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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of Acute Toxicity Studies with Tobacco Dust

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

To: Bruce Sidwell, Product Manager 72
Special Review and Reregistration Division (7508W)

The Ecological Effects Branch (EEB) has completed review of the three studies submitted to fulfill reregistration data requirements for tobacco dust. The two aquatic studies were conducted with end use product containing 0.5% nicotine (the active ingredient.) Both were found acceptable for reregistration of tobacco dust containing no more than 0.5% of nicotine. The avian oral toxicity study was not acceptable as the percent of active ingredient was not clearly specified. The Branch would like further clarification of the percent of nicotine contained in the test material used for the avian oral toxicity study MRID 42625501.

Based on test results presented, tobacco dust(containing 0.5 % nicotine) showed low acute hazard to freshwater invertebrates, freshwater fish, and avian wildlife. Based on the values presented for tobacco dust the active ingredient, nicotine, is estimated to have potentially moderate to high toxicity to aquatic species in it's pure form. Without adequate description of the test material used in the avian oral study an estimate nicotine toxicity is not possible for bobwhite quail.

Further questions regarding these reviews should be directed to Les Touart of our Branch at (703) 305-6143.

ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT

1. **Chemical:** Tobacco dust

2. **Test Material:** Tobacco dust, Lot Number 63092, >90% pure. Percent of the active ingredient (Nicotine) not listed

3. **Study Type:** 14-Day Oral Acute Toxicity study using bobwhite quail, *Colinus virginianus* and end use product.

4. **Study Identification:**
   Study Director: Pederson, Carol A.
   Study Laboratory: Biolife Associates, Ltd.
   Study Dates: October 16 - 30, 1992
   Study Identification: BLAL #131-001-03
   Study Sponsor: Faesy & Besthoff, Inc.
   EPA Identification: MRID 426255-01

5. **Reviewed by:** Brian Montague, Fisheries Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division (H507C)

   Brian Montague 11/4/93

6. **Approved by:** Les Touart, PhD, Section Supervisor
   Ecological Effects Branch
   Environmental Fate and Effects Division

   L. Touart 11/5/93

7. **Conclusions:** Tobacco dust appears to be nearly nontoxic to bobwhite quail on an oral acute basis. Tobacco dust ingested at 2150 mg/kg produced no mortality. Due to abnormality noted in feces of the high concentration (2150 mg/kg) birds, the NOEL is determined to be 1450 mg/kg. The LC_{50} for pure nicotine is estimated to be $\geq$ 11 mg/Kg based on results presented for the 0.5% product. This would place pure nicotine into a potentially highly toxic category for bobwhite quail. The test material active ingredient content needs to be better clarified in order that this study may be considered completely acceptable. The study will be considered only for registration of products containing 0.5% nicotine or less.

8. **Recommendations:** Registrant must clarify in writing the percent of active ingredient contained in the test material.

   Tag did — see attachment

9. **Submission Purpose:** Submitted to support Reregistration of tobacco dust as an animal repellant.

10. **Study Design and Protocol:** Protocol was believed to conform to EPA Good Laboratory Practices. No other sources were cited for protocol design.

Test Diet Preparations: Tobacco dust was placed in gelatin capsules and administered orally in two-dose ranges, 1,470 and 2,150 mg/kg (not based on active ingredient content).

Test Organisms: 30 bobwhite quail (15 males and 15 females) were used in definitive testing after initial range testing had been conducted with six birds. The birds were received on 9/1/92 from Sand Prairie Quail Farm and were 19 weeks old. Birds had been raised on 24% grower ration containing 20% Virginiamycin/ton. A 45-day quarantine was conducted.

Test Materials and Design: Biolife administered Purina Custom Game Bird Breeder Layena 28%. Wellwater was available ad libitum. Lighting was on 10 hours per day. Room temperature was maintained at 70°F. Only one female died out of the 140 birds held in quarantine. All other birds appeared normal. A 7-day range test was conducted from 10/8 to 10/15 with 2 birds at 1,470 and 2 birds at 2150 mg/kg dosages. No mortality was seen and the birds appeared normal. The definitive study was conducted with ten 25-week old birds per dose level and control group. The same dose levels were employed, 1450 and 2150 mg/kg. Birds were weighed on days 0, 3, 7, and 14. Feed consumption was recorded on days 3, 7 and 14. The 10 birds/level were housed in 61 x 53.3 x 38.1 cm steel wire pens. Leg bands identified each test group bird. Photo period was 10D/14N. Thermostatically controlled room temperature was 72°F (22°C) with an average humidity of 65%. Daily observations for mortality and behavior changes were made. At termination four birds were sacrificed from each test level for gross pathological examination.

