PART 5

Section 5 Fate and Behaviour in the Environment

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. Although based on a real submission, the data included in the following summary and evaluation have been amended to protect the commercial interests of the owner of the data.

Applicants should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the Country to which application is to be made.

Fate and Behaviour in Soil, Water and Air

Information is provided in the pages that follow with respect to the fate and the behaviour in soil, water and air of chemx, the active substance of a chemical herbicide intended for use in wheat. It is proposed that the plant protection product be applied as a post-emergent application in winter wheat, at a maximum rate of 20g as/ha. The studies concerning the fate and behaviour of chemx in the environment were conducted using one or both labelled forms of chemx. In the *Tier II* summaries that follow, reference is made to chem2 ring labelled chemx or simply Label 1, for the compound labelled with ^{14}C in the C-3 position of the chem2 ring, and chem3 ring labelled chemx or simply Label 2, for the compound labelled with ^{14}C in the C-5 position of the chem3 ring. The structure of chemx and the positions of the radiolabels were as follows:

Structures Omitted

IIA 7.1 Route of degradation in soil (laboratory studies)

IIA 7.1.1 Aerobic degradation

Report: IIA 7.1.1/01 - Grenfel RG 1996, The aerobic soil metabolism of ^{14}C-chemx, Chemco Report XX-14019

Guideline

BBA Guidelines for the official testing of plant protection products (Part IV, 4-1, Stage 1) ≅ Procedures for assessing the environmental fate and behaviour of pesticides, SETAC-Europe, 1995, ISBN Number 90-5607-002-9.

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England, conducted the study during the period August 1993 to December 1994.

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In a soil degradation study, the aerobic bio-transformation of ^{14}C-chemx was studied in loamy soil (Speyer 2.2 standard soil) for 225 days in a closed system in darkness at 19 to 22 °C, pH 5.8, and 40 % water holding capacity following application of 0.08 mg as/kg dry soil (≈ 80 g as/ha or 4 times the recommended field application rate). The total CO_2 and other volatile compounds released amounted to 2.2 % of the chem2-^{14}C applied and 13 % of the chem3-^{14}C applied.

The DT_{50} and DT_{90} values for degradation of chem2-^{14}C and chem3-^{14}C chemx in soil, based on pseudo first order reaction kinetics, were 51/ 54 days and 175/181 days, respectively. The mass balance for the study was > 93%. The major transformation products detected were desmethyl chemx (29 % of applied radioactivity).Other significant metabolites were formed by cleavage of the xxx bond of chemx to form xxxxxxx (metabolite 2) (9 % of applied radioactivity) and xxxx (metabolite 10) (5 % of applied radioactivity) where the chem2 labelled material was applied to soil and metabolite 3 (3.4 % of applied radioactivity) where the chem3 labelled material was applied to soil.

The amount of extractable radioactivity declined from 99 / 98 % of applied radioactivity at zero time to 79 / 70 % at day 100 and to 72 / 50 % after 225 days for chem2-^{14}C / chem3-^{14}C chemx respectively. Non-extractable radioactivity increased to 14 / 15 % of applied radioactivity at day 100 and to 41 / 33 % at day 225 for both radiolabels.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - chem2 ring labelled: ^{14}C in the C-3 position; specific activity 58.6 μCi/mmol;
chem3 ring labelled: ^{14}C in the C-5 position; specific activity 64.2 μCi/mmol;

Description:	White powder
Lot/Batch #:	NPD-9307-5385-T
Purity:	chem2 ring labelled: radiochemical purity ≥ 97 % chem3 ring labelled: radiochemical purity ≥ 97 %
CAS #:	16335-17-2
Stability of test compound:	The test material was stable for at least 7 days at room temperature.

2. Soil: A Speyer 2.2 standard soil, a loamy sand was used. Standard soils are collected from specific locations with restricted access managed to ensure that no pesticides or organic fertilizer treatments take place for at least five years prior to collection.

Table IIA 7.1.1-1 Soil Physicochemical Properties

Soil Origin	Soil Type	pH	OM %	Sand %	Silt %	Clay %	MWHC %	CEC meg / 100g	Biomass µg C/g soil
Speyer 2.2	Loamy Sand	*5.8	**3.94	82	13	#5.1	55.2	9.7	316 day 0 316 day 312

* measured in a 1 : 2.5 soil : water suspension # Less than the 10 % minimum recommended by BBA/SETAC
** Organic matter calculated as 1.72 x percent organic carbon

B. STUDY DESIGN

1. Experimental conditions

The aerobic soil metabolism of chemx was studied in using chem2 and chem3 ring labelled chemx applied at the rate of 0.08 mg/kg (equivalent to 80 g as/ha - 4 times the recommended field rate).

Portions of sieved soil, equivalent to 60 g dry soil, adjusted to 40 % maximum water holding capacity were transferred into storage columns and incubated for one week prior to test substance application. Solutions of each radiolabelled form of the test substance in acetonitrile were prepared, diluted with water and applied to the storage columns to give an application rate of 0.08 µg/g soil. The organic solvent was allowed to evaporate after which the soil was mixed to ensure homogeneity. Duplicate samples were incubated in darkness in an environmentally controlled room at a temperature 19 - 22°C for an incubation period of 100 days.

In a separate experiment conducted for the purposes of the identification of metabolites formed, further soil samples were treated with 4.4 mg/kg chemx (220 times the recommended field rate) and were maintained for 225 days under similar environmental conditions.

2. Sampling

Microbial biomass was determined at zero time and day 312 (Anderson and Domsch 1978). Soil samples were taken for analysis at zero time and 8, 16, 32, 64, 100, 181 and 225 days after application.

3. Description of analytical procedures

Sodium hydroxide solutions used to trap volatile components were replaced and analysed biweekly. Soil samples were subjected to the following extraction sequence:

a) 80 ml acetonitrile : 0.5% aqueous sodium chloride (65 : 35, V : V) - 3 extractions,
b) 100 ml acetonitrile : 2 M NaOH (50 : 50, V : V) - one extraction followed by separation of the organic layer and back extraction of the aqueous layer into acetonitrile.

After addition of the solvent, the samples were shaken using a sonic agitator for 10 minutes, and then shaken on an orbital shaker for 20 minutes at > 100 revolutions per minute. The soil was separated from the supernatant by centrifugation at approximately 1500 rpm for 10 minutes. That process was repeated following each extraction. Prior to analysis, the extracts were pooled (using only the organic layer from b), and their total volumes and radioactivity measured.

Soil samples were combusted and ^{14}C levels were measured using LSC. The radioactivity levels in extracts and residue were measured using LSC. The soil extracts were analysed using reverse phase HPLC (Spherisorb S5 ODS1, 25 cm x 10 mm id) eluted with a gradient of acetonitrile and water containing 1 % formic acid and 1.35 % triethylamine. The effluent was passed through an UV detector (254 nm) to detect reference standards and a radioactivity detector to determine the quantities of radiolabelled degradation products present. Chromatography using normal phase TLC and GC-MS were used to confirm the identity of the degradation products. The limit of detection (LOD) for chemx and metabolite 1 were x and x µg as/g soil, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 were y and y µg as/g soil, respectively.

II. RESULTS AND DISCUSSION

A. DATA: Table IIA 7.1.1-2 Degradation product distribution (expressed as % of applied radioactivity) over 225 days aerobic incubation of treated Speyer 2.2 soil (20°C)

% Applied Radioactivity								
Days after application	0	8	16	32	64	100	181	225
(a) chem2 ring ^{14}C labelled chemx								
Chemx	93	84	74	63	39	26	16	18
xxxxxxx (metabolite 2)	2.2	2.5	5.2	5.7	9.1	9.0	8.7	15
desmethyl chemx (metabolite 1)	1.0	3.7	11	20	21	28	23	19
xxxx (metabolite 10)	< 0.2	0.4	0.7	1.4	1.6	2.6	5.0	3.2
unidentified compounds *	< 2.7	2.2	< 1.7	< 3.9	5.8	10	< 6.4	8.7
polar compounds extracted **	ns	1	1	2	3	4	7	9
unextractable compounds	5.5	6.7	< 2.2	5.1	17	14	28	41
CO_2	ns	0.8	0.8	0.9	1.2	1.6	1.9	2.2
Total	105	101	93	101	97	95	94	115
					DT_{50}	51		
					DT_{90}	170		
(b) chem3 ring ^{14}C labelled chemx								
Chemx	94	89	79	60	43	24	25	23
metabolite 3	0.9	0.6	0.7	3.0	1.2	3.4	0.9	1.8
desmethyl chemx (metabolite 1)	0.9	4.6	10	29	26	29	18	19
unidentified compounds *	< 2.6	< 1.8	< 1.6	< 4.2	< 5.4	< 8.7	< 2.7	< 3.5
polar compounds extracted **	ns	1	1	2	3	5	6	8
unextractable compounds	3.2	2.7	2.4	4.5	18	15	38	33
CO_2	ns	2.6	3.2	4.0	5.8	8.1	12	13
Total	101	100	98	105	102	93	102	101
					DT_{50}	54		
					DT_{90}	181		

ns = not sampled

* unidentified compounds - 4 in number individually accounting for at maximum 2 - 4 % of applied radioactivity

** radioactivity in extracts not included in pools prepared for chromatography - mostly contain radioactivity not partitioned from 2M NaOH extracts

B. MASS BALANCE

The recovery level for radioactivity from Speyer 2.2 soil for all sampling points was > 93 % (93 - 115 %).

C. BOUND AND EXTRACTABLE RESIDUES

The amount of extractable radioactivity declined from 99 / 98 % of applied radioactivity at zero time to 79 / 70% at day 100 and to 72 / 50 % after 225 days for chem2-^{14}C / chem3-^{14}C chemx respectively. Non-extractable radioactivity increased to 14 / 15 % of applied radioactivity at day 100 and to 41 / 33 % at day 225 for both radiolabels.

D. VOLATILIZATION

Volatile radioactivity, identified as $^{14}CO_2$ represented 1.6 / 8.1 % of applied radioactivity at day 100 and 2.2 / 13 % at day 225 for chem2-^{14}C /chem3-^{14}C chemx respectively.

E. TRANSFORMATION OF PARENT COMPOUND

Over a period of 100 days incubation, levels of chemx in the soil extracts decreased to 26 / 24 % of applied radioactivity for chem2-^{14}C / chem3-^{14}C chemx respectively and declined further to 18 / 23% after 225 days.

Degradation half-life (DT_{50}) based on pseudo first-order reaction kinetics measured between day 0 and day 100 was 51 / 54 days while the DT_{90} values were 170 / 181 days for chem2-^{14}C / chem3-^{14}C chemx respectively.

It should be noted that the degradation half lives are derived from data generated using soil extracts which involved extraction with acetonitrile : 2 M NaOH (50 : 50), a very harsh extraction solvent which is considered likely to have resulted in the break down of soil structure, thereby releasing chemx which had been adsorbed to organic matter and clay and which would not have been bioavailable.

The degradation rate constant was calculated from the following equation:

$$\ln C = -Kt + \ln C_O$$

where K = rate constant
C = test substance concentration at t_O as a percentage of zero time radioactivity
C_O = test substance concentration at t_O as a percentage
t = time in days

Table IIA 7.1.1-3 Degradation rate constant calculations

Radiolabelled Form	Number of data points	r^2	$K(\times 10^{-4})$	DT_{50} (days)	DT_{90} (days)
chem2-^{14}C chemx	6	0.996	135.5	51	170
chem3-^{14}C chemx	6	0.988	127.4	54	181

The two major degradation products were formed in soil. Desmethyl chemx accounted for 28 / 29 % of applied radioactivity for chem2-^{14}C / chem3-^{14}C chemx respectively after 100 days incubation, declining to 19 % after 225 days for both radiolabels. In the chem2-^{14}C chemx treated soil xxxxxxx (metabolite 2) reached a maximum of 9 % of applied radioactivity after 100 days and increased to 15 % of applied radioactivity after 225 days, while xxxx (metabolite 10) reached a maximum of 5 % of applied radioactivity and then declined. In the chem3-^{14}C chemx treated soil metabolite 3 reached a maximum of 3.4 % of applied radioactivity after 100 days and declined to 1.8 % after 225 days.

Four unidentified compounds were detected, individually accounting for at maximum 2 - 4 % of applied radioactivity.

III CONCLUSIONS

It was found that chemx degrades in Speyer 2.2 soil at a moderate rate. The principle metabolite was desmethyl chemx (29 % of applied radioactivity). Other significant metabolites formed by cleavage of the xxx bond of chemx to form xxxxxxx (metabolite 2) (9 % of applied radioactivity) and xxxx (metabolite 10) (5 % of applied radioactivity) where the chem2 labelled material was applied to soil and metabolite 3 (3.4 % of applied radioactivity) where the chem3 labelled material was applied to soil.

The DT_{50} and DT_{90} values for this soil were found to be 51 - 54 days and 170 - 181 days respectively. A proposed degradation pathway in this soil is outlined in Figure IIA 7.1.1.1-1.

Figure IIA 7.1.1-1 Proposed metabolic pathway for chemx in soil

Pathway omitted

(Grenfel RG 1996a)

IIA 7.1.2 **Anaerobic degradation**

Data is not submitted with respect to anaerobic degradation, since the recommended .....*(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)*

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IIA 7.1.3 **Soil photolysis**

Report: IIA 7.1.3/01 - Sullivan R 1995, Photodegradation of ^{14}C-chemx in/on soil by natural sunlight, Chemco Report XX-14021

Guideline

EPA FIFRA Guideline § 161-3 ≅ Procedures for assessing the environmental fate and behaviour of pesticides, SETAC-Europe, 1995, ISBN Number 90-5607-002-9. The study was initiated prior to issuance of the SETAC guideline. It follows the basic principles of that guideline, with the exception that natural sunlight was used rather than a xenon arc lamp as a sunlight simulator. No difference in the outcome of the study is expected to have occurred since xenon arc illumination has been determined to be equivalent to sunlight for the measurement of aqueous photolysis rates. (Yager JE and CD Yue 1988). An advantage of natural sunlight for the measurement of soil photolysis is that the day-night cycle that occurs under natural sunlight is much closer to actual environmental conditions than continuous illumination that is sometimes used in xenon arc lamp exposures. The normal day-night cycle permits a more realistic evaluation of other processes, such as hydrolysis or soil microbial breakdown to occur within the same time frame as photodegradation.

Testing Laboratory and dates

The study was conducted during the period July 1994 to December 1994 by the Chemco Research Laboratory, Richmond, California.

GLP: Fully GLP compliant [17].

Executive Summary

In a soil photolysis study, the phototransformation of chemx was studied on an Elder sandy loam soil following 30 days exposure at 25 °C to natural sunlight (Richmond California). Test and control soil samples were treated with radiolabelled chemx (chem2-^{14}C and chem3-^{14}C) at a rate equivalent to 40 g as/ha (twice the recommended rate of application). The degradation profile of chemx was similar for both light exposed and dark control, indicating that degradation was not directly the result of photolytic processes however the rate of degradation was faster in light exposed samples. The phototransformation decline times (half-lives) of chem2-^{14}C labelled chemx on soil were 46 / 51 days and of chem3-^{14}C chemx were 51 / 117 days for irradiated and the dark control samples, respectively.

The mass balance averaged 99.8 % for chem2 ring labelled and 103.3 % for chem3 ring labelled chemx in both irradiated and control samples. Total CO_2 and volatile compounds accounted for 2 % of the applied radioactivity. The major transformation product(s) detected were xxxxxxx (metabolite 2) (~ 23 % of applied radioactivity) and metabolite 3 (*circa* 25 % of applied radioactivity), both products of hydrolytic cleavage.

While soil photo-degradation may contribute to the dissipation of chemx it is not a significant route of degradation. The calculated DT_{50} value was *circa* 49 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - chem2 ring labelled: ^{14}C in the C-3 position; specific activity 60.85 µCi/mmol;
chem3 ring labelled: ^{14}C in the C-5 position; specific activity 65.44 µCi/mmol;

Description: White powder
Lot/Batch #: NPD-9307-5385-T
Purity: chem2 ring labelled: radiochemical purity ≥ 99.6 %
chem3 ring labelled: radiochemical purity ≥ 99.6 %
CAS #: 16335-17-2
Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Soil: An Elder sandy loam soil, collected (0-15 cm depth) at Santa Cruz County, California was used. On arrival in the test house it was sieved through a 2 mm sieve to remove debris and was maintained under refrigerated conditions in the dark in tightly closed plastic bags, until required. The site from which the soil was taken had not received a pesticide treatment in the previous 5 years.

Table IIA 7.1.3-1 Physical and chemical properties of the soil used in the soil photolysis study

Parameter	
Series	Elder
Source	Santa Cruz County, CA, USA
Texture Class (USDA classification)	Sandy loam
% Sand	66.0
% Silt	16.0
% Clay	18.0
pH	5.5 *
% Organic carbon	1.1
% Organic matter	2.5
Cation exchange capacity (meg/100g)	14.7
% Moisture at 0.33 bar (Field Capacity)	15.3
Bulk Density (g/cm^3)	1.1

* measured in a 1 : 2.5 soil : water suspension

B. STUDY DESIGN

1. Experimental conditions

Duplicate samples of sieved soil were made into an aqueous slurry, placed in petri dishes allowed to air dry, treated with the equivalent of 40 g as/ha (2 x recommended field rate). The test solution (x mg as/L) was prepared in deionsed water containing x % acetonitrile, and x mL was added to x g of soil in petri dishes. The organic solvent was allowed to dry and the soil was mixed to ensure homogeneity and adjusted to 75% field moisture capacity. The treated plates were placed in temperature control chambers covered with quartz plates surrounded by a water circulating jacket and exposed to natural sunlight over

a 30 day period at 25°C. A parallel set of soil plates was maintained under the same conditions but with glass plates covered with a black rubber sheet to provide a dark control. The exposure phase was carried out at latitude 37.40°N, longitude 122.28°W, (Richmond California) between July and August with sunlight intensity and cumulative energy (250-700 nm integration range) recorded at 10 or 20 minute intervals throughout the study.

2. Sampling

The microbial viability of the test soil was evaluated by means of plate counts prior to initiation. Duplicate samples were taken for analysis at time zero and on five subsequent points (days 6, 10, 14, 20 and 30) for analysis. Soil samples were stored at x °C for x days prior to analysis.

3. Description of analytical procedures

Volatile organic compounds were trapped by passing the air flow through a polyurethane foam plug and an ethylene glycol trap and $^{14}CO_2$ were trapped using two 10 % NaOH traps. Soil samples were extracted three times with acetonitrile : 0.175 M NaCl (65 : 35) using a vortex agitator for one minute, sonication for 10 minutes and shaking for twenty minutes after which the samples were centrifuged for 10 minutes and the supernatants were combined.

After extraction soil residues were subjected to combustion to determine levels of unextracted radiocarbon which were quantified using LSC. All extracts were subjected to HPLC using a reverse phase column combined with radioactive detection or fraction collection followed by LSC to determine the distribution of parent and photoproducts in the extracts, with selective confirmation by TLC. The mobile phase used for the HPLC analysis was X : Y (x % / y %). The limit of detection (LOD) for chemx and metabolite 1 were x and x µg as/g soil, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 were y and y µg as/g soil, respectively.

II. RESULTS AND DISCUSSION

A. DATA: Table IIA 7.1.3-2 Composition of Radioactivity recovered following natural sun photolysis of treated Elder soil (25°C)

Days after application	0	6	10*	14	20	30
(a) chem2-^{14}C labelled chemx						
light exposed						
chemx	100.5	84.0	81.2	76.4	71.3	61.7
xxxxxxx (metabolite 2)	0	4.0	9.1	13.1	18.2	22.6
unidentified compounds**	1.4	0.9	1.6	4.3	2.9	3.8
unextractable compounds	1.3	5.8	7.1	8.5	7.9	1.8
CO_2	0	0.3	0.5	0.9	1.5	10.2
Volatile compounds (organic)	0	0	0	0	0	0
Total	103.2	95	99.5	103.2	101.8	100.1

Table IIA 7.1.3-2 (Continued)

Days after application	0	6	10*	14	20	30
Dark control						
Chemx	100.5	83.1	ns	78.1	72.5	66.9
xxxxxxx (metabolite 2)	0	1.5	ns	13.0	15.7	24.4
unidentified compounds**	1.4	0	ns	1.8	3.1	0.8
unextractable compounds	1.3	10.7	ns	8.6	7.5	8.3
CO_2	0	0	ns	0	0	0
Volatile compounds (organic)	0	0	ns	0	0	0
Total	103.2	95.3	ns	101.5	98.8	100.4
(b) chem3-^{14}C labelled chemx						
light exposed						
chemx	99.6	83.8	84.0	76.5	72.4	64.4
metabolite 3	0	6.9	9.1	11.5	16.3	24.9
xxx (metabolite 7)	0	1.4	0.9	3.0	3.2	3.1
unidentified compounds**	1.8	1.0	1.7	3.5	2.3	1.2
unextractable compounds	1.5	6.1	5.7	7.7	8.7	9.7
CO_2	0	0.4	0.7	1.0	1.5	2.0
Volatile compounds (organic)	0	0	0	0	0	0
Total	102.9	99.6	102.1	103.2	104.4	105.3
Dark control						
Chemx	99.6	88.4	ns	82.0	84.2	81.5
Metabolite 3	0	5.9	ns	9.2	11.8	15.7
xxx (metabolite 7)	0	0	ns	1.2	1.5	0
unidentified compounds**	1.8	0	ns	3.1	1.3	0.3
unextractable compounds	1.5	5.5	ns	8.9	8.1	6.7
CO_2	0	0	ns	0	0	0.3
Volatile compounds (organic)	0	0	ns	0	0	0
Total	102.9	99.8	ns	104.4	106.9	104.5

* One light exposed sample and both dark control samples were not analysed due to extraction error

** Several small peaks on HPLC - none greater than 4 % of the applied radioactivity

B. MASS BALANCE

On the basis of the mass balance compiled, the recovery of applied radioactivity averaged 99.8 % and 103.3 % for the two chem2 ring labelled and the chem3 ring labelled materials respectively.

C. BOUND AND EXTRACTABLE RESIDUES

In the light exposed samples bound residues comprised 10 % of the applied radioactivity, *circa* 10 % of which was extracted following further caustic extraction.

D. VOLATILIZATION

The level of CO_2 evolved was low at *circa* 2 %. No other volatile degradation products were detected.

E. TRANSFORMATION OF PARENT COMPOUND

Over the exposure period the average daily light energy was 12.22 W/min/cm^2 and the cumulative light energy 378.84 W/min/cm^2. Most of the radioactivity was recovered in the soil extracts with parent compound representing 61.7 % and 64.4 % of the applied radioactivity for the chem2-^{14}C chemx and chem3-^{14}C chemx labels after 30 days exposure.

The soil extract product profiles were similar in both light exposed and dark control samples for both radiolabels. In the chem2-^{14}C labelled chemx samples, the major photodegradate was xxxxxxx (metabolite 2) representing ~ 23 % of the applied dose after 30 days exposure with some 3.8 % of the applied radioactivity present in the form of unidentified compounds. In the chem3-^{14}C labelled chemx samples metabolite 3 was the major photodegradate representing ~ 25 % of the applied dose, while xxx (metabolite 7) was a minor degradation product (~ 3 % of applied radioactivity) and some 1.2 % of the applied radioactivity was present in the form of unidentified compounds.

The degradation rate of chemx in this soil was calculated on the basis of the amount of ^{14}C chemx in soil extracts. The degradation rate constant and half-life were calculated assuming pseudo first order kinetics using the following equation:

$$\ln C = -Kt + \ln C_O$$

where K = rate constant
C = test substance concentration at t_O as a percentage of zero time radioactivity
C_O = test substance concentration at t_O as a percentage
t = time in days

Table IIA 7.1.3-3 Degradation rate constants for photolytic degradation in soil

	chem2-^{14}C labelled chemx		chem3-^{14}C labelled chemx	
Sample	DT_{50} (days)	R^2	DT_{50} (days)	R^2
Light exposed	45.7	0.966	50.6	0.952
Dark control	55.1	0.917	117.3	0.704

III CONCLUSIONS

The degradation of chemx in viable Elder sandy loam soil exposed to natural sunlight was examined over a 30-day period. The degradation profile of chemx was similar for both light exposed and dark control, indicating that degradation was not directly the result of photolytic processes however the rate of degradation was faster in light exposed samples. The major degradation product identified using chem2-^{14}C labelled chemx was xxxxxxx (metabolite 2) (~ 23 % of applied radioactivity) the hydrolysis product, while the major degradation product

identified using chem3-^{14}C labelled chemx was metabolite 3 (*circa* 25 % of applied radioactivity) formed following hydrolytic cleavage and subsequent decarboxylation. A minor degradation product detected using chem3-^{14}C labelled chemx samples was xxx (metabolite 7) (~ 3 % of applied radioactivity). Unidentified components (< 4 % of applied radioactivity) were detected using both chem2-^{14}C labelled chemx and chem3-^{14}C labelled chemx.

These results indicate that while soil photodegradation may contribute to the dissipation of chemx in the soil, it is not a significant route of dissipation.

Figure IIA 7.1.3-1 Proposed pathway for photodegradation of chemx in soil

Pathway omitted

(Sullivan R 1995a)

IIA 7.2 Rate of degradation in soil (laboratory studies)

IIA 7.2.1 Aerobic degradation of chemx in soil

Report (first of two)**:** IIA 7.2.1/01 Grenfel RG 1995, Degradation of ^{14}C-chemx in three soils, Chemco Report XX-14020

Guideline

BBA Guidelines for the official testing of plant protection products (Part IV, 4-1, Stage 1) ≅ Procedures for assessing the environmental fate and behaviour of pesticides, SETAC-Europe, 1995, ISBN Number 90-5607-002-9, but with the following deviations: the last sampling was at 100 days, as specified by the BBA guideline, as opposed to the 120 days specified in the SETAC-Europe guideline (issued after the study was completed); one soil (Wick) had been collected almost one year before the start of the study whereas the guideline suggests the use of fresh soil and only short term storage - the two other soils were collected about three months before initiation of the study.

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England, conducted the study during the period February to November 1994.

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In a soil degradation study, the rate of degradation of chemx (80 mg as/kg dry soil ≅ 80 g as/ha, or four times the recommended application rate) was assessed in three UK soil types [Evesham clay loam (pH 7.9), Malham silt loam (pH 6.7) and a Wick sandy loam (pH 5.3)] following up to 100 days incubation at 20 °C and 40 % of the maximum water holding capacity. In addition the Evesham was incubated following application of the same rate of chemx, at 10 °C and 40 % moisture and at 20 °C and 70 % of the maximum water holding capacity.

