

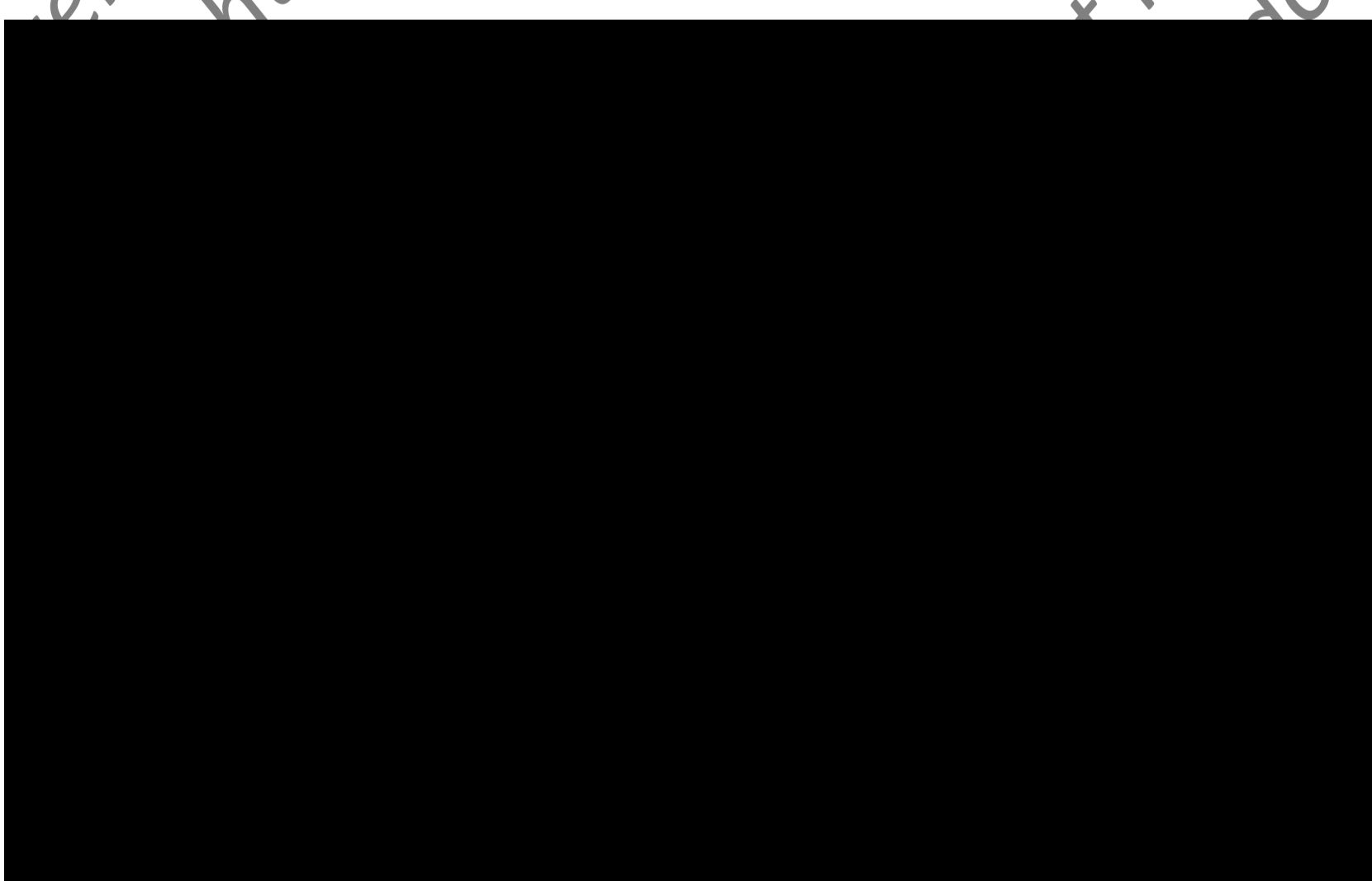
THE ACUTE ORAL AND CONTACT TOXICITY

TO HONEY BEES OF COMPOUND

