

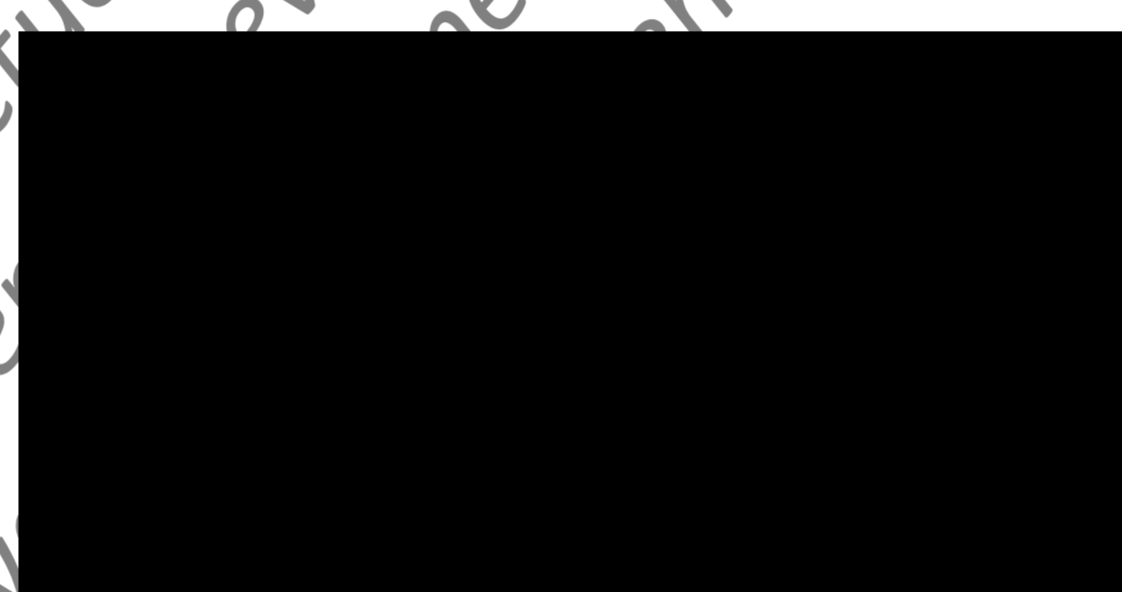
Study Title

Integrated Pest and Pollinator Investigations 1991  
(Including Honey Bee Toxicity of NTN 33893)

Data Requirement

Ecological Effects Requirements: Subdivision E  
40 CFR 158.145  
Supplemental to Guidelines 141-1 and 141-2

Authors



Study Completion Date

1991

Performing Laboratory

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Miles Report No.