Mass balance for the study was not completed as CO_2 and other volatile compounds released were not recovered. Decline of chemx (DT_{90}) at 20 °C and 40 % of the maximum water holding capacity increased with increasing soil pH and varied from 306 days in Wick sandy loam soil to 750 days in Evesham clay loam. Half-life (DT_{50}) ranged from 92 days in Wick sandy loam soil to 226 days in Evesham clay loam. Following incubation in Evesham clay loam soil at 70 % of the maximum water holding capacity, the DT_{50} was reduced to 192 days, while incubation in the same soil at standard moisture content and lower temperature (10°C) resulted in a much slower degradation (DT_{50} > 365 days).

The major metabolites found in soil were xxxxxxxx (metabolite 2) and metabolite 3 formed by cleavage of the xxx bond with maximum concentrations determined at 12.8 % and 10.6 % of the applied radioactivity after 100 days incubation, respectively. Desmethyl chemx was also formed (maximum concentration of 5.2 % of the applied radioactivity after 100 days).

The study indicated that degradation rate may be pH dependant, with faster degradation at low pH values.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - 1 : 1 mixture of chem2 ring labelled and chem3 ring labelled

chem2 ring labelled: ^{14}C in the C-3 position; specific activity 28.7 mCi/mmol;

chem3 ring labelled: ^{14}C in the C-5 position; specific activity 34.2 mCi/mmol;

Description: White powder

Lot/Batch #: NPD-9307-5385-T

Purity: chem2 ring labelled: radiochemical purity > 99 %

chem3 ring labelled: radiochemical purity ≥ 98 %

CAS #: 16335-17-2

Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Soils: Three soils were used for the study, soils which were chosen to represent arable farming conditions in respect of soil texture and pH. The soils were taken from pesticide free sites. Two of the soils were collected within three months of test initiation while the third soil (Wick) was stored for almost one year prior to testing, a deviation from testing guidelines. The Wick soil was representative of a low pH, low organic matter content and low biological activity soil.

Table IIA 7.2.1-1 Physical, chemical and microbiological properties of the soils used

	Soil Series Name		
Soil Property	**Evesham**	**Malham**	**Wick**
Particle size distribution (%) [a]			
Sand	35 (37)	8 (14)	67 (69)
Silt	34 (32)	73 (69)	21 (21)
Clay	31 (31)	18 (17)	12 (11)
Organic carbon (%)	1.2	5.1	0.8
Organic matter (%) [b]	2.1	8.8	1.4
Cation exchange capacity (meq/100g)	18.9	31.0	14.2
Classification	Clay loam	Silt/silty clay loam	Sandy loam
pH [c]	7.9	6.7	5.3
Microbial biomass (µg C/g soil) [d]	263 (431) [e]	892 (754)	63 (54)
Field capacity (1/3 bar %)	25.7	33.3	10.7
Maximum water holding capacity (%)	38.3	79.2	34.2

[a] Soil survey of England and Wales classification. USDA determination in parentheses.
[b] A factor of 1.72 was used in this study to convert % organic carbon to % organic matter.
[c] Measured in a 1 : 2.5 soil : water suspension
[d] Measurements are day 0 and day 100 (in parentheses).
[e] These biomass values are for flasks incubated at 20 °C and 40 % maximum water holding capacity (MWHC). Separate flasks were prepared for biomass analysis for Evesham soil incubated at 10 °C and 40 % MWHC and gave values of 301 and 364 µg C/g soil at Days 0 and 100 respectively. Further flasks were prepared for biomass analysis for Evesham soil incubated at 20 °C and 70% MWHC and these gave values of 322 and 280 µg C/g soil at Days 0 and 100, respectively.

B. STUDY DESIGN

1. Experimental conditions

Portions of sieved soil (100 g dry weight) were treated with test substance dissolved in acetonitrile to achieve a soil concentration of 0.08 µg/g equivalent to 80 g as/ha (4 fold recommended use rate). The organic solvent was allowed to evaporate after which the soil was mixed to ensure homogeneity. All soil samples were adjusted to 40 % maximum water holding capacity (MWHC) and were incubated in darkness at 20°C up to 100 days (this is below the maximum test period of 120 days specified in SETAC 1995). In addition further samples of the Evesham soil were incubated at 10°C (40 % MWHC) and at high soil moisture 70 % MWHC at 20°C.

Since the amount of volatile compounds and CO_2 collected in the soil metabolism study was low (< 10 % after 100 days of incubation - *cf* point IIA 7.1.1), volatile compounds and CO_2 were not collected in this study. Soil biomass levels were determined on all soils at day zero and day 100 using the methods described by Vance *et al* 1987, and Wu *et al* 1990.

Soil flasks were weighed at 1 to 2 week intervals. Water was added as necessary to maintain moisture levels.

2. Sampling

Soil samples were taken at time intervals 0, 1, 3, 7, 14, 30, 60 and 100 days for subsequent analysis and were stored at ≤ -15°C.

3. Description of analytical procedures

Soil extractions were carried out using the following solvent sequence:

a) 100 ml acetonitrile (two extractions)

b) 100 ml acetonitrile : water, 70 : 30, v : v (one extraction)

c) 100 ml acetonitrile : 2 M NaOH, 50 : 50, v : v (one extraction followed by separation of the organic layer, which was then pooled with the extracts from steps a and b)

After addition of the solvent, the samples were shaken using a sonic agitator for 10 minutes, and then shaken on an orbital shaker for 20 minutes at > 100 revolutions per minute. The soil was separated from the supernatant by centrifugation. That process was repeated following each extraction. Prior to analysis, the extracts were pooled (using only the organic layer from c), and their total volumes and radioactivity measured. The radioactive content of the NaOH extract was measured after neutralisation with HCL. The radioactivity levels in extracts and residue were measured using LSC. The soil extracts were analysed using reverse phase HPLC (Spherisorb S5 ODS1, 25 cm x 10 mm id) eluted with a gradient of acetonitrile and water containing 1 % formic acid and 1.35 % triethylamine. The effluent was passed through an UV detector (254 nm) to detect reference standards and a radioactivity detector to determine the quantities of radiolabelled degradation products present. Chromatography using normal phase TLC and GC-MS were used to confirm the identity of the degradation products detected.

II. RESULTS AND DISCUSSION

A. ANOMALIES ARISING FROM THE STUDY DESIGN

The soil extraction procedures used in the study included a harsh extraction solvent (2 N NaOH) which is considered likely to have broken down soil humus and have released humic and fulvic acids thereby releasing chemx normally adsorbed by organic matter and not extractable using normal solvent extraction methods. As a consequence the study results represent an extreme worst case, providing an exaggerated estimate of the bioavailability of chemx in soil. The harsh extraction procedure was conducted in order to characterise the nature of the chemx residues (approximately 30 - 50 % of the applied radioactivity) which were not extracted using normal extraction procedures.

B. MASS BALANCE

As volatile components were not recovered, a mass balance was not completed for the various incubation periods used in this study.

C. VOLATILIZATION

Volatile components were not recovered in this study.

D. TRANSFORMATION OF PARENT COMPOUND

Three main degradation products were formed, desmethyl chemx, formed by O-demethylation of chemx that accounted at maximum for 5.2 % of the applied radioactivity in Malham soil, xxxxxxxx (metabolite 2) that accounted for 12.8 % of the applied radioactivity in Wick soil and metabolite 3 that accounted for 10.6 % of

the applied radioactivity in Wick soil, both formed by hydrolysis of the xxx moiety following 100 days incubation. In all soils the content of chemx decreased steadily over the incubation period under normal incubation conditions and represented *circa* 66 % of the applied radioactivity after 100 days, except in the case of the Wick soil where it declined to *circa* 47 % of the applied radioactivity. The more rapid degradation observed in the Wick soil is surprising in view of its low microbial biomass (due to long-term storage) and is most likely the result of chemical hydrolysis at this low pH (5.3). Conversely the higher pH of the other soils particularly the Evesham soil may have resulted in slower hydrolysis and less breakdown.

Table IIA 7.2.1-2 Composition of radioactivity recovered following application of chemx to 3 soils under varying incubation conditions

	% Applied Radioactivity							
Days after application	0	1	3	7	14	30	60	100
Soil Malham 40 % MWHC / 20 °C								
Chemx	95.7	90.9	93.8	91.0	88.0	81.2	73.4	66.4
Desmethyl chemx (metabolite 1)	< 0.6	0.4	< 0.6	< 0.5	0.9	1.7	4.7	5.2
xxxxxxxx (metabolite 2)	0.7	1.1	0.6	< 0.5	0.8	1.7	3.1	3.7
xxxx (metabolite 10)	< 0.6	0.4	< 0.6	< 0.5	< 0.5	< 0.3	< 0.5	< 0.5
metabolite 3	< 0.6	0.7	< 0.6	< 0.5	< 0.5	0.8	1.2	1.3
unidentified compounds	< 2.4	2.6	< 2.4	< 2.0	< 2.0	< 1.2	< 2.0	< 3.9
microbial biomass (µg c/g soil)	892							754
Soil Wick 40 % MWHC / 20 °C								
Chemx	99.4	95.7	93.0	90.2	91.4	76.6	59.8	46.5
Desmethyl chemx (metabolite 1)	< 0.5	< 0.6	0.4	1.1	< 0.5	3.3	3.4	2.6
xxxxxxxx (metabolite 2)	1.0	1.5	2.3	2.2	2.7	4.6	9.8	12.8
xxxx (metabolite 10)	< 0.5	< 0.6	< 0.4	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
metabolite 3	< 0.5	1.0	2.2	2.0	1.9	4.2	8.5	10.6
unidentified compounds	< 2.0	< 2.4	< 1.6	< 2.0	< 2.0	< 2.0	< 2.2	< 5.6
microbial biomass (µg c/g soil)	63							54
Soil Evesham 40 % MWHC / 20 °C								
Chemx	95.8	95.2	92.7	93.9	90.6	85.8	79.1	70.0
Desmethyl chemx (metabolite 1)	< 0.5	< 0.5	< 0.5	< 0.6	< 0.6	0.6	1.9	1.9
xxxxxxxx (metabolite 2)	1.0	< 0.5	0.8	< 0.6	0.6	1.2	1.5	4.4
xxxx (metabolite 10)	< 0.5	< 0.5	< 0.5	< 0.6	< 0.6	< 0.6	< 0.6	< 0.5
metabolite 3	0.7	< 0.5	< 0.5	< 0.6	0.6	0.6	1.1	3.4
unidentified compounds	< 2.0	< 2.2	< 2.1	< 2.4	< 2.4	< 4.6	< 5.6	< 6.2
microbial biomass (µg c/g soil)	263							431
Soil Evesham 70 % MWHC / 20 °C								
Chemx	98.2	96.4	96.4	92.8	91.9	85.2	78.0	67.6
desmethyl chemx (metabolite 1)	< 0.5	< 0.4	< 0.5	< 0.5	< 0.5	1.1	2.7	2.2
xxxxxxxx (metabolite 2)	< 0.5	0.5	< 0.5	0.7	0.6	1.9	3.6	6.6
xxxx (metabolite 10)	< 0.5	< 0.4	< 0.5	< 0.5	< 0.5	< 0.4	< 0.4	< 0.4
metabolite 3	< 0.5	< 0.4	< 0.5	< 0.5	0.5	1.6	3.1	7.0
unidentified compounds	< 2.0	< 2.2	< 2.0	< 2.0	< 2.0	< 2.8	< 1.6	< 1.6
microbial biomass (µg c/g soil)	322							280

Table IIA 7.2.1-2 (Continued)

Days after application	% Applied Radioactivity							
	0	1	3	7	14	30	60	100
Soil Evesham 40 % MWHC / 10 °C								
Chemx	94.8	97.9	94.6	95.5	90.1	91.3	90.6	89.9
desmethyl chemx (metabolite 1)	< 0.6	< 0.9	< 0.7	< 0.7	< 0.7	< 0.9	< 0.7	0.8
xxxxxxxx (metabolite 2)	1.0	0.9	< 0.7	< 0.7	< 0.7	< 0.9	< 0.7	0.9
xxxx (metabolite 10)	< 0.6	< 0.9	< 0.7	< 0.7	< 0.7	< 0.9	< 0.7	< 0.8
metabolite 3	< 0.6	< 0.9	< 0.7	< 0.7	< 0.7	< 0.9	< 0.7	< 0.8
unidentified compounds	< 2.4	< 3.6	< 2.8	< 2.8	< 2.8	< 3.6	< 2.8	< 3.2
microbial biomass (µg c/g soil)	301							364

Incubation of the Evesham soil at an increased soil moisture content (70 % MWHC) resulted in an increased rate of degradation over the period, while incubation at a lower temperature 10°C substantially reduced the degradation rate (*circa* 90 % of the applied radioactivity present in the form of chemx after 100 days).

The degradation parameters DT_{50} and DT_{90} were calculated assuming pseudo first order reaction kinetics with best fit calculated by linear regression analysis. The degradation rate constant and half-life were calculated using the following equation:

$$InC = -Kt + InC_O$$

where K = rate constant
C = test substance concentration at t_O as a percentage of zero time radioactivity
C_O = test substance concentration at t_O as a percentage
t = time in days

Table IIA 7.2.1-3 DT_{50} and DT_{90} values for chemx in 3 UK Soils

Soil	Incubation Conditions	pH	DT_{50}	DT_{90}	r^2
			Days		
Malham	40 % / 20°C	6.7	194	643	0.972
Wick	40 % / 20°C	5.3	92	306	0.990
Evesham	40 % / 20°C	7.9	226	750	0.994
Evesham	70 % / 20°C	7.9	192	637	0.992
Evesham	40 % / 10°C	7.9	> 365*	*	*

* due to slow rate of degradation at 10°C over 100 days incubation, a DT_{50} > 365 days is quoted as 90 % chemx still present.

The rate of degradation of chemx in these three soils appeared to correlate with soil pH values with DT_{50} ranging from 92 to 194 and 226 days with increasing soil pH of from 5.3 to 6.7 and 7.9. These findings indicate that degradation in these soils is essentially a chemical rather than biological process. This study is indicative of relatively high DT_{50} values and correspondingly high DT_{90} values for chemx in the three soils studied.

III. CONCLUSIONS

The rate of degradation of chemx was studied in three soils under standard and modified incubation conditions. The half-life (DT_{50}) of chemx under the standard conditions varied from 92 days in Wick sandy loam soil to 226 days in Evesham clay loam soil. On incubation in Evesham clay loam soil at 70 % MWHC the DT_{50} was reduced to 192 days, while incubation in the same soil at standard moisture content and lower temperature (10°C) resulted in a much slower degradation (DT_{50} > 365 days).

The major metabolites found in soil were xxxxxxxx (metabolite 2) and metabolite 3 formed by cleavage of the xxx bond with maximum concentrations determined at 12.8 % and 10.6 % of the applied radioactivity after 100 days incubation, respectively. Desmethyl chemx was also formed (maximum concentration of 5.2 % of the applied radioactivity after 100 days).

This study indicated that degradation rate may be pH dependant, with faster degradation at low pH values.

(Grenfel RG 1995a)

Report (second of two)**:** IIA 7.2.1/02 - Sullivan R 1995, The aerobic soil metabolism of chemx, Chemco Report XX-13750

Guideline: EPA FIFRA Guideline § 162-1

Testing Laboratory and dates

The study was conducted during the period March 1993 to April 1994 by the Chemco Research Laboratory, Richmond, California.

GLP: Fully GLP compliant [17].

Executive Summary

In a soil degradation study, rate of aerobic degradation of chemx was assessed in two US soils [Elder sandy loam (pH 6.8) and Dupo silt loam (pH 7.6)] following up to 12 months soil incubation at 25 °C. The test substance was applied at a rate of 0.061 mg/kg for the Elder soil and 0.066 mg/kg for the Dupo soil, considered to be equivalent to 70g as/ha (3.5 fold recommended field use rate). Soil moisture was maintained between 65 and 85 % of maximum water holding capacity for the duration of the study.

CO_2 and other volatile degradation products were produced in extremely low quantities in both soils. Mineralization and volatilization clearly are not major routes of degradation for chemx in soil. Mass balance for the study ranged from 99.5 to 100.5 % for chem2 ^{14}C-labelled chemx and was *circa* 97.2 % for chem3 ^{14}C-labelled chemx. Chemx was found to be moderately persistent in both US soil types with DT_{50} values ranging 31 - 37 days and DT_{90} values ranging 206 - 262 days, using normal solvent extraction procedures.

Two major transformation products, xxxxxxx (metabolite 2) and metabolite 3 and two minor transformation products, desmethyl chemx and metabolite 6, were identified. The major route of degradation was through cleavage of the xxx bond leading to the formation of metabolite 3 and xxxxxxx (metabolite 2), with a minor route involving the oxidative demethylation of chemx to form desmethyl chemx which degraded further to metabolite 6.

At the end of the 365 d incubation period, 97 / 45% (chem2-^{14}C / chem3-^{14}C) of applied ^{14}C was present in the Elder soil as extractable residues, and 102 / 35% (chem2-^{14}C / chem3-^{14}C) of applied ^{14}C was present in the Dupo soil as extractable residues, while 0.1 / 55% (chem2-^{14}C / chem3-^{14}C) of applied ^{14}C was present in the Elder soil as bound residues, and 0.1/ 60 % (chem2-^{14}C / chem3-^{14}C) of applied ^{14}C was present in the Dupo soil as bound residues. These results are indicative of soil binding being a significant mechanism for dissipation of chemx in soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - chem2 ring labelled: ^{14}C in the C-3 position; specific activity 28.63 mCi/mmol;
chem3 ring labelled: ^{14}C in the C-5 position; specific activity 29.07 mCi/mmol;

Description: White powder
Lot/Batch #: NPD-9307-5385-T
Purity: chem2 ring labelled: radiochemical purity ≥ 99.1 %
chem3 ring labelled: radiochemical purity ≥ 99.1 %
CAS #: 16335-17-2
Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Soils: An Elder sandy loam soil and Dupo silt loam soil were used for the study. The two soils used from Monmouth Illinois and Litchfield California were freshly collected prior to use, the Elder sandy loam was freshly collected from the field and stored under refrigeration for less than 30 days prior to use, while the Dupo silt loam was obtained from the company outdoor bins immediately prior to use. The sites from which the soils were taken had not received a pesticide treatment in the previous 5 years. Both soils were equilibrated in metabolism flasks at 25°C for two weeks prior to use and their microbial viability was checked by means of plate counts.

Table IIA 7.2.1-4 Soil physiochemical properties

USDA Soil Series Name	Dupo	Elder
Source	St. Charles, Illinois	Litchfield, California
Textural Classification (USDA)	Silt loam	sandy loam
% Sand	29	72
% Silt	62	10
% Clay	9	18
pH (1:1 Soil:H_2O)	7.6	6.8
Cation Exchange Capacity (meq/100g)	9.6	13.5
% Moisture at 1/3 bar (Field Capacity)	29	15.56
Bulk Density (g/cm^3)	1.17	1.5
% Organic Matter	0.8	1.6

B. STUDY DESIGN

1. Experimental conditions

The degradation of chem2 ring labelled and chem3 ring labelled chemx was assessed under aerobic conditions in two US soils during a 12 month incubation periods at 25°C.

Samples of sieved soil (50g dry weight) were placed in 150 ml centrifuge tubes and adjusted to 85 % of water holding capacity at 0.33 bar. The tubes were placed in incubation chambers to which traps for the collection of volatile compounds and CO_2 were connected.

The test substance was applied at a rate of 0.061 mg/kg for the Elder soil and 0.066 mg/kg for the Dupo soil, considered to be equivalent to 70g as/ha (3.5 fold recommended field use rate). The test substance was dissolved in acetonitrile. The organic solvent was allowed to evaporate after which the soil was mixed to ensure homogeneity. For the purposes of the identification of metabolites, similar samples were treated at the exaggerated rate of 2.0 mg/kg (30 fold normal rate). Control and treatment incubation tubes were replicated six times. The treated soils were incubated for one year in darkness at 25°C and 80 % relative humidity (RH). Moisture content was maintained between 65 and 85 % of MWHC for the duration of the incubation period. Trapping solutions of ethylene glycol and ethanolamine were used to collect ^{14}C volatile compounds and $^{14}CO_2$.

2. Sampling

Duplicate samples were removed for analysis at 0, 1, 3, 7, 14, 30, 59, 90, 120, 181, 272/3 and 360/4 days after treatment.

3. Description of analytical procedures

Soils were extracted by adding 80 ml of solvent (acetonitrile: 0.175 % aqueous NaCl, 65 : 35) directly to the soil bottles. The soil / solvent mixture was shaken on a platform shaker for approximately one hour, and then centrifuged at 2600 rpm for 20 minutes. This procedure was repeated two or three times, depending upon the extraction recoveries from the previous extraction. Total extractability was determined by summing the percent of applied radioactivity present in the extracts from that sample, as determined by LSC of aliquots.

In samples taken 7 to 14 days after treatment, residues that were unextractable amounted to 15 to 20 % of the applied radioactivity. In order to release the unextractable residues, one or two additional extractions were conducted in the manner described above using acetonitrile : 2 N NaOH as the solvent. The acetonitrile layer was removed, the aqueous layer was extracted with additional acetonitrile, and the organic layers were combined. Separate HPLC analyses of the metabolite extracts and the residues released from the unextractable fraction were conducted using reverse phase chromatography (Spherisorb S5 ODS1, 25 cm x 10 mm id) eluted with a gradient of acetonitrile and water containing 1 % formic acid and 1.35 % triethylamine, with detection of the radioactive components by means of a flow-through radioactivity detector.

II RESULTS AND DISCUSSION

A. DATA: Table IIA 7.2.1-5 Average recovery of radioactivity and metabolite distribution (expressed as percent of applied radioactivity) following application of chemx to Elder and Dupo soils and aerobic incubation for up to 364 days at 25°C

	% Applied Radioactivity											
Days after application	0	1	3	7	14	30	59	90	118	181	272/ 273	360/ 364
Elder soil [chem2 ^{14}C-labelled chemx]												
Chemx	96.9	90.0	81.4	73.1	69.9	51.3	31.3	21.8	17.3	10.5	6.5	5.0
xxxxxxx (metabolite 2)	1.4	2.7	2.6	2.7	3.9	14.9	30.6	38.1	44.7	47.3	51.8	53.1
desmethyl chemx	0.0	0.0	0.0	0.0	0.0	2.2	2.5	2.3	2.4	2.1	1.4	1.0
metabolite 6	0.0	0.0	0.0	0.0	0.0	0.4	0.5	0.4	1.1	1.1	0.7	1.0
unextractable cpds*	1.8	5.3	15.5	21.05	24.8	30.4	45.6	36.3	42.8	35.3	38.02	35.5
volatiles (organic)	0.1	0.03	0.02	0.02	1.2	0.03	0.03	0.07	0.07	0.07	0.09	0.1
CO_2	0.0	0.0	0.01	0.01	0.01	0.01	0.03	0.04	0.1	0.3	0.7	1.03
Total	99.3	98.07	99.5	96.8	99.8	99.4	110.5	99.0	107.9	96.6	99.2	96.6
Dupo soil [chem2 ^{14}C-labelled chemx]												
Chemx	105.1	93.7	91.7	81.0	72.5	43.5	32.9	30.7	20.0	13.8	8.6	7.1
xxxxxxx (metabolite 2)	0.0	0.0	2.2	3.8	4.5	14.0	27.8	35.9	26.9	42.5	45.1	45.7
desmethyl chemx	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.7	1.5	1.3	1.4
metabolite 6	0.0	0.0	0.0	0.0	0.0	0.5	0.9	1.3	1.3	1.7	1.6	1.7
unextractable cpds*	1.40	4.5	6.4	13.4	23.9	39.8	30.74	33.7	34.3	36.1	37.9	44.5
volatile compounds (organic)	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.01	0.0	0.02	0.02	0.01
CO_2	0.0	0.0	0.01	0.01	0.06	0.2	0.4	0.7	0.8	1.07	1.3	1.6
Total **	106.5	99.4	100.4	98.2	101.3	98.4	93.1	104.2	95.9	97.2	96.4	102.8
Elder soil [chem3 ^{14}C-labelled chemx]												
Chemx	97.1	93.4	76.9	72.3	70.9	51.9	32.5	22.2	19.2	12.1	8.3	5.5
metabolite 3	0.9	1.8	1.1	1.7	2.0	9.6	22.9	28.9	36.8	35.2	39.4	34.1
desmethyl chemx	0.0	0.0	0.0	0.0	0.0	2.5	2.4	1.9	2.2	1.8	0.5	0.6
unextractable cpds*	2.1	5.0	13.9	23.03	23.8	34.9	45.5	39.1	35.5	46.4	46.0	55.4
volatile compounds (organic)	0.2	0.5	0.2	0.21	0.01	0.5	0.4	0.7	0.4	0.5	0.5	0.4
CO_2	0.0	0.01	0.01	0.01	0.1	0.1	0.3	0.4	0.5	0.7	1.3	1.6
Total **	100.3	100.74	91.7	97.8	98.06	99.6	104.0	88.2	94.6	96.7	96.1	99.9
Dupo soil [chem3 ^{14}C-labelled chemx]												
Chemx	92.1	94.9	89.7	81.2	72.8	45.8	32.0	25.6	21.0	13.0	8.0	6.9
metabolite 3	0.0	0.0	0.8	1.3	1.6	11.0	18.1	24.8	26.4	29.5	27.9	29.3
desmethyl chemx	0.0	0.0	1.0	1.3	1.2	1.0	1.1	1.3	1.0	1.1	0.4	0.0
unextractable cpds*	1.4	5.3	6.6	13.0	19.8	39.2	41.1	44.2	49.5	47.8	52.07	60.2
volatile compounds (organic)	0.0	0.01	0.04	0.0	0.0	0.01	0.6	0.03	0.04	0.2	0.05	0.04
CO_2	0.0	0.0	0.04	0.05	0.5	1.02	1.6	2.02	2.3	2.8	3.6	4.2
Total	93.5	100.2	98.1	97.6	95.9	98.5	94.8	98.3	100.7	94.7	92.5	100.7

* Unextractable - Amount remaining after normal extraction procedures

** The total presented is the sum of the amount readily extracted, volatiles, CO_2 and unextractable residues. This total does not necessarily correspond to the sum of the metabolites, unextracted residues and volatiles as presented in this table

Chromatography techniques involving use of normal phase TLC and GC-MS were used to confirm the identity of the degradation products detected.

B. MASS BALANCE

Overall recovery of applied radioactivity was good - average recovery ranged from 99.5 to 100.5 % for chem2 ^{14}C-labelled chemx and was *circa* 97.2 % for chem3 ^{14}C-labelled chemx.

C. BOUND AND EXTRACTABLE RESIDUES

Chemx was dissipated rapidly and steadily over the period of incubation with the formation of significant soil bound residues. At study end these unextractable residues comprised 35 / 45 % of the applied radioactivity for chem2 ^{14}C-labelled chemx and 55 / 60 % of the applied radioactivity for chem3 ^{14}C-labelled chemx. These results indicate that soil binding is a significant mechanism for dissipation of chemx in soils.

