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Profenofos/Lambda-Cyhalothrin

CGA15324/Lambda-Cyhalothrin EC (300/015) (A13735F) - 4-Hour Acute Inhalation Toxicity Study in Rats

Final Report

DATA REQUIREMENTS: EPA Health Effects Test Guidelines, OPPTS 870.1300 (1998)
OECD Guidelines for Testing of Chemicals, Procedure 403 (1997)

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STUDY COMPLETION DATE: July 8, 2005

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LABORATORY PROJECT ID: Report Number: CTL/HR2528/Regulatory/Report
Study Number: HR2528
Task Number: T003572-05

SUBMITTER/SPONSOR: Syngenta Crop Protection, Inc.
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VOLUME 1 OF 1 OF STUDY

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

1) *The following statement applies to submissions to regulatory agencies in the United States of America.*

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company: Syngenta Crop Protection, Inc.

Company Representative: Timothy E. Wilson, Ph.D.

Title: Regulatory Product Manager

Signature: Timothy E Wilson Date: Jan. 10, 2008

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STATEMENT OF GLP COMPLIANCE AND AUTHENTICATION

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

The study (HR2528) was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1999, Statutory Instrument No. 3106 as amended 2004, Statutory Instrument No. 994). These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

N J Rattray — Nicola J Rattray — 8 July 2005
Study Director — Date

Merrill Tisdel, B.S. — Date: JANUARY 10 2008
Representative of Submitter/Sponsor

Merrill Tisdel, B.S.
Representative of Submitter/Sponsor — Date

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QUALITY ASSURANCE STATEMENT

In accordance with CTL policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Audit/Inspection	Date of QA Report
03 May 2005	Protocol	03 May 2005
27 Jun 2005	Draft report	28 Jun 2005
07 Jul 2005	Final report review	08 Jul 2005

In addition, inspections associated with this type of study were made as follows:

05 Apr 2005	Atmosphere analysis	05 Apr 2005
06 Apr 2005	Bodyweights, clinical observations	06 Apr 2005
13 May 2005	Atmosphere generation, exposure, atmosphere collection	13 May 2005
24 May 2005	Post mortem	24 May 2005

Facilities and process based procedures associated with this type of study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, HR2528.

A R Tarry ..... 8 July 2005

(CTL Quality Assurance Unit)

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
N J Rattray	Study Director, (from date), CTL
S Goddard	Study technician
J Redwood	Chemical analyst
P Hext	Study reviewer

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TABLES

APPENDICES

This page is provided for the Regulatory Authority Reviewer's notes.

1. SUMMARY

1.1 Study design

A group of five male and five female Alpk:AP_fSD (Wistar-derived) rats was exposed nose-only for a single four-hour period to CGA15324/lambda-cyhalothrin EC (300/015) (A13735F) at a target formulation concentration of 2.5 mg/*l*. Test atmospheres were analysed for particulate concentration and CGA15324. The particle size distribution of the test atmosphere was analysed twice during the exposure period. Following exposure, the animals were retained without treatment for 14 days. Clinical observations and bodyweights were recorded throughout the study and at the end of the scheduled period, the animals were killed and subjected to a gross examination *post mortem*.

1.2 Results

The achieved test atmosphere had the following characteristics:

Target formulation concentration mg/*l*	Achieved formulation concentration mg/*l*	MMAD* µm	GSD^+
2.5	2.61	2.62 2.64	2.07 2.14

* Mass Median Aerodynamic Diameter (µm)
\+ Geometric Standard Deviation

There were no deaths. Clinical signs associated with restraint were seen during and immediately after exposure. Clinical signs indicative of mild toxicity were seen during and immediately after exposure. Clinical signs indicative of respiratory irritation were seen during, immediately after exposure and persisted in some animals to day 6 of the study. All animals had fully recovered by the end of the study. All animals had gained weight by the end of the study. There were no macroscopic abnormalities.

1.3 Conclusion

Nose-only exposure for 4 hours to a formulation concentration of 2.61 mg/*l* resulted in no mortalities and no adverse effects. It is concluded that the median lethal concentration of CGA15324/lambda-cyhalothrin EC (300/015) (A13735F) exceeds 2.61 mg/*l*.

2. INTRODUCTION

2.1 Purpose

The purpose of this study was to determine the 4-hour acute inhalation toxicity to male and female rats of CGA15324/lambda-cyhalothrin EC (300/015) (A13735F) and to monitor recovery over 14 days.

2.2 Regulatory guidelines

This study was conducted according to the following Regulatory Guidelines:

a) United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.1300 (1998): Acute Inhalation Toxicity.

b) US regulations relating to transportation (49 CFR Part 173 section 173 132).

2.3 Justification for test system selection

The rat was used because it is one of the species generally recommended for the assessment of Toxicity. The Alpk:AP$_f$SD, Wistar derived, strain of rat was used because of the substantial background data available for this strain, in this Laboratory, relating to studies of this type. Inhalation was chosen for administration of CGA15324/lambda-cyhalothrin EC (300/015) (A13735F) as this represents a possible route of exposure in humans.

2.4 Selection of atmospheric concentrations

A maximum atmospheric concentration of 2.5mg/*l* was selected as the target exposure level as this meets the requirements of the US regulations relating to transportation and satisfies US. EPA Guideline OPPTS 870.1300.

2.5 Study dates

The study was initiated on 29 April 2005. The experimental phase started on 16 May 2005 and was completed 13 June 2005.

2.6 Data storage

An original report, the study protocol, all raw data, samples and specimens, pertaining to this study are retained in the Archives, Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK.

