AUSTRIAN RESEARCH CENTERS
Toxikologie

SYN520453

SYN520453 EC (A15149AC) - Skin Sensitisation Study (Nine Induction Dose Buehler Test)

Final Report

DATA REQUIREMENT(S): OECD Test Guideline 406
Directive 96/54/EC, B.6.
EPA Health Effects Test Guidelines, OPPTS 870.2600
No.12-Nohsan-8147,24; November, 2000

AUTHOR(S): Mag. Katharina Eberhart-Sattler

STUDY COMPLETION DATE: 19 May 2008

PERFORMING LABORATORY: Austrian Research Centers GmbH-ARC
Business Field Toxikology
Seibersdorf/2444/Austria

LABORATORY PROJECT ID: Report Number: 2992
Study Number: SYN41
Task Number: T011329-05

SPONSOR: Syngenta Ltd
Jealott's Hill International Research Centre
Bracknell, Berkshire, RG42 6EY, United Kingdom

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Statement concerning the study:

SYN520453 EC (A15149AC) -
Skin Sensitisation Study (Nine Induction Dose Buehler Test)

This study meets the requirements of the Principles of Good Laboratory Practice of the OECD (Environment Health and Safety Publications, Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1, Paris 1998) with the exception that formulations of the test substance in vehicle were not analysed for concentration and homogeneity. The analysis of the test substance for purity and stability are the responsibility of the Sponsor.

ON HER BEHALF

R. L (DR. ROBERT HRUZA) 19 May 2008

Mag. Katharina Eberhart-Sattler — Date
Study Director
Toxicology

Performing Laboratory: Austrian Research Centers GmbH - ARC
Business Field Toxikology
Seibersdorf/2444/Austria

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Statement concerning the study:

SYN520453 EC (A15149AC) -
Skin Sensitisation Study (Nine Induction Dose Buehler Test)

The following inspections were made during this study:

Date of inspection	Inspected phases	Date of report to the management
22 February 2008	Protocol	22 February 2008
26 March 2008	Experimental phase	26 March 2008
06 May 2008	Draft report	06 May 2008

This report has been reviewed by the Quality Assurance Unit. The reported methods and procedures were found to describe those used and the results to constitute an accurate representation of the data recorded.

Josef Taferner
Quality Assurance Unit

Date 19 May 2008

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Katharina Eberhart-Sattler	Study Director
Josef Taferner	Quality Assurance Unit
Erna Stepan	Animal Technician
Ros Sheldon	Syngenta Study Manager

Study dates

Study initiation date: 21 February 2008.
Experimental start date: 22 February 2008.
Experimental termination date: 14 March 2008.

Deviations from the guidelines

None.

Retention of samples

All raw data, a copy of the original final report and the test substance are retained for 10 years. Neither the raw data nor the test substance will be destroyed without prior consent of the Sponsor.

Performing Laboratory Test Substance Reference Number

A15149AC.

Other

The archive is located at the Austrian Research Centers GmbH - ARC.

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

1.1. Study design

1.1.1. Aim

The aim of the study was to evaluate possible skin sensitising potential of SYN520453 EC (A15149AC). The "Buehler Test" was chosen as an appropriate method according to the Sponsor's request.

1.1.2. Method

20 female Hartley guinea pigs (Crl:HA) were used as a test substance group and another 10 females were used as a negative control group. There were nine epicutaneous induction exposures and one epicutaneous challenge exposure.

The concentration of the test substance was 100 % (i.e. undiluted) for all nine induction exposures.

For the challenge exposure, test substance concentrations of 100 % and 75 % (v/v) in deionised water were used. The concentrations for the challenge exposure refer to the findings of the pilot study, where 100% test substance concentration did not cause observable skin reactions.

For each exposure the areas of administration were covered occlusively for at least 6 hours.

1.2. Results

1.2.1. Skin reactions after the induction exposures

In the negative control group no adverse skin reactions were noted at any time.