Given the extent of soil bound residues recovered 55.4 - 60.2 % of the applied radioactivity with chem3 ^{14}C-labelled chemx, and 35.5 - 44.5 % of the applied radioactivity with chem2 ^{14}C-labelled chemx following aerobic incubation, further extractions using acetonitrile : 2 N NaOH (50 : 50) were performed on certain of the sampling dates in order to facilitate the further characterization of the bound residue.

These extraction conditions, which are more harsh than the conditions used for fractionation of soil organic matter into humic and fulvic acids, and humin (Kaufman *et al* 1976), destroy the humus layer by dissolving the humic and fulvic acids, and have the potential to alter the fine structure of the soil particles, thereby releasing residues which are bound to the soil organic matter, or which may be occluded or entrapped in the soil micropores. Examination of the distribution of components in this study reveals that chemx starts to move into this second "compartment" in the soil as early as three days after application. Initially, the main or sole component in the unextractable residues is parent chemx, but with time, the amount of chemx peaks, and an accumulation of metabolites occurs, indicating continued degradation with time.

Table IIA 7.2.1-6 Bound residues - distribution of metabolites in the acetonitrile : 2N NaOH extracts, as a percentage of applied radioactivity

Days after application	Unextractable*	Released following extraction	Chemx	Xxxxxxx (metabolite 2)	desmethyl chemx	metabolite 6
Elder soil [chem2 ^{14}C-labelled chemx]						
3	15.52	15.0	14.6	0.0	0.0	0.0
7	21.05	22.2	21.7	0.8	0.0	0.0
14	24.84	24.1	22.8	0.4	0.0	0.0
30	30.37	23.3	21.8	0.9	0.9	0.0
59	45.55	26.3	22.8	2.7	0.6	0.0
90	36.30	30.9	23.6	6.3	0.7	0.0
118	42.76	27.9	20.7	6.6	0.5	0.0
181	35.27	25.9	13.7	10.2	1.0	0.4
273	38.02	27.0	12.6	13.1	0.9	0.4
364	35.45	27.6	15.7	12.6	0.0	0.0

Table IIA 7.2.1-6 (Continued)

Days after application	Unextractable*	Released following extraction	Chemx	Xxxxxxx (metabolite 2)	desmethyl chemx	metabolite 6
Dupo soil [chem2 ^{14}C-labelled chemx]						
30	39.80	32.9	30.2	1.9	0.0	0.2
59	30.74	27.1	22.7	3.4	0.3	0.4
91	33.69	21.6	17.8	3.1	0.2	0.2
118	34.25	28.8	17.6	7.9	0.3	0.4
181	36.09	27.0	9.6	8.5	0.5	0.2
272	37.91	29.5	12.9	14.3	1.0	0.8
360	44.47	24.6	12.3	11.1	0.4	0.7
Elder soil [chem3 ^{14}C-labelled chemx]						
3	13.87	13.3	12.3	0.2	0.0	
7	23.03	21.5	20.9	0.0	0.0	
14	23.78	20.5	19.5	0.5	0.0	
30	34.91	27.4	22.7	3.3	0.3	
59	45.45	35.5	25.7	9.0	0.5	
90	39.13	35.4	21.2	13.5	0.4	
118	35.45	34.2	13.7	12.8	0.3	
181	46.40	33.1	13.1	22.4	0.0	
273	46.00	33.6	8.3	27.1	0.2	
364	55.36	34.1	8.6	25.3	0.0	
Dupo soil [chem3 ^{14}C-labelled chemx]						
30	39.23	32.4	29.1	1.1	0.2	
59	41.10	34.8	22.7	13.3	0.4	
91	44.19	33.3	17.8	15.3	0.0	
118	49.48	41.2	17.9	20.7	0.4	
181	49.84	43.5	16.7	25.9	0.3	
272	52.07	46.5	13.3	32.1	0.0	
360	60.2	44.6	11.4	32.7	0.3	

* percent of the applied radioactivity in the unextractable fraction which was released

In the unextractable residue extracts, metabolite 3 (chem3 ^{14}C-labelled chemx) constituted a larger proportion of the radioactivity (ratio 3 : 1 metabolite 3 : chemx) at day 364 than xxxxxxx (metabolite 2) (ratio 1 : 1) (chem3 ^{14}C-labelled chemx) at final sampling. This finding suggests that metabolite 3 is formed in soil more readily and moves to this second compartment where it tends to bind more strongly than other metabolites.

If account is taken of the residues in the "unextractable" residue fraction, the DT_{50} of chemx is approximately 75 days in Elder soil and 81 days in Dupo soil. This is approximately twice the level recorded when residues extracted by normal solvent procedures were used and confirms the extent to which soil binding is a significant mode of dissipation of chemx. The harsh extraction conditions required for release of these soil bound residues are not likely to occur under field conditions; consequently those bound residues are not readily bioavailable in soil.

The extent of the final soil residue burden of chemx based on the combined extraction procedures was calculated to be at maximum 21% of the applied amount.

Table IIA 7.2.1-7 Total residues of chemx in soil after 360 days aerobic incubation (25°C)

Label	Soil Type	chemx	
		mg/kg Soil*	% of Applied
chem2 ^{14}C-labelled chemx	Elder sandy loam	0.013	21.3
chem2 ^{14}C-labelled chemx	Dupo silt loam	0.013	19.7
chem3 ^{14}C-labelled chemx	Elder sandy loam	0.008	13.1
chem3 ^{14}C-labelled chemx	Dupo silt loam	0.012	18.2

D. VOLATILIZATION

Volatile degradation products were produced in extremely low quantities in both soils. Mineralization and volatilization clearly are not major routes of degradation for chemx in soil.

E. TRANSFORMATION OF PARENT COMPOUND

Chemx soil residues (parent compound) declined continuously over the test period and represented *circa* 50 % of applied radioactivity after 30 days incubation, *circa* 25 % of after 90 days incubation and only *circa* 6 % after 360 days.

The major soil metabolites found to be present were xxxxxxx (metabolite 2), 53 / 46 % of the applied radioactivity for chem2 ^{14}C-labelled chemx and metabolite 3, 34 / 29 % of the applied radioactivity for chem3 ^{14}C-labelled chemx following 364 days incubation, both of which were formed by cleavage of the xxx bond.

Minor metabolites found to be present were desmethyl chemx at < 3 % of the applied radioactivity (both labels) and metabolite 6 at < 2 % of the applied radioactivity (chem2 ^{14}C-labelled chemx), resulting from oxidative demethylation of chemx. Mineralization occurred to only a limited extent with a maximum value of 4 % of the applied radioactivity in the Dupo soil (chem3 ^{14}C-labelled chemx) and < 2 % of the applied radioactivity in the other incubations.

DT_{50} and DT_{90} values were calculated using a non-linear first order kinetic model, which is a two compartment model comprising initial linear first order kinetics followed by non linear degradation (Gustafson and Holden 1990) which provided a better fit to the data than did traditional least-squares linear regression analysis. The model used is based on the assumption of spatial variability in the dissipation process whereby the rate of dissipation is not constant over the degradation process.

On the basis of normal methods of extraction, soil residues of chemx were shown to dissipate rapidly in both soils with DT_{50} values of 31 - 37 days and DT_{90} values of 206 - 262 days.

Table IIA 7.2.1-8 DT_{50} and DT_{90} values for chemx in 2 US soils

Soil	Label Position	DT_{50}	DT_{90}	r^2
		Days		
Elder	Chem2 ^{14}C	32	206	Not stated
	Chem3 ^{14}C	37	232	Not stated
Dupo	Chem2 ^{14}C	31	262	Not stated
	Chem3 ^{14}C	32	254	Not stated

III. CONCLUSIONS

The rate of aerobic degradation of chemx was studied in two US soil types over a period of 364 days. Chemx was found to be moderately persistent in both soil types with DT_{50} values ranging 31 - 37 days and DT_{90} values ranging 206 - 262 days, using normal solvent extraction procedures.

Two major transformation products, xxxxxxx (metabolite 2) and metabolite 3 and two minor transformation products, desmethyl chemx metabolite 6, were identified. Neither mineralization to CO_2 nor volatilization was significant dissipation mechanisms in soil. The major route of degradation was through cleavage of the xxx bond leading to the formation of metabolite 3 and xxxxxxx (metabolite 2), with a minor route involving the oxidative demethylation of chemx to form desmethyl chemx which degraded further to metabolite 6.

Total soil residues of chemx were shown to decline over the incubation period of 364 days to a maximum level of 21 % of the applied amount for the two US soils concerned. The proposed degradation pathway in soil is as follows -

Figure IIA 7.2.1-1 Proposed degradation pathway of chemx in soil under aerobic conditions

Pathway omitted

The results of the separate analyses of readily extractable residues and unextractable residues carried out, show that inclusion of the unextractable residues in the DT_{50} calculation results in values which are more than twice those calculated when only the residues which were extracted by normal procedures are used. Because soil binding is a mode of dissipation for chemx, as it is for many other plant protection products, calculation in this way discounts the effect of soil binding and overestimates the amount of chemx which will be present at any time, leading to the calculation of a DT_{50} value which is more than twice DT_{50} which reflects the true rate of dissipation of chemx. The overestimation is exacerbated by the static conditions which occur in laboratory studies, conditions which are not as conducive to degradation as are those which occur in the field. It is generally recognised that since there are many mechanisms that contribute to dissipation in the environment, the results of a field study would provide much more realistic evidence as to the true rate of dissipation of chemx.

(Sullivan R 1995b)

IIA 7.2.2 **Aerobic degradation of the active substance at 10° C**

A study is not considered necessary since the degradation products .....*(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)*

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IIA 7.2.3 **Aerobic degradation of chemx metabolites**

In the aerobic degradation studies submitted (point IIA 7.2.1) three metabolites were found to occur at levels accounting > 10% of applied chemx, namely xxxxxxx (metabolite 2), metabolite 3 and desmethyl chemx. In order to assess the possible significance of these metabolites in soil, an estimate of maximum soil concentration expected based on the recommended application rate and the maximum levels of recovery for those metabolites in the aerobic degradation studies was prepared.

Table IIA 7.2.3-1 Maximum expected residue levels of metabolites in field soil

Metabolite	Maximum Detected % of chemx applied		*Maximum Concentration expected in field soil (mg/kg)	
Location	UK	US	UK	US
xxxxxxx (metabolite 2)	15	53	0.0010	0.0036
metabolite 3	11	39	0.0007	0.0026
desmethyl chemx	29	3	0.0019	0.0002

* Based on maximum label rate of 20 g as/ha, even distribution in 0 - 20 cm soil horizon and soil density of 1.5 g cm^3.

To permit assessment of the rate of degradation of the chemx metabolites which at any time during studies accounted for > 10 % of the amount of chemx applied, a kinetic analysis of the data was employed using Simu-Solv™, a computer modelling and simulation package in which the data were fit to a first-order kinetic model for the aerobic degradation of chemx. The data used for this analysis were from the route and rate of degradation studies (points IIA 7.1.1, 7.2.1, Grenfel 1996 and Grenfel 1995, XX-14019 and XX-14020). For the Speyer 2.2 soil, the data from the two labels was averaged, and truncated to use only those values up to 100 after application, in order to compare directly with the soils from the rate of degradation study which were incubated for only 100 days. The limited duration of the incubation period and the apparent slow rate of degradation of the metabolites led to cases in which the standard deviation of the rate constants was great enough to result in the calculated DT_{50} values for metabolites being considered to be an approximation rather than a definitive number. The DT_{50} values which were determined using this modelling approach are summarised in Table IIA 7.2.3-2.

The data generated is indicative of a slow rate of degradation of metabolites in all soils tested over the short (100 day) period for which data were available.

Table IIA 7.2.3-2 Calculated half lives in days for chemx metabolites resulting from aerobic degradation of chemx in soil

Soil Type	Evesham Clay Loam	Malham Silty Clay Loam	Speyer 2.2 Loamy Sand	Wick Sandy Loam
DT_{50} xxxxxxx (metabolite 2)	138	149	113	156
DT_{50} metabolite 3	138	144	142	147
DT_{50} desmethyl chemx	138	158	188	132

CONCLUSIONS

Although there are three metabolites which occur at levels ≥ 10 % of the applied dose, because of the low application rate of chemx, those metabolites are present at exceedingly low concentrations in the soil. On the basis of modelling using laboratory data, it was shown that these metabolites degrade slowly in soil.

IIA 7.2.4 **Anaerobic degradation of chemx**

Data is not submitted with respect to anaerobic degradation, since the .....*(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)*

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IIA 7.3 **Field studies**

IIA 7.3.1 **Soil dissipation**

IIA 7.3.1.1 **Soil dissipation studies**

Reports (one of six)**:** IIA 7.3.1/01, Lyons V 1997, Chemx terrestrial dissipation study - German field trials 1994-1996, Chemco Report: XX-30607

(two of six)**:** IIA 7.3.1/02, Lyons V 1997, Chemx terrestrial dissipation study - Belgian field trials 1995-1996, Chemco Report: XX-30606

(three of six)**:** IIA 7.3.1/03, Lyons V 1997, Chemx terrestrial dissipation study - German field trials 1995-1996, Chemco Report: XX-30608

(four of six)**:** IIA 7.3.1/04, Lyons V 1997, Chemx terrestrial dissipation study - UK field trials 1995-1996, Chemco Report: XX-30609

(five of six)**:** IIA 7.3.1/05, Lyons V 1997, Chemx terrestrial dissipation study - French field trials 1995-1996, Chemco Report: XX-30610

(six of six)**:** IIA 7.3.1/06, Brown JR 1996, Terrestrial field dissipation study with chemx in Canadian soils (1995 use season), Chemco Report: XX-14325

Guidelines

BBA Guidelines for the official testing of plant protection products Part IV, 4-1(1986), Procedures for assessing the environmental fate and behaviour of pesticides, SETAC-Europe, 1995, ISBN Number 90-5607-002-9, with the exception of Report IIA 7.3.1/06 which was conducted as required by the Pest Control Act of Canada and the Food and Drug Act of Canada.

GLP: Fully GLP compliant

Executive Summary

In a series of terrestrial field dissipation studies, soil dissipation of chemx under field conditions was assessed in bare plots or cropped plots at eleven sites in Belgium, France, Germany, and the UK, and at two sites in Canada. Maximum residue levels of chemx recovered in the 0 - 10 cm soil profile ranged from 0.0117 mg/kg at 20 g as/ha to 0.0191 mg/kg at 30 g as/ha applied to bare soil. There was no apparent correlation between soil type and degradation rate with chemx residues generally remaining in the 0 - 10 cm soil horizon.

Under field conditions at site 1 (Franc-Waret, Belgium), chemx was found to have a DT_{50} value of 12 days in soil. At sites 2, 3 and 4, (Saiguede, Endoufielle, and Villerbon, France), each of which involved a different soil type, the DT_{50} values for chemx were 11, 25, and 32 days respectively. At sites 5, 6, 7, 8 and 9 (Hilgermissen, Hanigsen, Wunstorf-Liethe, Konigslutter, and Riedstadt Germany) the DT_{50} values for chemx in the 5 different soils concerned were 18, 22, 26, 28 and 47 days. At sites 10 and 11 (Spalding and West-on-Trent, UK) the DT_{50} values for chemx in the 2 soils concerned were 23 and 25 days. Under the conditions prevailing at sites 12 and 13 (Alberta and Saskatchewan, Canada) the DT_{50} values for chemx were 13 and 52 days for the 2 soils at those sites.

At site 1, (Franc-Waret, Belgium) the DT_{90} value for chemx dissipation in soil was 131 days. At sites 2, 3 and 4, (Saiguede, Endoufielle, and Villerbon, France) the DT_{90} values for chemx in the 3 soils concerned were found to be 276, 302, and 358 days. At sites 5, 6, 7, 8, and 9 (Hilgermissen, Hanigsen, Wunstorf-Liethe, Konigslutter, and Riedstadt Germany) the DT_{90} values for chemx in the 5 soils concerned were found to be 197, 243, 247, 285, and 303 days. At sites 10 and 11, (Spalding and West-on-Trent, UK) the DT_{90} values for chemx in the 2 soils concerned were 252and 278 days. Under the conditions prevailing at sites 12 and 13 (Alberta and Saskatchewan, Canada) the DT_{90} values were 370 and 1190 days for the 2 soils at those sites. The mean DT_{50} for the eleven European sites was 24 days and the mean DT_{90} was 261 days.

The residue levels of chemx parent compound at the eleven European sites ranged from < 0.0005 to 0.0016 mg/kg in the 0 - 20 cm soil horizon three months after treatment and from < 0.0005 to 0.0007 mg/kg one year after a bare soil application at the maximum recommended rate of application of 20 g as/ha. Following post-emergent application at 20 g as/ha to a wheat crop, in accordance with the proposed recommendations for use, residues of chemx parent compound in soil 90 days after treatment ranged from < 0.0005 to 0.0007 mg/kg and one year after application, were from < 0.0005 to 0.0006 mg/kg.

The observation made on the basis of the results obtained in first of the aerobic soil degradation laboratory studies (point IIA 7.2.1 Grenfel RG 1995a) that degradation may be pH dependant with slower degradation in high pH soils, was not confirmed. In the two UK soil types pH 7.5 / 7.6 the DT_{50} values were 25 / 23 days respectively. The characteristics of the soils tested did not appear to influence dissipation rate.

The maximum levels of xxxxxxx (metabolite 2) and of desmethyl chemx (metabolite 1) observed in the 0 - 20 cm soil horizon during were low at 0.0034 mg/kg following bare soil application, and 0.0022 mg/kg following post-emergent application. In general a higher proportion of metabolite residue occurred in the 10 - 20 cm horizon as compared to parent compound residue. It is not anticipated that degradation products formed have any environmental significance.

The results of the field trials conducted showed that even when applied to bare ground at up to 30 g as/ha chemx did not significantly migrate down the soil profile. As anticipated, dissipation half-life (DT_{50}) in field soils is much faster than in laboratory studies - an average of 24 days for the eleven European sites and an average DT_{90} of 261 days. At higher use rates the ultimate dissipation of final soil residues may be prolonged as evidenced by studies at three German sites.

In soil dissipation studies conducted at two Canadian sites (Alberta and Saskatchewan) the dissipation of chemx and its major soil metabolite xxxxxxx (metabolite 2) was assessed following bare soil treatment. The half-life calculated (Gustafson and Holden, 1990) was 52 days (Alberta site) and 13 days (Saskatchewan site). In the main chemx residues were confined to 0 - 15 cm horizon but residues were detected in the 15 - 30 cm horizon at various sampling intervals. Residues of xxxxxxx (metabolite 2) were not detected in the Alberta site and only small amounts in the other site.

I. MATERIALS AND METHODS

1. Test Material: Chemx formulated as Chemx WG (80 % w/w as)

Description: Beige granule

Lot/Batch #: NPD-9403-5772-F

Purity: 80.4 % chemx

CAS # of as: 16335-17-2

Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Test sites

Locations indicated in column 2 of Table II 7.3.1-1, in Belgium, France, Germany, and the UK. Residue trials were also conducted at two Canadian locations, Alberta and Saskatchewan, (Brown, 1996). The management history of the test sites is provided in Table IIA 7.3.1-2.

3. Programme of studies

An extensive programme of soil dissipation studies was conducted in Europe to determine the residue levels of chemx parent compound and its xxxxxxx metabolite (metabolite 2) and desmethyl chemx (metabolite 1) and to provide estimates of the dissipation time (DT_{50} and DT_{90}) of chemx under field conditions. A summary of the European field programme is provided in Table IIA 7.3.1-1.

4. Experimental treatments

Chemx formulated as Chemx WG (80 % w/w as) was applied from April to early June, at the maximum recommended rate of application of 20 g as/ha to bare ground plots at sites in Belgium, France, Germany

and the UK and in addition in French sites to standing wheat crops at Zadoks growth stage 39 to 47 in accordance with the recommended GAP. In addition trials at 3 sites in Germany were conducted using an exaggerated application rate of 30 g as/ha on bare soil plots (1.5 times the maximum recommended rate).

Table IIA 7.3.1-1 Programme of European field dissipation studies with chemx (1994-1996)

Country/ Year	Location	Cropping Situation	Application Rate (kg as/ha)	Monitoring Period (days)	Report and Author
Belgium 1995/96	Franc-Waret	Bare soil	0.021	360	IIA 7.3.1/01 Lyons V 1997a Chemco Report: XX-30607
France 1995/96	Saiguede Endoufielle Villerbon	Bare and Cropped soil	0.0193 0.0201 0.0195	540	IIA 7.3.1/02 Lyons V 1997b Chemco Report: XX-30606
Germany 1995/96	Hilgermissen Hanigsen	Bare Soil	0.0208 0.0202	540	IIA 7.3.1/03 Lyons V 1997c Chemco Report: XX-30608
UK 1995/96	Spalding West-on-Trent	Bare Soil	0.02 0.02	510	IIA 7.3.1/04 Lyons V 1997d Chemco Report: XX-30609
Germany 1994/96	Wunstorf-Liethe Konigslutter Riedstadt	Bare Soil	0.0291 0.0296 0.0318	720	IIA 7.3.1/05 Lyons V 1997e Chemco Report: XX-30610

Table IIA 7.3.1-2 Management history of the test sites in the previous three years

Country	Location	Crops grown	Pesticides used	Fertilizers used	Report and Author
Belgium	Franc-Waret				IIA 7.3.1/01 Lyons V 1997a Chemco Report: XX-30607
France	Saiguede				IIA 7.3.1/02 Lyons V 1997b Chemco Report: XX-30606
	Endoufielle				
	Villerbon				
Germany	Hilgermissen				IIA 7.3.1/03 Lyons V 1997c Chemco Report: XX-30608
	Hanigsen				
UK 1995/96	Spalding				IIA 7.3.1/04 Lyons V 1997d Chemco Report: XX-30609
	West-on-Trent				
Germany	Wunstorf-				IIA 7.3.1/05 Lyons V 1997e Chemco Report: XX-30610
	Liethe				
	Konigslutter				
	Riedstadt				

In the Canadian trials, chemx WG (80 % w/w as) was applied in June 1995 at the target rate of 50 g as/ha (actual rate 43 g as/ha) onto bare ground.

Test material was added to water and applied using a xxxx sprayer. Plot size was xx meters by x meters and a distance of x meters separated plots on all sides. Bare soil treatments were maintained weed free by spray application of zzzzzzz. An aliquot of spray mixture was taken for analysis prior to and after each spraying. In addition, verification of the application rate was carried out using application monitors. Field spiking of soil samples was carried out by fortification of x g of soil with y mL test material containing xx µg as/mL.

5. Sampling

Fifteen soil cores (5 cm diameter) were taken from treated plots at intervals from application up to 360 days (8 samplings) 540 days (9 samplings) and 720 days (11 samplings) respectively to determine the rate of decline of chemx in soil.

Soil cores were divided into 0 - 10 cm and 10 - 20 cm layers, bulked and homogenised. A representative sub-sample of each depth was taken for residue analysis. Samples were stored at -18°C directly after sampling until analysed. A soil core taken at the different sampling points was used for the purposes of determining soil density (0 - 10 cm).

In the Canadian trials, the soils were sampled at 12 time intervals. Eighteen soil cores (3 cm diameter) per plot (3 from each of 6 subplots) to a depth of 30 cm were taken over a 150 day period in Alberta (due to ground conditions) and over 192 day period in Saskatchewan. Soil cores were segregated into the 0 - 15 and 15 - 30 cm horizons. In addition a composite of both horizons was taken from one core from each subplot. Soil samples were frozen within 6 hours of sampling and stored frozen until analysis.

6. Description of analytical procedure

Soil residue data were generated for chemx parent compound plus xxxxxxx metabolite (metabolite 2) and desmethyl chemx (metabolite 1), for chemx parent compound plus xxxxxxx metabolite (metabolite 2) and for chemx parent compound alone in the 0 - 10 cm layer and in the 0 - 20 cm layer (Table IIA 7.3.1-3). The analytical methods involved treating soil extracts with hydrochloric acid which gave a quantitative yield of a single analyte, an ethylsulphone (metabolite 7) which, depending on clean up, was quantified using HPLC and the residue was calculated as mg/kg of ethylsulfone accounting for -

a) chemx, xxxxxxx (metabolite 2) and desmethyl chemx (metabolite 1), expressed as chemx parent equivalent and identified as the total residue,

b) chemx and its xxxxxxx (metabolite 2), expressed as chemx parent, and

c) chemx parent compound.

The limit of quantification for the method was 0.0005 mg/kg chemx.

For the purposes of calculating the DT_{50} and the DT_{90} values, the residue of chemx parent compound found in the 0 - 10 and 10 - 20 cm soil layers were averaged to represent the chemx residues in the top 20 cm root zone.

Table IIA 7.3.1-3 Summary of the residue analysis programme for the European field dissipation studies with chemx (1994-1996)

Country, year	Trial location	Nature of the analyte(s) determined in the soil samples						Reference
		Chemx parent + metabolite 1 and 2 Method reference IIA 4.4.2/01		chemx parent + metabolite 2 Method reference IIA 4.4.1/01		chemx parent Method reference IIA 4.4.3/01		
		0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm	
Belgium, 1995-1996	Franc-Waret	Samples taken for analysis 0 to 360 days after treatment						IIA 7.3.1/02 Lyons V 1997b
Germany, 1995-1996	Hilgermissen	Samples taken for analysis 0 to 540 days after treatment						IIA 7.3.1/03 Lyons V 1997c
Germany, 1995-1996	Hanigsen	Samples taken for analysis 0 to 540 days after treatment						IIA 7.3.1/03 Lyons V 1997c
France, 1995-1996	Saiguede	Samples taken for analysis 0 to 540 days after treatment						IIA 7.3.1/05 Lyons V 1997e
France, 1995-1996	Endoufielle	Samples taken for analysis 0 to 540 days after treatment						IIA 7.3.1/05 Lyons V 1997e
France, 1995-1996	Villerbon	Samples taken for analysis 0 to 540 days after treatment, but the 420 days after treatment 10-20 cm sample was not analysed for chemx parent plus metabolite 2						IIA 7.3.1/05 Lyons V 1997e
UK 1995-1996	Spalding	Samples taken for analysis 0 to 510 days after treatment						IIA 7.3.1/04 Lyons V 1997d
UK 1995-1996	Weston-on-Trent	Samples taken for analysis 0 to 510 days after treatment						IIA 7.3.1/04 Lyons V 1997d
Germany, 1994-1996	Wunstorf-Liethe	-	-	samples taken for analysis 0 to 720 days after treatment				IIA 7.3.1/01 Lyons V 1997a
Germany, 1994-1996	Konigslutter	-	-	samples taken for analysis 0 to 720 days after treatment				IIA 7.3.1/01 Lyons V 1997a
Germany, 1994-1996	Riedstadt	-	-	samples taken for analysis 0 to 720 days after treatment				IIA 7.3.1/01 Lyons V 1997a

In the case of the Canadian trials, soil residue data were generated for chemx parent compound and for xxxxxxx (metabolite 2). The analytical procedure for the quantification of chemx and metabolites differed from that used for the European soils. It consisted of extraction initially with acetonitrile/water mixture, followed by centrifugation and a second extraction with an acetonitrile/NaOH mixture. These extracts, combined with a third acetonitrile extraction were subject to refluxing to convert chemx to a transformation product. Residues were determined quantitatively using HPLC. The limit of detection of this method was 0.0002 mg/kg for chemx parent compound and 0.0012 mg/kg for the xxxxxxx metabolite (metabolite 2).