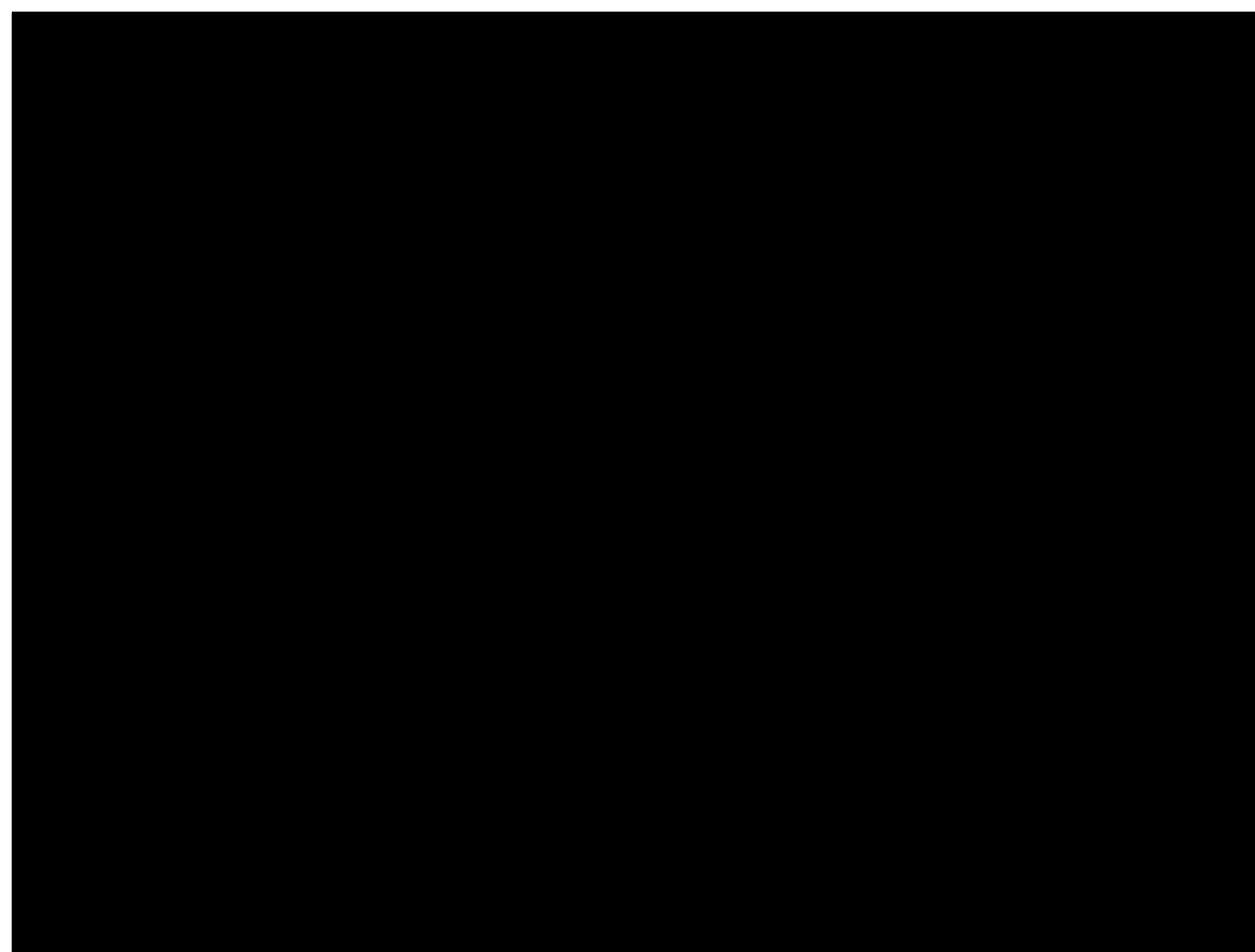
NTN.33893 TECHNICAL

Amendment 1
06.01.94

Addressee:



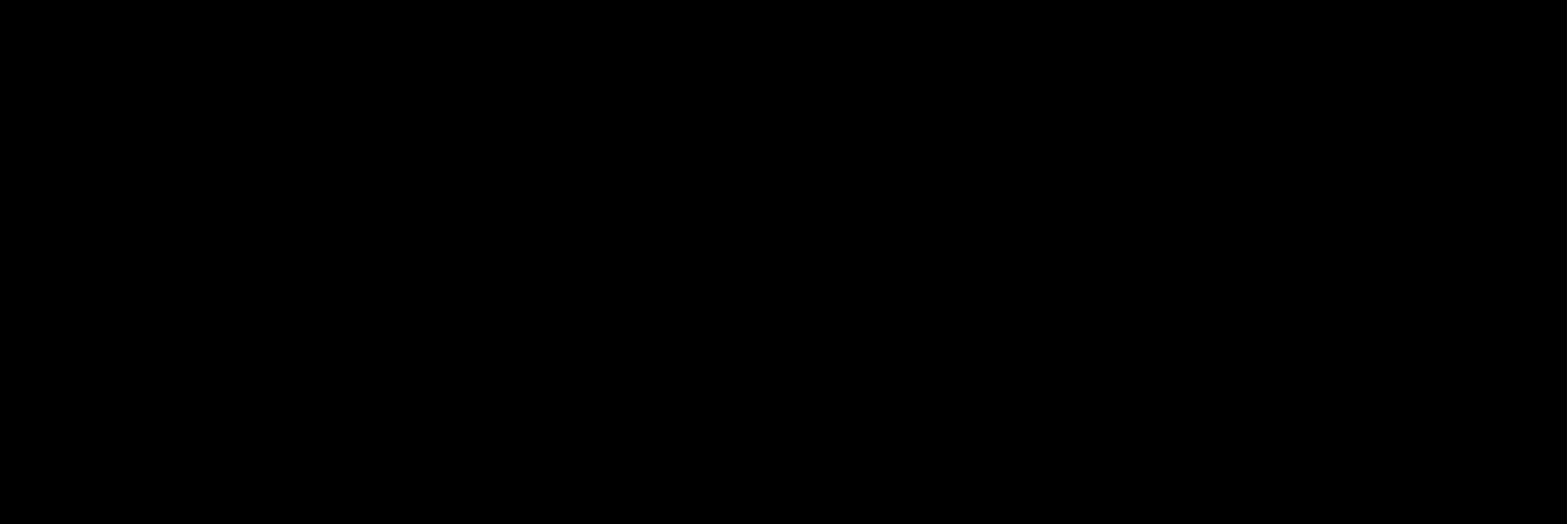
Authors:



Report issued 28 December 1990.



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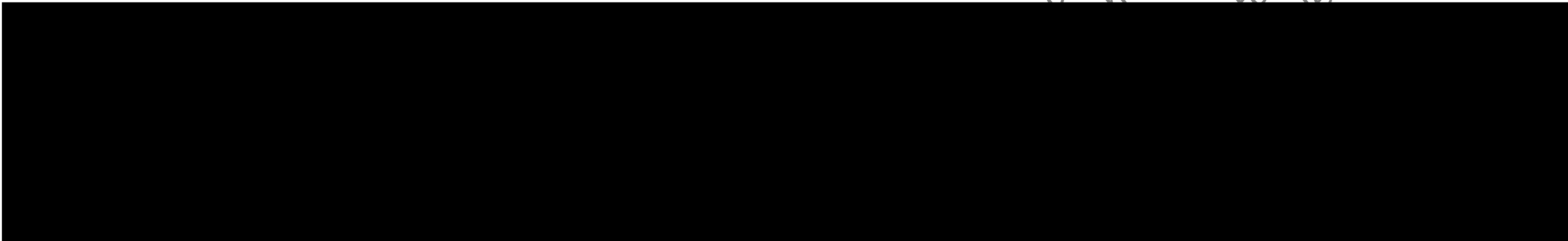


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DEPARTMENT OF QUALITY ASSURANCE