In 18/20 animals of the test substance group skin reactions (very slight to well defined erythema) were noted sometimes attended by eschars.

1.2.2. Skin reactions after the challenge exposure

The results of these skin examinations were decisive for the grading of the potential for sensitisation.

Group C (negative control):
No skin reactions in any animal at any reading time.

Group A (test substance):
No skin reactions in any animal at any reading time.

1.3. Conclusion

The net rate of positively reacting animals in the test substance group was 0%.
Referring to the OECD Guideline 406, 15% positively reacting animals is the threshold for regarding a test substance as a sensitiser.

Therefore the test substance was not a skin sensitiser under the conditions of the test.

2. INTRODUCTION

2.1. Purpose

A "Buehler Test" according to E. V. Buehler was performed to reveal a possible sensitising potential of SYN520453 EC (A15149AC). Investigations were in conformance with the following guidelines:

- OECD Guideline 406.
- Directive 96/54/EC, B.6.
- Directive 2001/59/EC for Classification.
- EPA Health Effects Test Guidelines, OPPTS 870.2600 Skin Sensitization, 1998.
- No.12-Nohsan-8147, 24 November, 2000 Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan, Skin Sensitization Studies 2-1-6.

3. MATERIALS AND METHODS

3.1. Test substance

3.1.1. Description by the Sponsor

Name	SYN520453 EC (A15149AC).
Batch ID	J8092/056
pH	> 6.0 <=8.0
Specific Gravity/Density	0.95 g/cm³ (20-25°C).
Appearance	Clear brown liquid.
Active Ingredient Content	13.4 % w/w SYN520453 corresponding to 128 g/L
Conditions of storage	0 < t< 40°C, keep away from direct sunlight.
Expiry date	December 2009.

3.1.2. Description and characterisation by the test facility

Date of receipt	24 January 2008.
Appearance	Brown liquid.
Extract from the label on the shipping container	N SYNGENTA EXPERIMENTAL COMPOUND GLP 526908 X A 15149AC SYN520453 EC (125)
Storage	Room temperature, keep away from direct sunlight.
Archive	A sample of the test substance will be archived at the end of the study, storage at ambient temperature in the dark.

The analyses of the test substance for purity and stability were the responsibility of the Sponsor.

3.1.3. Preparation and administration of the test substance

The test substance was thoroughly mixed before use.

For the induction exposures, the test substance was applied undiluted (100%). For the challenge exposure the test substance was applied undiluted (100%, anterior right flanks) and diluted with deionised water (75%, posterior right flanks).

All animals of the test substance group and the control group were treated in the same way. The test patch (Pur Zellin-Tupfer, Fa. Hartmann, A-2355 Wiener Neudorf, ca. 2 cm x 2 cm) was fully loaded with the test substance formulation or the vehicle, respectively, applied to the skin and fixed with a strip of "Fixomull Stretch®" (hypoallergenic tape, Beiersdorf AG, D-20245 Hamburg).

Occlusion was obtained by covering with teflon foil which was fixed with Guinea Pig Jackets (Hugo Sachs Elektronik- Harvard Apparatus GmbH, D-79232, March-Hugstetten, Gruenestrasse 1).

Applied amounts of the test substance formulation (per animal) were:
0.5 mL for each of the 9 induction exposures and for the challenge exposure.

Applied amounts of deionised water (per animal) were:
0.5 mL for each of the 9 induction exposures.

The dressings were removed at least 6 hours after each administration.

3.2. Experimental design

3.2.1. Test principle

Induction exposures:
Epicutaneous administrations to the left flanks of all animals.
Test substance group: Nine inductions with 100% test substance (undiluted).
Negative control group: Nine inductions with deionised water.

Challenge exposure:
All animals of both groups were treated in the same way: Administration of 100% test substance (undiluted) to the anterior right flanks, administration of 75% test substance in deionised water (v/v) to the posterior right flanks.