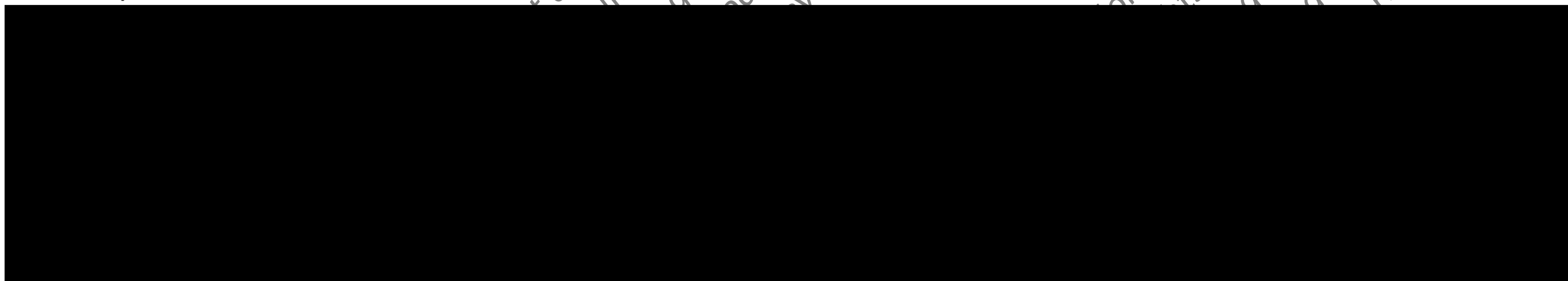
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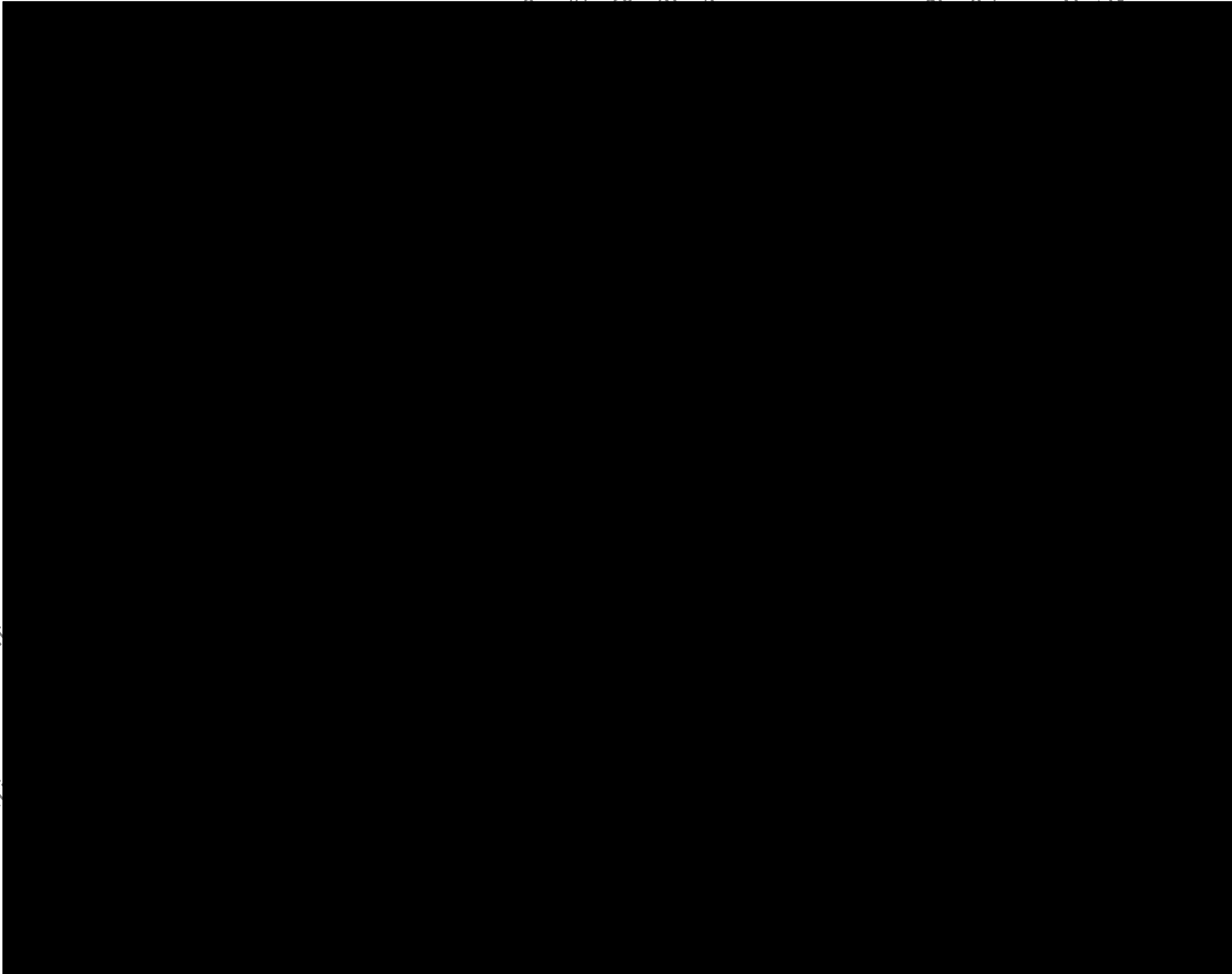
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Company: Miles Incorporated, Agriculture Division