11. **Reported Test Results:** No mortality was recorded at any dose level. Some chalkiness was noted in feces of the 2150 mg/kg group, but no behavioral signs were apparent. Generally birds in the treatment groups gained between one and 13 grams during the 14-day posttreatment observation period. Control birds, on the other hand, showed weight loss of three to 37 grams on three birds and gains of 0 to 11 grams on seven birds. Pathological examination revealed gaseous intestinal tracts in one control bird and one 2150 mg/kg bird. No other abnormalities were noted.

12. **Study Author's Conclusions:** "Since one-half of the excreta from the 2150 mg/kg test group were chalky and the other half normal in appearance after 19 3/4 hours post-dosing, the no-observed-effect level (NOEL) was determined to be 1,470 mg/kg of body weight. The acute oral LD_{50} of Tobacco dust was determined to be greater than 2150 mg/kg of body weight."

13. **Reviewer's Discussion:** The study was conducted under acceptable methodology and the Agency analysis agrees with the study author's conclusions. The registrant has not clarified what percentage of the tobacco dust is actually the active ingredient. A percentage of the active ingredient (nicotine) should be provided to the Agency to aid in determination of the actual amount of nicotine ingested by the test birds. As an end use product tobacco dust appears to demonstrate a very low degree of toxicity to bobwhite quail.

Adequacy of Study:

Category: ~~Supplemental~~ Core See attachment
Rationale: Agency cannot be sure about how much active ingredient was actually ingested based on the amount of information provided.
Repairable: Yes, with further clarification concerning % of active ingredient (nicotine) present in the tobacco dust used for this study.

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

FEB 15 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Review Clarification of Active Ingredient Content of Tobacco Dust

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division(H7507C)

Douglas J. Urban 2/15/94

TO: Tom Myers, Product Manager 51
Special Review and Reregistration Division (7508W)

Faesy and Besthoff, Inc. has submitted additional data to clarify the % of active ingredient used in Acute Oral Testing of Bobwhite Quail MRID 426255-01. The product contains 70% tobacco dust. Analysis of actual nicotine content was performed by Thorton Laboratories in Tampa, Fla. HPLC analysis revealed 0.27 to 0.34% Nicotine content. The acute oral study has tested at a high enough dosage to be equivalent to an oral ingestion of 0.50% pure nicotine (estimated) at which no mortality occurred. The Ecological Effects Branch is satisfied that the safety of this product has been demonstrated. The acute oral study is now reclassified to core and acceptable for registration purposes. The diet analysis will be attached to the original study review contained in our files. Further questions may be directed to Les Touart of our Branch at 305-6134.

ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT

1. **Chemical:** Tobacco dust -- active ingredient Nicotine

2. **Test Material:** Tobacco dust - Nicotine 0.5% Inerts 99.5%

3. **Study Type:** 48-hour static acute test using *Daphnia magna* and end use product.

4. **Study Identification:**
   Study Director: Ward, Timothy
   Study Laboratory: Envirosystems Division of Resource Analysts, Inc., Hampton, N.H.
   Study Dates: March 16-18, 1992
   Study Identification: Not provided
   Study Sponsor: Faesy & Besthoff
   EPA Identification: MRID 426255-02

5. **Reviewed by:** Brian Montague, Fisheries Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division (H7507C)

   Brian Montague 11/4/93

6. **Approved by:** Les Touart, PhD, Section Supervisor
   Ecological Effects Branch
   Environmental Fate and Effects Division

   L. Touart 11/5/93

7. **Conclusions:** The study results indicate that tobacco dust containing 0.5% nicotine as the active ingredient will display a low order of toxicity if introduced into aquatic environments. The estimated EC_{50} is 169 mg/L with a NOEL of <10 mg/L. This study is acceptable only for products containing less than or equal to 0.5% nicotine as the active ingredient. If the toxicity values for the 0.5 % product are utilized to estimate an EC_{50} for pure nicotine the resulting value would be approximately 0.84 mg/L for the pure active ingredient. This would place nicotine in the highly toxic category for *Daphnia magna*.

8. **Recommendations:** N/A

9. **Submission Purpose:** The study was submitted to satisfy data requirements for tobacco dust (ai 0.5% nicotine) which is used as a repellent near ornamental plants and gardens.

10. **Study Design and Protocol:** Study protocol was based on EPA testing guidelines and included in Envirosystems Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual.

