W. Nelson
H7505C
PM 20

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

NOV 12 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Azadirachtin Biochemical Pesticide. Response to Product Chemistry Data Deficiencies.
MRID Nos. 419232-00 to -14.
CB Nos. 8236, 8237, 8238, 8728.
DP Barcodes D166148, D169823.

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Thru: William Hazel, Ph.D., Head
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To: Willie Nelson, PM 18 Team
Insecticide Rodenticide Branch
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and

Thomas McClintock, Ph.D.
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

This review is being expedited at the request of Anne Lindsay, Director, Registration Division (memorandum to Penelope Fenner-Crisp, 10/4/91, memorandum not signed by Ms Lindsay). We note the reference in this memorandum to the original due date for this submission of Aug. 1, 1991, and point out that the Registration Division scheduled a meeting with the registrant to discuss the submission on September 10, 1991, and has transmitted new data to HED to be examined as part of this submission on September 20, 1991.

Conclusions and Recommendations

As discussed in the introduction, should RD determine that conditional registration for this product is appropriate, a

revised Confidential Statement of Formula for the end use product manufactured by the new manufacturing process is required.

Residue data are not required because the product contains a biochemical applied at ≤20 g a.i./A. If the company increases the application rate in the future, residue data may be required.

For the purposes of full registration of this product, we conclude the following:

Adequate data were submitted to fulfill the requirements for the following sections of Subdivision M of the Pesticide Assessment Guidelines for Azatin Technical:

151-12 151-17	for melting point, pH, vapor pressure and photostability only.

As discussed in detail above and in the Confidential Appendix, additional data are required for the following sections of Subdivision M:

151-10 151-11 151-13 151-15 151-16 151-17	Color, physical state, odor, density, oxidizing or reducing potential, flammability - flashpoint, storage stability, viscosity, corrosion characteristics, octanol\water partition coefficient, stability and solubility.

Toxicological considerations permitting, CBRS has no objection to a 1-year conditional registration of this product provided a revised CSF is submitted for the end use product. However, we recommend against extension of this conditional registration or full registration of this product until the remaining data deficiencies discussed above are resolved.

CBRS recommends that a copy of this review be provided to the submitter.

DETAILED CONSIDERATIONS

Introduction

AgriDyne Technologies, Inc. (formerly Native Plants, Inc., or NPI), responds to Product Chemistry data deficiencies for Azadirachtin Technical cited in the CBRS review of 4/25/91 (M. Metzger). The company provides 14 volumes of data (MRID Nos.

419232-00 to -14), and an additional volume with no MRID number assigned which was submitted in response to a meeting between the company and EPA in which data deficiencies were discussed (meeting held 9/10/91, attended by M. Metzger, CBRS, T. McClintock, SACB, W. Nelson, RD, J. Martineau, AgriDyne, E. Butts, AgriDyne consultant).

AgriDyne has provided labels for Azatin Technical Insecticide and Azatin EC. The label for the end use product, Azatin EC, allows application to numerous food crops including crops from most crop groupings (238 crops are listed on the label) to control numerous pests including ants, aphids, many types of worms, mites, flies, and others. Applications of the pesticide are to be made at rates of 8-21 fl. oz./A (maximum rate = 20 g a.i./A) in sufficient water to provide uniform and thorough coverage. Aerial or ground equipment may be used. No PHI is provided.

Since this pesticide will be applied at ≤20 g a.i./A, Residue Chemistry data are not required as specified in Subdivision M of the Pesticide Assessment Guidelines (Section 153-3(a)(ii)). If the label is modified to increase the application rate, residue chemistry data may be required.