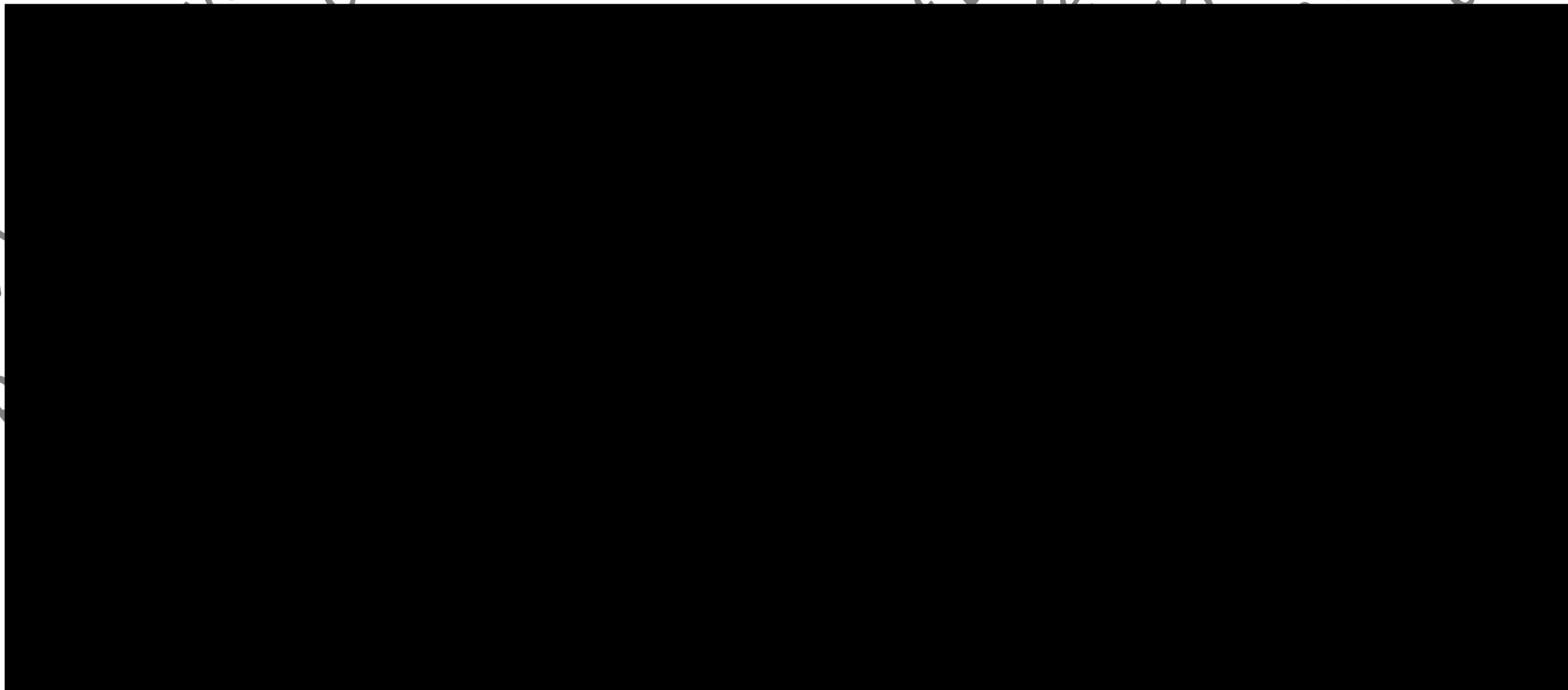
REPORT AUDIT STATEMENT

HRC Report No. **BAY 158/901384**



Date of reporting Audit Findings
to the Study Director
and HRC Management

15.10.90



19.10.90

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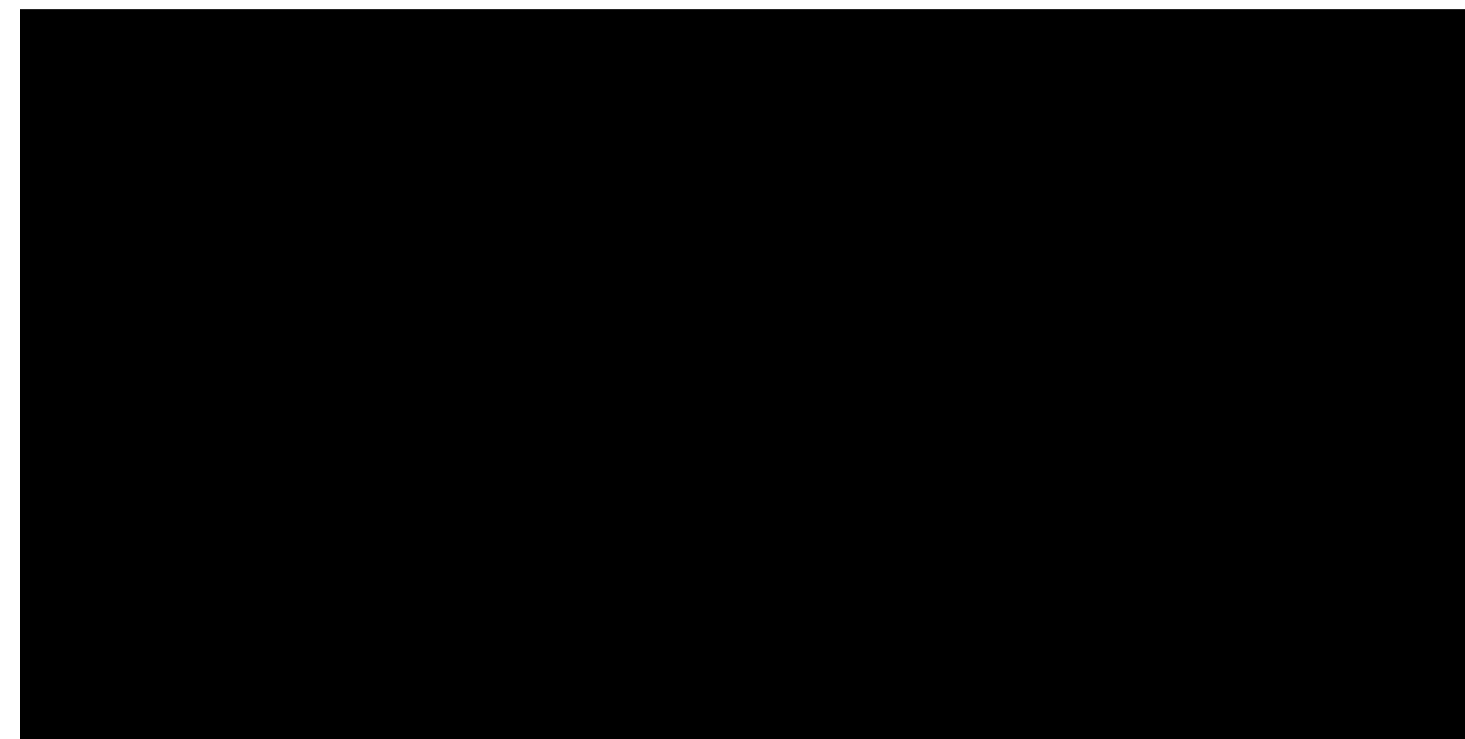
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Test insects

Species: Apis mellifera honey bee sterile females (workers).

Supplier:



Date supplied: 21 August 1990 for range-finding test.

23 August 1990 for final test.

The bees were dosed within 3 - 4 hours of removal from the hive.

Test substance

Name: NTN.33893 technical (Imidacloprid).

Chemical name: 1-[(6-chloro-3-pyrididyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine.

Product category: Insecticide.

Identification: Batch 890315 ELB 01.

Expiry date: March 1991.

Purity: 99.8%.

Appearance: Colourless crystals.

Stability: Refrigerated at 4°C.

Preparation: Solution in dimethylformamide (DMF).

Negative control: DMF without test substance.

Positive control: Not included in this study.

Test procedure

Containers:	Cylindrical wire mesh cages 11.5 cm long x 4.0 cm in diameter.
Loading:	10 bees per cage.
Dose range-finding test:	For each application route, 5 logarithmically spaced doses from 0.01 to 100 µg/bee using 2 groups of 10 bees at each dose and 2 untreated control groups were tested.