II. RESULTS AND DISCUSSION

A. APPLICATION VERIFICATION

Recoveries achieved on extraction and analysis of application monitors was in the range xx to xy %. Recovery achieved on analysis of filed spiked samples was xx %.

B. FINDINGS

Under field conditions, chemx parent compound dissipated rapidly in the 0 - 20 cm soil horizon. The dissipation values for each trial calculated using the mathematical model developed by Frehse, Timme and Laska, 1992, are summarised in Table IIA 7.3.1-4. In accordance with the best fit evaluation of the residue data, the half-life (DT_{50}) for the dissipation of chemx ranged from 11 to 47 days and the time for 90 % dissipation of chemx (DT_{90}) ranged from 131 to 358 days. No obvious correlation can be made between the rates of dissipation and soil properties

Table IIA 7.3.1-4 Soil dissipation of chemx under field conditions (0 - 20 cm horizon)

Country Year	Location	Soil characteristics					Statistical evaluation		
		Soil texture			% OM	pH	Best fit		
		% sand	% silt	% clay			Function	DT_{50} (days)	DT_{90} (days)
Belgium 1995-1996	Franc Waret	5.1	80	14.9	3.3	7.1	Sqrt 1 st order	12	131
Germany 1995-1996	Hilgermissen	18.6	55.1	26.3	1.4	6.1	Sqrt 1.5 st order	47	247
Germany 1995-1996	Hanigsen	70.6	25.5	3.9	1.9	6	Sqrt 1 st order	28	303
UK 1995-1996	Spalding	29.3	55.6	15.1	1.5	7.5	Sqrt 1 st order	25	278
UK 1995-1996	Weston-on-Trent	46.9	31.1	22	1.9	7.6	Sqrt 1 st order	23	252
France 1995-1996	Saiguede	16.9	71.6	10.2	1.3	6	Sqrt 1 st order	25	276
France 1995-1996	Endoufielle	16.5	49.6	32.1	1.8	6.4	Sqrt 1.5 st order	11	302
France 1995-1996	Villerbon	13.8	57.8	26.2	2.2	6	Sqrt 1 st order	32	358

In laboratory studies, there was some evidence that degradation was pH dependent - the longest DT_{50} was found in a soil with a pH of 7.9 (*cf* point IIA 7.2.1). The results obtained through field studies did not confirm that observation - at the two trials conducted on soils with highest pH values (UK Spalding pH 7.5 and UK Weston-on-Trent pH 7.6), the DT_{50} were 25 and 23 days, and the DT_{90} values were 278 and 252 days respectively (Lyons, V., 1997d).

The maximum content of parent chemx residues in the top 10 cm soil horizon, were 0.0117 mg/kg and 0.0191 mg/kg after application of, respectively, the maximum label use rate of 20 g as/ha and the exaggerated rate of

30 g as/ha applied as a bare soil treatment (Lyons, 1997a and 1997b). The initial theoretical content of chemx calculated for each trial, on the basis of an assumption of even distribution in the 0 - 10 cm layer, is summarised in Table IIA 7.3.1-5. The percent closure with the calculated initial concentration ranged from 33 % to 80 %.

The initial levels of chemx declined rapidly (within three months of treatment) to < 0.0005 to 0.0016 mg/kg in the 0 -20 cm soil horizon (Table IIA 7.3.1-6). One year after application, chemx residues (parent compound) ranged from < 0.0005 to 0.0007 mg/kg. These results relate to treated bare soil application. There was however a large variation between soil types in the extent to which residues of chemx parent declined. In the Franc-waret soil the content of chemx had declined to < 0.0005 mg/kg by day 90, while in the Hilgermissen soil a content of < 0.0005 mg/kg was not reached until 360 days after application and in that level was not reached until 525 days after application.

In Spalding soil (UK) a level of < 0.0005 mg/kg was not recorded until day 360 while in Weston-on-Trent soil (UK) that level was recorded on day 90. In the case of the three French soils, the period between application to bare soil and decline to < 0.0005 mg/kg was 90 days in the case of the Saiguede soil, 180 days in the case of the Endoufielle soil and 360 days in the case of the Villerbon soil.

Table IIA 7.3.1-5 Percent closure with respect to the theoretical initial concentration of chemx in field soil dissipation trials

Country Year	Location	Density (g/cm^3)	Application rate (kg as/ha)	Initial theoretical 0-10 cm residue (mg/kg)	Maximum 0-10 cm residue observed (mg/kg)	Percent closure
Belgium 1995	Franc Waret	1.11	0.021	0.0181	0.0117	65
Germany 1995	Hilgermissen	1.19	0.0208	0.0175	0.0116	66
Germany 1995	Hanigsen	1.14	0.0202	0.0176	0.0095	54
UK 1995	Spalding	1.08	0.02	0.0185	0.0087	47
UK 1995	Weston-on-Trent	0.99	0.02	0.0203	0.0071	35
France 1995	Saiguede	1.44	0.0193	0.00134	0.0060	45
France 1995	Endoufielle	1.41	0.0201	0.00143	0.0047	33
France 1995	Villerbon	1.33	0.0195	0.00146	0.0060	41
Germany 1994	Wunstorf-Liethe	1.18	0.0291	0.0247	0.0146	59
Germany 1994	Konigslutter	1.24	0.0296	0.0239	0.0191	80
Germany 1994	Riedstadt	1.26	0.0318	0.0252	0.0170	67
Mean percent closure						54

Table IIA 7.3.1-6 Decline with time of residue levels (mg/kg) of chemx in the 0 - 20 cm soil horizon

Country year	Germany 1995-1996 Reference IIA 7.3.1/03		France 1995-1996 Reference IIA 7.3.1/05					
Location Application rate Days after application	Hilgermissen 20 g as/ha	Hanigsen 20 g as/ha	Saiguede 20 g as/ha		Endoufielle 20 g as/ha		Villerbon 20 g as/ha	
			Bare soil	Cropped soil	Bare soil	Cropped soil	Bare soil	Cropped soil
0	0.0045	0.0053	0.0028	0.0017	0.0024	0.0009	0.0027	0.0011
1	0.0060	0.0055	0.0025	0.0015	0.0023	0.0009	0.0030	0.0027
3	0.0054	0.0040	0.0026	0.0015	0.0012	0.0008	0.0026	0.0034
7	0.0041	0.0044	0.0030	0.0013	0.0014	0.0012	0.0028	0.0032
30	0.0036	0.0027	0.0011	0.0015	0.0016	0.0011	0.0008	0.0017
90	0.0013	0.0016	<0.0005	<0.0005	0.0006	<0.0005	0.0007	0.0007
180	0.0009	0.0009	<0.0005	<0.0005	<0.0005	<0.0005	0.0007	0.0005
360	<0.0005	0.0007	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.0006
540	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005

Country Year	Belgium 1995-1995 Reference IIA 7.3.1/02	UK 1995-1996 Reference IIA 7.3.1/04		Germany 1994-1996 Reference IIA 7.3.1/01		
Location Application rate Days after application	Franc-Waret 20 g as/ha	Spalding 20 g as/ha	Weston-on-Trent 20 g as/ha	Wunstorf-Liethe 30 g as/ha	Konigslutter 30 g as/ha	Riedstadt 30 g as/ha
0	0.0028	0.0044	0.0036	0.0067	0.0059	0.0078
1	0.0037	0.0033	0.0017	0.0075	0.0086	0.0085
3	0.0059	0.0023	0.0028	0.0075	0.0096	0.0064
7	0.0051	0.0030	0.0029	0.0055	0.0061	0.0061
30	0.0016	0.0028	0.0020	0.0048	0.0033	0.0029
60				0.0032	0.0027	0.0023
90	<0.0005	0.0009	<0.0005	0.0012	0.0016	0.0013
180	<0.0005	0.0010	<0.0005	0.0008	0.0006	<0.0005
360	<0.0005	<0.0005	<0.0005	0.0005	0.0007	<0.0005
540	Ns	<0.0005	<0.0005	0.0005	<0.0005	<0.0005
720				<0.0005	<0.0005	<0.0005

The limit of determination was 0.0005 mg/kg of chemx

In the case of the German soils treated with 30 g as/ha final residues appeared to be more persistent with the decline to a content of < 0.0005 mg/kg not being recorded until 180 days after application in the case of the Riedstudt soil, 540 days in the case of the Konigslutter soil and 720 days in the case of the Wunstorf-Liethe soil.

Most of the chemx residues remained in the top 10 cm soil horizon, indicating minimal vertical mobility.

C. APPLICATION TO WHEAT CROPS

In the case of studies with treated wheat crops, chemx residues based on average soil residues recovered in the 0 - 20 cm soil horizon following treatment with 20 g as/ha at three sites, ranged from 0.0009 - 0.0017 mg/kg at day 0, 0.0012 - 0.0032 mg/kg at day 7, 0.0011 - 0.0017 mg/kg at day 30, ≤ 0.0005 - 0.0007 mg/kg at day 90 to < 0.0005 to 0.0006 mg/kg one year after application.

In the case of the 1 - 10 cm horizon, following post-emergent application to a wheat crop at 20 g as/ha, average soil residue measured directly after application was 0.0018 mg/kg to 0.0033 mg/kg, whilst it was 0.0047 mg/kg to 0.0055 mg/kg in the corresponding "bare soil" plots (Lyons 1997e). The results obtained, which are summarised in Table IIA 7.3.1-7, demonstrated that the crop intercepted approximately 53 % of chemx applied. Since application was made at late application timings (GS 39 and GS 47), the extent of interception by the wheat crop should be considered to be maximal.

Table IIA 7.3.1-7 Results of analysis of soils from test sites in which chemx was applied to wheat crops

Location	Application rate (kg as/ha)	Application timing[a]	Initial residue (mg/kg)		Percent of product intercept by the crop
			Bare soil	Cropped soil	
Saiguede	0.02	GS 39	0.0055	0.0033	40
Endoufielle	0.02	GS 39	0.0047	0.0018	62
Villerbon	0.02	GS 47	0.0053	0.0022	58
Mean			0.0052	0.0024	53

[a] Zadoks

D. TRANSFORMATION OF PARENT COMPOUND

The content of xxxxxxx (metabolite 2) and desmethyl chemx (metabolite 1) residues were obtained by the subtraction of content of chemx parent compound from the residue of the chemx parent compound plus its metabolites (Lyons 1997b, Lyons 1997c, Lyons 1997d, Lyons 1997e). The results obtained are summarised in Table IIA 7.3.1-8. Under bare soil conditions, the maximum content of xxxxxxx (metabolite 2) + desmethyl chemx (metabolite 1) ranged from 0.0019 to 0.0034 mg/kg in the 0 - 20 cm soil horizon and declined to 0.0009 to 0.0027 mg/kg 18 months after application, except in the case of the sites at sites at Franc-Waret and Villerbon where the initial and final concentrations were the same. When chemx was applied post-emergent onto wheat at the maximum label use rate of 20 g as/ha, the residues of the metabolites ranged from 0.0008 to 0.002 mg/kg at 18 months after treatment.

While the results are presented on the basis of residues arising in the plough layer (0 - 20 cm), a comparison of the content of chemx parent compound plus xxxxxxx (metabolite 2) and desmethyl chemx (metabolite 1) occurring in the 10 - 20 cm soil layer as compared to the content of chemx parent compound only, indicates somewhat greater propensity for both metabolites to migrate to the 10 - 20 cm soil layer over time.

In the case of chemx parent compound, most of the determinable soil residues in 10 - 20 cm soil horizon were at the limit of quantification while for the combined residue, residues in 10 - 20 cm soil horizon were generally quantifiable.

Table IIA 7.3.1-8 Residues in soil (mg/kg) of xxxxxxx (metabolite 2) and desmethyl chemx (metabolite 1) under field conditions

Country Year	Belgium 1995-1995 Reference IIA 7.3.1/02	Germany 1995-1996 Reference IIA 7.3.1/03		UK 1995-1996 Reference IIA 7.3.1/04		France 1995-1996 Reference IIA 7.3.1/05					
Location	Franc-Waret	Hilgermis-sen	Hanigsen	Spalding	Weston -on-Trent	Saiguede		Endoufielle		Villerbon	
Days after application						Bare soil	Croppe d soil	Bare soil	Croppe d soil	Bare soil	Croppe d soil
0	0.0022	<0.0005	<0.0005	0.0009	<0.0005	0.0014	<0.0005	0.0022	<0.0005	0.0021	0.0012
1	0.0013	<0.0005	<0.0005	0.0013	0.0008	0.0010	<0.0005	0.0008	<0.0005	0.0015	0.0014
3	0.0014	<0.0005	0.0018	0.0009	0.0005	0.0016	<0.0005	0.0019	0.0008	0.0020	0.0005
7	<0.0005	<0.0005	0.0008	0.0008	0.0009	0.0016	<0.0005	0.0022	<0.0005	0.0009	<0.0005
30	0.0010	<0.0005	0.0015	0.0012	<0.0005	0.0015	0.0016	0.0022	0.0014	0.0032	0.0011
90	0.0025	0.0032	0.0022	0.0033	0.0023	0.0019	0.0014	0.0021	0.0011	0.00023	0.0014
180	0.0024	0.0028	0.0027	0.0018	0.0012	0.0013	0.0022	0.0033	0.0007	0.0032	0.0018
360	0.0022	0.0024	0.0028	0.0034	0.0022	0.0009	0.0010	0.0029	<0.0005	0.0023	0.0013
540	-	0.0026	0.0022	0.0012	0.0027	0.0009	0.0008	0.0016	0.0009	0.0021	0.0020

E. CHEMX DISSIPATION IN SOIL UNDER COLD CLIMATIC CONDITIONS

Details of the soil characteristics for the two Canadian soils are provided in Table IIA 7.3.1-9.

Table IIA 7.3.1-9 Soil physiochemical properties - Canadian field dissipation studies

	Soil Texture			% OM	pH	CEC (meq/100g)	Field Capacity (% at $^1/_3$ Bar)	Bulk Density (g/cc)
	% Sand	% Silt	% Clay					
Alberta								
Sandy loam (0-15 cm)	60	20	20	2.3	8.0	35.2	20.7	1.07
Sandy clay loam (15-30 cm)	60	18	22	1.6	8.3	31.1	21.8	1.06
Saskatchewan								
Loam (0-15 cm)	36	38	26	2.0	6.7	21.2	35.1	1.07
Clay loam (15-30 cm)	38	34	28	0.8	7.7	29.5	32.4	1.04

During the study, method recovery data obtained from all locations and soil depths using fortified samples showed average recoveries of 80.5 % (0 - 15 cm horizon) and 75.8 % (15 - 30 cm horizon) for the Alberta site and 78.7 % (0 - 15 cm horizon) and 78.3 % (15 - 30 cm horizon) for the Saskatchewan site. Verification tests carried out with for each location with respect to the application of chemx demonstrated that the average application rate was 42.8 g as/ha or 85 % of the target rate.

The maximum residue level for chemx detected at day 0 in Alberta soil (0 - 15 cm horizon) was 0.0152 mg/kg, which had dissipated to 0.0045 mg/kg by day 150 (Table IIA 7.3.1-10). Levels of chemx in the 15 - 30 cm horizon were at or below the limit of detection starting from day 29 and dissipated to 0.0002 mg/kg by day 150. Residues of xxxxxxx (metabolite 2) were not detected in either horizon in this soil over the trial period, a finding that was consistent with results from radiolabelled studies carried out on soil from this location. The maximum residue level for chemx detected on day 1 in Saskatchewan soil (0 - 15 cm horizon) was 0.0276 mg/kg, which dissipated to 0.0041 mg/kg by day 192. Levels of chemx in the 15 - 30 cm horizon were very low with a maximum value of 0.0007 mg/kg on day 1 (may be due to contamination). Xxxxxxx residues in the 0 - 1.5 cm horizon were detected on day 29 at 0.0057 mg/kg and declined to 0.0013 mg/kg at day 192. A xxxxxxx residue was not detected in the 15 - 30 cm horizon.

The dissipation rates for chemx in both soils, based on parent residues detected (0 - 30 cm horizon) over the 150/192 experimental period, were estimated using a non-linear first order kinetic model developed by Gustafson and Holden 1990. The DT_{50} values computed were 52 days for the Alberta site and 13 days for the Saskatchewan site, with corresponding DT_{90} values of 1,190 and 370 days.

Table IIA 7.3.1-10 Residues of chemx and xxxxxxx (metabolite 2) residues in Canadian soils (mg/kg)

Location	Alberta				Saskatchewan			
Residue	Chemx		Xxxxxxx		chemx		xxxxxxx	
Depth of Sample (cm)	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
Days after application								
-3/-6	0.0000	0.0000	0.00	0.00	0.0000	0.0000	0.0000	0.00
0	0.0152	ns	0.00	ns	0.0272	ns	0.0000	ns
1	0.0128	0.0000	0.00	0.00	0.0276	0.0007	0.0000	0.00
7-8	0.0140	0.0000	0.00	0.00	0.0165	0.0003	0.0000	0.00
29	0.0083	0.0013	0.00	0.00	0.0094	0.0003	0.0057	0.00
58-59	0.0053	0.0010	0.00	0.00	0.0074	0.0003	0.0015	0.00
120	0.0047	0.0004	0.00	0.00	0.0050	0.0000	0.0013	0.00
150-192	0.0045	0.0002	0.00	0.00	0.0041	ns	0.0000	ns
*DT_{50}(days)	51.8				12.6			

* Gustafson and Holden (1990)

F. CARRY OVER OF RESIDUE

Carry over of chemx residues after 12 months was xx %.

III. CONCLUSIONS

Chemx dissipated rapidly in soil under field conditions. The dissipation rates calculated for eleven European trial sites following application to bare soil (soil residues 0-20 cm) calculated according to Timme, Frehse and Laske (1992), using the statistical function of best fit, produced half-life (DT_{50}) values for the dissipation of chemx ranging from 11 to 47 days and the periods required for 90 % dissipation (DT_{90}) ranged from 131 to 358 days with no apparent obvious correlation with soil properties. DT_{50} or DT_{90} calculations were not made following application to wheat crops as the soil residue levels were too low to allow reliable results to be obtained. The pattern of dissipation is shown in Figure IIA 7.3.1-1.

A summary of the results of the field soil dissipation studies carried out are provided in Table IIA 7.3.1-11 - the results of the laboratory studies conducted are included for comparative purposes.

Figure 7.3.1-1 Dissipation pattern of chemx under field conditions (20 g as/ha, 8 sites, application to bare soil)

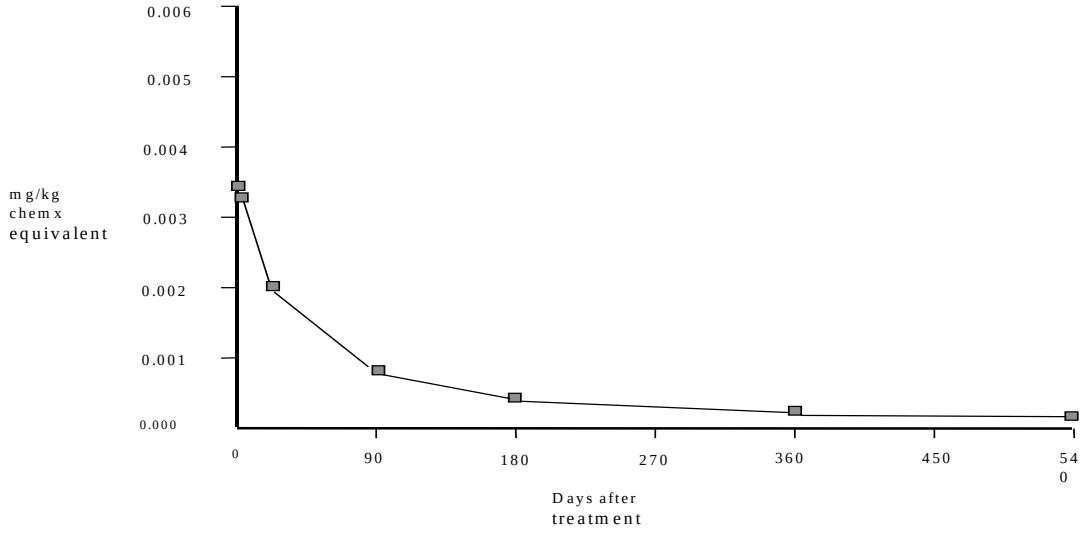

Table IIA 7.3.1-11 Summary of results of laboratory and field soil dissipation studies with chemx

Country/Year	Soil Characteristics						DT_{50} (days)	DT_{90} (days)
	Study	Soil Texture			% OM	pH		
		% sand	% silt	% clay				
Evesham (UK)	Laboratory	35	34	31	2.1	7.9	226	750
Malham (UK)	Laboratory	8	73	18	8.8	6.7	194	643
Wick (UK)	Laboratory	67	21	12	1.4	5.3	92	306
Elder (USA)[1]	Laboratory	72	10	18	1.6	6.8	35	219
Dupo (USA)[1]	Laboratory	29	62	9	0.8	7.6	32	259
Elder (USA)[1]	Laboratory	72	10	18	1.6	6.8	75[2]	nr
Dupo (USA)[1]	Laboratory	29	62	9	0.8	7.6	82[2]	nr
Belgium 1995/96	Field	5.1	80	14.9	3.3	7.1	12	131
Germany 1995/96	Field	18.6	55.1	26.3	1.4	6.1	47	247
Germany 1995/96	Field	70.6	25.5	3.9	1.9	6.0	28	303
UK 1995/96	Field	29.3	55.6	15.1	1.5	7.5	25	278
UK 1995/96	Field	46.9	31.1	22.0	1.0	7.6	23	252
France 1995/96	Field	16.9	71.6	10.2	1.3	6.0	25	276
France 1995/96	Field	16.5	49.6	32.1	1.8	6.4	11	302
France 1995/96	Field	13.8	57.8	26.2	2.2	6.0	32	358
Germany 1994/96	Field	57.8	35.3	6.9	1.9	6.5	26	285
Germany 1994/96	Field	11.2	71.1	13.7	1.7	6.7	22	243
Germany 1994/96	Field	44.6	44.8	10.6	2.2	7.1	18	197
Canada 1996	Field	60.0	20.0	20.0	2.3	8.0	52	1190
Canada 1996	Field	36.0	38.0	26.0	2.0	6.7	13	370

[1] EPA guideline study at 25°C
[2] DT_{50} includes unextracted residue fraction
nr not reported

The residue levels of chemx parent compound at the European sites were from < 0.0005 to 0.0016 mg/kg in the 0 - 20 cm soil horizon three months after treatment and ranged from < 0.0005 to 0.0007 mg/kg one year after a bare soil application at the maximum recommended rate of application of 20 g as/ha. Following post-emergent application at 20 g as/ha to a wheat crop, in accordance with the proposed recommendations for use, residues of chemx parent compound in soil 90 days after treatment were from < 0.0005 to 0.0007 mg/kg and one year after application, were from < 0.0005 to 0.0006 mg/kg.

The observation made on the basis of the results obtained in an aerobic soil degradation laboratory study (point IIA 7.2.1 Grenfel RG 1995a) that degradation may be pH dependant with slower degradation in high pH soils, was not confirmed. In the two UK soil types pH 7.5/7.6 the DT_{50} values were 25/23 days respectively. The characteristics of the soils tested did not appear to influence dissipation rate.

The maximum levels of xxxxxxx (metabolite 2) and of desmethyl chemx (metabolite 1) observed in the 0 - 20 cm soil horizon during were low at 0.0034 mg/kg following bare soil application, and 0.0022 mg/kg following post-emergent application. In general a higher proportion of metabolite residue occurred in the 10 - 20 cm horizon as compared to parent compound residue. It is not anticipated that degradation products formed have any environmental significance.

The results of the field trials conducted showed that even when applied to bare ground at up to 30 g as/ha chemx did not significantly migrate down the soil profile. As anticipated, dissipation half-life (DT_{50}) in field soils is much faster than in laboratory studies - an average of 24 days for the eleven European sites and an average DT_{90} of 261 days. At higher use rates the ultimate dissipation of final soil residues may be prolonged as evidenced by studies at three German sites.

In soil dissipation studies conducted at two Canadian sites (Alberta and Saskatchewan) the dissipation of chemx and its major soil metabolite xxxxxxx (metabolite 2) was assessed following bare soil treatment. The half-life calculated (Gustafson and Holden, 1990) was 52 days (Alberta site) and 13 days (Saskatchewan site). In the main chemx residues were confined to 0 - 15 cm horizon but residues were detected in the 15 - 30 cm horizon at various sampling intervals. Residues of xxxxxxx (metabolite 2) were not detected in the Alberta site and only small amounts in the other site.

(Lyons V 1997a; Lyons V 1997b; Lyons V 1997c; Lyons V 1997d; Lyons V 1997e; Brown JR 1996a)

IIA 7.3.1.2 **Storage stability of chemx residues in soil**

The study was conducted to demonstrate the storage stability of chemx residues in soils. The analytical method used was that used in field dissipation studies conducted in the United States rather than that used in the European field dissipation studies, however, storage stability is independent of analytical methodology.

Report: IIA 7.3.1/07 Brown JR 1996, The stability of residues of chemx and its major metabolite in soil during frozen storage, Chemco Report XX-14395

Guideline

EPA FIFRA Guideline § 171-4 (c)(l) (ii)- magnitude of the residue - storage stability

Testing Laboratory and dates

The study was conducted during the period February to November 1995 by the Chemco Research Laboratory, Richmond, California.

GLP: Fully GLP compliant [17].

Executive Summary

In a storage stability study, untreated soil (Dupo, St Charles, Illinois, silt loam as used in the second aerobic soil degradation study, point IIA 7.2.1) collected from control plots was used. 50g soil samples were maintained in plastic bottles in a frozen condition pending fortification. The soil samples were fortified at eight intervals with 0.2 mg/kg of test substance. No significant degradation of chemx or of xxxxxxx (metabolite 2) residues occurred in soil maintained in a frozen condition (< -12 °C) for more than 500 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Materials:	Chemx	xxxxxxx (metabolite 2)
Description:	White powder	White powder
Lot/Batch #:	NPD-9301-4706-T [18]	NPD-9301-4708-T [18]
Purity:	98 %	99 %
CAS # :	16335-17-2	16478-13-8

B. STUDY DESIGN

1. Experimental conditions

Untreated soil from the control plots of the aerobic soil metabolism study (Sullivan, R., 1995b, IIA 7.2.1/02, Report No XX-13750) was used as the test system for this study. Soil samples were prepared by weighing 50.0 ± 0.27 grams of soil into individual 4 oz. plastic bottles. The samples were placed in frozen storage pending fortification. Samples were fortified at eight different time points with chemx and xxxxxxx (metabolite 2) over a period of 510 days for chemx and 502 days for xxxxxxx. On the day of fortification, soil samples to be fortified were allowed to equilibrate to room temperature, and were then fortified by adding 1 ml of the fortification solution, which contained 10 µg/ml of the test substance in acetonitrile/ethanol (1 : 99 v/v). The fortification level was 0.20 mg/kg of the respective component. After fortification, samples were returned to frozen storage (< -12°C).