CBRS (M. Metzger) discussed the data requirements for this product in separate meetings with both Registration Division (W. Nelson) and SACB (T. McClintock, Ph.D., R. Engler, Ph.D.). Mr. Nelson stated that **Conditional Registration** of this product for a period of **1 year** may be appropriate (telecon, 10/16/91). During this 1 year period, outstanding Product Chemistry deficiencies would be addressed by the registrant. **If after 1 year these deficiencies have not been resolved, registration of the product would not be extended past the 1 year period.**

SACB (10/18/91) concluded that, based on the low toxicity of azadirachtin technical (batch 13), Conditional Registration for a 1 year period may be acceptable. However, additional Product Chemistry information must be provided prior to full registration of this product. Furthermore, it was stated that a short term toxicity study would be required for the batch of Azatin to be used over the 1 year conditional registration period, and possibly future batches of product depending on the identity of additional impurities found in the product. (The company provided a revised manufacturing process at the 9/10/91 meeting in which no technical azadirachtin is isolated; rather, the end-use product is manufactured in a continuous process starting from raw materials. The company representatives stated that only a single batch per year would be manufactured initially, and that this batch has not yet been made.) Batch to batch variation in impurities present and relative percentages of impurities indicate the necessity for additional toxicity and Product Chemistry data.

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As stated, the single batch of Azatin which would be used if conditional registration is granted has not yet been prepared. Therefore, data showing impurity levels resulting from this manufacturing process are not available. Since the new process is similar to the process used to isolate technical azadirachtin in the extraction and clean-up steps, a similar profile of active ingredient and impurities may be present in the batch made by the new process. If conditional registration is granted, a revised CSF must be submitted reflecting the possible composition of the product manufactured by the new process. Since the technical product contains 10 ± 1% a.i., and the end use product contains 3% a.i., in the absence of data for the new process it could be assumed that levels of impurities in the end use product are 30% of the levels found in the technical for which data are available. Therefore, prior to conditional registration, the company must submit a CSF for the end use product in which upper certified limits for impurities are 30% of those shown on the label for the technical product.

CBRS defers to RD regarding establishing conditional registration of this product since this is an administrative decision. We acknowledge the urgency of registering this product as stated in the 10/4/91 expedite request and in frequent communications with RD and the registrant. However, we point out that a batch of product has not yet been made using the proposed manufacturing process, and the resulting lack of information about the product. Numerous impurities remain unidentified in batches of product made using earlier manufacturing processes, and numerous other unresolved deficiencies remain. RD states (telecon with M. Metzger, CBRS, 10/29/91) that the registrant reports that one of the Product Chemistry studies was not performed in compliance with GLP regulations, and that a different study to fulfill the same guideline requirement which was performed in compliance with GLP regulations was not submitted. Finally, this product would be registered for use on virtually all commodities grown throughout the U.S. Should SACB determine that the available data are sufficient for 1 year conditional registration of this product, CBRS would have no objection provided the outstanding Product Chemistry data deficiencies are resolved prior to full registration. For Conditional Registration, submission of a revised CSF as discussed above is required. Should conditional registration be granted, the deficiencies listed in the remainder of this review must be resolved for full registration of this product.

Listed below, by Guideline number, are our previously cited Product Chemistry deficiencies, the company's response and CBRS comments.

151-10: Product Identity and Disclosure of Ingredients

Previous CBRS Response to 151-10

The data submitted do not adequately address the requirements of 151-10. Since numerous data requirements are not satisfied by this submission, we will not reiterate the Guidelines here but refer the submitter to the Pesticide Assessment Guidelines, Subdivision M, Section 151-10. In particular, the requirements for information on impurities must be addressed [Sections 151-10(c)(2)(i-viii), 151-10(c)(3), 151-10(c)(1)(i-vi) for any biochemicals present other than azadirachtin], a CSF must be submitted for Azatin™ Technical which contains all required information on the active ingredient as well as impurities in the technical product [Section 151-10(b)], and additional information regarding the host range and the mode of action must be provided [151-10(c)((1)(vi)].