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**Good Laboratory Practice Certification**

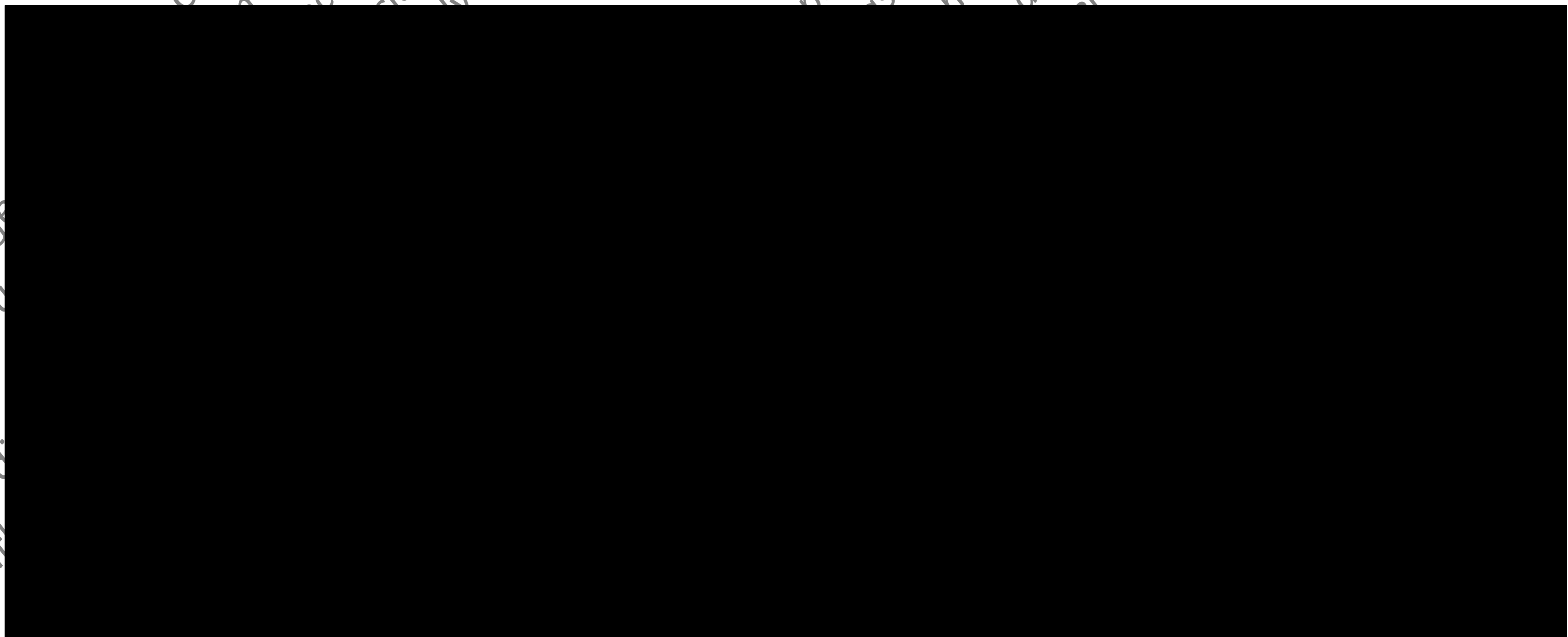


Dit document  
Publicat

Conseque



# INTEGRATED PEST & POLLINATOR INVESTIGATIONS 1991



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## RESIDUAL BEE POISONING BIOASSAY

Tests were conducted with insecticides applied with a R&D CO<sub>2</sub> pressurized sprayer at a rate of 26 gallons per acre using a hand-held boom with 4 (LF3) nozzles applied to 0.01 acre plots of first or second growth alfalfa. Field-weathered residual test exposures were replicated 4 times with 4 foliage samples per treatment and time interval. Samples consisting of about 500 cm of foliage taken from the upper 15 cm portions of plants and clipped to 1-inch lengths were placed into in each plastic petri dish (15 cm diameter) whose tops and bottoms were separated by a wire screen (6.7 meshes/cm) insert (45 cm long and 5 cm wide).