2. Sampling

On the final fortification day (Day 0), a set of samples including all previous time points was taken for analysis for chemx and xxxxxxx. One sample was analysed for each time point.

3. Description of analytical procedures

The analytical procedure used involved a three part extraction in which the sample was extracted first with acetonitrile/water, and then re-extracted with acetonitrile / 2 N NaOH. Finally, the filter cake was rinsed with acetonitrile. The total extract was then subjected to rotary evaporation to remove the acetonitrile, sodium hydroxide was added, and the sample was refluxed for one hour to convert chemx to a transformation product. The extract was then acidified, and the analytes were partitioned into methylene chloride. The extract was purified using a Florisil® cleanup column, concentrated, adjusted to final volume, and analysed by HPLC using fluorescence detection.

II. RESULTS AND DISCUSSION

The values reported are the results of single determinations and are expressed in percent of the amount fortified. A statistical analysis was performed on the storage stability data. The logarithm of the ratio of the measured concentration to the amount fortified was regressed against days in storage. The resulting slope estimate was used as a measure of the trend, with data analysis using the SAS statistical package. Because of the low recoveries for chemx at 82 and 137 days of storage, a second method of regression, robust to potential outliers, was used to analyse the data. The impact of the two low points for chemx did not appear sufficiently great to justify excluding them from statistical analysis; therefore, the original least-squares analyses with all data included were retained.

The recovery of chemx residues ranged from 83.54 % at day zero to 73.56 % at day 502. While recovery on day 82 and 137 were low relative to other time points, statistical analysis did not show those data points to be outliers. The recovery of xxxxxxx (metabolite 2) residue was more consistent and ranged from 85.42 % at day zero to 83.6 % at day 510.

Table IIA 7.3.1.2-1 Summary of Results - Stability of chemx and xxxxxxx (metabolite 2) residues in soil during frozen storage

Chemx		xxxxxxx (metabolite 2)	
Days of Storage	Percent of Initial Residue	Days of Storage	Percent of Initial Residue
0	83.54	0	85.42
82	54.82	90	82.36
137	65.91	145	83.16
194	80.88	202	82.49
250	83.85	258	87.85
321	84.84	329	85.80
403	76.26	411	82.47
502	73.56	510	83.60

III. CONCLUSIONS

There is no significant degradation of chemx or of xxxxxxx (metabolite 2) in soil under conditions of frozen storage.

(Brown JR 1996b)

IIA 7.3.2 **Soil residue testing**

A study is not considered necessary since the .....*(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)*

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.

IIA 7.3.3 **Soil accumulation studies**

The field dissipation studies presented included studies relevant to eleven sites in four European countries. DT_{90} values calculated in those studies ranged from 131 to 358 days with a mean value of 261 days (Lyons V 1997a, Lyons V 1997b, Lyons V 1997c, Lyons V 1997d, Lyons V 1997e). The residue levels of chemx recovered 360 days after treatment with 20 g as/ha to bare soil ranged from < 0.0005 to 0.0007 mg/kg with only two sites having residues above the limit of quantification.

A similar result was evident in field trials with chemx applied in accordance with the proposed recommendations for use of chemx. At the maximum rate of application proposed (20 g as/ha) it was evident that residue levels of chemx in soil were greatly reduced within one year of application. Since the DT_{90} value obtained is < 1 year and residual traces present are not expected to have any phytotoxic effects in succeeding crops nor are they expected to have unacceptable effects on the environment, it is not necessary that soil accumulation studies be conducted.

IIA 7.4 Mobility studies with chemx and its degradation products

IIA 7.4.1 Adsorption and desorption of chemx

Report: IIA 7.4.1/01 Grenfel RG 1995, ^{14}C-chemx, adsorption and desorption, Chemco Report XX-13701

Guideline

OECD Test Guideline 106 ≅ EPA FIFRA Guideline § 163-1

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England, conducted the study during the period August 1993 to November 1993.

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In an adsorption / desorption study, 2 US soil types and 3 UK soil types (pH range of 5.3 - 7.9) were used to assess the adsorption behaviour of chemx in soil. In all soil types tested chemx was mobile. There was little correlation between adsorption constants and soil organic matter, but some positive correlation with soil pH. Determined K_{oc} values ranged 5.3 to 89 (average 33) indicating high potential soil mobility for chemx based on the ASTM classification system. Once adsorbed by soil colloids chemx was not readily desorbed (K_{oc} = 400). During the equilibration phase some degradation of chemx occurred, due presumably to hydrolysis - appropriate adjustments were made in the calculations reported. The mass balance at the end of the study ranged from 87 to 99 %.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** Chemx - chem2 ring labelled: ^{14}C in the C-3 position; specific activity 28.6 mCi/mmol;

Description:	White powder
Lot/Batch #:	NPD-9307-5385-T
Purity:	radiochemical purity ≥ 98 %
CAS #:	16335-17-2
Stability of test compound:	The test material was stable for at least 7 days at room temperature.

2. **Soils:** The study was conducted with five different soil types (three European and two from the US). These soils were collected from the top 0-15cm layer in fields that had not been treated with a pesticide for at least three years. Air dried soils were stored at ambient temperatures for x weeks prior to experimentation. A summary of the physical and chemical properties of the soils is provided in Table IIA 7.4.1-1. The

percent sand, silt and clay are quoted on the basis of the Soil Survey of England and Wales (SSEW) classification. Values using the USDA classification are provided in brackets.

Table IIA 7.4.1-1 Soil physiochemical properties

Soil Name Origin	Elder USA	Hanford USA	Evesham UK	Sandiacre UK	Wick UK
Textural class	Sandy loam	Loamy sand	Clay loam	Sandy/silt loam	Sandy loam
% Sand	70	82	35	42	67
% Silt	16	15	34	38	21
% Clay	14	3	31	20	12
% OC*	0.9	0.3	1.2	4.3	0.8
CEC (meq/100g)	13.5	6.4	18.9	21.1	14.2
pH #	6.8	7.3	7.9	7.1	5.3
% Moisture ($^1/_3$ bar)	15.56	6.3	**	**	**

* OM./1.72 = OC # measured in a 1 : 2.5 soil : water suspension ** Only determined on dried soil

B. STUDY DESIGN

1. Experimental conditions

The pH of the equilibrium solution was x.x. Stock solutions of chem2 ^{14}C labelled chemx in acetonitrile were prepared and aliquots added to portions of 0.01 M $CaCL_2$ solution to give a concentration range of 0.04, 0.08, 1.05 and 4.7 µg/ml ensuring that the concentration of acetonitrile in aqueous solution did not exceed 0.1 % by volume. The appropriate solution to soil ratio was determined in preliminary testing at 2 : 1 (*circa* 10 % adsorption). Portions of test solution (10 ml) were shaken at ~ 25°C with samples of test soil (5 g dry weight) for a 24 hour equilibration period in darkness. Following centrifugation (x rpm for y minutes) the supernatant was decanted and duplicate aliquots were prepared for radioassay.

A control experiment was also performed to assess potential adsorption to glass test vessels. Following the adsorption phase fresh 0.01 M aqueous $CaCL_2$ (20 ml) was added to each test vessel, equilibrated for 24 hours at 25°C, solutions and soils separated, quantified and subject to a further desorption phase. Soil extracts from the highest concentration tested were further extracted twice using 15 ml acetonitrile and the extracts used to assess the degree of degradation of chemx during equilibration. Results were corrected for the slight degradation observed.

2. Description of analytical procedures

Radioactivity was determined by LSC, and both aqueous supernatants and soil extracts obtained after equilibration were analysed by reverse phase HPLC of the highest test concentration (4.7 µg/ml) samples. Chromatography using normal phase TLC and GC-MS were used to confirm the identity of the degradation products detected. The limit of detection (LOD) for chemx and metabolite 1 were x and x µg as/g soil, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 were y and y µg as/g soil, respectively.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Recovery of radioactivity in aqueous supernatant and soil extracts on completion of adsorption ranged from 87 - 99 % of applied amount. Unextractable soil residues were calculated by subtraction. Recoveries following desorption ranged from 101 - 114 %.

B. TRANSFORMATION OF PARENT COMPOUND

During the 24-hour equilibration period, chemx was degraded to varying degrees depending on the soil.

The major degradation product was xxxxxxx (metabolite 2) which represented 0.1 (Hanford) to 0.6 % (Sandiacre/Elder) of the applied radioactivity in the soil extracts, and 0.8 % (Evesham) to 8% (Wick) applied radioactivity in the aqueous supernatant. Each of the other degradation products accounted for < 1% of the applied radioactivity.

C. FINDINGS

Adsorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

Table IIA 7.4.1-2 Adsorption/desorption constants and correlation coefficients for chemx in soil at 25°C

				Adsorption				First Desorption			
Soil type	OM %	OC %	pH	K_a	R	K_{om}	K_{oc}	Kd	r	K_{om}	K_{oc}
Elder sandy loam	1.5	0.9	6.8	0.359	0.985	24	40	4.42	0.986	300	490
Hanford loamy sand	0.5	0.3	7.3	0.076	0.895	15	25	1.90	0.926	380	630
Evesham clay loam	2.1	1.2	7.9	0.079	0.982	3.8	6.6	2.59	0.996	120	220
Sandiacre sandy/silty loam	7.4	4.3	7.1	0.230	0.937	3.1	5.3	2.82	0.940	38	66
Wick sandy loam	1.4	0.8	5.3	0.710	0.991	51	89	4.69	0.997	340	590

K_a/Kd Freundlich adsorption/desorption distribution coefficient r Correlation coefficient
K_{om}/K_{oc} Coefficient of adsorption/desorption per unit organic matter/organic carbon

The Freundlich adsorption/desorption plots obtained showed good linearity, with slopes 1/Na : 1/Nd generally close to unity indicating both adsorption and desorption was linearly proportional to soil concentration over the range tested. The Freundlich adsorption constants ranged from 0.076 to 0.710 for the five test soils showing that chemx was poorly bound to all five soils. The Freundlich desorption constants were larger than those obtained for adsorption, with the first desorption constants in the range 1.90 - 4.69 and Koc values in the range 66 - 630 indicating that once adsorbed, chemx is not as readily desorbed. The % adsorbed and desorbed chemx at each concentration is provided in Table IIA 7.4.1-3.

Table IIA 7.4.1-3 Adsorbed and desorbed chemx at each concentration tested

Soil type	Adsorbed concentration (µg as/mL)				Desorbed concentration (µg as/mL)			
	0.04	0.08	1.05	4.7	0.04	0.08	1.05	4.7
Elder sandy loam								
Hanford loamy sand								
Evesham clay loam								
Sandiacre sandy/silty loam								
Wick sandy loam								

III. CONCLUSIONS

The adsorption constants did not appear to correlate with the organic carbon content of the soils tested - K_{oc} values ranged from 5.3 to 89. Adsorption of chemx appears to increase as the pH decreases. On the basis of the results obtained it appears that chemx has a high potential soil mobility (ASTM classification system) (American Society for Testing and Materials, 1988 Annual Book of ASTM Standards, pp 731 - 737, Designation: E 1195 - 87, "Standard Test Method for Determining a Sorption Constant (K_{oc}) for an Organic Chemical in Soil and Sediments".

(Grenfel RG 1995b)

IIA 7.4.2 **Adsorption and desorption of chemx degradation products**

Report (first of three)**:** IIA 7.4.2/01 Cranwel R 1996, Soil adsorption/desorption of ^{14}C-xxxxxxx (metabolite 2), by the batch equilibrium method, Chemco Report XX-14409

Guideline

OECD Test Guideline 106 ≅ EPA FIFRA Guideline § 163-1

Testing Laboratory and dates

The Chemco Research Laboratory, Richmond, California, conducted the study during the period November to December 1995.

GLP: Fully GLP compliant [17]

Executive Summary

In an adsorption / desorption study, the adsorption / desorption characteristics of xxxxxxx (metabolite 2) was studied using the batch equilibrium method, for 24 h in 2 US soil types and 2 UK soil types over the pH range 5.5 - 8.1. Adsorption partition coefficients (K_d values) ranged from 0.524 to 2.070 with corresponding

adsorption constants (K_{oc} values) ranging 60.9 to 260.8 for the 4 test soils. Desorption partition coefficients (K_d values) ranged from 1.792 to 5.514 with corresponding desorption constants (K_{oc} values) ranging 208.4 to 590.2 for the 4 test soils

The percent of the applied amount adsorbed in soils ranged from 14.77 % (Wick sandy loam) to 40.54 % (Evesham clay loam), while the percent desorbed ranged from 34.84 % (Evesham clay loam) to 63.45 % (Wick sandy loam). The average desorption K_{oc} value was 383.9. The desorption K_{oc} values were higher than those obtained for adsorption. The average materials balance from supernatants, extracts and combusted soil was 98.3 % ± 2.0 %.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** xxxxxxx (metabolite 2) - chem2 ring labelled: ^{14}C in the C-3 position; specific activity 116.35 µCi/mmol;

Description: White powder
Lot/Batch #: CP 180422
Purity: radiochemical purity ≥ 98.65 %
CAS #: 16478-13-8
Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. **Soils:** The adsorption/desorption characteristics of the xxxxxxx (metabolite 2) was determined in four soil types - two of UK origin and two USA origin (Table IIA 7.4.2-1) using the batch equilibrium method. These soils were collected from the top 0-15cm layer in fields that had not been treated with a pesticide for at least three years. Air dried soils were stored at ambient temperatures for x weeks prior to experimentation.

Table IIA 7.4.2-1 Soil physiochemical properties

Soil Name Origin Textural class	Wick UK Sandy loam	Evesham UK Clay loam	Dupo USA Silt loam	Sarpy-91 USA Sandy loam
% Sand	65.7	34.4	2.8	63.6
% Silt	16.7	28.0	81.6	22.8
% Clay	17.6	37.6	15.6	13.6
% OC*	0.86	1.96	0.57	0.41
% OM	1.46	3.34	0.97	0.70
CEC (meq/100g)	5.69	16.63	7.16	6.37
Bulk Density**	1.52	1.26	1.21	1.26
pH #	5.5	7.1	7.1	8.1
Field Capacity ($^1/_3$ bar)	15.86	26.57	28.84	16.11

* OC = OM/1.72 # measured in a 1 : 2.5 soil : water suspension ** Determined using disturbed soils

B. STUDY DESIGN

1. Experimental conditions

The pH of the equilibrium solution was x.x. Prepared stock solutions of xxxxxxx in acetonitrile were mixed with aqueous 0.01 M $CaCL_2$ to give test solution concentrations of 0.04, 0.10, 0.25 and 1.0 µg/ml (*circa* 0.078 % acetonitrile). Data from a preliminary study indicated an absorption range of 25 - 58 % using a 3 : 1 solution to soil ratio (10 g soil : 30 ml aqueous solution). Based on the results of the preliminary testing this optimised ratio was chosen for the definitive study, which involved 24 hours equilibration at 25°C in darkness.

Following equilibration the test samples were centrifuged (x rpm for y minutes), supernatant volumes measured and aliquots taken for radioassay. Desorption studies were performed on the soil remaining following removal of the adsorption solution, with the addition of 0.01 M $CaCL_2$ solution equivalent to the volume removed during the adsorption phase. Tubes were again equilibrated by placing them in a shaking water bath for an additional 24 hours in darkness at 25°C. Following equilibration, the tubes were centrifuged and the supernatant solutions were decanted, their volumes were measured and aliquots were taken for radioassay.

2. Description of analytical procedures

Samples of the solutions from the highest test concentration from both the adsorption and desorption studies were analysed by HPLC to assess stability during equilibration. LSC was used to determine the concentrations of the adsorption and desorption solutions and for the HPLC recovery analysis. Radioactivity in residual soil samples was determined by combustion followed by LSC. Chromatography using normal phase TLC and GC-MS were used to confirm the identity of the degradation products detected. The limit of detection (LOD) for xxxxxxx (metabolite 2) was x µg as/g soil. The limit of quantification (LOQ) for xxxxxxx (metabolite 2) was y µg as/g soil.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The average materials balance from supernatants, extracts and combusted soil was 98.3 % ± 2.0 %. The percent absorbed ranged from 14.77 % (Wick sandy loam) to 40.54 % (Evesham clay loam), while the percent desorbed ranged from 34.84 % (Evesham clay loam) to 63.45 % (Wick sandy loam)

B. FINDINGS

Adsorption partition coefficients (Kd values) ranged from 0.524 - 2.070 with corresponding adsorption constants (Koc values) ranging 60.9 - 260.8 for the 4 test soils. Desorption partition coefficients (Kd values) ranged from 1.792 - 5.514 with corresponding desorption constants (Koc values) ranging 208.4 - 590.2 for the 4 test soils (Table IIA 7.4.2-2). The % adsorbed and desorbed xxxxxxx (metabolite 2) at each concentration is provided in Table IIA 7.4.1-3.

Table IIA 7.4.2-2 Adsorption/desorption constants and correlation coefficients for xxxxxxx (metabolite 2) in four test soils

				Adsorption				Desorption			
Soil Type		%OC	pH	Kd	r	Koc	$^1/_n$	Kd	R	Koc	$^1/_n$
Wick	Sandy loam	0.86	5.5	0.524	1.000	60.9	1.123	1.792	0.992	208.4	0.972
Evesham	Clay loam	1.96	7.1	2.070	1.000	105.6	1.115	5.514	0.999	281.3	1.067
Dupo	Silt loam	0.57	7.1	1.485	1.000	260.5	1.160	3.364	0.997	590.2	1.006
Sarpy-91	Sandy loam	0.41	8.1	0.914	0.999	222.9	1.076	1.869	0.997	455.7	0.900
Mean						162.5				383.9	

Kd = partition coefficient
Koc = organic carbon normalised Kd
$^1/_n$ = 1/slope of linear regression of Freundlich equation
r = correlation coefficient

Table IIA 7.4.2-3 Adsorbed and desorbed xxxxxxx (metabolite 2) at each concentration tested

Soil type	Adsorbed concentration (µg as/mL)				Desorbed concentration (µg as/mL)			
	0.04	0.10	0.25	1.0	0.04	0.10	0.25	1.0
Wick sandy loam								
Evesham clay loam								
Dupo silt loam								
Sarpy-91 sandy loam								

III. CONCLUSIONS

Adsorption appeared to correlate with soil organic matter content and was more pronounced at alkaline pH values and was retarded at low pH as evidenced by adsorption in the Wick soil. Desorption was inversely related to the percent adsorbed.

Based on Koc values recorded, xxxxxxx is likely to be highly mobile in Wick and Evesham soil types (UK) and moderately mobile in Dupo and Sarpy-91 (USA).

(Cranwel R 1996a)

Report (second of three)**:** IIA 7.4.2/02 Cranwel R 1996, Soil adsorption/desorption of ^{14}C-desmethyl chemx (metabolite 1), by the batch equilibrium method, Chemco Report XX-14410

Guideline

ECD Test Guideline 106 ≅ EPA FIFRA Guideline § 163-1

Testing Laboratory and dates

The Chemco Research Laboratory, Richmond, California, conducted the study during October 1995.

GLP: Fully GLP compliant [17]

Executive Summary

In an adsorption / desorption study, the adsorption / desorption characteristics of desmethylchemx (metabolite 1) was studied for 24 h, using the batch equilibrium method, in 2 US soil types and 2 UK soil types over the pH range 5.5 - 8.1. The adsorption partition coefficients (K_d values) for ^{14}C-desmethyl chemx ranged from 0.316 to 0.732 with corresponding K_{oc} values ranging from 36.7 to 116.0 for the four test soils. Desorption partition coefficients (K_d values) ranged from 2.195 to 5.252 and the corresponding K_{oc} values were 255.2 to 819.6 for the four test soils.

The percent of the applied amount adsorbed in soils ranged from 17.25 % (Wick sandy loam) to 32.48 % (Evesham clay loam). The average desorption K_{oc} value was 485.8. The percent of adsorbed amount desorbed in soils ranged from 23.11% (Evesham clay loam) to 40.00% (Wick sandy loam). The desorption K_{oc} values were higher than those obtained for adsorption. The mass balance for the study amounted was 100.2 % ± 2.8 %.

Desmethylchemx exhibited greater potential mobility than xxxxxxx (metabolite 2), K_{oc} = 37 in UK soils and ~ 110 in US soils. Based on the desorption constants obtained (K_{oc} values of 255.2 - 819.6 for the four test soils, it would appear that desmethylchemx once adsorbed has greater soil binding capacity than xxxxxxx (metabolite 2).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** chem3 ring labelled desmethyl chemx (metabolite 1): ^{14}C in the C-5position; specific activity 103.5 µCi/mmol;
   - **Description:** White powder
   - **Lot/Batch #:** CP 187619
   - **Purity:** radiochemical purity 100 %
   - **CAS #:** 16478-13-8
   - **Stability of test compound:** The test material was stable for at least 7 days at room temperature.

2. **Soils:** The adsorption/desorption characteristics of desmethyl chemx was determined in four soil types - two of UK origin and two USA origin the same soils as used in the study with xxxxxxx (metabolite 2) (reference IIA 7.4.2/01, Cranwel R 1996a), using the batch equilibrium method.

B. STUDY DESIGN

1. Experimental conditions

The pH of the equilibrium solution was x.x. Stock solutions of desmethyl chemx were prepared in dimethyl formamide and mixed with 0.01 N1 $CaCL_2$ to give test solution concentrations of 0.04, 0.10, 0.25 and 1.0 µg/ml (*circa* 0.085 % dimethyl formamide). Data from a preliminary study indicated an absorption range of 18 - 35 % using a 1.5 to 1 solution to soil ratio (20 g soil : 30 ml aqueous solution. Based on the results of the preliminary testing, this optimised ratio was chosen for the definitive study, which involved 24 hours equilibration at 25°C in darkness.

Following equilibration the test samples were centrifuged (x rpm for y minutes), supernatant volumes measured and aliquots taken for radioassay. Desorption studies were performed on the soil remaining following removal of the adsorption solution, with the addition of 0.01 M $CaCL_2$ solution equivalent to the volume removed during the adsorption phase. Tubes were again equilibrated by placing them in a shaking water bath for an additional 24 hours in darkness at 25°C. Following equilibration, tubes were centrifuged and the supernatant solutions were decanted, their volumes measured and aliquots were taken for radioassay.

2. Description of analytical procedures

Samples of the solutions from the highest test concentration from both the adsorption and desorption studies were analysed by HPLC to assess stability during equilibrium. LSC was used to determine the concentrations of the adsorption and desorption solutions and for the HPLC recovery analysis. Radioactivity in residual soil samples was determined by combustion followed by LSC. Chromatography using normal phase TLC and GC-MS were used to confirm the identity of the degradation products detected. The limit of detection (LOD) for desmethyl chemx (metabolite 1) was x µg as/g soil. The limit of quantification (LOQ) for desmethyl chemx (metabolite 1) was y µg as/g soil.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The average materials balance from supernatants, extracts and combusted soil was 100.2 % ± 2.8 %. The percent absorbed ranged from 17.25 % (Wick sandy loam) to 32.48 % (Evesham clay loam), while the percent desorbed ranged from 23.11 % (Evesham clay loam) to 40.00 % (Wick sandy loam).

B. FINDINGS

The adsorption partition coefficients (Kd values) for ^{14}C-desmethyl chemx ranged from 0.316 - 0.732 with corresponding Koc values of 36.7 - 116.0 for the four test soils (Table IIA 7.4.2-4). Desorption partition coefficients (Kd values) ranged from 2.195 - 5.252 and corresponding Koc values 255.2 - 819.6 for the four test soils. The % adsorbed and desorbed desmethyl chemx (metabolite 1) at each concentration is provided in Table IIA 7.4.1-5.

Table IIA 7.4.2-4 Adsorption/desorption constants and correlation coefficients for desmethyl chemx (metabolite 1) in four test soils

				Adsorption				Desorption			
Soil Type		%OC	pH	Kd	r	Koc	$^1/_n$	Kd	r	Koc	$^1/_n$
Wick	Sandy loam	0.86	5.5	0.316	0.999	36.7	1.169	2.195	0.983	255.2	0.898
Evesham	Clay loam	1.96	7.1	0.732	1.000	37.3	1.183	5.252	0.993	288.0	0.860
Dupo	Silt loam	0.57	7.1	0.661	1.000	116.0	1.132	4.672	0.999	819.6	0.996
Sarpy-91	Sandy loam	0.41	8.1	0.428	1.000	104.4	1.082	2.380	0.999	580.5	1.030
Mean						73.6				485.8	

Kd = partition coefficient
Koc = organic carbon normalised Kd
$^1/_n$ = 1/slope of linear regression of Freundlich equation
r = correlation coefficient

Table IIA 7.4.2-5 Adsorbed and desorbed desmethyl chemx (metabolite 1) at each concentration tested

Soil type	Adsorbed concentration (µg as/mL)				Desorbed concentration (µg as/mL)			
	0.04	0.10	0.25	1.0	0.04	0.10	0.25	1.0
Wick sandy loam								
Evesham clay loam								
Dupo silt loam								
Sarpy-91 sandy loam								

III. CONCLUSIONS

The adsorption pattern for desmethyl chemx was similar to that for xxxxxxx (metabolite 2) but desmethyl chemx, once adsorbed appeared to be more tightly bound than xxxxxxx.

Based of the Koc values determined, it can be concluded that desmethyl chemx is very mobile in Wick and Evesham (UK) soils and moderately mobile in Dupo and Sarpy-91 (USA) soils (ASTM classification).

(Cranwel R 1996b)

Report (third of three)**:** IIA 7.4.2/03 Somerville A 1996, Adsorption/desorption of ^{14}C-metabolite 3, a soil metabolite of chemx, Chemco Report XX-14437

Guideline

OECD Test Guideline 106 ≅ EPA FIFRA Guideline § 163-1

Testing Laboratory and dates

The Chemco Research Laboratory, Richmond, California, conducted the study during the period March to May 1991.

GLP: Fully GLP compliant [17]

Executive Summary

In an adsorption / desorption study, the adsorption / desorption potential of metabolite 3 was studied using the batch equilibrium method, for 24 h in 4 US soil types. Adsorption coefficients (K_d values) for metabolite 3 ranged from 2.32 - 165.6 with corresponding K_{oc} values ranging from 259.98 - 8279.97. Desorption partition coefficients (K_d values) ranged from 4.88 - 193.97 for first desorption and from 7.94 - 210.36 for second desorption and followed a similar order.

Both K_d and K_{oc} values appeared to increase with increasing organic carbon content. The results obtained suggest moderate mobility in two soil types, Spinks loamy sand and Sarpy silt loam, low mobility in Drummer silt loam and immobility in Sable silt/clay loam.

