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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: November 2, 1999

SUBJECT: Review of Isoxaflutole Contaminated Irrigation Studies on Five Plant Species
(Chemical # 123000; DP Bar Code D257678)

TO: Joanne Miller (PM 23)
Herbicide Branch
Registration Division (7505C)

FROM: Michael Davy, Agronomist *Michael Davy*
Environmental Risk Branch 2
Environmental Fate and Effects Division (7507C)

THROUGH: Jean Holmes, Acting Branch Chief *Jean Holmes*
Environmental Risk Branch 2
Environmental Fate and Effects Division (7507C)

EFED has reviewed non-target plant field study submitted by Rhone Poulenc. This study is titled *Effect of Irrigation Contamination on a Range of Crops*, MRID 448397-05. The crops tested are sugar beets, tomato, cabbage, turnip, radish, and lettuce. These crops were tested to act as surrogate for other sensitive non-target plants. The study is part of the required data set for the conditional registration of Isoxaflutole.

EFED considers this study to be **invalid** for the following reasons:

1. Design of the trial may lead to the controls being contaminated by drift and/or runoff from nearby treated plots.

2. Small design of the plots lead to competition between plots as was observed with tomatoes and cabbage.
3. Analysis of samples of cabbage plant in a control plot reveals residues of RPA202248. No other crop controls have been sampled and analyzed. Due to the lack of data, one must assume that other controls may have been contaminated or otherwise compromised as was the cabbage.
4. Population of plants in trials is too small to have effective statistical analysis.
5. Plant tissue and water solutions from a control plot were not analyzed for RPA202248.

If you have any questions, please do not hesitate to contact Michael Davy at 305-7081.

Data Evaluation Record

Chemical: Isoxaflutole (PC 123000)

Author: Parsons, R. G.

MRID: 44839705; D Barcode: D257678

Nontarget Plant Field Study: *RPA202248: Effect of Irrigation Contamination on a Range of Crops*

Study Carried Out At: Rhone Poulenc Research Farm, Essex, England

Study Completed: April 16, 1999

Good Laboratory Practice Statement - "this study was performed in accordance with OECD Principles of Good Laboratory Practice . . . as accepted by . . . regulatory authorities throughout . . . the United States of America."

1. Materials

Test Substance:

RPA202248, primary transformation product of isoxaflutole

Tested Plants:

Sugar Beet (*Beta vulgaris*), Roberta variety (Pelleted seed)

Cabbage (*Brassica oleracea*), First Early Market variety

Tomato (*Lycopersicon esculentum*), Moneymaker variety (Pelleted seed)

Turnip (*Brassica rapa*), Royal Crown variety

Radish (*Raphanus sativa*), Juliette variety

Lettuce (*Lactuca sativa*), Saladina variety (Pelleted seed)

Irrigation Water:

Water supply was from a deep aquifer. Section 4.4.4 of page 14 in authors' report is attachment showing water quality. Section 4.4.5 to 4.4.7 of page 15 in authors' report is attachment showing description of irrigation equipment.

2. Methods

Plot Size:

Plots were 2 x 2 meters with a 1.5 meter space between plots. Plots were randomized to seven treatments (1-7) with four replicates (A-D) per treatment. The plants were planted per plot as follows: sugar beet - 1 row with 20 plants, cabbage - 1 row with 24 plants, tomato - 1 row with 32 plants, turnip - 2 rows with 48 plants, radish - 2 rows with 62 plants, and lettuce - 4 rows with 40 plants. Seeds were planted on July 8, 1998. Fertilizer was applied on July 8 & 29, 1998 and on August 7, 1998. Each of the plot and their replicates were randomized. It is not known what the location of the randomized plots are and whether there are high dose plots adjacent to the control plots.