Worker honey bees (*Apis mellifera*) (HB) were obtained from the top frames of colonies and anesthetized with CO<sub>2</sub> to facilitate handling. Alfalfa leafcutting bees (*Megachile rotundata*) (LB) were emerged in an incubation chamber at 85° F., allowed to fly in the lab, and collected off the windows. Alkali bees (*Nomia melanderi*) (AB) were collected from nesting sites and chilled at 35° F. to facilitate handling. Residual test exposures were replicated 4 times by caging 30 to 40 worker HB, 20 to 25 LB or 20 to 25 AB with each of four foliage samples per treatment and time intervals. Bees in cages were fed syrup (1:1 ratio) in a wad of cotton (5 x 5 cm), and the bees held at 75 degrees F. for 24 hour mortality counts.

Results:

When 8 hour residues cause less than 25% mortality the material is probably safe to use around bees if applied in late evening after bees have quite foraging for the day.

Andalin 2EC at 0.125 and 0.25 lb(AI)/acre was non-hazardous to honey bees if applied late evening. (Table 1).

EXP 60434A 80WG at 0.012 and 0.025 lb(AI)/acre was non-hazardous to alkali and alfalfa leafcutting and honey bees if applied in late evening (Table 1). At 0.1 lb(AI)/acre and 0.2 lb(AI)/acre it was moderately hazardous to honey bees.

ICI 0321A 1E at 0.01, 0.015 and 0.02 lb(AI)/acre was non-hazardous to alkali and honey bees if applied late evening (Table 1). All 3 rates were somewhat hazardous to alfalfa leafcutting bees.

Lock-On at all 3 rates was highly hazardous to honey bees (Table 1).

NTN 33893 240FS at 0.025, 0.05 and 0.1 lb(AI)/acre was non-hazardous to alkali, alfalfa leafcutting and honey bees if applied in late evening (Table 1).

San 839 was non-hazardous to honey bees if applied in late evening (Table 1).

RH-9999 was non-hazardous to honey bees if applied in late evening (Table 1).



Table 1. Mortalities of alkali bees (AB), alfalfa leafcutting bees (LB), and honey bees (HB) exposed to different age residues of insecticide applied to 0.01 acre plots of alfalfa. [REDACTED] 1991.

**10381!**

Treatment	lb(AI)/a	24 hr % mortalities of bees caged with treated foliage.					
		age of residues					
		AB		LB		HB	
		2 hr	8 hr	2 hr	8 hr	2 hr	8 hr
Andalin 2EC	0.125	--	--	--	--	3	15
Andalin 2EC	0.25	--	--	--	--	6	10
EXP 60434A 80WG	0.012	1	4	7	14	7	17
EXP 60434A 80WG	0.025	1	4	8	6	10	18
EXP 60434A 80WG	0.1	4	3	30	14	22	43
EXP 60434A 80WG	0.2	--	--	--	--	47	69
ICI 0321A 1E	0.01	27	4	14	28	7	12
ICI 0321A 1E	0.015	38	0	15	25	10	14
ICI 0321A 1E	0.02	25	7	13	19	17	13
Lock-On 1.6 EC	0.25	--	--	--	--	100	100
Lock-On 1.6 EC	0.5	--	--	--	--	100	100
Lock-On 1.6 EC	1.0	--	--	--	--	100	100
NTN 33893 240FS	0.025	20	2	13	18	12	13
NTN 33893 240FS	0.05	19	8	9	15	14	20
NTN 33893 240FS	0.1	28	3	16	11	14	19
SAN 839 5%EC	36 gr	--	--	--	--	2	13
RH-9999 20% WP	0.1	--	--	--	--	2	13
RH-9999 20% WP	0.2	--	--	--	--	3	7
Untreated check	--	0	1	1	4	1	2

### LD<sub>50</sub> TOPICAL DROP INSECTICIDE TESTS ON BEES

This study was designed to determine the lethal dose (LD<sub>50</sub>) for the honey bee (*Apis mellifera*), the alfalfa leafcutting bee (*Megachile rotundata*) and the alkali bee (*Nomia melanderia*) for different insecticides.