Some 94 - 96.5 % of applied radioactivity from the Sarpy and Spinks soils was accounted for (determined on 0.97 µg/ml samples), while in the Drummer and Sable soils recoveries averaged 82 % and 62 % respectively. A second analysis of the Drummer and Sable soils treated with 0.1 µg/ml showed recoveries of 95 % and 96.8 % (the research workers involved suggested that some of the test soil radioactivity might have been lost during vacuum soil drying, resulting in low initial recoveries in these soils).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** chem3 ring labelled metabolite 3 of chemx: ^{14}C in the C-5position; specific activity 27.4 mCi/mmol;
   Description: White powder
   Lot/Batch #: CP 1893419
   Purity: radiochemical purity ≥ 99.53 %
   CAS #: 16478-13-8
   Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. **Soils:** The study comprised part of a larger study conducted to support the registration of another chemical. The soils used differed from those used for the aerobic soil metabolism studies and for the determination of the adsorption constants of chemx

and its two main metabolites. The soils used nevertheless represented an appropriate range of characteristics (Table IIA 7.4.2-6). It therefore was not considered necessary to repeat the study using different soils. The soils used were collected from the top 0-15cm layer in fields that had not been treated with a pesticide for at least three years. Air dried soils were stored at ambient temperatures for x weeks prior to experimentation.

Table IIA 7.4.2-6 Soil physiochemical parameters

Soil Type	Drummer	Sarpy	Spinks	Sable
Origin	USA	USA	USA	USA
Textural class	Silt loam	Sandy loam	Loamy sand	Silt/Clay loam
% Sand	23	59	77	7
% Silt	53	31	19	62
% Clay	24	10	4	31
% OM*	2.1	0.8	1.8	3.5
% OC	1.78	0.58	1.15	2.0
CEC meq/100g	22.1	10.3	6.3	30.3
pH **	6.9	8.0	6.8	5.8
% Moisture ($^1/_3$ Bar)	27.10	12.99	9.48	29.5
Bulk density	1.11	1.11	1.25	1.13

* Determined by colourimetry # measured in a 1 : 2.5 soil : water suspension

B. STUDY DESIGN

1. Experimental conditions

The pH of the equilibrium solution was x.x. Metabolite 3 in acetonitrile was used to prepare two test solutions one using pH 5.5 buffer, the other pH 7.7 buffer. The pH 5.5 buffered solutions were used in the experiments with Drummer, Sable and Spinks soils, while the pH 7.7 buffered solutions were used with Sarpy soil, in order not to modify soil pH. Aqueous test solution concentrations with the pH 5.5 buffer : 0.01 M $CaSO_4$, were 0.97, 0.68, 0.35 and 0.10 µg/ml. The concentrations of the pH 7.7 buffer : 0.01 M $CaSO_4$ adjusted to pH 7.7 with 0.01 M $Ca(OH)_2$, were 0.97, 0.68, 0.35 and 0.10 µg/ml.

On the basis of preliminary experiments, an optimised solution to soil ratio of 5 : 1 (1 g soil : 5 ml solution) and an equilibration period of six hours at ambient temperature was established. Samples were centrifuges for x minutes at y rpm. The stability of metabolite 3 in the test system during the equilibration period was determined by HPLC analysis. The adsorption desorption experiments consisted of one adsorptive cycle followed by two desorptive periods of equal length.

2. Description of analytical procedures

Following each adsorption/desorption phase, samples were centrifuged and radioactivity in the supernatant was determined by LSC. Radioactivity remaining in soil samples was quantified by combustion analysis, and radioactivity in HPLC eluate was quantified using a flow through detector. Chromatography using normal phase TLC and GC-MS were used to confirm the identity of the degradation products detected. The limit of detection (LOD) metabolite 3 was x µg as/g soil. The limit of quantification (LOQ) for metabolite 3 was y µg as/g soil.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Some 94 - 96.5 % of applied radioactivity from the Sarpy and Spinks soils was accounted for (determined on 0.97 µg/ml samples), while in the Drummer and Sable soils recoveries averaged 82 % and 62 % respectively. A second analysis of the Drummer and Sable soils treated with 0.1 µg/ml showed recoveries of 95 % and 96.8 % (the research workers involved suggested that some of the test soil radioactivity might have been lost during vacuum soil drying, resulting in low initial recoveries in these soils).

B. FINDINGS

Adsorption coefficients (Kd values) for metabolite 3 ranged from 2.32 - 165.6 with corresponding Koc values ranging from 259.98 - 8279.97. Desorption partition coefficients (Kd values) ranged from 4.88 - 193.97 for first desorption and from 7.94 - 210.36 for second desorption and followed a similar order. The % adsorbed and desorbed metabolite 3 at each concentration is provided in Table IIA 7.4.1-8.

Table IIA 7.4.2-7 Adsorption/Desorption constants for metabolite 3 in four US Soils

			Adsorption			Desorption	
Soil Type	% OC	pH	Kd	Koc	$^1/_n$	Kd_1	Kd_2
Drummer silt loam	1.78	6.9	18.56	1042.43	0.72	28.05	35.54
Sarpy silt loam	0.58	8.0	2.32	399.55	0.68	4.88	7.94
Spinks loamy sand	1.15	6.8	2.99	259.98	0.79	7.10	12.19
Sable silt/clay loam	2.0	5.8	165.60	8279.97	0.56	193.97	210.36
Mean				2495			

Kd = Partition coefficient
Kd_1 = Partition coefficient (first desorption)
Kd_2 = Partition coefficient (second desorption)
Koc = Kd/% O.C* 100
$^1/_n$ = Reciprocal of the Freundlich exponent constant

Table IIA 7.4.2-8 Adsorbed and desorbed metabolite 3) at each concentration tested

Soil type	Adsorbed concentration (µg as/mL)				Desorbed concentration (µg as/mL)			
	0.10	0.35	0.68	0.97	0.10	0.35	0.68	0.97
Drummer silt loam								
Sarpy silt loam								
Spinks loamy sand								
Sable silt/clay loam								

III. CONCLUSIONS

Both adsorption coefficients (Kd values) and Koc values appear to increase with increasing organic carbon content of soils.

Metabolite 3 can be classified as having moderate mobility in Sarpy and Spink soils, low mobility in Drummer soil and as being immobile in Sable soil (ASTM classification).

(Somerville A 1996)

IIA 7.4.3 Column leaching studies with the active substance

Column leaching studies were not conducted since reliable adsorption coefficient values were obtained in the adsorption/desorption study reported (point IIA 7.4.1) (Grenfel RG 1995b).

IIA 7.4.4 Column leaching studies with relevant metabolites, degradation and reaction products

Column leaching studies were not conducted since reliable adsorption coefficient values were obtained in the adsorption/desorption studies reported (point IIA 7.4.2) (Cranwel R 1996a, Cranwel R 1996b, Somerville A 1996).

IIA 7.4.5 Aged residue column leaching studies

Although it could be argued that it is not necessary to conduct and report such a study since reliable adsorption coefficient values were obtained for the main metabolites of chemx in the adsorption/desorption studies reported (point IIA 7.4.2) (Cranwel R 1996a, Cranwel R 1996b, Somerville A 1996), a study was conducted and submitted.

Report: IIA 7.4.5/01 Grenfel RG 1995, ^{14}C-chemx - Soil column leaching of aged residues, Chemco Report XX-13702

Guideline

BBA Guidelines for the official testing of plant protection products (Part IV, 4-2) ≅ Procedures for assessing the environmental fate and behaviour of pesticides, SETAC-Europe, 1995, ISBN Number 90-5607-002-9. The SETAC guidelines specify that the soil used should have an organic matter content of 1.5 to 2.5 %. The soil actually used had an organic matter content of 1.2 %. A second deviation from the SETAC guidelines was that the soil was incubated for 100 days after treatment rather than the SETAC-specified “one half-life or 30 days (whichever is shorter)”.

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England conducted the study during the period September 1993 to May 1994.

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In an aged soil column leaching study, the leaching behaviour of chemx and its transformation products was studied for 100 days using a Speyer 2.1 standard German soil in 5 cm soil columns. The soils were treated with a rate of xx mg as/kg soil (equivalent to 40 g as/ha - twice the recommended field rate) and aged for 100 days under aerobic conditions at 20 °C and 12.3 % moisture. Most of the applied radioactivity remained in the soil columns (64 – 67 %) or leached through the soil columns (30 – 39 %). At the end of the 100-day study period, 13 % of applied radioactivity was present as unextracted soil residue.

Analysis of soil column sections showed that most of the applied parent compound remained in the top soil layer (23 % of applied amount in the 0-5 cm depth) or was distributed throughout the column. The transformation product(s) desmethylchemx, xxxxxxx (metabolite 2), metabolite 3, and xxxx metabolite 10 remained mostly in the top soil layer (3 % of the applied dose in 0-5 cm depth) or were distributed throughout the soil column. The mass balance at the end of study amounted to 91 % of applied radioactivity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - 1 : 1 mixture of chem2 ring labelled and chem3 ring labelled
chem2 ring labelled: ^{14}C in the C-3 position; specific activity 28.63 mCi/mmol;
chem3 ring labelled: ^{14}C in the C-5 position; specific activity 34.22 mCi/mmol;

Description: White powder
Lot/Batch #: NPD-9307-5385-T
Purity: chem2 ring labelled: radiochemical purity > 98 %
chem3 ring labelled: radiochemical purity ≥ 99 %
CAS #: 16335-17-2
Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Soils: The soil used was a Spayer 2.1 standard German soil with properties as indicated in Table IIA 7.4.5-1.

Table IIA 7.4.5-1 Soil physiochemical properties

Soil Type	% Sand	% Silt	% Clay	% OC	% OM	Bulk Density (g/cm^3)	pH	CEC (meq/100g)	% MWHC	Microbial Biomass
Speyer 2.1	87.4	9.1	3.5	0.7	1.2*	1.5	5.9	4.9	24.6	82**

* Below SETAC guideline 1.5 - 2.5 %
** Low level of microbial biomass

B. STUDY DESIGN

1. Experimental conditions

Samples of 2 mm-sieved Speyer 2.1 soil (equivalent to 100 g air-dried weight) were weighed into each of nine flasks. Sufficient water was added to bring the soil in each flask to 50 % of its maximum water holding capacity (12.3 % moisture content). The flasks were incubated in the dark for seven days prior to application of the test substance as a methanol/water solution (20 / 80 v/v). Four of the flasks were treated with the test substance solution four days prior to application to the four of the remaining flasks that were for use in evaluating the extent of degradation prior to preparation of the soil columns. One flask, which had not been treated with the test substance solution, was taken for microbial biomass analysis. Following application of chemx at a rate of 40 g as/ha (twice the recommended field use rate) the soil was incubated at 20 °C for 100 days in darkness.

Single vessels were taken immediately and at 26 days after application to permit measurement of the any degradation of chemx under the experimental conditions used. After 100 days, duplicate flasks were analysed to determine the proportions of chemx and metabolites present in the remaining flasks.

At 100 days, the contents of two of the remaining flasks were transferred to the tops of duplicate columns packed with Speyer 2.1 soil - sectioned soil columns (30 cm x 5 cm x 6 sections). The columns had been prepared with test soil infiltrated with HPLC grade water until saturated and allowed to drain under gravity. Following transfer of the contents of the aged incubation flasks to the top of the soil columns, the columns were eluted with HPLC water at a rate of 8 ml/hour (total volume of 394 ml, equivalent to 20 cm rainfall), and the leachate was collected. Columns, each containing xx g of soil, were maintained at ambient temperature and were protected from light using aluminium foil.

Following elution soil columns were sectioned into 5 cm sections which were separately extracted.

2. Description of analytical procedures

Soil sections were extracted x times with y mL of acetonitrile. The soil extracts were pooled and concentrated under vacuum. The column leachate was filtered through a x µm filter and analysed. Following extraction the recovery of applied radioactivity in the extracts was determined using LSC and in the residue by combustion. Reverse phase HPLC and normal phase TLC were performed on all samples - radiolabelled degradation products were characterised using co-chromatography with authentic standards. Quantification of radioactivity was done using LSC in the case of liquid samples, combustion in the case of soils and using a linear analyser for plates. The limit of detection (LOD) for chemx and metabolite 1 were x and x µg as/g soil, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 were y and y µg as/g soil, respectively.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Radioactivity recovered decreased from 103 % of applied radioactivity at zero time to 91 % after incubation for 100 days. Radioactivity recovered in soil extracts declined from 93 % of applied radioactivity to 78 % following the 100 day incubation period, while unextracted radioactivity increased from 10 % of applied radioactivity to 13 %.

B. FINDINGS

The proportion of radioactivity accounted for by chemx declined from 93 % at zero time to 62 % at day 100 days, demonstrating a slow degradation rate for chemx in this soil (Table IIA 7.4.5-2). Two unidentified components A and C were detected which represented 1 - 8 % of the applied radioactivity (mean values).

Table IIA 7.4.5-2 Content of parent compound and degradation products in chemx treated Speyer 2.1 soil at zero time and after ageing for 100 days

Radioactive Component	% Applied Radioactivity		
	Zero days	100 Days	
		Sample 1	Sample 2
Chemx	93	62	62
Metabolite 3	<1	<1	<1
Desmethyl chemx	<1	1.4	1.0
xxxx (metabolite 10)	<1	<1	<1
xxxxxxx (metabolite 2)	<1	1	2.2
Component A	<1	11	4.8
Component C	<1	1	<1

The results obtained demonstrated that most of the applied radioactivity was retained in the soil columns. Some 30 and 39 % of the applied radioactivity was recovered in the column leachates (Table IIA 7.4.5-3). The majority of the retained radioactivity (47 and 49 % of the applied radioactivity) was in the top 0 - 5 cm section of the columns, with the remainder evenly distributed throughout the rest of the columns. In all, 64 and 67 % of the applied radioactivity was present in the soil columns and overall recoveries were 94 and 106 % of the applied radioactivity.

Table IIA 7.4.5-3 Distribution of radioactivity in Speyer 2.1 soil columns following leaching

Soil Column	% Applied Radioactivity	
Depth (cm)	Column 1	Column 2
0 – 5	47	49
5 – 10	2.1	2.3
10 – 15	2.5	2.4
15 – 20	3.0	2.7
20 – 25	4.4	4.2
25 – 30	5.1	6.7
Total soil sections	64	67
Total leachate	30	39
Recovery	94	106

The volume of leachate collected was x mL. Analysis of the various components (parent compound and degradation products) present in the top 5 cm of soil and in the leachate, showed that chemx was the major component recovered with a maximum value of 39 % of applied radioactivity in the column leachate (Table IIA 7.4.5-4). Both desmethyl chemx and xxxxxxx (metabolite 2) represented individually *circa* 2 % of the applied radioactivity in the leachate.

The distribution of parent compound and degradation products in the soil columns and leachate is provided in Table IIA 7.4.5-5.

Table IIA 7.4.5-4 Proportions of components in column leachates and soil column sections (0 - 5 cm) from Speyer 2.1 soil following aged leaching

	% Radioactivity applied to soil column			
Radioactive	Soil column extract (0-5cm)		Soil column leachate	
Component	Column 1	Column 2	Column 1	Column 2
Chemx	19	23	25	39
Metabolite 3	0.41	0.51	<1.7	<0.4
Desmethyl chemx	1.1	0.62	2.7	<0.4
xxxx (metabolite 10)	0.62	0.32	<1.7	<0.4
xxxxxxx (metabolite 2)	0.89	0.92	2.0	<0.4
Component A	0.86	0.62	<1.7	<0.4
Component C	0.62	0.43	<1.7	<0.4

Table IIA 7.4.5-5 Distribution of chemx and its degradation products in the soil column and leachate

Soil layer	% of applied radioactivity						
	Chemx	desmethyl chemx (metabolite 1)	xxxxxxx (metabolite 2)	Metabolite 3	xxxx (metabolite 10)	Component A	Component C
0-5							
5-10							
10-15							
15-20							
20-25							
25-30							
Leachate							

III. CONCLUSIONS

The results obtained indicated that chemx exhibits mobility in Speyer 2.1 soil columns although up to 23 % of the applied radioactivity was retained in the surface layer. The study also confirmed that both desmethyl chemx and xxxxxxx (metabolite 2) are somewhat mobile in this soil while metabolite 3 has limited mobility.

These findings are generally in accordance with the results of the adsorption/ desorption studies previously reported.

Since there was very limited degradation of chemx in the test soil (DT_{50} > 100 days), the study is not regarded as a good indicator of metabolite mobility. The results obtained were possibly influenced by the low microbial biomass of the test soil.

(Grenfel RG 1995c)

IIA 7.4.6 **Leaching (TLC)**

TLC leaching studies were not conducted since reliable adsorption coefficient values were obtained in the adsorption/desorption studies reported (points IIA 7.4.1 and IIA 7.4.2) (Grenfel RG 1995b, Cranwel R 1996a, Cranwel R 1996b, Somerville A 1996).

IIA 7.4.7 **Lysimeter studies**

In the light of the results obtained in the adsorption/desorption studies and the aged column leaching study, it was concluded that lysimeter or field leaching studies conducted under conditions which reflect the proposed recommendations for use of chemx would be required. Accordingly a 3-year field lysimeter study using radiolabelled chemx was initiated in April 1994. The summary and evaluation that follows is based on an interim report of the study covering the first two years of leachate collection. On completion of the third year of leachate collection, the lysimeter will be removed and sectioned in preparation for analysis of the soil. The final report will be submitted when it becomes available in March 1998.

Report: IIA 7.4.7/01 Lockrel S 1997, ^{14}C-chemx - Mobility and degradation in soil in outdoor lysimeters (Interim Report), Chemco Report XX-14576

Guideline

BBA Guidelines for the official testing of plant protection products (Part IV, 4-3), February 1990 and Modification of the Lysimeter Guideline (Part IV, 4-3), September 1991.

Testing Laboratory and dates

The study was conducted during the period April 1994 to January 1997 by the Chemco Research Laboratory, Bonn, Germany.

GLP: Fully GLP compliant.

Executive Summary

In a three-year lysimeter study conducted using a soil type that is susceptible to leaching, preliminary results were reported. The study, which involved use of radiolabelled material, was undertaken to clarify and quantify more fully the extent of mobility of chemx and its soil degradation products under practical use conditions.

A recovery of *circa* 8 % of the applied radioactivity was obtained in the leachate. On analysis the leachate was shown to contain significant amounts of chemx (parent compound) at peak times together with a known soil metabolite xxxxxx (metabolite 6) (M9) and an unknown component (M10) both of which were present in quantities exceeding 0.1 µg/l.

None of the main soil metabolites identified - xxxxxxx (metabolite 2), desmethyl chemx - were detected in this study. Taken together with the low level of leaching of parent compound, these results suggest that strong binding to soil occurs, which is contrary to predictions made on the basis of the adsorption/desorption and column leaching studies. The absence of metabolite 3 in soil leachates is consistent with predictions based on adsorption/desorption studies - average K_{oc} = 2495 (Somerville A 1996).

The preliminary results reported for the lysimeter study suggest a low mobility of both chemx and its common soil metabolites in this sandy soil. Most of the radioactivity recovered in the leachate consisted of polar metabolites most of which are unidentified, apart form xxxxxx (metabolite 6) an identification that has yet to be confirmed using spectroscopic and other means. Final conclusions from this study must await the assessment of the final report of the study and in particular an examination of the leachate data for the third year and examination of the residue data for the soil segments.

The study involved treatment with 30 g as/ha on both lysimeters while the proposed maximum rate of application for chemx is 20 g as/ha which proportionally should result in concentrations in the leachate being reduced by a third under normal use conditions (Volkel W and A Burgener 1997). Accordingly, following application at the recommended field rate (20 g as/ha) and assuming the leachate would contain residues in the same proportion as recorded in this study the expected mean concentrations of the two components M9 and M10 in the leachate would be 0.07 and 0.08 µg/L after two years.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - 1 : 1 mixture of chem2 ring labelled and chem3 ring labelled
chem2 ring labelled: ^{14}C in the C-3 position; specific activity 30.5 and 29.3 mCi/mmol;
chem3 ring labelled: ^{14}C in the C-5 position; specific activity 30.5 and 29.3 mCi/mmol;

Description: White powder
Lot/Batch #: NPD-9307-5385-T
Purity: chem2 ring labelled: radiochemical purity > 99 %
chem3 ring labelled: radiochemical purity ≥ 99 %
CAS #: 16335-17-2
Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Soils: Soil was taken from agricultural land in Borstel, Lower Saxony, Germany. The physical, chemical and biological properties of the soil are provided in Table IIA 7.4.7-1. Undisturbed soil monoliths taken were taken from a site that had not been treated with a pesticide for at least three years.

Table IIA 7.4.7-1 Soil physicochemical properties

Parameter	Horizon [cm]			
	0-30	30-60	60-90	90-120
pH (KCl)	6.1	5.9	6.1	7.3
Organic carbon (%)	1.05	0.49	0.14	0.00
Cation exchange capacity (meq/100 g dry soil)	5.62	4.06	2.51	0.94
Particle size (mm)[a]				
a) Classification (ISSS)				
< 0.002 (clay) %	5.6	2.6	3.2	0.3
0.002-0.02 (silt) %	6.4	7.3	3.5	0.0
> 0.02 (sand) %	88.0	90.1	93.3	99.7
b) Classification (USDA):				
< 0.002 (clay) %	5.6	2.6	3.2	0.3
0.002-0.05 (silt) %	10.9	8.4	5.8	0.1
> 0.05 (sand) %	83.5	89.0	91.0	99.6
Soil density (g/cm^3)	1.34	1.50	1.62	1.41
Field capacity (FC)[b]	17.9	15.5	11.6	7.4
Max. water holding capacity (MWC)[b]	34.5	28.6	23.2	23.6
Biomass (mg C/100 g dry soil)[c]				
Spring, 1994	24.1	np[d]	np	np
Spring 1995	33.1	Np	np	np
Spring 1996	22.4	Np	np	np

[a] Particle size analysis was performed using the pipette method (International and USDA classification, Soil Taxonomy, 1975)
[b] g water/100 g dry soil
[c] Determined by the method of Anderson and Domsch, *Soil Bio. Biochem.*, 10 215-221.
[d] np = not performed

B. STUDY DESIGN

1. Experimental conditions

Two open field lysimeters containing sandy soil monoliths of low organic carbon content (depth 1.2 m, surface area 1.0 m^2), surrounded by a small field plot of about 2.2 by 4 meters were chosen for treatment to represent field conditions. A third control lysimeter was used for determinations of soil temperatures and for generation of biomass data. The filled lysimeter containers were allowed to settle for 2 years prior to starting the study.

Fertilization, seedbed preparation, sowing and harvest were carried out at normal times for the corresponding crop and were in accordance with normal agricultural practice. Shortly before sowing, the topsoil of the lysimeters and the surrounding plot was weeded manually. The topsoil was ploughed using a spade to a depth of about 10 cm and was prepared for sowing using a rake. The standard fertilization regime for winter wheat was followed. Pesticide treatments (mecoprop-P and pirimicarb)

were used for weed and insect control but were kept to a minimum to avoid possible interaction with the test substance.

The test material was applied post-emergence to wheat at the Zadoks growth stage of 30 to 31 at an application rate equivalent to 30 g as/ha (1.5 times the recommended field use rate) using an automatic spraying device with a full cone nozzle. As specified in the modified guideline, one of the lysimeters received a second application one year after the first application, again at a rate equivalent to 30 g as/ha. Untreated areas cultivated with the same crops as used for the lysimeters surrounded the lysimeters. After cultivation of winter wheat for two seasons, winter barley was grown. All lysimeters and surrounding plots were subject to normal crop cultivation.

In the first year following treatment, precipitation amounted to 1106 mm, and in the second year 1036 mm with irrigation applied once in April using 6 litres of water/m^2. Air temperature, soil temperature, air humidity and wind speed data were collected over the experimental period.

2. Sampling

Leachate samples were collected monthly or more frequently if necessitated by the volumes of leachate.

3. Description of analytical procedures

Leachate samples containing > 0.05 µg/l parent equivalents after CO_2 stripping were analysed by two HPLC methods, with total radioactivity determined by LSC, and TLC chromatograms used to confirm the presence of chemx. Standards of some 10 known degradation products were used as reference compounds during analysis. Spiked control samples with test concentrations of 0.05 µg/L and 0.5 µg/L were used to assess the recovery of chemx obtained that averaged *circa* 97 %. The limit of quantification for leachate extracts using HPLC analysis was 0.01 µg/L and the limit of detection was < 0.01 µg/L. The limit of quantification for combusted plant and soil samples was 0.05 µg/kg and the limit of detection was < 0.01 µg/kg.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Treatment application was made in April 1994 and lysimeters discharges commenced in May 1994. During the summer period no leachate was discharged since evapo-transpiration exceeded precipitation. On average 1 sample per month was collected from autumn to spring except for periods of heavy precipitation. Precipitation over the two years averaged 106 % of the long-term average for the area for the first year and 99 % for the second year. In the second year precipitation was supplemented by irrigation during April.

Recovery volumes of leachate were higher in the first year than the second year but in terms of total radioactivity recovered were comparable 7.9 % as against 7.0 % for the two lysimeters.

B. FINDINGS

In lysimeter 4 a total of 3.5 % of the applied radioactivity, equivalent to 0.17 µg/L, was recovered in leachates after the first year, which increased to 4.38 % of the applied radioactivity, equivalent to 0.28 µg/L in the second year despite receiving only one application. In lysimeter 6 a total of 4.1 % of the applied radioactivity, equivalent to 0.21 µg/L was recovered in first year leachate. In the second year, following the repeat application 4.94 % of the applied radioactivity, equivalent to 0.69 µg/L, was recovered in the leachate. The annual percentages of total radioactivity recovered in the leachate from lysimeter 6 are not additive because of the two treatments.

In total *circa* 8 % of the applied radioactivity, equivalent to 0.21 µg/L chemx equivalents and 7 % of the applied radioactivity, equivalent to 0.42 µg/L chemx equivalents, was leached from both lysimeters over the two years of the study. The mineralization of leached $^{14}CO_2$ was negligible in both lysimeters.

Table IIA 7.4.7-2 Leachate volumes and mass balance of radioactivity for chemx treated lysimeters

		Lysimeter 4				Lysimeter 6			
Application 1994 Application 1995		2.920 mg no treatment				2.919 mg 2.882 mg			
Year	Precipitation + Irrigation (mm)	Volume (L)	Leachate Total Radioactivity (%)	(µg/L[1])	$^{14}CO_2$ (%)	Volume (L)	Leachate Total Radioactivity (%)	(µg/L[1])	$^{14}CO_2$ (%)
1	1105.7	621.5	3.53	0.17[2]	0.05	561.0	4.10	0.21[2]	0.04
2	1042.0	455.9	4.38	0.28[2]	0.01	416.9	4.94	0.69[2]	0.01[3]
Total	2147.7	1077.4	7.91	0.21[2]	0.06	977.9	7.00[3]	0.42[2]	0.03[3]

[1] Calculation of concentration based on molecular weight of parent (includes $^{14}CO_2$)
[2] Average content
[3] Related to 1994 and 1995 radioactivity

The amount of radioactivity taken up from the system by plants was quantified by combustion, and was found mainly in the straw. In total this accounted for 1.75 % of the radioactivity applied to lysimeter 4 and 2.39 % of the radioactivity applied to lysimeter 6.