Skin reactions after the challenge exposure were compared between the test substance group and the negative control group.

3.2.2. Time schedule

3.2.2.1. Preliminary study

The test substance was applied on 22 February 2008.

3.2.2.2. Definitive study

Day 0 was 25 February 2008.

Day 0: recording of body weight, clipping of hair, 1st induction exposure, removal of dressings 6 hours after application.
Day 1: scoring.
Day 2: scoring, clipping of hair, 2nd induction exposure, removal of dressings 6 hours after application.
Day 3: scoring.
Day 4: scoring, clipping of hair, 3rd induction exposure, removal of dressings 6 hours after application.
Day 5: scoring.
Day 7: clipping of hair, 4th induction exposure, removal of dressings 6 hours after application.
Day 8: scoring.
Day 9: scoring, clipping of hair, 5th induction exposure, removal of dressings 6 hours after application.
Day 10: scoring.
Day 11: scoring, clipping of hair, 6th induction exposure, removal of dressings 6 hours after application.
Day 12: scoring.
Day 14: clipping of hair, 7th induction exposure, removal of dressings 6 hours after application.
Day 15: scoring.
Day 16: scoring, clipping of hair, 8th induction exposure, removal of dressings 6 hours after application.
Day 17: scoring.
Day 18: scoring, clipping of hair, 9th induction exposure, removal of dressings 6 hours after application.
Day 19: scoring.
Day 28: clipping of hair, challenge exposure, removal of dressings 6 hours after application.
Day 29: approx. 21 hours after removing the patch the hair clipped on the application area, approx. three hours later scoring.
Day 30: scoring, recording of body weight, sacrifice of animals, end of test.

3.2.3. Clipping of the hair

On the respective days (see also time schedule), the hair of the animals was clipped on the appropriate application sites with an Aesculap GH204 electric hair clipper with a 0.1 mm cutter head.

3.2.4. Groups, animal numbers, substances and concentrations

I 9 induction exposures
Ch challenge exposure
TS test substance
lf left flank
arf anterior right flank
prf posterior right flank

Group	Animal Nos.	Sex	Exposure		Substances
C	61 - 70	F	I	lf	Deionised water
			Ch	arf	TS, 100 % (undiluted)
				prf	TS, 75 % (v/v) in deionised water
A	71 - 90	F	I	lf	TS, 100 % (undiluted)
			Ch	arf	TS, 100 % (undiluted)
				prf	TS, 75 % (v/v) in deionised water

3.2.5. Rationale for the selection of the concentrations

According to the Guidelines, the concentrations of the test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest non-irritant one.

To obtain the appropriate concentrations of the test substance for the definitive study, a preliminary test was carried out with 3 female guinea pigs. Four different concentrations of the test substance were administered epicutaneously.

The mode of application was the same as in the definitive study. The duration of the epicutaneous exposure was 6 hours. The test substance was applied undiluted and diluted with deionised water (v/v).

Results (scores) after the epicutaneous exposure of the preliminary tests (for scale see Table 3):

Test Substance Concentration (v/v)	Scores for animal Nos. [a)]		
	101	102	103
100 %	0/0	0/0	0/0
75 %	0/0	0/0	0/0
50 %	0/0	0/0	0/0
25 %	0/0	0/0	0/0

[a)] Scores 24/48 hours after the end of the epicutaneous exposure. For scoring scheme see Table 3.

For the main study, the following concentrations of SYN520453 EC (A15149AC) were therefore selected:
100 % test substance for all nine epicutaneous induction exposures, 100 % test substance and 75 % (v/v) in deionised water for the challenge exposure.

3.2.6. Vehicle, reference substance

Deionised water was used as a reference substance and as a vehicle for the challenge exposure.