Preparation of solutions. All insecticides were dissolved in acetone, and solutions were prepared so that a given quantity, 2 microliters (μl), of the solution contained a know amount of the active ingredient (ai) of the insecticide. The amount of insecticide solution applied to each bee throughout these tests was 2 μl. The most concentrated solution was prepared first, and appropriate dilutions were made from it.



Collection and holding of bees. Worker honey bees were collected from the top frames of colonies in a holding box and taken to the lab. Alfalfa leafcutting bees were emerged in an incubation chamber at 85° F., allowed to fly in the lab and collected off the windows in groups of 30 into plastic vials. Alkali bees were collected from nesting sites and chilled at 35° F. and placed into plastic petri dishes. All bees were held in a refrigerator (4-5° C.) until activity ceased and they could be handled. Tests were conducted as soon after collection of bees as possible and the period of refrigeration kept to a minimum to reduce possible cold-related mortality.

Application of insecticides. Just prior to treatment, groups of 30 bees were removed from the cold, and arranged on a petri dish with their dorsal sides up. Insecticide solutions were applied using a calibrated Eppendorf microsyringe and disposable tips. For each bee, 2 µl of the solution was drawn into the tip and then gently dispensed onto the dorsal surface of the bee's thorax. (mesoscutum). Thirty bees were treated with each solution. One group of control bees was refrigerated as described above, and treated with 2 µl of acetone. A second control groups was refrigerated for the same length of time as the chemical treated bees but was not treated with acetone. After treatment each group of 30 bees were transferred to screen cages made from 15-cm plastic petri dishes whose tops and bottoms were separated by a wire screen (6.7 meshes/cm) insert (45 cm long and 5 cm wide). Bees in cages were fed syrup (1:1 ratio) in a wad of cotton (5 x 5 cm), and the bees held at 75 degrees F. for 24 hour mortality counts.

### Results:

Table 1. Toxicity of insecticides applied topically to honey bee (HB) alkali (AB) and alfalfa leafcutting (LB) female bees, [REDACTED] 1991.

Bee	Insecticide	LD <sub>50</sub> (µg/bee)	95% Confidence Limits (µg/bee)	Slope
AB	Ambush 2EC	0.0107	0.019, 0.006	-1.015
AB	Cymbush 3E	0.0054	0.010, 0.003	-0.989
AB	Baythroid 2E	0.0027	0.005, 0.001	-1.028
AB	Danitol 2.4EC	0.0191	0.040, 0.009	-0.680
AB	Pydrin 2.4EC	0.0104	0.019, 0.006	-0.888
AB	Spur 2FL	0.0462	0.122, 0.018	-1.188
LB	Ambush 2EC	0.0312	0.044, 0.022	-1.950
LB	Baythroid 2E	0.0084	0.015, 0.005	-1.253
HB	Spur 2FL	0.8393	1.074, 0.656	-2.057
HB	NTN 33893 240FS	0.0439	0.063, 0.031	-1.551

### **LC<sub>50</sub> MICROSPRAYER INSECTICIDE TESTS ON ADULT BEES**

This study was designed to determine the lethal concentration (LC<sub>50</sub>) for the honey bee (*Apis mellifera*), the alfalfa leafcutting bee (*Megachile rotundata*) and the alkali bee (*Nomia melanderia*) for different insecticides.