At each sampling period the concentrations of non-volatile radioactivity recovered was characterised on the basis of parent material and up to twelve degradation products for each lysimeter, and the results obtained were expressed on a yearly basis. The mean concentrations of radioactive fractions from the two lysimeters treated with chemx are provided in Table IIA 7.4.7-3.

Chemx (parent compound) was found in low concentrations in both lysimeter leachates with peak concentrations of 0.01 µg/L in the first year and of 0.02 µg/L in the second year and mean concentration of 0.01 µg/L for the two year period in lysimeter 4. In lysimeter 6 which was given two applications, chemx (parent compound) occurred more consistently with peak concentrations of 0.09 µg/L in the first year and of 0.06 µg/L in the second year and a two year mean of 0.03 µg/L.

In lysimeter 4 the main radioactive component (M9) which corresponded by co-chromatography with xxxxxx (metabolite 6) was found in concentrations of 0.13 µg/L in first year and 0.21 µg/l in second year and a mean concentration of 0.10 µg/l over the two year period. In lysimeter 6 peak concentration of xxxxxx (metabolite 6) in the first year was 0.06 µg/L and in the second year was 0.24 µg/L with a two year mean of 0.07 µg/L.

The main radioactive component in lysimeter 6 was M10 (unidentified but close to xxxxxx on the basis of HPLC) which occurred at peak concentrations of 0.13 µg/L in the first year and 0.71 µg/L in the second year with two year mean of 0.12 µg/L. In lysimeter 4 the peak concentration of this fraction was 0.12 µg/L in the first year and 0.07 µg/L in the second year and the mean concentration of this fraction was 0.03 µg/L over the two year period.

Table IIA 7.4.7-3 Mean concentrations (in mg parent equivalents/L) of radioactive fractions of leachates from the lysimeters treated with chemx

	Mean Concentration (µg parent equivalents/L)													
Year	Chemx	M1[a]	M2	M3	M4	M5	M6	M7	M8	M9 [b]	M10	M11	M12	Total
	Lysimeter 4													
1st yr	<0.01	<0.01	nd	nd	nd	0.01	0.02	0.01	0.02	0.06	0.04	<0.01	<0.01	0.16
2nd yr	<0.01	Nd	<0.01	<0.01	nd	0.06	0.01	<0.01	nd	0.17	0.03	<0.01	<0.01	0.28
1 & 2 yr	<0.01	<0.01	<0.01	<0.01	nd	0.03	0.02	<0.01	0.01	0.10	0.03	<0.01	<0.01	0.21
	Lysimeter 6													
1st yr	0.03	0.02	<0.01	0.01	<0.01	0.02	0.03	<0.01	<0.01	0.03	0.04	<0.01	nd	0.21
2nd yr	0.03	Nd	0.02	0.06	nd	0.10	0.11	nd	nd	0.13	0.22	0.01	nd	0.68
1& 2 yr	0.03	0.01	0.01	0.03	<0.01	0.06	0.06	<0.01	<0.01	0.07	0.12	0.01	nd	0.41

[a] The designations M1, M2, etc. are used to specify a radioactive component at a particular retention time in this lysimeter study only. They do not correspond to any other component designations found elsewhere in this summary.
[b] This fraction has tentatively been identified as xxxxxx (metabolite 6).
nd = not detected

Ten additional unknown radioactive fractions were detected in leachates from both lysimeters with mean concentrations ≤ 0.03 µg/L and ≤ 0.06 µg/L.

III. CONCLUSIONS

The result from this lysimeter study, which was conducted using a soil type that is susceptible to leaching, showed following treatment with ^{14}C chemx, a recovery of *circa* 8 % of the applied radioactivity in the leachate. On analysis the leachate was shown to contain significant amounts of chemx (parent compound) at peak times together with a known soil metabolite xxxxxx (metabolite 6) (M9) and an unknown component (M10) both of which were present in quantities exceeding 0.1 µg/l.

None of the main soil metabolites identified - xxxxxxx (metabolite 2), desmethyl chemx - were detected in this study. Taken together with the low level of leaching of parent compound, these results suggest that strong binding to soil occurs, which is contrary to predictions made on the basis of the adsorption/desorption and column leaching studies. The absence of metabolite 3 in soil leachates is consistent with predictions based on adsorption/desorption studies - average Koc = 2495 (Somerville A 1996).

The preliminary results reported for the lysimeter study, suggest a low mobility of both chemx and its common soil metabolites in this sandy soil. Most of the radioactivity recovered in the leachate consisted of polar metabolites most of which are unidentified, apart form xxxxxx (metabolite 6) an identification that has yet to be confirmed using spectroscopic and other means. The other main metabolite found in leachate following the second application (M10) has yet to be characterised (Table IIA 7.4.7-4).

Final conclusions from this study must await the assessment of the final report of the study and in particular an examination of the leachate data for the third year and an examination of the residues data for the soil segments.

Table IIA 7.4.7-4 Peak and year end concentrations of major radioactive fractions detected in the first two years of the lysimeter study

Radio active fraction	Lysimeter No.	µg parent equivalents/l				
		Year 1		Year 2		
		Peak value	Year end value	Peak value	Year end value	2 year mean value
Chemx	4	0.01	< 0.01	0.02	< 0.01	<0.01
	6	0.09	0.03	0.06	0.03	0.03
M9	4	0.12	0.06	0.21	0.17	0.10
	6	0.06	0.03	0.24	0.12	0.07
M10	4	0.12	0.04	0.07	0.03	0.03
	6	0.13	0.04	0.71	0.22	0.12

The study involved treatment with 30 g as/ha on both lysimeters while the proposed maximum rate of application for chemx is 20 g as/ha which proportionally should result in concentrations in the leachate being reduced by a third under normal use conditions (Volkel W and A Burgener 1997). Accordingly, following application at the recommended field rate (20 g as/ha) and assuming the leachate would contain residues in the same proportion as recorded in this study the expected mean concentrations of the two components M9 and M10 in the leachate would be 0.07 and 0.08 µg/L after two years.

(Lockrel S 1997)

IIA 7.4.8 **Field leaching studies**

A study was not conducted since a lysimeter study that addresses all relevant issues arising was provided.

IIA 7.5 Hydrolytic degradation of relevant metabolites, degradation and reaction products

In accordance with point IIA 2.9.1, tests on the hydrolysis of chemx at 25 °C and at 40°C, using ^{14}C labelled chemx in sterile buffer solutions at pH 4, 5, 7 and 9 in the absence of light were submitted (White, T., 1995).

Report: IIA 2.9.1/01 White T 1995, The hydrolysis of ^{14}C-chemx, Chemco Report XX-13700

Guideline

BBA Guidelines for the official testing of plant protection products (Part IV, 4-3), February 1990 and Modification of the Lysimeter Guideline (Part IV, 4-3), September 1991.

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England, conducted the study during the period April to June 1994.

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In an hydrolysis study, the hydrolysis of chemx in the dark was studied at 25 °C and at 40 °C in sterile aqueous buffered solutions at pH 4, pH 5, pH 7 and pH 9. The nominal concentrations of chemx were 0.5 mg as/mL for pH 4 and 3 mg as/mL for pH 5, 7 and 9. Acetonitrile (0.1 %) was used as a cosolvent. Total recovery of radioactivity was in the range of 95 – 105 %.

The major hydrolysis products were Xxxxxxx (metabolite 2) and metabolite 3. Xxxxxxx (metabolite 2) was the major radioactive component detected (other than the parent) representing 93 % at pH 4, 34 % at pH 5, 13 % at pH 7 and 15 % at pH 9) of the applied radioactivity after 30 days incubation at 25 °C. The half-lives of chemx, calculated assuming first order kinetics were 7, 48, 168, and 156 days at pH 4, pH 5, pH 7 and pH 9, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - 1 : 1 mixture of chem2 ring labelled and chem3 ring labelled

chem2 ring labelled: ^{14}C in the C-3 position; specific activity 58.5 mCi/mmol;

chem3 ring labelled: ^{14}C in the C-5 position; specific activity 30.5 mCi/mmol;

Description: White powder

Lot/Batch #: C-1872.1

Purity: chem2 ring labelled: radiochemical purity ≥ 98.9 %

chem3 ring labelled: radiochemical purity ≥ 99.4 %

CAS #: 16335-17-2

Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Buffers: 0.1M buffer solutions in HPLC grade water were prepared at pH 4 and 5, using acetic acid and NaOH, pH 7 using KH_2PO_4 and KOH and pH 9 using H_2BO_3 and NaOH.

B. STUDY DESIGN

1. Experimental conditions

In separate experiments, the hydrolysis of chem2 ring and chem3 ring labelled chemx was studied at pH 4 (acetate buffer), pH5 (acetate buffer), pH7 (tris buffer) and pH 9 (borate buffer) at 25 °C, in darkness. In addition, the hydrolysis of chem2 ring labelled chemx was studied at 40 °C at each pH. The test solutions (x mL) were sterilized by autoclaving and were placed in 100 mL glass flasks. The nominal concentrations of chemx were 0.5 mg as/mL for pH 4 and 3 mg as/mL for pH 5, 7 and 9. Acetonitrile (0.1 %) was used as a cosolvent. Samples (x mL) were taken at 0, x , xx, xxx, — x_n day. Samples were incubated for a maximum of 30 days. Total ^{14}C was measured using LSC. HPLC (x column; 2% acetonitrile mobile phase; with UV (xx nm) and radio monitor detectors) was used for the identification and quantitative analysis of chemx and hydrolysis products. The limit of detection (LOD) for chemx and metabolite 1 were x and y µg as/mL, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 were xx and yy µg as/mL, respectively.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Total recovery of applied radioactivity was typically in the range 95-105%.

B. FINDINGS

Hydrolysis of chemx occurred over the pH range 4-9 and was most rapid at lower pH. Hydrolysis rate increased substantially at the higher temperature (40°C). In test solutions at pH 4 (25°C) the mean proportion of applied radioactivity declined rapidly from 97 % at zero time to 5 % at 30 days. Similarly in test solutions at pH 4 (40°C) the mean proportion of applied radioactivity decreased from 98 % at zero time to < 0.2% at 14 days. Assuming first order kinetics the hydrolysis half-life for chemx (25°C) was 7 days at pH 4, 48 days at pH 5, 168 days at pH 7 and 156 days at pH 9.

Table IIA 7.5-1 Hydrolysis of chemx in aqueous buffer solutions

Temperature	25°C		40°C	
pH	Half-life (days)	r^2	Half-life (days)	r^2
4	7.0	1.000	0.83	0.998
5	48	0.997	6.0	0.998
7	168	0.925	16	0.996
9	156	0.790	15	0.998

In the chem2 ring labelled ^{14}C-chemx solutions only one major radioactive component other than parent compound was detected - xxxxxxx (metabolite 2) representing 93 % at pH 4, 34 % at pH 5, 13 % at pH 7 and 15 % at pH 9) of the applied radioactivity after 30 days incubation at 25°C. A similar profile was evident following incubation at 40°C, but the amounts formed were greater.

In the chem3 ring labelled ^{14}C-chemx solutions a single major degradation product was similarly evident after 30 days incubation at 25°C - metabolite 3, representing 92 % of the applied radioactivity at pH 4, 31 % at pH 5, 12 % at pH 7 and 15 % at pH 9. Other minor components detected represented < 1% of the applied radioactivity individually.

Table IIA 7.5-2 Hydrolysis of chemx as a percentage of the applied radioactivity at pH 4

Compound	Sampling times							
	0	t1	t2	t3	t4	t5	t6	tn
Chemx								
desmethylchemx (metabolite 1)								
xxxxxxx (metabolite 2)								
metabolite n								
Total % recovery								

Table IIA 7.5-3 Hydrolysis of chemx as a percentage of the applied radioactivity at pH 7

Compound	Sampling times							
	0	t1	t2	t3	t4	t5	t6	tn
Chemx								
Desmethylchemx (metabolite 1)								
xxxxxxx (metabolite 2)								
metabolite n								
Total % recovery								

Table IIA 7.5-4 Hydrolysis of chemx as a percentage of the applied radioactivity at pH 9

Compound	Sampling times							
	0	t1	t2	t3	t4	t5	t6	tn
Chemx								
desmethylchemx (metabolite 1)								
xxxxxxx (metabolite 2)								
metabolite n								
Total % recovery								

III. CONCLUSIONS

In aqueous hydrolysis studies conducted using sterile buffer solutions at 25°C the hydrolytic half life of chemx was found to be 7, 48, 168 and 156 days at pH 4, 5, 7 and 9 respectively. Hydrolysis of chemx was found to be most rapid at acidic pH values and under elevated water temperatures. Xxxxxxx (metabolite 2) and metabolite 3 were the only significant hydrolysis products formed.

(White T 1995)

IIA 7.6 **Photochemical degradation**

In accordance with point IIA 2.9.2, tests involving the photolysis of chemx at 25 °C using ^{14}C labelled chemx in sterile buffer solutions at pH 7 were submitted (White T 1993a). In a separate study conducted in accordance with the requirements of point IIA 2.9.3, the quantum yield of direct phototransformation was determined (White T 1993b).

Report (first of two)**:** IIA 2.9.2/01 White T 1993, Aqueous photolysis studies of chemx, Chemco Report No. XX-12898

Guidelines

US EPA FIFRA Guideline § 161-2. The study also complies with the requirements specified of the Procedures for assessing the environmental fate and behaviour of pesticides, SETAC-Europe, 1995, ISBN Number 90-5607-002-9. This study was conducted to determine the photolytic half-life of chemx under aqueous conditions and to identify photolytic degradation products that accounted for ≥ 10 % of the radioactive distribution at any time during the irradiation. The photolysis experiments were conducted only at pH 7.0 because the spectra of chemx at pH 5, 7 and 9 are virtually identical.

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England, conducted the study during the period February to April 1993.

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In an aqueous phototransformation study, the aqueous phototransformation of chemx was studied under continuous artificial light for up to 144 hours at 25 ºC in sterile aqueous buffered solutions at pH 7 at initial concentrations of 12.64 mg/kg and 17.98 mg/kg. The photolytic half-live for decomposition of chemx was calculated assuming a first-order rate of decomposition, with determined values expressed on the basis of 12 hour sunlight days. The DT_{50} for continuous irradiation was found to be 36.3 hours (3.2 DT_{50} 12 hour equivalents/day) for chem2 ring labelled ^{14}C-chemx and 33.0 hours (2.8 DT_{50} 12 hour equivalents/day) for chem3 ring labelled ^{14}C-chemx at pH 7. The mass balance for the chem2 ring labelled ^{14}C-chemx was 96.8 and

98.2 % for the irradiated and control samples. The mass balance for the chem3 ring labelled ^{14}C-chemx was 96.5 and 104.7 % for the irradiated and control samples.

Evolution of CO_2 accounted for 6.2 % of the radioactivity in the case of chem2 ring labelled ^{14}C-chemx and 1.3 % in the case of chem3 ring labelled ^{14}C-chemx irradiated solutions, with volatile organic compounds accounted for < 1% for both labels. Six photolytic degradation products that were formed in amounts ≥ 10% of applied radioactivity were identified (three from each label) during irradiation, while a further seven unidentified photolytic degradation products comprising < 10 % of applied radioactivity at any time point (maximum 8.74 % at 144 hours) were detected, 3 following irradiation of chem2 ring labelled ^{14}C-chemx and 4 following irradiation of chem3 ring labelled ^{14}C-chemx.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** Chemx - chem2 ring labelled: ^{14}C in the C-3 position; specific activity 58.5 mCi/mmol;
   chem3 ring labelled: ^{14}C in the C-5 position; specific activity 30.5 mCi/mmol;

   Description: White powder
   Lot/Batch #: C-1872.1
   Purity: chem2 ring labelled: radiochemical purity ≥ 98.9 %
   chem3 ring labelled: radiochemical purity ≥ 99.4 %
   CAS #: 16335-17-2
   Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. **Buffers:** 0.1M buffer solution in HPLC grade water, at pH 7 was prepared using KH_2PO_4 and KOH.

B. STUDY DESIGN

1. Experimental conditions

The two radiolabelled forms of the test substance, were used in separate studies at concentrations of 12.64 mg/kg and 17.98 mg/kg respectively. The sterile aqueous solutions were buffered to pH 7.0 with 0.05 M potassium KH_2PO_4-NaOH and contained acetonitrile (< 1 % by volume) as a co-solvent.

Portions (*circa* 60 %) of the resulting solutions were transferred to cylindrical photolysis cells fitted with Pyrex® filters at both ends of the cell to filter out light with wavelengths < 290 nm. Each cell (7.5 cm diameter x 10.5 cm length) contained an internal glass coil through which water was circulated to maintain the irradiation solution at a temperature of 25 ± 1 °C. The remaining test substance solutions were used as dark controls, which were also maintained at a temperature of 25 °C. Test samples were irradiated with artificial light from a Xenon arc lamp. A comparison of the emission spectrum of midday sun in Oxford with Xenon arc lamp emission spectrum over the wavelengths 300 - 750 nm showed excellent overlap. The photolysis cell and dark control cells were fitted with trapping towers for CO_2 and volatile compounds. The trapping solutions used were 0.01 N NaOH for CO_2 and ethylene glycol for volatile organic compounds.

Samples were irradiated continuously for up to 144 hours and the photolytic half-life for decomposition of chemx was calculated, assuming a first-order rate of decomposition, with determined values expressed on the basis of 12 hour sunlight days. Samples (x mL) were taken at x day intervals and were stored for two weeks at 4 °C prior to analysis.

2. Description of analytical procedures

Samples were removed periodically over the exposure period, filtered and analysed by LSC to determine solution concentration at each time point and by HPLC/RAD for characterisation of isolated fractions. Peaks for non-photolyzed chemx and corresponding photolytic degradation products that attained levels of ≥ 5% of the radioactive distribution were quantified. The mobile phase used for HPLC analysis was X : Y (x % / y %). The limit of detection (LOD) for chemx and metabolite 1 were x and x µg as/mL, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 were y and y µg as/mL, respectively.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Overall recoveries of applied radioactivity for chem2 ring labelled ^{14}C-chemx was 96.8 % (test solutions) and 98.2 % (dark control) and for chem3 ring labelled ^{14}C-chemx was 96.5 % (test solutions) and 104.7 % (dark control).

B. FINDINGS

Evolution of CO_2 accounted for 6.2 % of the radioactivity in the case of chem2 ring labelled ^{14}C-chemx and 1.3 % in the case of chem3 ring labelled ^{14}C-chemx irradiated solutions, with volatile organic compounds accounted for < 1% for both labels. On the basis of linear first order kinetic reactions the decomposition half-life of chemx was calculated (Table IIA 7.6-1).

Six photolytic degradation products that were formed in amounts ≥ 10% of applied radioactivity were identified (three from each label) during irradiation, while a further seven unidentified photolytic degradation products comprising < 10 % of applied radioactivity at any time point (maximum 8.74 % at 144 hours) were detected, 3 following irradiation of chem2 ring labelled ^{14}C-chemx and 4 following irradiation of chem3 ring labelled ^{14}C-chemx

The structures of those photylitic degradation products which have been identified, their common names and their maximum relative distribution are shown in Figure 7.6-1.

Table IIA 7.6-1 Photolytic half-life of chemx

Label	1st order rate constant (h^{-1})	DT_{50} (hours)	DT_{50} (12 hour equivalents/day)	r^2
chem2 ring labelled ^{14}C-chemx	0.01907	36.3	3.2	0.9969
chem3 ring labelled ^{14}C-chemx	0.02101	33.0	2.8	0.9992

Table IIA 7.6-2 Characterisation of the photolytic degradation products of chemx identified

Photoproduct	^{14}C Label	Maximum Product Distribution %	Time Point of Maximum Distribution (hours)	mg/kg
metabolite 3	chem3 ring	28.31	144	4.99
xxxx (metabolite 9)	chem3 ring	20.95	144	3.69
N-hydroxy (metabolite 12)	chem3 ring	14.98	48	2.69
zzzz metabolite 11	chem3 ring	5.09	96	0.91
xx (metabolite 7)	chem3 ring	8.96	144	1.58
xxxyx (metabolite 10)	chem2 ring	15.62	144	1.74
xyxx (metabolite 5)	chem2 ring	14.59	96	1.69
metabolite 8	chem2 ring	28.34	96	3.28

Figure IIA 7.6-1 Proposed aqueous photolytic degradation pathway for chemx

Structures, names and maximum concentrations Omitted

Table IIA 7.6-3 Photodegradation of chemx at different sampling times (5 applied radioactivity)

Compound	Sampling times							
	0	T1	t2	t3	t4	t5	t6	tn
Chemx								
metabolite 3								
xxxx (metabolite 9)								
N-hydroxy (metabolite 12)								
xx (metabolite 7)								
xxxyx (metabolite 10)								
xyxx (metabolite 5)								
metabolite 8								
CO2								
Volatile organic compounds								
Total recovery (%)								

III. CONCLUSIONS

Chemx was degraded by photolysis to significant extent following exposure to artificial light equivalent to mid day summer sunshine with a DT_{50} *circa* 3 days (12 hour sunshine equivalents). The photolytic degradation route was complex with up to thirteen photodegradation products detected of which six represented ≥ 10 % of the applied radioactivity (Table IIA 7.6-2). Kinetic studies on these identified photolytic degradation products indicated that they would degrade further with half-lives in the range of 22 - 346 hours. The study demonstrated that chemx will degrade rapidly in aquatic systems under sunshine conditions to mainly photolabile metabolites.

(White T 1993a)

Report (second of two)**:** IIA 2.9.3/01 White T (1993), Determination of the direct phototransformation of ^{14}C-chemx in water, Chemco Report No. XX-14474

Guidelines

BBA Guidelines Part IV, 6-1 and draft OECD Guideline. The half-life determined in the quantum yield study was considerably shorter than that determined in the aqueous photolysis study (White, T., 1993a). The reasons for the difference may be attributed to several factors:

- the solutions for the quantum yield study were contained in small vessels (approximately 20 ml volume) with a short path length, compared to the *circa* 400 ml of solution in the vessel used for the main photolysis study;
- the concentration of the solution for the isolation and identification of metabolites in the photolysis study was much greater due to the necessity to isolate sufficient quantities of material to permit spectral identification.
- a comparison of the apparatus used for the two studies suggests that the vessels in the quantum yield study were closer to the light source than in the initial aqueous photolysis study.

The differences in the half-lives determined in the two studies should not be of any consequence. The tendency of chemx to degrade on irradiation can best be compared to that of other compounds on the basis of its quantum yield, a characteristic that is independent of conditions of irradiation.

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England, conducted the study between January and April 1996.

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In a phototransformation study, the aqueous phototransformation of chemx was studied at 20 ºC in distilled water at an initial concentration of 5.45 mg/L under artificial light for 24 hours. The photolytic half-life (DT_{50}) calculated assuming first order (non-linear) reaction kinetics was 5.7 hours (rate constant 0.1212 hour^{-1}, r^2

0.997) and the DT_{90} was 18.9 hours. The quantum yield of chemx was calculated to be $\phi = 1.81 \times 10^{-3}$. Mass balance at each sampling time ranged from 87.7 % to 100.0 % over the 24 hour period (no volatile compounds were trapped). The identification of the major photolytic transformation products was not attempted.

The differences in the photolysis half-lives determined in the two studies is ascribed to the lower solution concentration and the shorter path length in the quantum yield apparatus. When the quantum yield determination was used to provide an estimate of the environmental life time of chemx in Rhine river water, half life estimates were 2.4 - 3.8 days for summer conditions at a depth of 0 - 30 cm and 21.7 - 33.4 days for winter conditions at a depth of 0 - 30 cm. Aqueous photolysis is likely to be a significant mechanism for dissipation of chemx in aquatic environments.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Chemx -	chem2 ring labelled:	^{14}C in the C-3 position; specific activity 30.5 mCi/mmol;
		chem3 ring labelled:	^{14}C in the C-5 position; specific activity 29.3 mCi/mmol;
Description:		White powder	
Lot/Batch #:		C-1865.6	
Purity:		chem2 ring labelled:	radiochemical purity ≥ 99.81 %
		chem3 ring labelled:	radiochemical purity ≥ 99.4 %
CAS #:		16335-17-2	
Stability of test compound:		The test material was stable for at least 7 days at room temperature.	

B. STUDY DESIGN

1. Experimental conditions

Aliquots of test solution (20 ml of a solution containing 5.45 mg/l in double distilled water) were placed in quartz covered cylindrical glass vessels and irradiated in a “Suntest” apparatus fitted with Xenon arc light source filtered to cut off light below 290 nm. The vessels were irradiated continuously in sockets of a water-cooled steel tank (20°C) placed within the photolysis apparatus. Irradiated samples were exposed to a mean light intensity in the wavelength range 300 - 400 nm of about 32 W/M^2 comparable to the light intensity of natural daylight in summer.

2. Description of analytical procedures

Samples were taken from the irradiated solutions at intervals over a 24 hour irradiation period and radioactivity was determined by LSC, with characterisation of the radioactivity by TLC and HPLC. The mobile phase used for HPLC analysis was X : Y (x % / y %). The limit of detection (LOD) for chemx and metabolite 1 were x and x µg as/mL, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 were y and y µg as/mL, respectively.

A uranyl nitrate/oxalic acid actinometer was used to determine the number of photons penetrating the test solution.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Recoveries of applied radioactivity at each sampling time ranged from 87.7 % to 100.0 % over the 24 hour period (no volatile compounds were trapped).

B. FINDINGS

Parent compound present in the irradiated samples declined steadily from 100 % at time zero to 4.8 % after 24 hours. There was no degradation in the dark control sample during the period of the experiment. The identity of the photolytic degradation products was not attempted. The photolytic half-life (DT_{50}) calculated assuming first order (non-linear) reaction kinetics was 5.7 hours (rate constant 0.1212 $hour^{-1}$, r^2 0.997) and the DT_{90} was 18.9 hours.

The quantum yield of chemx was calculated to be $\phi = 1.81 \times 10^{-3}$.

Validation of the quantum yield calculation was achieved using the reference compounds pentachlorophenol and 2,4-dichlorophenol (reproducible ECETOC yield data).

III. CONCLUSIONS

The calculated quantum yield was used to estimate the environmental lifetimes of the test substance under direct photodegradation in water. Theoretical lifetime was calculated by considering the direct phototransformation for mid-day sunlight conditions in the top millimetre of a natural aquatic system. Real lifetime was calculated following considering of two types of factor which determine sunlight intensities available for direct photolysis: solar light intensity incident upon the upper layer of water; and penetration of light into the water and the absorption of light by water dissolved organic matter (pond water).