Dilution Water and Test Solution Preparations: Well water collected at the EnviroSystems Laboratory site was utilized as dilution water. The water had a hardness of 160-180 mg/L as $CaCo_3$ and was aerated prior to use. The test material was not prepared as a solution but simply added as a dust to the test vessels.

Test Organisms: Daphnids used in the study were obtained from laboratory-reared cultures and were less than 24 hours old at test initiation.

Test Materials and Design: Ten daphnids were indiscriminately distributed to each of the four 1 liter glass beaker containing dilution water at a 12-cm depth. Daphnids were confined within Nitex screen enclosures placed in the beakers. Test beakers were arranged in a water bath to maintain test temperature 20 $\pm 0.5^{\circ}C$. Only one replicate was used at each of the 4 test concentrations. Due to O_2 depletion problems a second aerated test vessel was used at the 1000 mg/L test concentration. A single control replicate was employed. The test substance was applied as a dust to the test vessels at the appropriate volumes to obtain ratios of 1, 10, 100, and 1000 mg tobacco dust/Liter of dilution water. Observation of daphnia was made daily as was recordation of pH, temperature, conductivity and dissolved oxygen measurements for each test replicate. Daphnids were not fed during the test period. The second test using the 1000 mg/L concentration in an aerated test vessel was conducted from March 19-21. A 16D/8N photoperiod was maintained for all study phases.

11. **Reported Test Results:** No mortality occurred in the control vessel. Due to oxygen depletion 100% mortality was seen at 1000 mg/L when unaerated. The 48-hour mortality experienced was 0%, 50%, 0%, 30% and 100% in controls, 1 mg/L, 10 mg/L, 100 mg/L, and 1000 mg/L (aerated) test groups, respectively. The test material did not solubulize well in the dilution water. As it is a cellulose-based material this is not unexpected. Test material was observed floating on the surface, midwater and coating the bottom of all treated test vessels, thus indicating the insoluble nature of tobacco dust in water. Water quality parameter ranges were as follows: conductivity; 620-780 umhos/cm, DO; 8.7 - 6.0 (except at 1000 mg/L unaerated), temperature; 19.5 -20.3°C, and pH from 7.1 to 8.1.

12. **Study Author's Conclusions:** The 24-hour LC_{50} for daphnids exposed to tobacco dust is greater than 1000 mg/L if test vessels are aerated and 100 to 1000 mg/L if they are not aerated. The 48-hour LC_{50} is 100 to 1000 mg/L whether aeration is employed or not. No sublethal effects were noted during the test except at 1000 mg/L and the NOEC is 10 mg/L.

13. **Reviewer's Discussion:** The study employed a relatively insoluble (in water) end use product. As the study employed an end-use product containing only a 0.5% nicotine, the results should be used to support only those products containing this or lesser percentages of nicotine. Based on the observed mortality EEB estimates the 48-hour EC_{50}, for tobacco dust (0.5% nicotine) on *Daphnia magna* to be approximately 169 (0 - 1000 mg/L). The reviewer does not believe that mortality seen at 1 ppm is dose related due to lack of mortality at 10 ppm of tobacco dust. Though this value is an approximation it does appear to indicate that the EC_{50} would fall somewhere above 100 mg/L. For registration purposes the above value is adequate for risk assessment with tobacco dust products, but not with nicotine if used in its pure form.

Adequacy of Study

Category: Core
Rationale: Study produced a lethal concentration range with 0% and 100% mortality levels.
Repairable: N/A

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B.Montague Tobacco dust Acute 48 hour Daphnid

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1000	10	10	100	9.765625E-02
100	10	3	30	17.1875
10	10	0	0	9.765625E-02
1	10	5	50	62.30469

THE BINOMIAL TEST SHOWS THAT 0 AND 1000 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 169.1514

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	26.42292	8.720904	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .4788067

95 PERCENT CONFIDENCE LIMITS =-1.982414 AND 2.940028

LC50 = 55.94783

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .1245535

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT

1. **Chemical:** Tobacco dust 0.5% ai

2. **Test Material:** Tobacco dust
   Active Ingredient - Nicotine 0.5%
   Inert Ingredients 95.5%

3. **Study Type:** 96-Hour Acute Toxicity Study Using Rainbow Trout ,*Oncorhyncus mykiss* using end use product

4. **Study Identification:**
   Study Director: Ward, Timothy
   Study Laboratory: Envirosystems Division, Resource Analysts, Inc.
   Study Dates: March 10 - 14, 1992
   Study Identification: None provided
   Study Sponsor: Faesy & Besthoff, Edgewater, N.J.
   EPA Identification: MRID 426255-03

