MEMORANDUM July 9, 1998

Re: Validation of Analytical Method:
"Method of Analysis for Isoxaflutole and its Metabolites in Water"

TO: James Breithaupt
EFED/ERBI

FROM: Thuy L. Nguyen, Chemist
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THROUGH: Daniel Rieder, Chief
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Conclusion:
The studies were conducted by three independent laboratories (**ABC Laboratories** of Columbia, MO, **Centre Analytical Laboratories**, Inc. of State College, PA and **Covance Laboratories Inc.** of Madison, Wisconsin) and the internal laboratory of **Rhone-Poulenc** in Research Triangle Park, North Carolina. Together, they have successfully validated the analytical method "Method of Analysis for isoxaflutole and its metabolites in Water", RPAC File Number 45390. Overall, the method accuracy criteria (70 to 120%) were satisfied at the LOQ levels, and the precision criteria (RSD ≤20%) were satisfied at the 10 x LOQ levels. This analytical method, which includes a sample preparation step prior to the analyses by LC/MS and LC/MS/MS, could therefore be used to determine the concentration of Isoxaflutole and its metabolites in ground, surface and tap waters. The limit of quantitation (LOQ) was set at 40ppt for the parent compound and RPA 202248, and 400 ppt for RPA 203328).

Findings:
Several minor findings are listed below. Although they require some explanations/clarifications, these findings do not have any negative impact on the validity of the studies.

Method (study number EC-97-396): The actual method description was included in each study. It was well written and easy to follow.

Suggestions/Actions Required:

1. In section 2.3.1, the reagent blanks for the determination of the LODs are to be analyzed according to the analytical procedure described in section 3.6 (extraction + instrumental analysis). However, it is unclear to whether or not the LOD standards were treated in the same manner as the blanks. Please note that, it is imperative that the LOD standards be treated exactly under the same analytical procedure as the blanks. Please supply explanations.

2. The range of the freezer temperature (± 3 °C) is slightly wide (± 75%). It is recommended that the freezer be kept at 4 °C ± 2 °C. The integrity of the standards used in this project should not be greatly affected, as the length of the study was brief.

3. A typographical error was noted in the fifth paragraph of 2.3.1 section of the method entitled "Limit of Detection and Limit of Quantitation". The units for the target LOQs should read "ng/mL" instead of "ng/L".

Comments/Remarks:

1. It is not understood why the water sample was recorded by weight and not by volume. No impact on the actual results should be seen, except for the reported units (pg/g vs. pg/L).

2. The LOD (Limit of Detection) of each analyte was defined as the concentration at which the analyte gives at least a 3:1 ratio of the signal to the average noise of the reagent blanks. This is acceptable, however technically, LODs should be determined by the lowest amount of standard which could be detected by the instrument at a signal to noise ratio greater than 9:1.

3. According to section III.A., approximately 0.1000g (4 numbers after the decimal point) of the neat standards was weighed out in the preparation of the fortification and calibration standards. However, section II.B (Equipment and Supplies) lists two analytical balances of accuracy ±0.1mg and ±0.1g (1 number after the decimal point).

All studies listed below were received by the Agency on October 21, 1997

MRID# 443999-001 - Rhone Poulenc Ag Inc.
The study protocol was signed by the Study Director on September 5, 1997. The experimental termination date was September 22, 1997 and the study completion date was September 26, 1997.

Suggestions/Action Required:

1. The ranges for the standard concentrations of the calibration curves as mentioned in page 14, were from 1 ng/mL to 8 ng/mL for IFT and RPA 202248, and from 10 to 80 ng/mL for RPA 203328. According to the figures 2 to 4 (pages 33 to 34), the ranges were from 2 ng/mL to 6 ng/mL and 20 ng/mL to 60 ng/mL, respectively. Please clarify.

2. According to section 9.3, Sample Handling, paragraph 2, page 20, it is recommended that samples extracts should not be stored for more than 48 hours. However, on pages 74 to 79, Raw Data Sheets for the precision and accuracy study, there was as much as a 7-day gap between the date extracted and date analyzed. Please clarify.

3. According to section 9.1, paragraph 2 (page 16), reagent blanks were run through the extraction method, and then chromatographed. However, based on the raw data sheets (Appendix B, pages 68 to 70), there was no evidence that the blanks underwent the extraction procedure (no date extracted, no sample weight, no final volume,...). Please clarify.

Comments/Remarks:

1. All average recoveries were within limits, however several individual recoveries at the LOQ level were noted to be below the specified 70% limit, and one %RSD above 20%.