Using the EPA (GCSOLAR) computer program, direct photolysis at the surface of pure water at a location at 10° longitude and 50° latitude (Central Europe) with mid-day sunlight and typical ozone concentrations, the theoretical life time (days) at the surface of the water were calculated as indicated in Table IIA 7.6-4.

Table IIA 7.6-4 Theoretical life time (days) of chemx at water surface following irradiation

Season	Spring	Summer	Autumn	Winter
Theoretical half-life (days)	3.4	2.4	8.2	22

Using the same computer program, geographic and atmospheric conditions and using the adsorption spectra of a typical Rhine water sample, the real life environmental half-life (0 - 30 cm depth pond water) was calculated as indicated in Table IIA 7.6-5.

Similar results were obtained using the Frank and Klopfer Model (Ecotox. Env. Safety 17.323. 1989).

Table IIA 7.6-5 Real life environmental half-life (0 - 30 cm water column) following irradiation

Depth (cm)	Season	0	10	20	30
Environmental Half-life (days)	Summer	2.4	2.8	3.2	3.8
	Winter	21.7	25.3	29.2	33.4

(White T 1993b)

IIA 7.7 Ready biodegradability of chemx

A study was not conducted since ample evidence of the biodegradability of chemx in aqueous aerobic and anaerobic systems is available of other studies reported (White T 1995; White T 1993a; White T 1993b). The water / sediment study provides a basis for a comprehensive assessment of the degradation of chemx in aqueous environments (White T 1996).

IIA 7.8 Degradation in aquatic systems

IIA 7.8.1 Aerobic degradation in aquatic systems

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IIA 7.8.3 Water / sediment studies

Report: IIA 7.8/01 White T 1996, Chemx: Degradation and metabolism in aerobic aquatic systems, Chemco Report No. XX-14023

Guidelines: BBA Guidelines Part IV, 5-1.

Testing Laboratory and dates

The Chemco Research Laboratory, Oxford, England, conducted the study during the period October 1994 to June 1995

GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

Executive Summary

In a water / sediment study, degradation and metabolism of chemx was studied in a river water / sediment system (pH 7.48) and in a pond water / sediment systems (pH 7.1) under aerobic conditions for 100 days at 20 °C. The test material used was a mixture of chem2 ring labelled (^{14}C in the C-3 position) and chem3 ring labelled (^{14}C in the C-5 position) chemx. Mass balance for the aerated systems at 20 °C was 100.9 % (95.4 - 108.3 %) for the river system and 99.3 % (90.7 - 108.0 %) for the pond system. The mean recovery for the pond system at low temperature was 101.9 % (98.8 - 108.0 %) and for the sterile flasks was 103.1 % for the river system and 101.5 % for the pond system.

In both systems, degradation of chemx was rapid at ambient temperature (20°C) with DT_{50} values of 16.1 and 19.5 days. In the case of the pond system maintained at low temperature (5.7°C), degradation was much slower with a DT_{50} value of 58.3 days. In sterile systems degradation of chemx was much slower. Volatile substances (CO_2 or organic volatile compounds) did not exceed 1.3 % of the applied radioactivity in any test system. In these systems the decline of chemx from the aqueous phase was accompanied by a corresponding increase in sediment residues which represented > 50 % of applied material after 100 days incubation. Further extraction of the bound sediment residues confirmed that most of the applied radioactivity was associated with the humin, fulvic acid and humic acid fractions. In the total water sediment systems the main metabolite detected was desmethylchemx *circa* 13 % of applied radioactivity, with smaller amounts of xxxxxxx (metabolite 2) *circa* 6 % of applied radioactivity and metabolite 3 *circa* 1.5 % of applied radioactivity, which is consistent with the occurrence of hydrolytic breakdown followed by microbial degradation.

In the supernatant water of both test systems, desmethylchemx comprised *circa* 10 % of the applied radioactivity, xxxxxxx (metabolite 2) comprised *circa* 4 % of applied radioactivity and metabolite 3 comprised *circa* 1.3 % of applied radioactivity, at peak concentrations. In the sediment layer of both systems chemx comprised *circa* 15 % of applied radioactivity, desmethyl chemx comprised *circa* 3 % of applied radioactivity, xxxxxxx (metabolite 2) comprised *circa* 2 % of applied radioactivity and metabolite 3 comprised 0.5 % of applied radioactivity at peak concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chemx - 1 : 1 mixture of chem2 ring labelled ^{14}C in the C-3 position : chem3 ring labelled ^{14}C in the C-5 position, of specific activity 30.1 mCi/mmol;

Description: White powder
Lot/Batch #: NPD-9307-5385-T
Purity: radiochemical purity ≥ 99.07 %
CAS #: 16335-17-2
Stability of test compound: The test material was stable for at least 7 days at room temperature.

2. Water / Sediment: Two freshly collected water / sediment systems were used, one from a river, the Rhine, and the other from a pond. Prior to use the water was filtered through a 0.2 mm sieve and the sediment was sieved through a 2.0 mm screen.

Table IIA 7.8-1 Physiochemical parameters of the water / sediment systems

System	Rhine River		Pond Water	
Water phase	Before Start	At End	Before Start	At End
Total OC (mg c/l)	9.3	9.1	7.4	7.1
pH	7.48**	8.14*	7.76**	7.96*
Oxygen concentration (mg/l)	5.0**	7.4*	7.1**	7.5*
Redox potential (mV)	216**	225*	211**	216*
Sediment				
Redox potential (mV)	166	-201*	-155	-182*
pH	7.04	nd	6.98	nd
OC (%)	0.98	nd	1.69	nd
Biomass (mgC/100g dry sediment)	56.2	33.6	111.9	83.9
% Clay	5.9	nd	24.8	nd
% Silt	19.7	nd	40.4	nd
% Sand	74.4	nd	34.8	nd
USDA Classification	Sandy loam		Loam	

* Value of pooled control samples at day 100
** Mean value of control samples at day 0

B. STUDY DESIGN

1. Experimental conditions

The study was performed in an open gas flow system using one litre glass metabolism flasks (10.6 cm internal diameter) containing 530 ml water and either 250 g river sediment or 200 g pond sediment. Flasks were filled to a height of about 6 cm for water and a depth of 2.0 - 2.5 cm for the sediment layer, which were then allowed to equilibrate for 33 days before application of the test material.

The test systems were maintained at 20°C ± 2°C for the experimental period. An additional set of flasks containing the pond systems were changed to low temperature conditions (average 5.7°C ± 0.7°C) 14 days before application of test substance. Untreated controls were similarly maintained. One set of treated flasks was ventilated with CO_2 free air with simultaneous gentle agitation of the water column by stirring from the top with a suspended magnetic stirrer. Another set was maintained under sterile conditions.

Radiolabelled chemx was applied to the surface of both systems at a rate of 10.34 µg as/l corresponding to a field rate of 31 g as/ha. The study was conducted in the dark over a 100 day incubation period.

2. Sampling

Volatile compounds were collected in 2 N NaOH traps and CO_2 in ethylene glycol traps. Duplicate samples were collected at sampling intervals 0, 6, 24 and 48 hours, 7, 14, 30, 61 and 100 days for the aerated systems maintained at 20°C, at 30 and 100 day sampling intervals for the sterile systems and at 7, 14, 30, 61 and 100 day intervals for the flasks incubated at low temperature.

3. Description of analytical procedures

The water in the water phase was drawn off from the sediment by pipette. Any water remaining in the sediment was thereafter treated as sediment. The radioactivity in the water was partitioned with dichloromethane at pH 2 - 4. After partitioning, the volume of organic solvent was reduced and radioactivity was quantified by LSC. Radioactive components extracted were characterised using two TLC systems. The remaining water phase was analysed for radioactivity. From day 30 to 100 the partitioned aqueous phase was lyophilised, and analysed by LSC, TLC and HPLC.

After removal of the water phase, the sediment was exhaustively extracted at room temperature twice (for all except the 0 and 6 hour samples) with acetonitrile / 0.5 % aqueous NaCl (65 : 35, v : v). The organic solvent was removed using a rotary evaporator, and the resultant aqueous solution was partitioned at pH 3 - 4 using dichloromethane. The remaining water phase samples were lyophilised at days 30, 61 and 100, and the residue was dissolved in methanol for analysis by LSC and TLC. The organic phases were concentrated, and analysed by LSC and TLC.

Beginning with day 7 (or day 30 for the low temperature portion), the sediment still containing radioactivity after exhaustive extraction was further extracted with acetone using a Soxhlet apparatus. These extracts were analysed by LSC, but TLC was not performed because of the low amounts of radioactivity extracted. Sediment samples from day 100 were submitted to fractionation into humin, humic and fulvic acids.

The limit of detection (LOD) for chemx and metabolite 1 in water was x and y µg as/mL, respectively, while in soil it was x and y µg as/g soil, respectively. The limit of quantification (LOQ) for chemx and metabolite 1 in water was xx and yy µg as/mL, respectively, while in soil it was xx and yy µg as/g soil, respectively.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The mean recoveries of radioactivity for the aerated systems at 20°C were 100.9 % (95.4 - 108.3 %) for the river system and 99.3 % (90.7 - 108.0 %) for the pond system. The mean recovery for the pond system at low temperature was 101.9 % (98.8 - 108.0 %) and for the sterile flasks was 103.1 % for the river system and 101.5 % for the pond system.

B. FINDINGS

The level of radioactivity in river water at 20°C decreased from 101.2 % of the applied radioactivity at day 0 to 25.9 % at day 100, and in pond water it decreased from 105.3 % to 22.5 % over the same period.

In the sterile systems, radioactivity in the river water decreased to only 68.3 % of the applied radioactivity at day 100 and to 64 % of the applied radioactivity in the corresponding pond system (20°C). In the low temperature pond system (5.7°C), radioactivity in the pond water decreased from 105.3 % at day 0 to 44.9 % at day 100.

In river sediment maintained at 20°C, the level of radioactivity increased from 1.2 % of the applied radioactivity at day 0 to 67.9 % of the applied radioactivity at day 100, and in pond sediment from 1.0 % of the applied radioactivity to 67.7 % of the applied radioactivity in the same period. In the sterile systems the radioactivity in the river sediment increased to only 35.1 % of the applied radioactivity at day 100 and to

32.0 % of the applied radioactivity in the corresponding pond sediment (20°C). In the low temperature (5.7°C) pond sediment, radioactivity increased from 1.0 % of the applied radioactivity at day 0 to 55.7 % of the applied radioactivity at day 100. Volatile substances (CO_2 or organic volatile compounds) never exceeded 1.3 % of the applied radioactivity in any test system.

Overall, in the river system (water plus sediment) the amount of chemx decreased from 99.2 % to 11.8 % of applied radioactivity after 100 days incubation, and in the total pond system decreased from 102.9 % to 11.3 % after 100 days incubation at 20°C.

Overall, in the sterile systems (water plus sediment), chemx decreased to 70.2 % and 61.9 % for the river and pond systems respectively. In the water of the pond system incubated at low temperature (5.7°C) chemx decreased to 53.2 % of applied after 100 days incubation.

A summary of the recoveries obtained and of the distribution of the residues expressed as µg parent equivalent per litre at each sampling time interval is provided in Table IIA 7.8-2.

In water (20°C) the concentration of chemx (equivalents) declined rapidly at a similar rate in both river water / sediment and pond water / sediment systems from *circa* 10.5 µg/L to *circa* 2.5 µg/L. This decline was associated with an increase in concentration in the sediments that reached 7.0 µg/L by day 100. Once in sediment chemx (equivalents) remained tightly bound with 5.8 µg/L being unextractable after 100 days representing > 50 % of the radioactivity applied.

The dissipation rate was similar in both water sediment systems but was somewhat slower in the pond water / sediment at maintained at low temperature (5.7°C).

In the sterile water / sediment systems chemx (equivalents) in water declined from *circa* 10.5 µg/L to *circa* 7.0 µg/L following incubation for 100 days with an associated increase in sediment levels to *circa* 3.5 µg/L (Table IIA 7.8-3).

When the non extractable radioactivity at day 100 was further fractionated into humin, humic acid and fulvic acid fractions according to Stevenson 1982, the remaining radioactivity was mainly associated with the humin fraction *circa* 23.8 % for the river system and 30.5 % for the pond system. Radioactivity in the fulvic acid fractions amounted to 20.5 % in the case of the river system and 14.4 % in the case of the pond system, while radioactivity in the humic acid fractions was 11.7 % in the case of the river system and 9.8% in the case of the pond system. Attempts to back-extract any released radioactivity were not fruitful.

Details of the concentrations of metabolites in the viable water / sediment systems at 20 °C, the viable pond system at low temperature, and the sterile system at 20 °C, respectively are provided in Tables IIA 7.8-4 and IIA 7.8-5. There was no significant difference in the distribution pattern for metabolites between the two viable systems with the three metabolites present in each in similar proportions. Desmethyl chemx was the major metabolite detected in both river and pond systems comprising 14.0 / 12.9 % of the applied radioactivity at day 30, while xxxxxxx (metabolite 2) comprised 5.3 / 6.1 % of applied radioactivity at day 100 and metabolite3 comprised 1.7 / 1.4 % of applied radioactivity at day 30 / 61 respectively.

In the sterile system, degradation of chemx was much slower, with 7.3 µg/L of parent compound remaining after 100 days. The concentrations of metabolites formed were correspondingly lower. Apart from detection at extremely low levels in two samples, desmethyl chemx was not found in the sterile systems. The major degradation products in all systems were xxxxxxx (metabolite 2) and metabolite 3, the products of hydrolytic and microbial cleavage of the xxx bond. Desmethyl chemx was only detectable after 100 days in this system indicating that this metabolite is mainly the result of microbial degradation.

Table IIA 7.8-2 Recovery of radioactivity in viable water / sediments systems expressed in µg chemx parent equivalent per litre

	µg parent compound equivalents per litre								
Incubation Time (days)	0	0.25	1	2	7	14	30	61	100
River water/sediment (20°C)									
Water	10.5	10.0	9.4	8.4	7.3	6.7	5.3	3.6	2.7
Sediment Extractables *	< 0.01	0.2	1.2	1.8	2.3	2.4	1.9	1.5	0.9
Soxhlet Extraction	np	np	np	np	0.3	0.3	0.4	0.3	0.3
Non-extractables**	0.1	0.1	0.2	0.3	0.5	1.1	3.2	4.6	5.8
Sediment Total	0.1	0.3	1.3	2.0	3.1	3.8	5.5	6.4	7.0
Volatile organic compounds	np	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
$^{14}CO_2$	np	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1
Total System	10.6	10.3	10.8	10.5	10.4	10.5	10.9	10.1	9.9
Pond water/sediment (20°C)									
Water	10.9	9.9	9.8	9.2	7.8	6.7	5.4	2.7	2.3
Sediment Extractables*	< 0.1	0.3	0.7	1.2	1.7	2.0	1.4	1.2	1.1
Soxhlet Extraction	np	np	np	np	0.2	0.1	0.3	0.2	0.3
Non-extractables**	< 0.1	0.1	0.1	0.2	0.6	1.5	3.1	5.4	5.7
Sediment Total	0.1	0.5	0.8	1.3	2.4	3.6	4.8	6.8	7.0
Volatile organic compounds	np	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	<0 .1
$^{14}CO_2$	np	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1
Total System	11.0	10.3	10.5	10.6	10.3	10.4	10.2	9.7	9.5
Pond water/sediment (5.7°C)									
Water	10.9	-	-	-	10.2	8.3	7.1	5.9	4.6
Sediment Extractables*	< 0.1	-	-	-	0.3	1.7	2.1	2.1	2.3
Soxhlet Extraction	np	-	-	-	np	np	0.2	0.2	0.2
Non-extractables**	< 0.1	-	-	-	0.1	0.4	0.9	2.2	3.2
Sediment Total	0.1	-	-	-	0.4	2.1	3.2	4.5	5.8
Volatile organic compounds	np	-	-	-	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
$^{14}CO_2$	np	-	-	-	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total System	11.0	-	-	-	10.6	10.5	10.3	10.4	10.5

* Extractions with acetonitrile/0.5 NaCl ** Determined by combustion np Not performed

Both xxxxxxx (metabolite 2) and metabolite 3 were found as degradation products in all tests (aerobic, sterile and low temperature) confirming that hydrolysis of the xxx bond is the major degradation route followed in importance by microbial degradation.

Degradation does not result in complete mineralization as only small amounts of CO_2 were found.

The degradation rate constants were determined for both aquatic systems by application of the best fit reaction kinetic model, Levenspiel 1972; Timme and Frehse 1980; Time *et al* 1986. Chemx disappeared rapidly from water with calculated half-lives of 19.5 and 16.1 days in the river and pond systems at 20°C. It is interesting to note that degradation was rapid even though the water phase pH ranged from 7.5 to 8.1 at

the beginning and the end of the study. The pH of the sediment at the beginning of the study was approximately neutral.

The degradation rates for the total systems were similar with half lives of 32.2 and 19.8 days respectively. In the pond system at low temperature the degradation rates were correspondingly low with half lives of 58.3 days in water and 104.8 days in the water / sediment system. The DT_{90} values for water and for the total river and pond systems at *circa* 100 days except following low temperature incubation when the calculated values were extremely high and must be regarded as being tentative in view of the limited number of time points used.

Table IIA 7.8-3 Recovery of radioactivity in sterile water / sediments systems expressed in µg chemx parent equivalent per litre

	Incubation time in days		
	0	30	100
River water/sediment system	µg parent equivalents per litre		
Water	10.5	7.6	7.1
Sediment Extractables[a]	< 0.1	2.5	2.7
Soxhlet (acetone)	np	np	Np
Non-extractables[b]	0.1	0.6	1.0
Sediment (total)	0.1	3.1	3.6
Volatile organic compounds	np	< 0.1	< 0.1
$^{14}CO_2$	np	< 0.1	< 0.1
Total System Recovery	10.6	10.7	10.7
Pond water/sediment system			
Water	10.9	7.4	6.6
Sediment Extractables[a]	< 0.1	2.6	2.4
Soxhlet (acetone)	np	np	Np
Non-extractables[b]	< 0.1	0.4	1.0
Sediment (total)	0.1	3.1	3.3
Volatile organic compounds	np	< 0.1	< 0.1
$^{14}CO_2$	np	< 0.1	< 0.1
Total System Recovery	11.0	10.5	10.0

[a] Extractions with acetonitrile/0.5% NaCl (65/35; v/v) [b] Non-extractables determined by combustion np = not performed

Table IIA 7.8-4 Distribution of radioactive components in total viable water/sediment systems over an incubation period of 100 days, expressed as µg parent equivalents/litre water

	µg chemx (parent equivalents)/litre water								
Incubation Time (days)	0	0.25	1	2	7	14	30	61	100
River System (20°C)									
Chemx	10.3	9.8	10.1	9.8	8.6	7.5	4.7	2.6	1.2
Desmethyl chemx	nd	nd	nd	< 0.1	0.2	0.1	1.5	1.0	0.8
xxxxxxx (metabolite 2)	nd	nd	nd	< 0.1	0.1	0.2	0.3	0.5	0.6
metabolite 3	nd	nd	nd	< 0.1	0.1	0.1	0.1	0.1	0.1
Not identified	0.2	0.2	0.4	0.3	0.7	1.1	0.7	0.9	1.0
Pond System (20°C)									
Chemx	10.7	9.5	10.2	10.0	8.5	6.5	4.4	1.8	1.2
Desmethyl chemx	nd	nd	nd	< 0.1	0.1	0.1	1.3	0.5	0.7
xxxxxxx (metabolite 2)	nd	nd	nd	<0.1	0.1	0.5	0.3	0.5	0.6
metabolite 3	nd	0.1	<0.1	nd	< 0.1	0.1	0.2	0.1	0.1
Not identified	0.2	0.2	0.3	0.4	0.7	1.5	0.6	1.0	0.8
Pond System (5.7°C)									
Chemx	10.7	-	-	-	10.1	9.6	8.2	6.7	5.5
Desmethyl chemx	nd	-	-	-	nd	0.1	0.3	0.6	0.5
xxxxxxx (metabolite 2)	nd	-	-	-	nd	0.1	< 0.1	0.1	0.3
metabolite 3	nd	-	-	-	nd	< 0.1	< 0.1	< 0.1	0.1
Not identified	0.2	-	-	-	0.1	0.3	0.7	0.3	0.6

nd Not detected

Table IIA 7.8-5 Distribution of radioactive components in total sterile water/sediment systems over an incubation period of 100 days, expressed as µg parent equivalents/litre water

	Incubation time in days		
	0	30	100
	µg parent equivalents/litre water		
Sterile River System			
Chemx	10.3	8.9	7.3
Desmethyl chemx	nd	nd	<0.1
Xxxxxxx (metabolite 2)	nd	0.4	1.2
Metabolite 3	nd	0.2	0.4
Not identified	0.2	0.6	0.9
Sterile Pond System			
Chemx	10.7	8.9	6.4
Desmethyl chemx	nd	nd	0.1
Xxxxxxx (metabolite 2)	nd	0.4	1.0
xxx (metabolite 5)	nd	nd	<0.1
Metabolite 3	nd	0.2	0.5
Not identified	0.2	0.6	1.0

nd = not detected

Table IIA 7.8-6 Degradation parameters for chemx in water / sediment systems

System	Phase	Kinetic Order	Rate Constant	Correlation Coefficient	DT_{50} (days)	DT_{90} (days)
River (20°C)	Water	1.5	0.0022	0.998	19.5	101.8
	Total	1.0	0.0093	0.998	32.2	107.0
Pond (20°C)	Water	1.5	0.0026	0.995	16.1	83.9
	Total	1.5	0.0021	0.994	19.8	103.5
Pond (5.7°C)	Water	2.0	0.0002	0.996	58.3	524.5
	Total	2.0	0.0001	0.999	104.8	942.9

III. CONCLUSIONS

The study provided evidence that chemx degrades rapidly in water bodies through degradation to polar metabolites and through binding to sediment. It can be anticipated that in viable streams degradation will be most rapid and will be aided by the processes of photodegradation. At recommended use rates chemx is unlikely to result in any significant environmental loading of the aquatic environment.

(White T 1996b)

IIA 7.9 **Degradation in the saturated zone**

Data is not required, since the interim results of the lysimeter study (Lockrel S 1997) indicated that .....…*(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)*

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IIA 7.10 **Route and rate of degradation air**

The vapour pressure of chemx is 3.05 x 10^{-8} Pa at 20°C and Henry's Law Constant (20°C) values calculated were 8.15 x 10^{-7} Pa/M^3/mol at pH 5 and 8.83 x 10^{-9} Pa/M^3/mol at pH 7 (White T 1996a). Since .....…*(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)*

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IIA 7.11 **Definition of the residue**

In the aerobic soil metabolism study (Grenfel RG 1996a) and in the degradation rate studies (Grenfel RG 1995; Sullivan R 1995b) three metabolites were identified in soil and were present at amounts which accounted for > 10 % of the applied compound. These metabolites were recovered at various time points: xxxxxxx (metabolite 2) 15 % 225 days after application; desmethyl chemx 29 % 100 days after application and metabolite 3 11 % 100 days after application.

These three metabolites were also detected in the water / sediment study (White T 1996b). In the field dissipation studies (Lyons V 1997a, Lyons V 1997b, Lyons V 1997c, Lyons V 1997d, Lyons V 1997e, Brown JR 1996a) following application at the maximum recommended use rate of 20 g as/ha to bare soil, maximum residues of chemx recovered in 0 - 20 cm soil horizon after 3 months was 0.0017 mg/kg and after one year < 0.001 mg/kg. Following post emergence application of chemx at 20 g as/ha the maximum residue levels of chemx in soil after 3 months were < 0.001 mg/kg. The levels of the xxxxxxx (metabolite 2) and desmethyl chemx metabolites observed in soil after 3 months were 0.0033 mg/kg in bare soil plots and 0.0014 mg/kg in cropped soil and the maximum levels observed during the course of the study were 0.0034 mg/kg in bare soil and 0.0022 mg/kg in cropped soil in the 0 - 20 cm soil horizon.

The method of analysis proposed for the determination of residues of chemx and metabolites in soil is based on acid hydrolysis and quantitative conversion into a single analyte, metabolite 7. Residues of chemx, xxxxxxx (metabolite 2) and desmethyl chemx metabolites are determined as mg/kg of metabolite 7 expressed as chemx parent equivalent and defined as the total residue. A second method for the determination of chemx parent compound in soil, involves clean-up on an aluminium oxide column, then on a florisil column, followed by acid hydrolysis and quantitative conversion into a single analyte, metabolite 7. The limit of quantification for both methods is 0.0005 mg/kg.

On the basis of the maximum levels detected in laboratory studies, the maximum concentrations of metabolites expected in field soil following treatment at the recommended rate of 20 g as/ha in the case of xxxxxxx (metabolite 2) is 0.001 mg/kg, in the case of desmethyl chemx is 0.0019 mg/kg and in the case of metabolite 3 is 0.007 mg/kg. At such levels, these metabolites may be considered as not being environmentally significant. The only significant residue is chemx (parent compound). Accordingly, the residue definition proposed is: chemx (parent compound), determined as metabolite 7, expressed as chemx.

IIA 7.12 **Monitoring data**

Plant protection products containing chemx are not yet authorized for use. Accordingly no monitoring data is available from any source.

Attachment

CHEMX METABOLITE KEY : NAMES, STRUCTURES & STUDIES WHERE FOUND

[Number] in *Tier II* text refers to ID Number below

ID No	Trivial Name	Chemical Abstracts Name	Structure	Where Found
1	Chemx			Wheat forage, wheat straw
2	Xxxxxxx Metabolite 2			Hydrolysis, aerobic soil, aquatic sediment, wheat forage, wheat straw, rotation crops, rat (urine and faeces)
3	Metabolite 3			Hydrolysis, aerobic soil, aquatic sediment, aqueous photolysis
4	desmethyl chemx Metabolite 1			Aerobic soil, aquatic sediment, wheat forage, wheat straw, rat (urine and faeces)
5	Xxxx Metabolite 10			Aerobic soil, aqueous photolysis
6	Xxxxxx Metabolite 6			Lysimeter leachate, wheat forage, wheat straw
7	Xxx Metabolite 5			Aqueous photolysis, soil photolysis, wheat forage, wheat straw
8	Metabolite 7			Aqueous photolysis, Common chemophore or analyte for residue method

Attachment (Continued)

ID No	Trivial Name	Chemical Abstracts Name	Structure	Where Found
9	Metabolite 10			Aqueous photolysis, wheat forage, wheat straw
10	Xxyx Metabolite 8			Aqueous photolysis, wheat forage, wheat straw
11	Metabolite 9			Aqueous photolysis
12	N-hydroxy Metabolite 5			Aqueous photolysis
13	5-Hydroxy-OEC 1000			Rat (urine and faeces)
14	Rearrangement product			Chemophore (analyte) for analysis of OEC 1000 in water and in soil storage stability
15	Metabolite 11			Rat (urine)