3. TEST SUBSTANCE

Name:	CGA15324/lambda-cyhalothrin EC (300/015) (A13735F)
Source:	EZA Münchwilen
Colour:	Orange Yellow to Brown
Physical state:	Liquid
Batch reference number:	SVR4J135162
CTL test substance reference number:	Y03088/038
Concentrations of active ingredients	Profenofos (CGA15324) 310 g/*l* corresponding to 28.2%w/w
	Lambda-cyhalothrin 17.3 g/*l* corresponding to 1.57%w/w
Storage conditions:	Ambient temperature (<30°C) in the dark
Expiry date:	March 2007
Formulation reference number:	A13735F

The sample was tested as supplied.

From information supplied by the Sponsor, the test substance was used within the stated expiry date.

A certificate of analysis, dated12 April 2005, is retained in the CTL Archives.

The test substance characterisation was carried out by Syngenta Ltd..

4. EXPERIMENTAL PROCEDURES

4.1 Atmosphere generation

4.1.1 Trial generation

Trial generations were carried out prior to the start of the study in order to:

i) determine the appropriate generation system and conditions

ii) determine that the appropriate target concentration could be achieved, or, if not, what was the maximum stable attainable concentration

iii) obtain data on the aerodynamic particle size of the atmosphere generated

iv) determine an appropriate method of analysis of CGA15324 in the particulate phase of the test atmosphere

4.1.2 Generation conditions

The test atmosphere was generated using a glass concentric - jet atomiser. The test substance was pumped to the atomiser using a peristaltic pump supplied by Watson Marlow. Clean, dry air (dried and filtered using equipment supplied by Atlas-Copco, Sweden) was passed through the atomiser at a nominal flow rate of 28*l*/minute (at normal temperature and pressure) and carried the atmosphere to the exposure chamber, having an internal volume of 27.6 litres, in order to achieve a minimum of 12 air changes per hour. Since diluting air was not employed, the flow rate through the exposure chamber was the same as that employed in the generation of the test atmosphere. Air flows were monitored continuously and recorded at least 3 times using variable area flowmeters (KDG Flowmeters, Burgess Hill, Sussex, UK) and were altered as necessary to maintain the target concentration.

4.2 Atmosphere sampling and analysis

4.2.1 Nominal atmosphere concentration

The nominal concentration is a concentration based on the total weight of test substance used during the exposure period. This represents the maximum concentration to which the animals could be exposed, assuming no losses within the generation or exposure systems. The nominal concentration of the test substance during the exposure generation period was calculated from the following formula:

$$\text{Concentration (mg/}l\text{)} = \frac{\text{weight loss(mg)}}{\text{time (minutes) x airflow (l/minute)}}$$

4.2.2 Particulate concentration

The particulate concentration of the test atmosphere, close to the animals' breathing zone, was measured gravimetrically 9 times during exposure. This was done by drawing the test atmosphere, at a known flow rate, for a known time, through a 25mm diameter, polyvinyl chloride (PVC) GLA 5000 filter housed in a Delrin open-faced filter holder (both filters and holders supplied by Gelman Sciences Limited, Northampton, UK). The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

$$\text{Concentration (mg/}l\text{)} = \frac{\text{post wt(mg) - pre wt (mg)}}{\text{time (minutes) x airflow (l/minute)}}$$

pre wt = weight of filter prior to sampling
post wt = weight of filter after sampling

4.2.3 Aerodynamic particle size distribution

The aerodynamic particle size distribution was measured twice during the exposure period, using a Marple Cascade Impactor (supplied by Schaefer Instruments, Wantage, Oxon., UK) which aerodynamically separated airborne particles into pre-determined size ranges. The amount of aerosol, by weight, in each size range, was then used to calculate the aerodynamic particle size distribution of the aerosol. Using a spreadsheet, the data were transformed using a log/probit transform and a linear regression derived from the cumulative data.

Using this regression line, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated. Definitions of particle size are given in Appendix A.

All filters and the stages of the cascade impactors were stored in sealed bottles after weighing prior to subsequent analysis of CGA15324 concentration.

4.2.4 Analysed atmospheric concentration

The atmospheric concentration of CGA15324was determined by analysis of the material collected on the GLA 5000 filter and the stages of the cascade impactors.

The analytical method is given in Appendix B.

4.2.5 Total formulation concentration

The total formulation concentration represents the total atmosphere to which the rats were exposed, based on the chemical analysis of CGA15324in the particulate phase, and compensates for volatilisation of solvent components. The total formulation concentration was calculated and derived from the analysed CGA15324 concentration in the particulate phase of the test atmosphere and the actual concentration of CGA15324 in the formulation:

$$\text{Total formulation concentration (mg/}l\text{)} = \text{Analysed CGA15324concentration} \times \frac{100}{A}$$

where A = % CGA15324 in the formulation

4.3 Experimental design

4.3.1 Animals

Species	Rat
Strain	Alpk:AP$_f$SD (Wistar-derived)
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK
Sex/Numbers:	5 Males and 5 females plus 2 males and 2 females for a trial exposure
Specification:	7-8 weeks old on arrival at CTL 8-9 weeks old at start of study Within 379.2±16.8 g (males); 242.2±18.7 (females) giving a weight range at the start of the exposure within 20% of the mean for each sex.

4.3.2 Accommodation and husbandry

Animals were housed at a maximum of 5 per cage, sexes separately in cages under environmental conditions with the following specification:

Temperature:	22±3°C
Relative Humidity	30-70%
Air Changes:	At least 15 changes/hour
Light Cycle	Artificial giving 12 hours light/12 hours dark.