We note that identification of components and determination of certified limits for all components present at ≥0.1% (as required by the Guidelines) for an extract of this type may not be feasible because of the large number of components which may be present, and because of the large batch to batch variation which likely occurs. However, at a minimum, chromatographs must be supplied in which all major peaks are identified, and certified limits must be determined for these peaks taking into account the batch to batch variation resulting from different neem seed sources, different manufacturing processes, and any other factor which may affect the percentages of these impurities in the technical material. Additionally, the weight percentage of the impurities which have been identified in the technical material must be provided. Should TOX consider it necessary that additional information regarding the identity and levels of impurities be provided, these data will also be required. (Note: the toxicological studies submitted for Azadirachtin utilized the technical material, NPI-720; personal communication with T. McClintock, Ph.D., 3/19/91.) We refer the submitter to the Pesticide Assessment Guidelines, Subdivision D (Product Chemistry), Sections 61, 62, and 63 for additional information.

NPI's Response

The following additional information was submitted in support of 151-10: a revised Confidential Statement of Formula (CSF) and a detailed discussion of the mode of action of azadirachtin (MRID No. 419232-07).

The revised CSF contains additional information for impurities in azadirachtin technical. The following required information was not included on the revised CSF:

- CAS Registry Numbers for impurities.

A very detailed description of the mode of action of azadirachtin is presented. Briefly, azadirachtin functions as an insect anti-feedant and as an antagonist to ecdysone, the natural molting hormone for insects. Antifeedant activity is both sensory and metabolic. Upon ingestion, insects become quiescent as if beginning the early stages of molt. Feeding slows or ceases, giving protection from further insect damage. Insects fail to successfully complete the molt and usually die within a two to fourteen day period. It is not known to cause insect death as a result of inherent toxic properties associated with the molecule.

CBRS Comments

Certified limits for additional impurities are required.

CAS numbers for the active ingredient and all impurities for which certified limits are required must be included on the CSF.

Since a revised manufacturing process has been submitted in which no technical material is isolated, CSFs submitted in the future must be for the end use product, not the technical. For the purposes of conditional registration, a CSF for the end use product must be submitted in which certified limits for impurities in the end use product should be 30% of the values on the CSF for the technical product (see introduction). For full registration, the registrant must identify and establish certified limits for all impurities at levels >1% in any batch of technical azadirachtin or >0.3% in the end use product.

Refer to the discussion of 151-13 and 151-15 for additional information.

151-11: Manufacturing Process

Previous CBRS Response to 151-11

The submitted information is not adequate to fulfill the requirements for Manufacturing Process.

NPI's Response

The submitter has provided a revised manufacturing process. See the Confidential Appendix for details.

CBRS Comments

Additional information are required for full registration of this product. See the Confidential Appendix for details.

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151-12: Discussion of the Formation of Unintentional Ingredients

Previous CBRS Response to 151-12

We cannot determine the adequacy of the discussion of the formation of unintentional ingredients until additional information regarding the manufacturing process is provided. However, it is unlikely that the information submitted fulfills the requirements of 151-12 since the product is extracted from the seeds of Neem trees, and the presence of extraneous host residues are likely. We refer the submitter to the Guidelines (151-12) for information on what types of chemical components must be considered in this discussion. We note that the published article regarding the chemistry of Neem trees provides some interesting and potentially useful information which might be useful to the submitter in putting together this discussion, but the article alone is not sufficient to fulfill the data requirement since it refers to the Neem tree rather than the technical product.

NPI's Response

The submitter did not respond specifically to this deficiency. However, since the product is extracted from neem seeds without further chemical modification, and since considerable additional information has been provided regarding components which might coextract with azadirachtin, no further information will be required.

CBRS Comments

This deficiency is resolved.

151-13: Analysis of Samples

Previous CBRS Response to 151-13

The requirements for 151-13 have not been satisfied. See the Confidential Appendix for details.

NPI's Response

NPI has submitted nine volumes of information to fulfill the outstanding deficiencies related to this guideline. These are reviewed separately and summarized in the Confidential Appendix.

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CBRS Comments

The requirements for 151-13 have not been satisfied. Additional information is required for full registration of this product. See the Confidential Appendix for details.

151-15: Certification of Ingredient Limits

Previous CBRS Response to 151-15

The requirements for 151-15 have not been satisfied. See the Confidential Appendix for details.

