUPAC Name: 3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS Name: 3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS Number: 74115-24-5.
SMILES String: Clc1ccccc1c2nnc(c3ccccc3Cl)nn2 (EPI Suite, v3.12 SMILES).

Unlabeled



[Tetrazine-3,6-^{14}C]Clofentezine



* = Location of the radiolabel.

Attachment 2: Illustration of Test System

3.3 Preparation and treatment of soil leaching columns

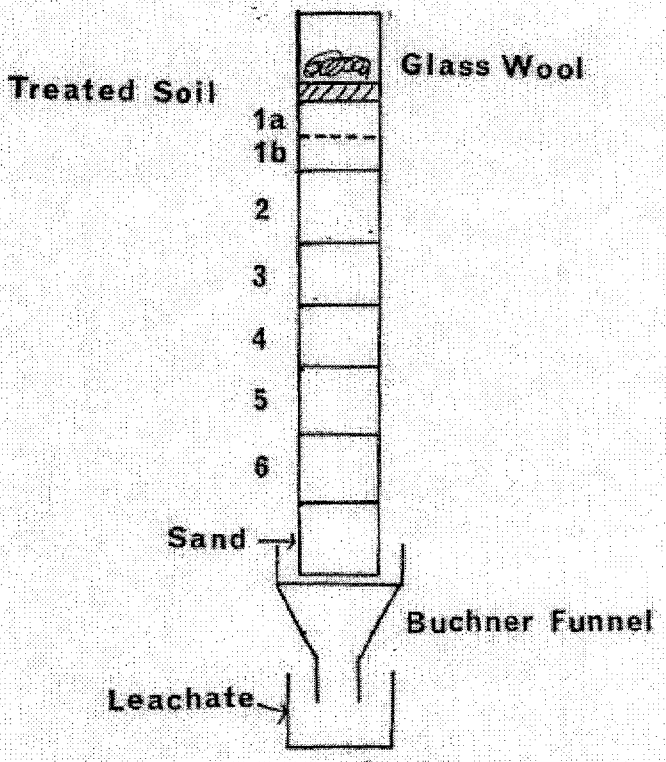
Ten columns were prepared by binding together with water proof adhesive tape 8 aluminium rings (5 cm i.d. x 5 cm) to form a cylinder of total height 40 cm. Nylon gauze was taped across the bottom ring before filling it with sand (40-100 mesh) and placing it in a Buchner funnel to enable collection of column leachate.

Duplicate, or in the case of "Cottenham" sandy loam and "Willingham" silt loam soils, triplicate columns of each soil type were packed, one segment at a time, to a constant bulk density (see Table 2) up to a total height of six soil segments. The top soil segment was divided into 2 x 2.5 cm segments above which an eighth ring was placed to hold the treated soil layer and above this a layer of glass wool to assist dispersion of the eluate (see Figure 1).

The columns were conditioned by applying 0.01 M $CaCl_2$ at a rate of approximately 50 ml/column per day for 2 days using a peristaltic pump (Watson and Marlow 202U), until leachate was freely draining from the soils.

Figure 1

Construction of columns



10/18