Temperature and relevant humidity were monitored and recorded daily throughout the study. The recorded values were within the specified ranges.

During the study the animals were given environmental enrichment consisting of nylabones, cardboard tubes and paper. A metal tray provided a solid area within the cage. Details of suppliers and certificates of analysis for these items are retained in the CTL Archive.

Diet (RM1) supplied by Special diets Services Limited, Witham, Essex, UK and mains water, were supplied by an automatic system *ad libitum*, except during exposure.

Each batch of diet and the water supply are routinely analysed for composition and the presence of any contaminants that could affect the purpose or integrity of the study. Certificates of analysis are retained in the CTL Archive.

4.3.3 Acclimatisation

Animals were acclimatised for at least 5 days before the start of the study.

4.3.4 Animal identification

On arrival, the animals were assigned to the study. The animals were individually identified with their allocation number by ear punching.

On the front of each cage of animals was a card identifying the contained animals by exposure concentration, group number, individual number, sex and study.

4.3.5 Atmospheric concentration

The study consisted of one main study group of five male and five female rats. A preliminary group (identified with permanent marker as A, B, C and D) of 2 males and 2 females used for trial exposures to the target concentration.

Group	Target Concentration (mg/*l*)	Male Numbers	Female Numbers
1	2.5	21-25	26-30

4.3.6 Exposure system

Animals were exposed nose-only to the test atmosphere. Animals were restrained in polycarbonate tubes supplied by Battelle, Geneva, Switzerland. These were inserted into a PERSPEX exposure chamber (Appendix C). The chamber was covered with an aluminium cone and stood on an aluminium base. Before exposure of the test animals, the atmosphere

was shown to have been acceptably stable (Appendix D). During this period the holes of the exposure chamber were plugged. The animals were exposed for 4 hours.

The temperature and relative humidity in the chamber was recorded 8 times during exposure using a portable, digital temperature and relative humidity monitor; they were within the range of 20.5-20.7°C and 20-26% respectively.

4.4 Clinical observations

Prior to the start of the study all rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure, they were observed frequently and, at the end of the 4-hour exposure period, each rat was given a detailed clinical examination. The animals were also subjected to detailed clinical observations, included the finding of no abnormalities detected, daily during the 14 day observation period.

4.5 Bodyweights

The bodyweight of each rat was recorded on day -1 (to ensure animals of one sex were within a similar weight range), 1, 8 and prior to termination on day 15.

4.6 Investigations *post mortem*

4.6.1 Termination

All rats were killed by over exposure to halothane Ph Eur. vapour followed by exsanguination.

4.6.2 Macroscopic examination

All animals were subjected to a gross examination *post mortem*. This involved an external observation and a careful internal examination of all thoracic and abdominal viscera.

5. RESULTS

5.1 Atmosphere analysis

5.1.1 Nominal concentration

The concentration from the weight loss of test substance was calculated to be 12.66 mg/*l*.

5.1.2 Particulate concentration

The mean concentration (± standard deviations) was as follows:

Target concentration (mg/*l*)	Measured particulate concentration (mg/*l*) Mean ± SD
2.5	2.56±0.18

5.1.3 Analysed atmospheric concentration

The atmospheric concentration of CGA15324determined by chemical analysis was as follows:

Target formulation concentration (mg/*l*)	Analysed concentration of CGA15324 (mg/*l*)	
	Mean ± SD	% Total particulate ± SD
2.5	0.737±0.055	28.8±0.4

Individual values are given in Table 1.

5.1.4 Total formulation concentration

The mean total formulation concentration (± SD) was calculated to be as follows:

Target concentration (mg/*l*)	Total formulation concentration (mg/*l*)
2.5	2.61

Unless stated otherwise, **total formulation concentration** is used subsequently to identify the exposure levels.

5.1.5 Aerodynamic particle size distribution

The aerodynamic particle size distribution of the total particulate was extrapolated to be as follows:

Time into exposure (minutes)	Median size (MMAD) (µm)	GSD
65	2.62	2.07
185	2.64	2.14

Individual values are given in Table 2.

The percentages of the particulate on the impactor stages were similar to the corresponding amounts of CGA15324 determined by chemical analysis.

5.2 Clinical observations

5.2.1 Mortality

There were no deaths during the exposure or observation periods.

5.2.2 Observations during exposure

Abnormalities generally associated with restraint: wet fur and salivation were seen in all animals during exposure.

Abnormalities associated with respiratory tract irritation: breathing rate reduced and breathing depth increased were seen in all animals during exposure.

Abnormalities associated with mild toxicity: reduced response to sound was seen in all animals during exposure (Table 3).

5.2.3 Observations immediately after exposure

Abnormalities generally associated with restraint: (wet fur, hunched posture and salivation were seen in all animals, piloerection was seen in 3 males and 2 females and chromodacryorrhea in 1male) post exposure.

Changes indicative of mild toxicity: decreased activity was seen in all males and 4/5 females. Other changes, including pinna reflex absent, shaking, tip toe gait, reduced response to sound, reduced righting reflex and reduced foot withdrawal reflex were seen in some animals.

Changes indicative of respiratory tract irritation, increased breathing depth and decreased breathing rate were seen in all animals Gasping was seen in 1 male and 1 female animal (Tables 4 and 5).

5.2.4 Observations during the maintenance period

The clinical condition of the animals had greatly improved by day 2 of the study. Mild respiratory irritation, shown by the presence of abnormal respiratory noise was present in some animals to day 6 of the study. All animals had fully recovered by day 7 of the study (Tables 4 and 5).