NPI's Response

A revised CSF has been submitted and is attached at the end of the Confidential Appendix.

CBRS Comments

The deficiencies related to 151-15 have not been resolved. Additional information is required for full registration of this product. See the Confidential Appendix for details.

151-16: Analytical Methods for Certified Limits

Previous CBRS Response to 151-16

The information provided is not adequate to fulfill the requirements of 151-16. Analysis of the technical product, not analytical grade material, must be demonstrated, and the method(s) must be capable of determining azadirachtin as well as any other component for which certified limits are required (see Section 62-2 of Subdivision D of the Pesticide Assessment Guidelines for information regarding establishment of certified limits). We refer the submitter to Subdivision M (Section 151-16) and Subdivision D (Product Chemistry, Section 62-2 and 62-3) of the Pesticide Assessment Guidelines for further information. We note that supporting raw data must be submitted (e.g. chromatographs).

NPI's Response

NPI submitted additional HPLC chromatographs and standard curves for analyses of batches of Azatin Technical.

CBRS Comments

A sufficient number of chromatographs were submitted in which batches of the technical material were analyzed using the

analytical method discussed. However, standard curves must be provided for azadirachtin, per se, for the five batches of technical azadirachtin discussed in MRID No. 419232-03. The submitter must demonstrate that the stated 100% (representing peak area) on the HPLC chromatogram printouts represents 100% (weight percent) of the injected material; i.e., that the impurities represented by the peaks on the chromatographs represent all impurities present in the technical material. When the unidentified impurities present at >1% are identified, the submitter must demonstrate the applicability of this method to these impurities.

151-17: Physical and Chemical Properties

Previous CBRS Response to 151-17

A discussion of the information submitted for Physical and Chemical Properties is provided in the Confidential Appendix. Adequate data were submitted to fulfill the following requirements for Azatin™ Technical:

Color	Physical state
Odor	Melting point
pH	

Data were submitted but were found deficient for the following properties (see Confidential Appendix for details):

Density
Solubility
Stability

Data are required but were not submitted for Azatin™ Technical for the following properties (see the Pesticide Assessment Guidelines, Subdivision M, Section 151-17, Subdivision D, Sections 63-9 and 63-11):

Vapor pressure
Octanol-water partition coefficient

NPI's Response

Five additional volumes were submitted discussing physical and chemical properties. These are discussed individually below.

Determination of the Density of NPI-720 and NPI-720F (MRID No. 416264-13)

Density determination was made for two lots of Azatin Technical (NPI-720) and two lots of Azatin EC (NPI-720F). The method used for Azatin Technical was ASTM Method D70-82 entitled, "Standard

Test Method for Specific Gravity and Density of Semi-Solid Bituminous Material". The method used for Azatin EC was ASTM Method D1429-86 entitled, "Standard Test Methods for Specific Gravity of Water and Brine". The results are as follows:

Test Material	Density, g/ml
NPI-720	1.379 @ 24°C
NPI-720	1.450 @ 24°C
NPI-720F	1.069 @ 24°C
NPI-720F	1.060 @ 23°C

The data provided in this report adequately fulfill the data requirement for density determination for Azatin Technical.

Determination of Solubility of NPI-720 (MRID No. 419232-09)

This report describes determination of the solubility of Azatin Technical in acetone, ethanol, methanol and water. The following procedure was used for the organic solvents.

> As a starting point, 100 mgms of NPI-720 was placed in a test tube and 1 ml of the solvent was added; the process was repeated for each of the solvents. The test tubes were vortexed in a shaker for 5 minutes and observed by both the naked eye and through a magnifying glass and results were noted. Based on the above results, the next step was to reduce the amount of material in order to arrive at an approximate solubility value. The volume of the solvent was increased until an approximate clear solution was obtained. This study was carried out for all the solvents at all the temperatures [10°C, 25°c, 50°C]. The observations were graded relatively and confirmed by a second person. Also, even in the clear solution, there were small particles which were thought to be impurities (fibre, hair, etc.) rather than particles of NPI-720. To confirm the above results and insure that saturation had occurred, a range, above and below the estimated solubility value, was weighed and the experiments were conducted a second time using the same protocol. A water bath (Model Lauda Brinkman RC20) with a variable heat controller was used to conduct the experiments at 50°C. Experiments at 10°C were conducted by placing the set up in a refrigerator. The samples in both cases were agitated for a period of 60 minutes.