2. According to the time stamped on the chromatograms of figure 7 (page 38), the calibration standards were analyzed in random: 4.0/40ng/mL before 3.0/30 ng/mL standard, and 6.0/60 ng/mL before 2.0/20 ng/mL standard. Although no apparent cross-contamination was observed, it is a good practice to analyze standards from low level to high level, or to insert an instrument blank after a high level run to prevent any carry-over into a low level run.

3. There is no clear indication that the LOD samples/standards underwent the extraction procedure. Therefore the experimental LODs are technically IDLs (Instrument Detection Limit). However, since the precision studies were good (thus indicating good extraction efficiency), it could be assumed that LODs are equivalent to IDLs, for the purpose of estimating LOQs.

4. All calibration standards and fortified samples were spiked from a stock solution prepared from one source. For future studies, it is strongly recommended that standards and fortified samples are prepared from different sources (neat material from different lot numbers), or if several sources of neat standards were not available, from different stock solutions.

MRID# 443999-002 - Centre Analytical Laboratories, Inc.
The study was initiated on September 9, 1997. The experimental start date was September 12, 1997 and the termination date was September 28, 1997. No major deficiencies were noted.

MRID# 443999-003 - ABC Laboratories, Inc.
The study protocol was inspected by the Study Director on September 8, 1997. The experimental start date was September 9, 1997 and the termination date was September 14, 1997. The study was completed on September 22, 1997. No major deficiencies were noted.

Comments/Remarks:

1. The water bath temperature of the rotor evaporator should be kept below 40 °C (preferably at 35 °C) to better regulate the evaporation step and minimize sample loss due to dryness.

MRID# 443999-004 - Covance Laboratories, Inc.
The study protocol was inspected by the Study Director on September 8, 1997. The experimental start date was September 12, 1997 and the termination date was September 22, 1997. The study was completed on October 07, 1997. The laboratory apparently has difficulties in setting up the instrument and in performing the method performance study.

Comments/Remarks:

1. For the precision and accuracy study, the laboratory has difficulty in recovering several samples at the LOQ level during the first two trials. During the third attempt, recoveries were successful at 1.7 times the LOQs, instead of at the LOQ level. The data did not meet the validation criteria, however are usable when used in conjunction with the above studies.

Table I. Comparison of Method Validation Studies for Parent Isoxaflutole, RPA 202248, and RPA 203328 in Water submitted by Rhone Poulenc for Section 3 Registration.

Laboratory	Analytical Details	Compound		
		Parent Isoxaflutole	RPA 202248 (Phytotoxic degradate)	RPA 203328 (Terminal Degradate)
ABC Laboratories (MRID 44399903)	Calculated Limits of Detection (ppt)	Not reported	Not Reported	Not Reported
	Limit of Quantitation (ppt)	40	40	400
	Lowest Spike Level (ppt)	40	40	400
	Average Recovery (%)	80	75	88
	Precision (reported standard deviation, %)	3	1	3
Laboratory	Analytical Details	Compound		
		Parent Isoxaflutole	RPA 202248 (Phytotoxic degradate)	RPA 203328 (Terminal Degradate)
Covance Laboratories (MRID 44399904)	Calculated Limits of Detection (ppt)	Not Reported	Not Reported	Reported
	Limit of Quantitation (ppt)	40	40	400
	Lowest Spike Level (ppt)	67.6	68	696
	Average Recovery (%)	90	97	97
	Precision (reported relative standard deviation, %)	5.6	5.9	4.1

Laboratory	Analytical Details	Compound		
		Parent Isoxaflutole	RPA 202248 (Phytotoxic degradate)	RPA 203328 (Terminal Degradate)
Centre Analytical Laboratories (MRID 44399902)	Calculated Limits of Detection (ppt)	Not Reported	Not Reported	Not Reported
	Limit of Quantitation (ppt)	40	40	400
	Lowest Spike Level (ppt)	40	40	400
	Average Recovery (%)	86	108	91
	Precision (standard deviation, %)	10	6	9
Laboratory	Analytical Details	Compound		
		Parent Isoxaflutole	RPA 202248 (Phytotoxic degradate)	RPA 203328 (Terminal Degradate)
Rhone Poulenc Laboratories (MRID 44399901)	Calculated Limits of Detection (ppt)	10	10	15
	Limit of Quantitation (ppt)	39[a] /40[b]	3[a] /40[b]	39[a] /400[b]
	Lowest Spike Level (ppt)	40	40	400
	Average Recovery (%)	83	86	93
	Precision (relative standard deviation, %)	14	21	5

[a] - Calculated LOQ

[b] - Method LOQ