Preparation of solutions. All insecticides were dissolved in acetone, and solutions were prepared so that a given amount, 80 ml, of the solution contained



AUTHOR: [REDACTED] STUDY: <INSECTICIDE> DATE: 09/09/91  
TEST NO.: 454-91-069 ATTACHMENT: (Y) (M) CROP: ALFALFA  
TITLE: NTN RESIDUAL BEE POISONING BIOASSAY OBJ/PROT#: 3210

LOCATION: TOWN <PROSSER> STATE <WA> TRIAL TYPE: (R)ield, (L)ab, (G)rn  
COOPERATOR: NAME [REDACTED] RATING (X)(2); STATUS (I)(P)(F)  
AFFIL. <WASHINGTON ST UNIV> STATE <WA> PLANT AGE: 4 (8)(H)(B)

CROP VARIETY: FORTRESS DESIGN LAYOUT: RCB  
PLANTING DATE: \_\_\_ / \_\_\_ / \_\_\_ \*V CODE: \_\_\_ PLOT SIZE: 432 UNIT: S  
EMERGENCE DATE: \_\_\_ / \_\_\_ / \_\_\_ NO. REPS: 4  
PLANT DEPTH: \_\_\_ UNIT: (I)nch, (C)m TEXT: 12 FT X 36 FT  
ROW SPACING: \_\_\_ UNIT: (I)nch, (F)t, (C)m, (X)m

SOIL TEXTURE: (C)oarse, (M)edium, (F)ine YIELD: CHECK: \_\_\_ / \_\_\_ UNIT: -  
Specific - SIL METHOD: \_\_\_ REL TO: -  
SOIL OM%: 1.5 SOIL pH: 6.5 WEATHER: HALF/1st: \_\_\_ 2nd: DATA  
SOIL CONDITION: (F)ine, (M)ormal, (Y)(M)  
(C)loddy, (M)uddy (1)DRY(2)WET/(3)HOT(4)COLD  
SOIL MOISTURE: (D)ry, (M)ormal, (W)et TEMPERATURE: (H)ot, (M)ormal, (C)old  
MOISTURE: (D)ry, (M)ormal, (W)et

SPECIAL CONDITIONS: \_\_\_\_\_

APPLIC :	1	2	3	4	5
TYPE	FOLIAR				
EQUIPMENT:					
CODE	HP				
DESCRIP	R & D CO2 PLOT SPRAYER				
NOZZLE	LF3				
PRESSURE	30				
VOLUME	25				
UNITS:	PRESSURE (R)si, (A)tm, (K)pl				VOLUME (B)pa, (L)/ha

STANDARDS: None

COMMENTS: APISME = honey bee worker  
MEGCRO = alfalfa leafcutting bee  
CODE-1 = alkali bee (Nomia melanderi)  
HEADER KEY:  
- 7/10 = foliage residue 2 hour old  
- 7/11 = foliage residue 8 hour old

Suggestions: Weather conditions, pest codes, sample size and pest pressure.

COMMON EQUIPMENT CODES: HP=Hand CO2, RP=Knapsack CO2, KP=Bicycle CO2,  
SD=Hand granule, HG=Trailer type, SA=Tractor applicator,  
SZ=High clearance, BZ=Seed treating drum, TA=Drip irrigation.  
PLOT SIZE UNIT: A=Acre, B=No. of trees, J=No. of pests, S=Sqft,  
T=No. of pots, H=Hectares, M=SqMeters.







09/12/91  
INSECTICIDEMOBAY AG CHEM R&D - BIOLOGICAL DEVELOPMENT  
PRODUCT TRIAL SUMMARY

Page 3

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AUTHOR: [REDACTED]  
TEST NO.: 454-91-069DATE: 09/09/91  
CROP: ALFALFA

=====

Pollinating bees were exposed to NTN treated alfalfa foliage to evaluate poisoning risk. NTN 33893 240 FS was sprayed onto second growth alfalfa at 0.025, 0.05, and 0.1 lb. AI/A. Foliage samples were placed in petri dishes, part with 2 hour old residue and part with 8 hour old residue. Pollinating bees were placed in the petri dishes and % mortality recorded after 24 hours. Four replications each of three different bees were made. The pollinators included honey bee workers (APISME), alfalfa leafcutting bees (MEGCRO), and alkali bees (Nomia melanderi). Bee repellency was not evaluated in this study.