3.2.7. Test system (animals)

Species, strain	Guinea pigs, Hartley, Crl:HA
Supplier	Charles River, D-97633
Sex, specification	Female, healthy, young, non pregnant adults
Age	5 - 7 weeks at the first application
Weight range	294 g to 367 g at the first application
Number of animals in the definitive study	20 animals for the test substance group 10 animals for the control group

3.2.8. Animal maintenance

Hygiene:	Optimal hygienic conditions.
Room number:	EH1-19.
Room temperature:	Mean of 22.0°C (continuous control and registration). The room temperature was permanently within the specified limits.
Relative humidity:	Mean of 45.8 % (continuous control and registration). The relative humidity was permanently within the specified limits.
Air exchange:	Approx. 12/h.
Light:	Artificial light from 6.00 a.m. to 6.00 p.m.
Cages:	Group caging in plastic containers (46 cm x 105 cm x 36 cm), partly shaded, 10 animals per container.
Feed:	Ssniff Ms-H (Guinea Pig Maintenance Diet V2233), including ascorbic acid (2400 mg/kg), *ad libitum*, offered in stainless steel containers. Analysis of the feed for ingredients and contaminants are performed randomly by Ssniff Spezialdiäten GmbH, Ferdinand-Gabriel-Weg 16, 59494 Soest, Germany.
Bedding material:	Wood chips (aspen) from Fa. ABEDD Dominik Mayr KEG, A-8580 Köflach. Reduction of microorganisms by autoclaving.
Water:	Tap water offered in Makrolon bottles with stainless steel canullae *ad libitum*. Random samples of the water are analysed by the "AGES", A-1226 Vienna, to assure that the water fulfils the requirements for drinking water for humans.
Animal identification:	Number tattooed in the pinna of the right ear.
Acclimatisation:	11 days.

3.3. Investigations

3.3.1. General observations

All animals were observed once daily for behavioural changes or signs of toxicity.

3.3.2. Body weights

The body weight of each animal was recorded on days 0 and 30.

3.3.3. Skin reactions

Skin was examined 24 and 48 or only 24 hours after the start of each induction exposure as well as 24 and 48 hours after the end of the challenge exposure (blind reading of test and control animals). Findings on the test substance and on the control areas were scored according to the scheme of Table 3.

3.4. Data evaluation

3.4.1. Bias control

The individual animals were allocated to their groups by random numbers. The scoring after the challenge exposure was performed "blind", i.e. the animals were randomly presented for scoring (computer generated random lists) and the numbers of the animals were not visible to the person scoring.

3.4.2. Evaluation of results

After the challenge exposure, the number of animals with skin reactions on the test substance treated areas was recorded.

The potential of the test substance to cause skin sensitisation was established by subtracting the percentage of animals with positive skin reactions in the control group from the percentage of positively reacting test substance group animals.

3.4.3. Positive control

The sensitivity and the reliability of the experimental procedure is checked separately, twice a year, using α-hexyl cinnamic aldehyde as a sensitiser and the same strain of animals and the same experimental procedure as in the present study (see Appendix 1).

3.4.4. Unforeseen events

No unforeseen events occurred.

4. RESULTS AND DISCUSSION

4.1. Mortality

All animals survived until the end of the study.

4.2. Body weights

No abnormalities were observed.

4.3. General observations

Immediately after the beginning of all exposures (inductions, challenge), the activities of all animals were increased. This is a common finding in studies of this type due to the dressings which restrict the freedom of movement. Soon afterwards the behaviour returned to normal.

Alterations at the induction sites, increasing in incidence with time, were attributed to mechanical irritation from test substance removal. These alterations are not considered to demonstrate evidence of toxic or sensitising properties of the test substance.

Except for the observations described above no abnormal behaviour or clinical signs in the animals were detected.

4.4. Skin reactions

See Table 2.

4.4.1. Skin reactions after the induction exposures

Group C (negative control):
No skin reactions in any animal at any reading time.

Group A (test substance):
In 18/20 animals of the test substance group skin reactions (very slight to well defined erythema) were noted sometimes attended by eschars.