Final test:	The range-finding tests showed that the oral LD ₅₀ of NTN.33893 was less than 0.1 µg/bee and the contact LD ₅₀ was about 0.1 µg/bee. Final test concentrations of 0.0015, 0.0031, 0.0063, 0.0125 and 0.025 µg/bee for the oral route and 0.025, 0.05, 0.10, 0.20 and 0.40 µg/bee for the contact route were used. Two groups of 10 bees were dosed with each concentration and the control treatment for both oral and contact tests.
Temperature:	25°C ± 1°C.
Lighting:	The tests were conducted in darkness except for essential procedures.
Feeding:	20% sucrose in water <u>ad libitum</u> , except for the oral test in which the bees were not fed before dosing.
Dose administration:	
(a) Contact (cuticular absorption):	One cage of bees at a time were lightly anaesthetised with carbon dioxide and a 1.0 µl droplet of the appropriate dilution of test material was placed on the ventral surface of the thorax of each bee, using a micrometer syringe. The bees were then replaced in the cage.
	Control groups were treated with 1.0 µl droplet of DMF only.

(b) Oral (normal feeding):

The appropriate concentration was administered as a single dose of 0.2 ml to each group of 10 bees in a cage. The dose was introduced with a syringe into a glass tube 50 x 8 mm with a 1.5 mm opening. The tube was inserted open end down through the top of the cage.