RESLUTS: There appeared to be no separation of % mortality by rate. The alkali bee was the only one of the three to show increased mortality from the 2 hr old residue vs the 8 hr old residue. Mortality, after 24 hr exposure ranged as follows: alkali bee 2 - 28%, leafcutting bee 9 - 18%, honey bee 12 - 20%, and UTC 0 - 4%.

WSU guidelines suggest when 8 hour residues cause less than 25% mortality the compound is probably safe to use around bees if applied in late evening after bees have quit foraging for the day. NTN as tested would qualify as non-hazardous to these three pollinating bees if applied in late evening.

NTN 33893 240 FS Batch No. = 1033004

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09/12/91  
INSECTICIDE

MOBAY AG CHEM R&D - BIOLOGICAL DEVELOPMENT  
PRODUCT TRIAL SUMMARY

AUTHOR: [REDACTED]  
TEST NO.: 454-91-069

DATE: 09/09/91  
CROP: ALFALFA

TREATMENT	FORM	RATE	UN 1/ IT NO.	REF	APPLICATION TYPE	NO	INT	NO.	OBJ SP KEY	%INJURY	%PHYTO	%PHYTO	APISME	APISME
											07/11/91	07/11/91	07/10/91	07/11/91
1. UNTREATED													( 1 )	( 2 )
2. NTN33893	240 FS	0.025	6	1	FOLIAR					0	1		12	13
3. NTN33893	240 FS	0.05	6	1	FOLIAR					0	1		14	20
4. NTN33893	240 FS	0.1	6	1	FOLIAR					0	1		14	19

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1/ Rate units: 6=1b/A

09/12/91  
INSECTICIDE

MOBAY AG CHEM R&D - BIOLOGICAL DEVELOPMENT  
PRODUCT TRIAL SUMMARY

AUTHOR: [REDACTED]  
TEST NO.: 454-91-069

DATE: 09/09/91  
CROP: ALFALFA

TREATMENT	FORM	RATE	IT	NO.REF	UN	1/ APPLICATION	OBJ	SP	APISME ADULTS DESTROYD /PERTI 0-9SCALE	MEGCRO ADULTS DESTROYD /PERTI %CONTROL	MEGCRO ADULTS DESTROYD /PERTI %CONTROL	MEGCRO ADULTS DESTROYD /PERTI 0-9SCALE
1. UNTREATED										(1)	(4)	
2. NTN33893	240 FS	0.025	6	1		FOLIAR			7	13	18	7
3. NTN33893	240 FS	0.05	6	1		FOLIAR			7	9	15	7
4. NTN33893	240 FS	0.1	6	1		FOLIAR			7	16	11	7

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1/ Rate units: 6=1b/A



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AUTHOR: [REDACTED]  
TEST NO.: 454-91-069

DATE: 09/09/91  
CROP: ALFALFA

TREATMENT	FORM	RATE	UN	1/ IT NO.	REF	TYPE	NO	INT	NO.	SP	OBJ	KEY	CODE-1	CODE-1	CODE-1	O-9SCALE
													ADULTS	ADULTS	ADULTS	
													DESTROYD	DESTROYD	DESTROYD	
													/PERTI	/PERTI	/PERTI	
													%CONTROL	%CONTROL	%CONTROL	
													07/10/91	07/11/91	07/11/91	
1. UNTREATED													(0)	(1)		
2. NTN33893	240 FS	0.025	6	1		FOLIAR	1						20	2	7	
3. NTN33893	240 FS	0.05	6	1		FOLIAR	1						19	8	7	
4. NTN33893	240 FS	0.1	6	1		FOLIAR	1						28	3	7	

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