4.4.2. Skin reactions after the challenge exposure

These were the reactions of interest for the grading of the allergenic potency of the test substance. Results are shown in Table 1.

Group C (negative control):
No skin reactions in any animal at any reading time.

Group A (test substance):
No skin reactions in any animal at any reading time.

4.5. Discussion

None of the animals induced with the test substance (0/20 - that is 0%) had a positive response at the challenge exposure. This is below the threshold of 15% positively responding animals, when a substance is regarded to be a sensitiser.

SYN520453 EC (A15149AC) is therefore not regarded as a sensitiser.

5. CONCLUSIONS

The net rate of positively reacting animals in the test substance group was 0%. Referring to OECD Guideline 406, 15% positively reacting animals is the threshold for regarding a test substance as a sensitiser.

Therefore the test substance was not a skin sensitiser under the conditions of the test.

6. REFERENCES

None.

TABLES SECTION

TABLE 1 Results of the Challenge Exposure.

These results were decisive for the grading of the allergenic potency of the test substance.

Group	Number of animals at challenge exposure	Number of animals with positive skin reactions	Net rate of animals with positive skin reactions (%)
C Negative control	10	0	-
A Test substance	20	0	0

TABLE 2 Skin Reactions after Induction Exposures and after Challenge Exposure - Visual Examination

Negative control group C: individual data. Skin reactions 24 and 48 or only 24 hours after the start of each induction exposure and 24 and 48 hours after the end of the challenge exposure. For scoring scheme see Table 3.

	Skin reaction after induction															Skin reaction after challenge with 100% test substance		Skin reaction positive	Skin reaction after challenge with 75% test substance		Skin reaction positive
	1st		2nd		3rd	4th		5th		6th	7th		8th		9th						
Animal No.	24 h	48 h	24 h	48 h	24 h	24 h	48 h	24 h	48 h	24 h	24 h	48 h	24 h	48 h	24 h	24 h	48 h	(yes/no)	24 h	48 h	(yes/no)
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
66	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no

TABLE 2 **Skin Reactions after Induction Exposures and after Challenge Exposure - Visual Examination. Continued**

Test substance group A: individual data. Skin reactions 24 and 48 or only 24 hours after the start of each induction exposure and 24 and 48 hours after the end of the challenge exposure. For scoring scheme see Table 3.

	Skin reaction after induction															Skin reaction after challenge with 100% test substance		Skin reaction positive	Skin reaction after challenge with75% test substance		Skin reaction positive
	1st		2nd		3rd	4th		5th		6th	7th		8th		9th						
Animal No.	24 h	48 h	24 h	48 h	24 h	24 h	48 h	24 h	48 h	24 h	24 h	48 h	24 h	48 h	24 h	24 h	48 h	(yes/no)	24 h	48 h	(yes/no)
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
72	0	0	0	1	0	1	1	1	1	1	0	0	1	1	0	0	0	no	0	0	no
73	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	no	0	0	no
74	0	0	0	0	0	1	1	1	2*	1	0	0	1	1	1	0	0	no	0	0	no
75	0	0	0	1	1	1	1	0	1	0	1	1	0	1	1	0	0	no	0	0	no
76	0	0	0	0	0	1	2*	2*	2*	1	1	2	0	1	1	0	0	no	0	0	no
77	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	no	0	0	no
78	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	no	0	0	no
79	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	no	0	0	no
80	0	0	0	0	0	1	1*	1*	1*	1	0	0	0	0	0	0	0	no	0	0	no

* Skin reaction attended by eschars.

TABLE 2 **Skin Reactions after Induction Exposures and after Challenge Exposure - Visual Examination Continued**

Test substance group A: individual data. Skin reactions 24 and 48 or only 24 hours after the start of each induction exposure and 24 and 48 hours after the end of the challenge exposure. For scoring scheme see Table 3.