5.3 Bodyweights

Three females had gained weight by day 8 of the study. All animals had gained weight by the end of the study (Table 6).

5.4 Investigations *post mortem*

There were no macroscopic abnormalities.

6. DISCUSSION

The purpose of this study was to investigate the 4-hour acute inhalation toxicity of CGA15324/lambda-cyhalothrin EC (300/015) (A13735F) and this was accomplished successfully. The test atmosphere generated was acceptable with regard to its general stability and physical characteristics.

There were no deaths and only transient toxicity and respiratory irritation was seen.

7. CONCLUSION

Nose-only exposure for 4 hours to a formulation concentration of 2.61 mg/*l* resulted in no mortalities and no adverse effects. It is concluded that the median lethal concentration of CGA15324/lambda-cyhalothrin EC (300/015) (A13735F) exceeds 2.61 mg/*l*.

TABLE 1 - ATMOSPHERIC CONCENTRATION OF CGA 15324

Time into exposure (hrs:mins)	Analysed concentration (mg/l)	Gravimetric concentration (mg/l)	Total particulate (%)	Total formulation concentration (mg/l)
00:12	0.769	2.68	28.7	2.73
00:42	0.652	2.27	28.7	2.31
01:02	0.790	2.71	29.2	2.80
01:30	0.720	2.55	28.2	2.55
02:05	0.763	2.67	28.6	2.71
02:30	0.644	2.23	28.9	2.28
03:00	0.757	2.68	28.2	2.68
03:20	0.744	2.57	28.9	2.64
03:50	0.792	2.69	29.4	2.81
Mean	0.737	2.56	28.8	2.61
Standard deviation	0.055	0.18	0.4	0.2

TABLE 2 - AERODYNAMIC PARTICLE SIZE DISTRIBUTION

Group Number	RUN 1		RUN 2	
	Time into exposure : 01hr 05min		Time into exposure : 03hr 05min	
Size range	% by weight in range		% by weight in range	
(µm)	Analysed	Gravimetric	Analysed	Gravimetric
≥9.8	2.7	3.2	2.8	3.4
9.8-6.0	10.5	11.4	13.7	13.6
6.0-3.5	27.7	27.3	27.2	26.8
3.5-1.55	43.4	39.2	40.6	37.1
1.55-0.93	9.5	9.6	8.6	8.9
0.93-0.52	4.5	5.9	4.7	6.7
≤0.52	1.8	3.2	2.4	3.6

Gravimetric: $\frac{\text{weight trapped at each size range} \times 100}{\text{total weight trapped}}$

Analysed: $\frac{\text{analysed CGA 15324 concentration at each size range} \times 100}{\text{total CGA 15324 analysed concentration}}$

TABLE 3 - CLINICAL OBSERVATIONS DURING EXPOSURE (SEXES COMBINED)

Group	Time into Exposure: (minutes)	Clinical abnormalities
1 (2.61 mg/*l*)	30	All animals WET (S-M), SAL (S-M)
	55	All animals WET (M), SAL (M)
	80	All animals WET (M), SAL (M), SENS (R), BD (I), BR (R)
	115	All animals WET (M), SAL (M), SENS (R), BD (I), BR (R)
	145	All animals WET (M), SAL (M), SENS (R), BD (I), BR (R)
	175	All animals WET (M), SAL (M), SENS (R), BD (I), BR (R)
	195	All animals WET (M), SAL (M), SENS (R), BD (I), BR (R)
	210	All animals WET (M), SAL (M), SENS (R), BD (I), BR (R)

WET	wet fur
SAL	salivation
SENS (R)	sensitivity to sound reduced
BR (R)	breathing rate reduced
BD (I)	breathing depth increased
S	slight
M	moderate

GLOSSARY FOR TABLES 4-6

Key:-

The following abbreviations may appear in these Tables.

NO. OF OBS	-	Number of observations. This may represent the recording of an observation on more than one occasion during the day for any individual animal.	
NO	-	number	
NAD	-	no abnormalities detected	
S	-	slight	
M	-	moderate	
E	-	extreme	
X	-	noted	
N	-	abnormality no longer present	
I	-	increased	⎫ applies to breathing rate/depth
R	-	reduced	⎭ and response to sound
R	-	right	applies to eyes
SUBS	-	substance	
WITHDRWL REF	-	Withdrawal reflex	
RESPIRAT/Y	-	respiratory	
CURV'URE	-	curvature	
(D)	-	dead	

For Table 4, note that breathing rate was reduced, breathing depth was increased, and response to sound was reduced.

TABLE 4 - GROUP CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 SEX: MALES

mg/l

		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED																
SEVERITIES	M	4														
	S	1														
TOTAL NO. OF OBS.		5														
BREATHING DEPTH																
SEVERITIES	I	5														
TOTAL NO. OF OBS.		5														
BREATHING RATE																
SEVERITIES	R	5														
TOTAL NO. OF OBS.		5														
CHROMODACRYORRHEA																
SEVERITIES	R	1														
TOTAL NO. OF OBS.		1														
REDUCED FOOT WITHDRWL REF																
SEVERITIES	M	2														
	S	1														
TOTAL NO. OF OBS.		3														
GASPING																
SEVERITIES	X	1														
TOTAL NO. OF OBS.		1														
HEAD HELD TWISTED TO SIDE																
SEVERITIES	L	2														
TOTAL NO. OF OBS.		2														
HUNCHED																
SEVERITIES	M	2														
	S	3														
TOTAL NO. OF OBS.		5														
KILLED TERMINATION																
SEVERITIES	X															5
TOTAL NO. OF OBS.																5