Pesticides application:

<u>crop</u>	<u>pesticide</u>	<u>time of application</u>
all crops	Quintozene and Lindane	7/6/98
all crops	PBI Slug Pellets	7/21/98
all crops	cypermethrin and pirimicarb	8/7/98
lettuce	cymoxanil and mancozeb	8/19,25/98

Protection from wildlife and weeds:

Grosheild non-woven spun polyethylene fleece removed at emergence.

Anti-bird netting (¾ inch mesh) on steel hoops (irrigation treatments applied through nets).

Electric fence (0.5 m tall and 75 mm mesh) against rabbits.

Humming line bird scarcer.

Scarey man bird scarcer .

Weeds were removed frequently by hand and trial kept weed-free.

Treatment preparation:

0.2487 grams of RPA202248 were dissolved in 250 ml to provide a stock solution of 0.9948 mg/ml. 25 ml of this stock solution was diluted in another 250 ml flask to provide a dilution stock of 0.0995 mg/ml. 0.25 ml of dilution stock was made up to 5 liters with tap water for treatment 2. 1 ml of dilution stock was made for treatment 3 and 4 ml for treatment 4. 1.6 ml of the original stock was diluted into 5 liters for treatment 5, 3.2 ml for treatment 6 and 6.4 ml for treatment 7. The treatment solutions were poured carefully into dilutors and labeled accordingly.

Analysis of treatment solutions show that the concentrations in the dilutors degrade after about 10 days. Therefore, fresh treatment solutions were made up every 10 days.

The treatment solutions were analyzed and the following information was derived:

<u>Treatment Number</u>	<u>Nominal Concentrations</u>	<u>Analyzed Concentrations (+ S.D.)</u>	<u>Mean % deviation from Target</u>
1	Control (0)	---	---
2	25 ppt	26.5 ppt (7.4)	+7
3	100 ppt	86.7 ppt (11.5)	-13
4	400 ppt	294 ppt (49)	-27
5	1600 ppt	1298 ppt (241)	-19
6	3200 ppt	2380 ppt (525)	-26
7	6400 ppt	5100 ppt (1057)	-20

Application of treatments:

The author found that the most water that can be applied over a plot at one time is 5 mm (20 L) before runoff starts to occur. Therefore, 5 mm was applied over 4 meters square per each application. Before application was made for each plot, the dilutor and associated equipment were connected to the stand-pipe for the plot and flushed briefly using the bleed on the filter and the lance. Water meters were not available before August 5. Therefore, the flow through each dilutor and lance was regulated to 1 bar at the pressure gauge and calibrated using graduated buckets in which 20 L was found to take 35 seconds. Therefore, each plot was watered for 35 seconds from the hose, lance and rose. **No information was given as to the height of the application.**

The control plots were watered with untreated water which was **delivered with the same apparatus that was used for the treated plots** but the water did not pass through a dilutor. It is possible at this point that the hose, lance and rose may have some small amounts of contaminated residues that were delivered to the controls.

After August 5, water meters readable to 0.1 L were used to measure the amount of water applied. The meters were calibrated on August 20 by weight and found to be reading between 0.5 and 3.5% under the 20 liters.

The weather pattern in the summer of 1998 was cool and damp. Applications could not frequently be made more than twice a day. In all, 26 applications were made, a total of 130 mm or 520 liters was made to each plot.

Analysis of treatment solution samples

Samples of the treatment solutions were made on 13 of the 26 applications. The replicate samples were commingled. The samples were sent to the laboratory where they were analyzed for RPA202248. Samples of control water were tested for pH, free chlorine content and temperature. It appears that the control solutions were not tested for the presence of RPA202248.

Plant Assessment

Growth stages, disease and pest problems were reported. Lettuce on Plot 5B was infected with downy mildew. The yield of lettuce in this plot was lowered because of the infection.

As radish, lettuce, and turnip reached a marketable growth stage, they were harvested and weighed on a per weight basis. Cabbage, sugar beets and tomatoes were grown in small numbers with visual and weight assessments made. Harvest yield was not made for cabbage, sugar beets, and tomatoes. For sugar beets and turnips, roots were weighed individually, and the shoots weighed as total per plot. For non-root crops, the fresh weight of shoots measured individually.