	Skin reaction after induction																Skin reaction after challenge with 100% test substance		Skin reaction positive	Skin reaction after challenge with75% test substance		Skin reaction positive
	1st		2nd		3rd	4th		5th		6th	7th		8th		9th							
Animal No.	24 h	48 h	24 h	48 h	24 h	24 h	48 h	24 h	48 h	24 h	24 h	48 h	24 h	48 h	24 h		24 h	48 h	(yes/no)	24 h	48 h	(yes/no)
81	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0		0	0	no	0	0	no
82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	no	0	0	no
83	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0		0	0	no	0	0	no
84	0	0	0	0	0	0	0	1*	1*	1	1	1	1	1	0		0	0	no	0	0	no
85	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0		0	0	no	0	0	no
86	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0		0	0	no	0	0	no
87	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0		0	0	no	0	0	no
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		0	0	no	0	0	no
89	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0		0	0	no	0	0	no
90	0	0	0	2	1	0	0	1	0	0	1	1	0	0	0		0	0	no	0	0	no

* Skin reaction attended by eschars.

TABLE 3 Scheme for Scoring of Skin Reactions

Skin reactions	Score	Graded as
No reactions	0	normal
Very slight erythema	1	mild
Well defined erythema	2	moderate
Severe erythema and/or oedema	3	severe

TABLE 4 Body Weights of the Animals on Days 0 and 30

Individual body weights (b.w.) in grams of the animals of the negative control group C and of the test substance group A (means, standard deviations (SD) and number of animals per group (n)).

Group C(negative control)			Group A (test substance)		
	b.w. (g) on Day			b.w. (g) on Day	
Animal No.	0	30	Animal No.	0	30
61	333	554	71	338	515
62	302	469	72	330	535
63	322	481	73	333	516
64	331	487	74	310	447
65	338	477	75	321	476
66	342	535	76	315	477
67	315	483	77	327	517
68	333	539	78	340	554
69	332	491	79	315	473
70	309	460	80	303	436
			81	329	543
			82	340	509
			83	350	558
			84	323	467
			85	322	496
			86	367	598
			87	294	450
			88	351	601
			89	328	481
			90	325	506
mean	352.7	497.6	mean	328.0	507.8
SD	13.2	32.7	SD	17.0	46.8
n	10	10	n	20	20

APPENDICES SECTION

APPENDIX 1 Positive Control Data

To check the sensitivity of the strain of animals used to detect a possible sensitising potential of a test substance as well as the reliability of the experimental technique, the relevant OECD- and EC-guidelines require periodic checks with known sensitisers.

The last check was performed with "HEXYL CINNAMIC ALDEHYDE" (HCA):

Time schedule:

Dosing:	first epicutaneous induction exposure:	30 January 2008
	second epicutaneous induction exposure:	06 February 2008
	third epicutaneous induction exposure:	13 February 2008
	epicutaneous challenge exposure:	27 February 2008

Positive control substance:

Data obtained from the manufacturer:

Name:	"HEXYL CINNAMIC ALDEHYDE".
Synonym:	α-HEXYLCINNAMIC ALDEHYDE.
CAS No.:	101-86-0.
Molecular formula:	$C_6H_5CH=C[(CH_2)5\text{-}CH_3]CHO$.
Manufacturer:	ALDRICH, D-89555, Steinheim.
Cat. No.:	29,128-5.
Identity:	-.
Purity:	techn. 85 %.
Molecular weight:	216.33.
Label on shipping container:	F:W. 216.33 $C_6H_5CH=C[(CH_2)5\text{-}CH_3]CHO$ b.p. 174 - 176 °C d 0.950 α-Hexylzimtaldehyd techn. 85 %. α-Hexylcinnamaldehyde, techn. 85 % Katalog-Nr.: 29,128-5 Stabilized with 0.3 ppm BHA Severe irritant.
Storage:	Room temperature, in the dark.

APPENDIX 1 Positive Control Data - Continued

Characterisation by the test facility:
Date of receipt: 14 November 2007.
Appearance: Yellow liquid.