TABLE 4 - GROUP CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l — SEX: MALES

		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PILOERECTION																
SEVERITIES	S	3														
TOTAL NO. OF OBS.		3														
PINNA REFLEX ABSENT																
SEVERITIES	X	2														
TOTAL NO. OF OBS.		2														
ABNORMAL RESPIRAT/Y NOISE																
SEVERITIES	X	5	5	5	5	2	2									
TOTAL NO. OF OBS.		5	5	5	5	2	2									
SALIVATION																
SEVERITIES	E	4														
	M	1														
TOTAL NO. OF OBS.		5														
SIGNS OF DIARRHOEA																
SEVERITIES	X						1									
TOTAL NO. OF OBS.							1									
RESPONSE TO SOUND																
SEVERITIES	R	4														
TOTAL NO. OF OBS.		4														
SHAKING																
SEVERITIES	X	4														
TOTAL NO. OF OBS.		4														
TIP TOE GAIT																
SEVERITIES	S	1														
TOTAL NO. OF OBS.		1														
WET FUR																
SEVERITIES	E	4														
	M	1														
TOTAL NO. OF OBS.		5														

TABLE 4 - GROUP CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l — SEX: FEMALES

		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED																
SEVERITIES	M	3														
	S	1														
TOTAL NO. OF OBS.		4														
BREATHING DEPTH																
SEVERITIES	I	5														
TOTAL NO. OF OBS.		5														
BREATHING RATE																
SEVERITIES	R	5														
TOTAL NO. OF OBS.		5														
REDUCED FOOT WITHDRWL REF																
SEVERITIES	M	2														
	S	1														
TOTAL NO. OF OBS.		3														
GASPING																
SEVERITIES	X	1														
TOTAL NO. OF OBS.		1														
HUNCHED																
SEVERITIES	M	1														
	S	4														
TOTAL NO. OF OBS.		5														
KILLED TERMINATION																
SEVERITIES	X															5
TOTAL NO. OF OBS.																5
PILOERECTION																
SEVERITIES	S	2														
TOTAL NO. OF OBS.		2														
PINNA REFLEX ABSENT																
SEVERITIES	X	5														
TOTAL NO. OF OBS.		5														

TABLE 4 - GROUP CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l — SEX: FEMALES

		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ABNORMAL RESPIRAT/Y NOISE																
SEVERITIES	X	5	5	5	5	2	1									
TOTAL NO. OF OBS.		5	5	5	5	2	1									
REDUCED RIGHTING REFLEX																
SEVERITIES	S	1														
TOTAL NO. OF OBS.		1														
SALIVATION																
SEVERITIES	E	4														
	M	1														
TOTAL NO. OF OBS.		5														
SHAKING																
SEVERITIES	X	1														
TOTAL NO. OF OBS.		1														
WET FUR																
SEVERITIES	E	4														
	M	1														
TOTAL NO. OF OBS.		5														

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 ANIMAL NO: 21

mg/l MALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	M	N													
RESPONSE TO SOUND	R	N													
KILLED TERMINATION															X
HUNCHED	M	N													
SALIVATION	E	N													
WET FUR	E	N													
HEAD HELD TWISTED TO SIDE	L	N													
REDUCED FOOT WITHDRWL REF	S	N													
PINNA REFLEX ABSENT	X	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
GASPING	X	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	X	X	N								

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 22
MALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	M	N													
RESPONSE TO SOUND	R	N													
SHAKING	X	N													
KILLED TERMINATION															X
CHROMODACRYORRHEA	R	N													
HUNCHED	M	N													
PILOERECTION	S	N													
SALIVATION	E	N													
WET FUR	E	N													
HEAD HELD TWISTED TO SIDE	L	N													
PINNA REFLEX ABSENT	N														
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	N										

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 23 MALE

DAY NUMBERS

CONDITION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	M	N													
RESPONSE TO SOUND	R	N													
SHAKING	X	N													
KILLED TERMINATION															X
HUNCHED	S	N													
PILOERECTION	S	N													
SALIVATION	M	N													
WET FUR	M	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	N										

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 24 MALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	S	N													
SHAKING	X	N													
KILLED TERMINATION															X
TIP TOE GAIT	S	N													
HUNCHED	S	N													
SALIVATION	E	N													
WET FUR	E	N													
REDUCED FOOT WITHDRWL REF	M	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	X	X	N								

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 25
MALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	M	N													
RESPONSE TO SOUND	R	N													
SHAKING	X	N													
KILLED TERMINATION															X
SIGNS OF DIARRHOEA						X	N								
HUNCHED	S	N													
PILOERECTION	S	N													
SALIVATION	E	N													
WET FUR	E	N													
REDUCED FOOT WITHDRWL REF	M	N													
PINNA REFLEX ABSENT	X	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	N										

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 26
FEMALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	S	N													
SHAKING	X	N													
KILLED TERMINATION															X
HUNCHED	S	N													
SALIVATION	E	N													
WET FUR	E	N													
REDUCED FOOT WITHDRWL REF	M	N													
PINNA REFLEX ABSENT	X	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	N										

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 27 FEMALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	M	N													
KILLED TERMINATION															X
HUNCHED	S	N													
SALIVATION	E	N													
WET FUR	E	N													
REDUCED FOOT WITHDRWL REF	S	N													
PINNA REFLEX ABSENT	X	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	N										

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 28
FEMALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
KILLED TERMINATION															X
HUNCHED	S	N													
SALIVATION	E	N													
WET FUR	E	N													
PINNA REFLEX ABSENT	X	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
GASPING	X	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	X	N									