The bees are known to share the 0.2 ml among themselves and so would have received similar amounts of 20 µl each. A solution of the appropriate concentration of the test material in water (1 part) was mixed with 20% sucrose in water (19 parts). When the bees had taken all the test solution after approximately 4 hours the dosage tubes were replaced by tubes containing 20% sucrose.

RESULTS

The final test mortalities were recorded after 24 and 48 hours and are given in the table of results.

The 48-hour LD₅₀ values were computed from the mortality data by probit analysis* with Abbots correction for control mortality.

Oral LD₅₀ 0.0037 µg/bee (95% confidence limits 0.0026 - 0.0053)
 Contact LD₅₀ 0.0081 µg/bee (95% confidence limits 0.0055 - 0.0119)

NTN.33893 technical is highly toxic to bees.

Records

The study protocol, raw data, a sample of the test compound and the final report will be retained in the HRC Archives for at least 10 years.

* Thompson, W.R. and Weil, C.S. (1952) *Biometrics* 8, 51 - 54.

NTN.33893 technical

Mortality of bees after oral and contact treatment using
10 bees per group

Route	µg/bee	Group	24 hour		48 hour	
Oral	0.025	1	9	95%	10	100%
		2	10		10	
	0.0125	1	8	75%	10	90%
		2	7		8	
	0.0063	1	5	45%	5	65%
		2	4		8	
0.0031	1	4	40%	6	50%	
	2	4		4		
0.0015	1	1	15%	1	20%	
	2	2		3		
Control	1	0	5%	0	5%	
	2	1		1		
Contact	0.40	1	6	75%	9	95%
		2	9		10	
	0.20	1	7	55%	9	80%
		2	4		7	
	0.10	1	3	35%	4	55%
		2	4		7	
	0.05	1	2	20%	3	30%
		2	2		3	
	0.025	1	3	15%	4	20%
2		0		0		
Control	1	0	0%	0	0%	
	2	0		0		



REPORT AMENDMENT

Amendment No.: 1

HRC Report No. BAY 158/901384

Date Final Report issued 28 December 1990

Date of amendment 5 January 1994

Study Director

Amendment requested by

Company Miles Inc., Agriculture Div.

Authorisation signatures

HRC Management*

Date

Quality Assurance

Date

6 January 1994

6 January 1994

Details of Amendment

Reason for Amendment

1 Contact LD₅₀ value and 95% confidence limits

Typographical error.

0.081 µg/bee (limits 0.055-0.12)

previously reported as:

0.0081 µg/bee (limits 0.0055-0.0119)

2 Department of Quality Assurance

Omitted during report binding.

Dates of Study Inspections page to be included.