5. **Reviewed by:** Brian Montague, Fisheries Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division(H7507C)

   Brian Montague 11/4/93

6. **Approved by:** Les Touart, PhD, Section Supervisor
   Ecological Effects Branch
   Environmental Fate and Effects Division

   L. T. 11/5/93

7. **Conclusions:** The study has produced an estimated LC_{50} of approximately 316 (100-1000) mg/L for the end use product, Tobacco dust containing 0.5% nicotine. The NOEL for this study is 100 mg/L. The study is useable for tobacco dust (0.5% nicotine content) only and not for registration of other nicotine products with higher percentages of nicotine as the active ingredient. Based on the reported results the LC_{50} for pure nicotine is estimated to be approximately 1.5 mg/L which would classify the active ingredient as moderately toxic to rainbow trout.

8. **Recommendations:** N/A

9. **Submission Purpose:** Submitted to satisfy data requirements for tobacco dust. The submission is for tobacco dust only and not for pure nicotine the active ingredient.

10. **Study Design and Protocol:** The Envirosystems Aquatic Toxicology Laboratory Standard Operating Procedures Manual was based on US EPA Good Laboratory Practice Guidelines.

Dilution Water and Test Solutions Preparations: Well water obtained from the Laboratory's own water supply system was utilized. Water was adjusted to a hardness of 40-48 mg/L as $CaCo_3$. Analysis did not reveal any pesticide or heavy metal contamination above accepted levels. The test material was not dissolved, but instead applied in dust form to the test vessels in amounts estimated to produce concentrations of 1, 10, 100, and 1000 mg of tobacco dust/Liter of dilution water.

Test Organisms: Rainbow trout were obtained from Aquatic Research Organisms Division of Resource Analysts, Inc. The juvenile trout were acclimated for 14 days prior to test initiation. No signs of disease or injury were noted. Test fish were not fed during testing.

Test Materials and Design: The pure test material was introduced into the tanks at initiation. Ten Rainbow trout were randomly distributed to each of the 5 twenty-liter glass aquaria. Aquaria contained 15 liters of test solution and were not aerated. All test vessels were placed in a chilled water bath which maintained water at 12.6 to 12.9°C. Nominal estimated concentrations were not verified by any type of analysis. A 16D/8N photoperiod was maintained. Daily observations for abnormal behavior and/or mortality were conducted. Daily measurements of pH, DO, conductivity, and temperature were recorded for each test vessel containing fish.

11. **Reported Test Results:** Test material was observed floating on the surface of the water and on the bottom of the aquaria as well as suspended midwater at all concentrtion levels. Water quality parameters were as follows: Conductivity 680 - 860 umhos/cm, dissolved oxygen - 6.2 to 9.0 mg/L, temperature - 12.6 to 12.9°C, and pH - 7.0 to 7.7. Control fish displayed no symptoms of toxic response. After 24 hours the 1000 mg/L dose level experienced 20% mortality. This same group experienced 100% mortality after 48 hours. No other mortality or symptoms were observed in the other test concentrations or in the control vessel.

12. **Study Author's Conclusions:** "The 24-hour LC_{50} for Rainbow Trout exposed to tobacco dust is greater than 1000 mg/L and the 48, 72, and 96-hour LC_{50} are all 100 to 1000 mg/L. No sublethal effects were noted during the test and the NOEC is 1000 mg/L."

13. **Reviewer's Discussion:** The study author has made the conclusion (under VIII Results, pg. 10) that the NOEC is 1000 mg/L of tobacco dust despite 100% mortality at this concentration. He may have meant 100 mg/L. The calculated LC_{50} is 316 (100 - 1000) mg/L. This value is approximate due to the wide confidence limits and lack of mortality at any other concentration. In addition, the test material is practically insoluble. The toxicity probably resulted from a small portion of the active ingredient which dissolved into the dilution water. This study is only useable in risk assessment for the tobacco dust containing 0.5% nicotine. It is not useable for risk assessment of other products containing higher percentages of nicotine or for nicotine itself. Though the laboratory has indicated that the test fish were juvenile, actual weight or length measurements are preferred.

Adequacy of Study

Category: Core for tobacco dust, 0.5% nicotine.
Rationale: The study has produced 100% and 0% mortality. Though the undissolved test material would normally invalidate the study the insoluble nature of tobacco dust warrants special consideration.
Repairable: N/A

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B.Montague Tobacco dust Acute 96 Hour Rainbow Trout

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1000	10	10	100	9.765625E-02
100	10	0	0	9.765625E-02
10	10	0	0	9.765625E-02
1	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 100 AND 1000 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 316.2279

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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