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 29
FEMALE

CONDITION	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	M	N													
KILLED TERMINATION															X
HUNCHED	M	N													
PILOERECTION	S	N													
SALIVATION	M	N													
WET FUR	M	N													
REDUCED FOOT WITHDRWL REF	M	N													
PINNA REFLEX ABSENT	X	N													
REDUCED RIGHTING REFLEX	S	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	X	X	N								

TABLE 5 - INDIVIDUAL CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

2.61 mg/l ANIMAL NO: 30 FEMALE

DAY NUMBERS

CONDITION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ACTIVITY DECREASED	M	N													
KILLED TERMINATION															X
HUNCHED	S	N													
PILOERECTION	S	N													
SALIVATION	E	N													
WET FUR	E	N													
PINNA REFLEX ABSENT	X	N													
BREATHING DEPTH	I	N													
BREATHING RATE	R	N													
ABNORMAL RESPIRAT/Y NOISE	X	X	X	X	N										

TABLE 6 - BODYWEIGHTS (g)

DOSE: 2.61 mg/l

ANIMAL NUMBER	DAY 1	DAY 8	DAY 15
MALES			
21	373	373	411 (D)
22	397	385	426 (D)
23	397	394	428 (D)
24	361	356	380 (D)
25	368	346	395 (D)
MEAN	379.2	370.8	408.0
S.D.	16.8	19.9	20.5
FEMALES			
26	247	279	291 (D)
27	265	255	276 (D)
28	213	231	236 (D)
29	245	256	255 (D)
30	241	269	282 (D)
MEAN	242.2	258.0	268.0
S.D.	18.7	18.1	22.3

APPENDIX A - PARTICLE SIZE CLASSIFICATION

1. Mass median aerodynamic diameter (MMAD)

The mass median aerodynamic diameter (MMAD) of an aerosol, or part of an aerosol, is the diameter of a unit density sphere having the same terminal settling velocity as a particle shown to divide the size distribution of the aerosol in half when measured by mass. The algebraic symbol commonly used for the MMAD is "D_{50}".

2. Geometric standard deviation (GSD)

The GSD of an aerosol is the ratio of the mean of the distribution to the mean ± 1 standard deviation i.e.:

$$GSD\ (\delta g) = \frac{D50}{D16} = \frac{D84}{D50} = \sqrt{\frac{D84}{D16}}$$

This relationship is only valid for aerosols with a log normal distribution, which is considered to be the case in this study.

APPENDIX B - THE DETERMINATION OF LAMBDA-CYHALOTHRIN IN SAMPLES TAKEN FOR TOTAL PARTICULATE AND PARTICLE SIZE DETERMINATION

METHOD SUMMARY

Samples were extracted with acetonitrile, and aliquots of resulting solutions were diluted with acetonitrile, as appropriate, to give sample solution concentrations typically within the range of calibration standards used. Samples and standards were analysed by High Performance Liquid Chromatography (HPLC).

CHEMICALS AND REAGENTS

Acetonitrile, HPLC grade

Water, Milli Q+ grade (Millipore)

Methanol, HPLC grade

Orthophosphoric acid, Analytical grade

Orthophosphoric acid 0.1% (v/v) - orthophosphoric acid, 1ml, to 1 litre water.

CALIBRATION STANDARDS

CGA 15324/lambda-cyhalothrin EC (300/015) (A13735F), CTL Reference Y03088/038, with a CGA 15324 content of 28.2% w/w was used. This value of CGA 15324 was taken into account in the preparation of standard solutions.

Nominally 177.3mg of CGA 15324/lambda-cyhalothrin EC (300/015) (A13735F), was accurately weighed into a 50mL volumetric flask and diluted to volume with acetonitrile (nominally 1.0mg/mL CGA 15324).

Further appropriate dilutions were made with diluting solvent using volumetric glassware, to produce a range of solutions nominally within 1µg/mL to 50µg/mL CGA 15324.

APPENDIX B - THE DETERMINATION OF LAMBDA-CYHALOTHRIN IN SAMPLES TAKEN FOR TOTAL PARTICULATE AND PARTICLE SIZE DETERMINATION

PROCEDURE

Sample preparation

A known volume of acetonitrile was added to each filter or stage in the container provided with the sample. The samples were sonicated for approximately 5 minutes to extract the CGA 15324 and further diluted with diluting solvent, as required, to a concentration within the range of the calibration standards selected.

High Performance Liquid Chromatography Conditions

Pump	:	600 Series (Waters)	
Mobile phase	:	Orthophosphoric acid 0.1% (v/v)	20%
		Acetonitrile,	70%
		Methanol,	10% (v/v/v)
Flow rate	:	1mL/min	
Detector	:	486 Series UV detector (Waters)	
Detector wavelength	:	240nm	
Column	:	15cm x 4.6mm ID Luna C8 5um (Phenomenex)	
Column temperature	:	Ambient	
Sample introduction	:	717 plus (Waters)	
Injection volume	:	40µL	
Data handling	:	Millennium 32 CDS (Waters)	

CALIBRATION

The analysis system was calibrated using a range of CGA 15324 standards to determine the linearity of response. An appropriate standard of known concentration was interspersed at intervals throughout the analysis.