The reported results are a range of concentrations at each temperature accompanied by a qualitative description of the mixture of NPI-720 with solvent (e.g. "thin suspension", "turbid, yellow").

Solubility in water was determined by adding a triturated sample to water, stirring with a magnetic stirrer for 45 minutes, and evaluation of the presence of suspended particles using a Tyndall beam. The following results were obtained:

	10°C	25°C	50°C
Acetone	2.0	6.2	9.5
Ethanol	0.05	0.12	3.8
Methanol	0.01	0.1	4.2
Water	2.8×10^{-5}	5×10^{-5}	3×10^{-4}

Determination of the solubility of NPI-720 in a non-polar organic solvent was not provided and is required.

Supplement to "Thermal Stability Study for NPI-720"-MRID #416264-16. This MRID # is the Same as One Assigned to the Storage Stability of NPI-720F (Azatin EC)" (MRID No. 419232-10)

The data submitted in this report are meant to be supplemental raw data to a thermal stability report submitted previously (see M. Metzger, 4/25/91).

Previous CBRS Response to Thermal Stability

The following procedure was used to determine the thermal stability of the active ingredient in Azatin™ Technical:

> The temperature of a thermostatically controlled oven was monitored for 24 hours. Ten grams of the test article was weighed and placed in a brown screw-cap bottle. The bottle was sealed and placed in a pre-heated oven at 50°C ± 2°C for 14 days. The temperature of the oven was recorded daily in a log book. At 14 days the bottle was removed from the oven and placed in a freezer for subsequent HPLC analysis.
>
> The HPLC analysis was performed as detailed in SOP 23. A standard curve was generated from analytical grade azadirachtin (NPI-100) from which to base the thermal decomposition calculations. The thermal stability samples were ground with a pestle, weighed and diluted with acetonitrile to give concentrations between 5 and 11 mg/ml.

Percent Azadirachtin		
Initial	Final	Net Loss
10.3%	7.32%	28.9%
23.2%	22.5%	3.0%

The information provided is not sufficient to fulfill this data requirement. Additional raw data must be submitted including sample chromatographs and additional data supporting the validity of the analytical method as described in the CBRS response to Guideline 151-16.

Additionally, the "stability" requirement calls for data regarding the sensitivity of the active ingredient to metal ions and metal, and to sunlight, as well as thermal stability. These requirements remain outstanding (see Subdivision D of the Pesticide Assessment Guidelines, Section 63-13).

NPI's Response

Raw data summarizing the results of the thermal stability study were submitted. These data included several notebook pages showing HPLC chromatographs and a standard curve for azadirachtin.

CBRS Comments

Data were submitted only for the study showing 3% net loss of azadirachtin (23.2% to 22.5% azadirachtin content). The 22.5% value is an average of two samples with azadirachtin contents of 23.8% and 21.3%. Comparison of the HPLC chromatogram obtained before storage at 50°C with those taken after 14 days storage showed minimal azadirachtin decomposition although modification of other components was observed.

The company must submit raw data for the experiment in which a 28.9% net loss of azadirachtin was observed, or else discuss the reasons for omitting these data.

Photostability of NPI-720 and Derivatives Thereof (MRID No. 419232-11)

This report presents the results of a photostability study using Azatin Technical. Solutions of NPI-720 containing 5 mg/ml were transferred to a petri dish and allowed to evaporate to dryness. These samples were placed under lights rated to provide 7000 uW/cm^2 at 15 feet. Analytical samples were removed every 16 hours for HPLC analysis. Raw data/chromatographs were provided in the document submitted following the 9/10/91 meeting with the company.