3. Results

Foliar Symptoms

Turnip - 2 plants at 5100 ng/l (plot 7B) were bleached, one severely with purple discoloration and another had 2 leaves showing 10% discoloration which eventually went away. The plants remained stunted. The author feels that these plants were on the edge of a plot and touched fiberglass rod thereby causing the bleaching.

Cabbage - severe damage was noticed on plot 7A, 7B, 7C, and 7D (5000 ng/l) with ranges from 21% to 70% of occurrence with 3% to 14% of leaf area having chlorosis. Treatment Plot 6D (2380 ng/l) showed 5% occurrence of damage covering <1% of leaf area.

Cabbage samples of chlorotic and normal leaves taken at harvest were extracted and analyzed by LC-MS/MS for RPA202248. The results of the analysis were inconclusive. This may be due to the fact that the samples were taken 21 days after application.

No other symptoms on other species were displayed.

Fresh Weights

Fresh weights for radish, turnip, cabbage, and lettuce were reported and shown on pages 21-23 of authors' report.

Tomatoes suffered badly from competition with cabbage.

Data for tomatoes and sugar beets were not provided.

Author's Discussion

A total of 130 mm was applied to the crops despite a rather late start in a cool and wet summer. Turnip and cabbage showed damage at 5100 ng/l - turnip showed bleached symptoms, typical of the herbicide and cabbage showed a more chronic type of general chlorosis. The cabbage variety has dark green leaves with an open growth habit and the lettuce variety, Saladin, (an iceberg type) was light colored. Only outer leaves were visible and inner leaves were blanched. If lettuce had been chlorotic, it would have been difficult to spot. Yield results of the crops were not statistically significant. The author also indicated that this study was "a novel experiment, methods were developed as the work progressed and could be improved in any future work. This study should be regarded to some extent as a pilot exercise."

Reviewer's Discussion

The author acknowledges that this study should be regarded as a pilot exercise. This study was begun on July 8, 1998 and the report completed on April 16, 1999. EFED has provided a response on April 29, 1999 to the registrant's rebuttal of protocols for plant field studies for simulated irrigation and drift. This study was begun 9 months prior to approval of protocol by EFED for this study. EFED has requested that registrant not begin study until the protocol was approved.

The design of the plots (4m² plots separated by 1.5 m between plots) limited the plant population and created some uncertainty about the controls being exposed to the contaminated irrigation water from drift, runoff and/or possible contaminated applicator. In addition, with the small plots, there was competition between plots as was observed between the cabbage and the tomatoes. The design of such small plot dimensions and proximity of plots to each other and especially to the controls.

The treated irrigation water was tested for RPA202248 but the control irrigated water from the hose, lance, and rose water was not. This is necessary to show whether the controls were being contaminated from improper cleaning of non-dedicated application apparatus.

No data were provided for sugar beets or tomatoes; no raw data were provided for any of the crops (only summarized data); and no plot layout or map was provided to ascertain the proximity of the controls to the plots.

On page 59 of authors' report, there is a graph of mass chromatograph for RPA202248 in cabbage leaf samples. The first graph for sample 1C is for a control cabbage plant. RPA202248 was found in this sample. Since there are no data on other controls or treated samples of other crops, there appears to be a great uncertainty as to the extent that the other controls have been contaminated.

EFED has determined that this study should be classified as **invalid** due to the following reasons:

1. Design of the trial may lead to the controls being contaminated by drift and/or runoff from nearby treated plots.
2. Small design of the plots lead to competition between plots as was observed with tomatoes and cabbage.
3. Analysis of samples of cabbage plant in a control plot reveals residues of RPA202248. No other crop controls have been sampled and analyzed. Due to the lack of data, one must assume that other controls may have been contaminated or otherwise compromised.
4. Population of plants in trials is too small to have effective statistical analysis.
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