APPENDIX B - THE DETERMINATION OF LAMBDA-CYHALOTHRIN IN SAMPLES TAKEN FOR TOTAL PARTICULATE AND PARTICLE SIZE DETERMINATION

CALCULATION OF RESULTS

Total particulate samples:

$$\text{Analysed atmosphere concentration (mg/L) } Ca = \frac{Cs \times Df}{Va \times 1000}$$

Where Cs = Sample concentration of CGA 15324 from data system (µg/mL)
Df = Dilution factor
Va = Atmosphere sample volume (L)
= Sample collection time (min) x flow (L/min)

$$\text{\% Total particulate} = \frac{Ca \times 100}{Cg}$$

Where Cg = Gravimetric atmosphere concentration

Particle size distribution:

$$\text{\% Mass on stage by analysis} = \frac{Ms \times 100}{Mt}$$

Where Ms = Cs x Df
Mt = Total mass on all stages by analysis

$$\text{Total formulation concentration (mg/L)} = \frac{Ca \times 100}{\text{\% CGA 15324 in formulation}}$$

FILTER RECOVERY

The extraction and subsequent analysis of filters spiked with known quantities of CGA 15324 have been shown to give recoveries of 100%.

LIMIT OF DETECTION

The limit of detection was calculated to be approximately 0.16µg/mL CGA 15324 in the analysed solution, corresponding to an atmosphere concentration of 0.003mg/L.

APPENDIX C - THE DESIGN AND USE OF THE CIRCULAR NOSE-ONLY EXPOSURE CHAMBER AND BATTELLE RESTRAINING TUBES FOR RATS

1. Purpose

The nose-only method of exposure was used since deposition on the fur of the test particulate and subsequent ingestion during grooming can result in substantial quantities of test substance entering the body by the oral route, exacerbating and possibly masking any effects produced by inhalation.

2. Design (Figure 1)

2.1 The chamber

The chamber consisted of sections of PERSPEX tubing (6mm wall thickness) with an internal diameter of 28cm and height of 15cm (approximate volume 9.2 litres). Each section was drilled with ten equidistant holes of 28mm diameter into which the restraining tubes were pushed to give a good seal. There was also one sampling port.

The chamber located on to a base-plate, fitted with castors for manoeuvrability. A conical aluminium lid ensured good distribution of the atmosphere across the chamber, the atmosphere having being generated from above. The conical lid and the base together had a volume of approximately 9.2 litres. In this study two sections were connected, giving a volume of approximately 27.6 litres.

APPENDIX C - THE DESIGN AND USE OF THE CIRCULAR NOSE-ONLY EXPOSURE CHAMBER AND BATTELLE RESTRAINING TUBES FOR RATS

2.2 The restraining tubes

The restraining tubes were made by Battelle, Geneva and consisted of a polycarbonate tube, one end of which was tapered and fitted into the exposure chamber. In the other end a spring loaded plunger was fitted which was contoured to fit a rat and fitted over the base of the tail. The top of the tube had apertures to prevent excessive build-up of heat and water vapour which might make the animal unduly uncomfortable, while similarly there was a groove in the bottom of the tube for drainage of urine.