Number of animals:
10 females for the positive control substance group.
5 females for the negative control group.

Exposures:
3 induction exposures.
1 epicutaneous challenge exposure.

Concentrations of HCA:
100% (undiluted) for the 3 induction exposures.
75% (v/v) diluted with acetone for the challenge exposure.

Results:
Negative control group for positive control:

Vehicle site:	No adverse skin reactions in any animal at any time.
Test substance site:	No adverse skin reactions in any animal at any time.

No animal had a positive skin reaction.

Positive control group:

Vehicle site:	No adverse skin reactions in any animal at any time.
Test substance site:	Very slight erythema in 5/10 animals 24 and/or 48 hours after the end of the challenge exposure.

These 5/10 animals, i.e. 50% of the animals of the positive control group, were regarded as sensitised.
The results prove the sensitivity of the strain of animals used and the reliability of the experimental technique.

APPENDIX 2 GLP Certificate

Bundesministerium für Land- und Forstwirtschaft,
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Certificate

It is hereby certified that the test facility

ARC Seibersdorf research GmbH
Bereich Umwelt- und Lebenswissenschaften
GLP- Prüfstelle
A-2444 Seibersdorf

is subject to the Austrian GLP Compliance Monitoring Programme for Chemicals and Plant Protection Products since February 7th, 1989, and that the last inspection by the Competent Authority regarding compliance with the Principles of Good Laboratory Practice on the basis of the corresponding guidelines of the OECD and the European Union was carried out on September 13th - 15th 2005.
On the basis of this inspection the test facility was found to be compliant with the OECD Principles of Good Laboratory Practice.

Areas of expertise:
1. Physical-chemical testing
2. Toxicity studies
3. Mutagenicity studies
4. Environmental toxicity studies on aquatic and terrestrial organism
5. Studies on behavior in water, soil and air, bio-accumulation
8. Analytical and clinical chemistry
9. Other: stability testing

Vienna, March, 17th, 2006

On behalf of the Federal Minister

Gottfried Gidaly

(Dr. Gottfried Gidály)

APPENDIX 3 Certificate of Analysis

syngenta

GLP Testing Facility JH
Analytical Development & Product Chemistry

Jealott's Hill International Research Centre,
Bracknell, Berkshire
RG42 6EY
United Kingdom

Certificate of Analysis

Formulated Material
SYN520453(125 g/L) EC

Batch Identification	**J8092/056**
Design Code	**A15149AC**
Other Product Code(s)	-
Chemical Analysis (Active Ingredient Content)	
– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of SYN520453 ***	**13.4% w/w corresponding to 128 g/L.**
	4.1% w/w SYN534968 corresponding to 39 g/L)
	9.3% w/w SYN534969 corresponding to 89 g/L))
Methodology used for Characterisation / Reanalysis	Capillary GC
The Active Ingredient(s) content is within the FAO limits.	
Physical Analysis	
– **Appearance**	**A uniform mobile clear brown liquid**
– **Density ***	**0.956 g/cm^3**
Stability:	
– **Storage Temperature**	**0 < t < 40°C, keep away from direct sunlight**
– **Expiry date**	**December 2009 (Temperate and sub-tropical climates)**

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

This Certificate of Analysis summarizes data which originates either from a single study or from several individual studies. Tests marked with an asterisk (*) have been conducted under GLP protocol. Raw data, documentation, study plans and reports pertaining to this/these study/studies are stored under the study number(s) referenced below within the archives of the GLP Testing Facility JH at Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY.

Study Number(s):	08AS001, NS00979
IDS Report Number(s):	10343850
Supplementary Information:	Initial characterisation January 2008.
	Where applicable, spray tank dilutions should be used within one working day.
	Handling of the material will follow the guidelines within the appropriate MSDS

Authorisation:

P. Clarke — P M Clarke

15 Jan 2008 — Date