The results showed almost complete degradation of azadirachtin within 80 hours in an approximately linear fashion.

The requirements for this portion of the guideline have been met.

Storage Stability of Various Formulations of NPI-720F (MRID No. 419232-12)

This report discusses the storage stability of formulated products of Azadirachtin which were not manufactured by the most recently submitted, continuous manufacturing process. Data must be provided showing the storage stability of the end use product produced by this new manufacturing process.

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Vapor Pressure

The company requests a waiver of the vapor pressure requirement stating "based on the Pesticide Assessment Guidelines, Subdivision D, Product Chemistry, Series 63.9 which states that 'If the melting point of the pure form of the a.i. in a product is equal to or less than 30°C, then the determination of the vapor pressure of the pure form of such active ingredient is required by 40 CFR 158.120.'" The pure form of azadirachtin has a melting point of 154 to 158 deg C (The Merck Index, 11th Edition). Therefore, determination of the vapor pressure is not required.

Octanol/Water Partition Coefficient

The company requests a waiver from the octanol water partition coefficient requirement "based on the Pesticide Assessment Guidelines, Subdivision D, Product Chemistry, Series 63.11 which states 'a determination of the octanol/water partition coefficient for the pure grade of each nonpolar organic active ingredient in a product' is required to support the registration of each manufacturing and end use product. AgriDyne has determined the solubility of pure (99.31+/-0.69%) azadirachtin to be 2100 mg/l in water and 610 mg/l in octanol. Because of the high solubility of pure azadirachtin in water and low solubility in octanol, azadirachtin is considered polar in nature."

Because of the solubility of azadirachtin in both polar and non-polar solvents, solubility alone is not sufficient to indicate how the compound will partition among various environmental and/or biological systems. CBRS concludes that determination of the octanol/water coefficient is required.

Summary of CBRS Comments Regarding Physical and Chemical Properties

Since another new, revised, continuous manufacturing process has been proposed by the company (no technical azadirachtin isolated), we must revise our previous conclusions regarding the adequacy of the data for physical/chemical properties as follows.

Adequate data have been submitted to fulfill data requirements for the following physical/chemical properties:

Melting point
pH
Vapor pressure (no data required)
Photostability

Data have been submitted but were found to be deficient for the following properties:

Solubility - solubility of the technical material in a polar solvent must be determined.
Stability (thermal) - the company must submit raw data for the experiment in which 28.9% net loss of azadirachtin was observed, or else discuss the reasons for omitting these data. Additionally, a study of stability of the technical material to metal and metal ions must be submitted. (The company stated that they require guidance on how to carry out a study showing stability to metal and metal ions. We recommend that the company develop a detailed protocol and submit it to the Agency for comment prior to initiation of the study.)

New studies must be submitted for the following properties in which material obtained from a batch of azadirachtin manufactured by the newest manufacturing process is utilized:

Color (63-2)
Physical state (63-3)
Odor (63-4)
Density (63-7)
Oxidizing or reducing potential (63-14)
Flammability - flashpoint (63-15)
Storage stability (63-17)
Viscosity (63-18)
Corrosion characteristics (63-20)

Studies must be submitted using the pure form of the active ingredient for the following properties:

Octanol\water partition coefficient (63-11)

Attachment: Confidential Appendix
cc without attachment: Circu
cc with attachment: M. Metzger (CBRS), Azadirachtin SF, RF, C. Furlow (PIB/FOD, H7506C)
RDI;W.Hazel:WH:10/30/91:EZ:10/30/91
H7509C:CBRS:M.Metzger:MM:Rm810f:CM#2:10/30/91

CONFIDENTIAL APPENDIX

RIN 6265-94 PP2F4046

Page____ is not included in this copy.

Pages 16 through 33 are not included.

The material not included contains the following type of information:

✓ Identity of product inert ingredients.

____ Identity of product impurities.

✓ Description of the product manufacturing process.

✓ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

____ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.