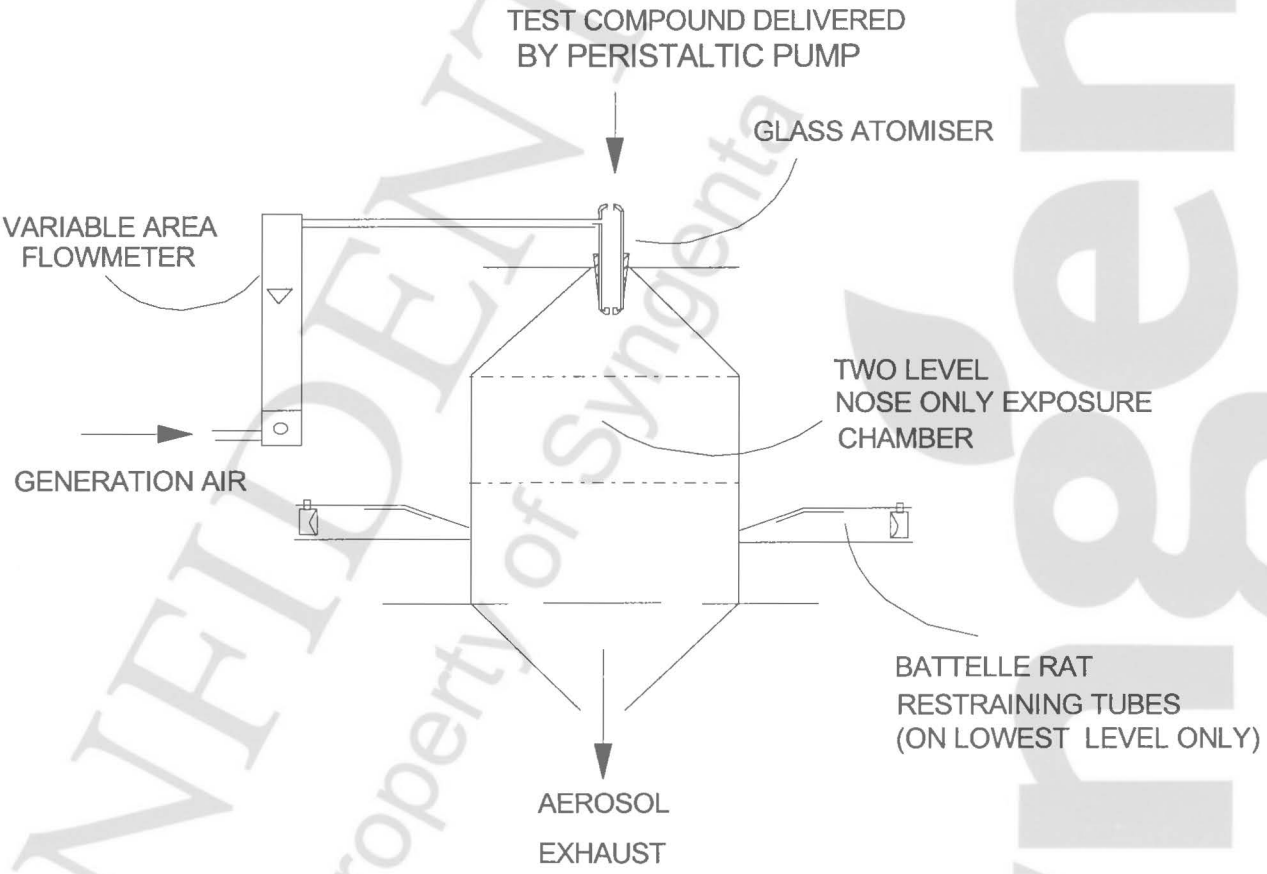
3. Sampling

Atmospheric concentrations were determined by sampling through the sampling port. While atmospheres were being set up prior to animal exposure the holes for the restraining tubes were plugged.

APPENDIX C - THE DESIGN AND USE OF THE CIRCULAR NOSE-ONLY EXPOSURE CHAMBER AND BATTELLE RESTRAINING TUBES FOR RATS

FIGURE 1

DIAGRAM OF 2-TIERED EXPOSURE CHAMBER
WITH CONCENTRIC GLASS JET ATOMISER



APPENDIX D - CHAMBER EQUILIBRATION TIME

The total volume of the chamber was 27.6 litres. Silver (1946) showed that the time taken for a chamber to reach a point of equilibrium was proportional to the flow rate of atmosphere passing through the chamber and the chamber volume. From this, the concentration/time relationship during the 'run-up' and 'run-down' phase can be expressed by the equation:

$$tx = \frac{k * V}{F}$$

where tx = time required to reach x% of the equilibrium concentration
k = a constant of value determined by the value of x
V = chamber volume
F = chamber flow rate

The t_{99} value is quoted usually with respect to the performance of exposure chambers, representing the time required to reach 99% of the equilibrium concentration and providing an estimate of chamber efficiency. Thus, at maximum efficiency, the theoretical value of k at t_{99} is 4.605 and the closer to this the results of evaluation of actual chamber performance falls, the greater the efficiency and the better the design of the chamber. Bennett (1990) conducted a detailed evaluation of this chamber design and showed that it performed close to the predictions of Silver.

Using the above formula for the chamber volume and flow rates used in this study (27.6 litres and 28 litres/min respectively):

$$T_{99} = \frac{4.605 * 27.6}{28}$$

$$= 4.5 \text{ mins.}$$

This means that within 4.5 minutes of switching on of the generation system the chamber reached 99% of the equilibrium stage. In this study, atmosphere generation started at 07 10am on the day of exposure. According to the equation, t_{99} would have been achieved after 4.5 minutes. This was confirmed by a sample taken after 15 minutes showing a gravimetric atmosphere concentration of 2.42 mg/l. Since the target concentration was 2.5 mg/l, the generation rate was increased slightly at this time 25 minutes after start of generation the concentration was 2.5 mg/l. This was considered acceptably stable to commence exposure of

APPENDIX D – CHAMBER EQUILIBRATION TIME

the test animals since further adjustments to the generation system would be required once the animals were introduced to the exposure chamber and during the exposure period. Exposure started at 07.40 am and all samples taken during the exposure phase and the time at which they were taken after the exposure commenced are reported in Table 1. 4-Hours after the start of the exposure the rats were removed from the chamber prior to switching off of the generation system. Hence the rats were exposed for a full 4 hours to the test atmosphere.

8. REFERENCES

Bennett I.P. (1990). The variability of particulate atmospheres. M.Phil Thesis. Dept. of Chemical Engineering, South Bank polytechnic, London.

Silver, S.D. (1946). Constant flow gassing chambers: principles influencing design and operation. J. Lab. Clin. Med. 31, 1153-1161

CERTIFICATE OF ANALYSIS

syngenta

GLP Testing Facility EZA
Analytical Development &
Product Chemistry GS2131

Syngenta Crop Protection Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

A13735F
CGA15324/lambda-cyhalothrin EC (300/015)
SVR4J135162

Batch Identification	**SVR4J135162**
Product Code	**A13735F**
Other Product Code(s)	CGA15324/lambda-cyhalothrin EC (300/015)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of :**	
profenofos *	**310 g/l corresponding to 28.2 % w/w**
lambda-cyhalothrin *	**17.3 g/l corresponding to 1.57 % w/w**
Methodology used for Characterization / Reanalysis	GC

The Active Ingredient(s) content is within the FAO limits.

Physical Analysis

– **Appearance**	**yellowish liquid**
– **Density ***	**1100 kg/m^3**

Stability:

– **Storage Temperature**	**< 30°C, keep away from direct sunlight**
– **Reanalysis date**	**March 2007**

The stability of this test substance will be controlled by reanalysis of material held in the inventory at Syngenta Crop Protection Münchwilen AG at the appropriate time.

This Certificate of Analysis is summarizing data which originate either from a single study or from several individual studies which have been performed in compliance with GLP. Tests marked with an asterisk (*) have been conducted within a single study/as individual studies. Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these studie(s) are stored under the study number(s) referenced below within the archives of the GLP Testing Facility EZA at Syngenta Crop Protection Münchwilen AG. No GLP compliance is claimed for this certificate.

Characterisation: 114379 Reanalysis:

Authorisation: April 12, 2005

S.Voellmin
Analytical Development & Product Chemistry

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