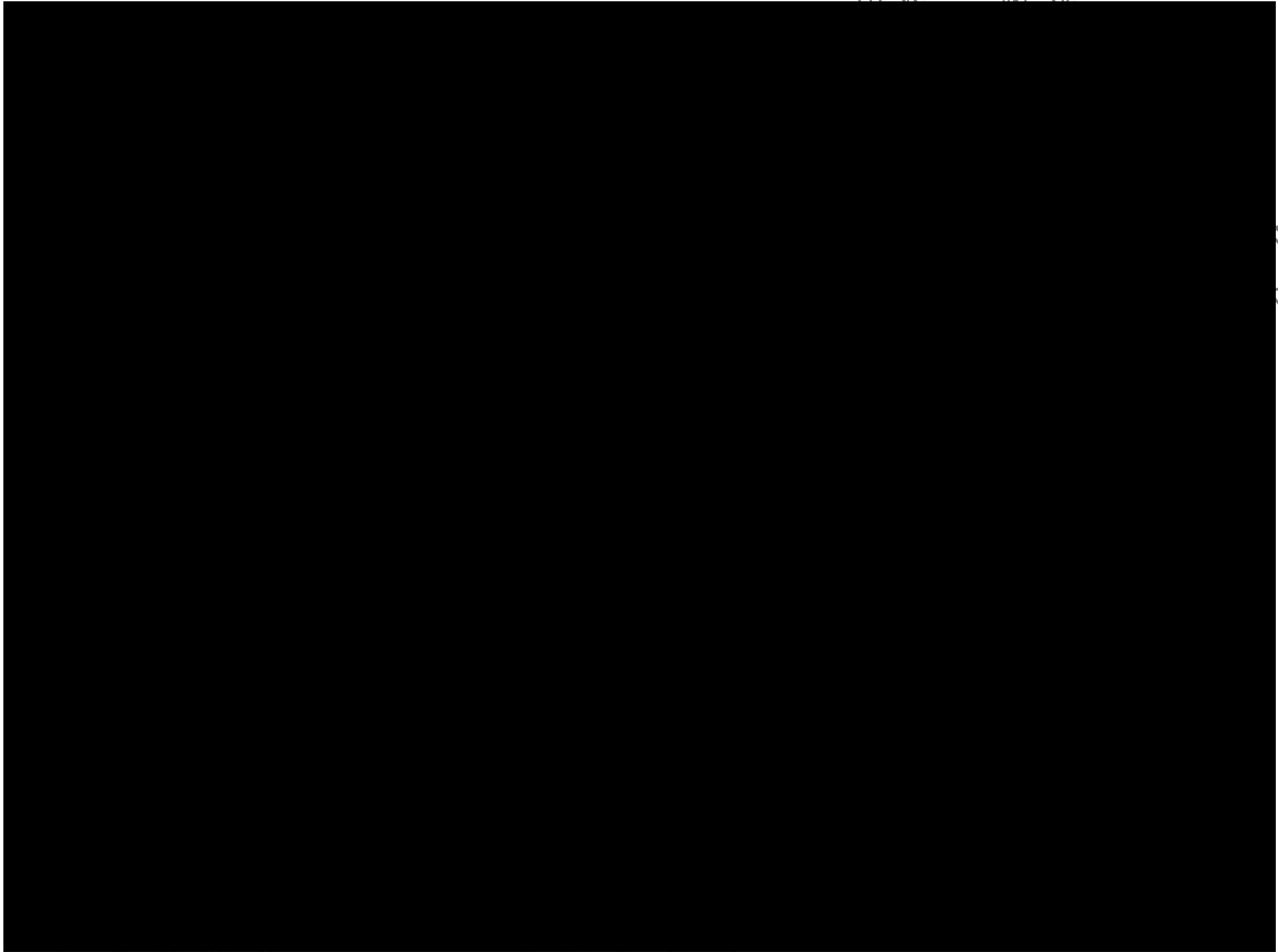
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*Authorisation for these changes is given by HRC Management in the absence of the Study Director who is no longer employed by HRC.

DEPARTMENT OF QUALITY ASSURANCE

DATES OF STUDY INSPECTIONS

HRC Report No. BAY 158/901384



Senior Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

* Week beginning. This has been adopted to avoid the presentation of excessive dates in instances of an inspection being conducted over several days.

Dates on which any 'process-based' inspections were made while the study was in progress are not included in the above listing.

This study was conducted on behalf of Bayer UK Ltd., to determine the acute toxicity to honey bees of NTN.33893 technical by the United Kingdom Control of Pesticides Regulations 1986 protocol.

This protocol also satisfies the EPA Pesticide Assessment Guidelines for Non-target Insects, Subdivision L, Series 141-1.

Preliminary dose range finding tests indicated that NTN.33893 was highly toxic to bees with an oral LD₅₀ of less than 0.1 µg/bee and a contact LD₅₀ of about 0.1 µg/bee.

This was confirmed in a final test using 2 groups of 10 bees each at concentrations of 0.0015 - 0.025 µg/bee for the oral route and 0.025 - 0.40 µg/bee for the contact route.

The 48-hour LD₅₀s with 95% confidence limits were found to be:

Oral LD ₅₀	0.0037 µg/bee (limits 0.0026 - 0.0053)
Contact LD ₅₀	0.081 µg/bee (limits 0.055 - 0.12)

It is concluded that NTN.33893 technical is highly toxic to bees by both oral and contact routes.

INTRODUCTION

Objective

To determine the 48-hour LD₅₀ values of NTN.33893 technical to honey bees by the protocol described in Working Document 7/3 of the United Kingdom Control of Pesticides Regulations, 1986.

Protocol approval

Study Director:	1 June 1990.
HRC Management:	1 June 1990.
Sponsor:	7 June 1990.

Dates of study

The study was conducted from 21 - 25 August 1990.

(b) Oral (normal feeding):

The appropriate concentration was administered as a single dose of 0.2 ml to each group of 10 bees in a cage. The dose was introduced with a syringe into a glass tube 50 x 8 mm with a 1.5 mm opening. The tube was inserted open end down through the top of the cage.

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