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TYPE PRODUCT(S) HERBICIDE

DATA ACCESSION NOS. 410302-03 thru 06

PRODUCT MANAGER NO. L. Schnaubelt (23)

PRODUCT NAME(S) clethodim products, RE-45601, (Select)

COMPANY NAME Valent (Chevron Chem. Co.)

SUBMISSION PURPOSE Proposed registration of new chemical technical, and end use products for soybeans and cotton

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% AI

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Proposed Registration of a New Pesticide, Clethodim

FROM: James W. Akerman
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Larry Schnaubelt, PM-23
Herbicide/Fungicide Branch
Registration Division (H7505C)

Attached is the registration standard review for the registration of clethodim herbicide technical, and clethodim formulated as Select herbicide for weed control in soybeans and cotton.

The available toxicity information for technical clethodim indicates that the proposed cotton and soybean use patterns will result in minimal hazard to nontarget and endangered beneficial insect, avian, freshwater fish, and mammal species. The formulated product (Select) contains a high percentage of an "inert" ingredient considered by EEB to be potentially toxic to terrestrial and aquatic organisms. Based on this concern, an acute honeybee study using the TEP (typical end-use product) is needed.

The EEB cannot assess potential hazards to aquatic invertebrates or non-target plants because of outstanding data. Per registrant correspondence, the acute Daphnia magna study [72-2] will be repeated using the formulated (Select) product. Because clethodim will be applied aerially to soybeans and cotton, the non-target phytotoxicity studies [123-1 Seed Germination/Seedling Emergence], [123-1 Vegetative Vigor], [123-2 Aquatic plant growth (Selenastrum capricornutum, Lemna gibba, Skeletonema costatum, Anabaena flos-aquae, and a freshwater diatom)], [201-1 Drift], and [201-2 Drift] are required using the TEP.

In the future, new use patterns and increased rates may dictate the need to repeat [71-2 Avian dietary LC50 for upland game bird and a waterfowl] and [72-1 Freshwater fish LC50, warmwater species only] due to solubility limits for the tests conducted.

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The need for additional acute, chronic, or higher tier testing will be determined after receipt of the studies listed above.

If you have any questions, please contact Richard Petrie at 557-7358.

Attachments- 2 DER's (Avian Reproduction Studies)

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CLETHODIM (SELECT) HERBICIDE

Ecological Effects Topical Summary

Effects on Birds

Five studies have been submitted and evaluated. Of the five three were required and two were not.

Required:

Author	Date	MRID No.
Hinken	1986	409745-25
Hinken	1986	409745-27
Hinken	1986	409745-26

These three studies are acceptable for use in a hazard assessment.

Not Required, but Submitted:

Author	Date	MRID No.
Beavers	1988	410302-06
Beavers	1988	410302-05

One of these two studies is acceptable for use in hazard assessments (410302-05). The other study, 410302-06 is invalid.

In order to assess the toxicity of clethodim to birds the following tests are required using the technical grade material:

a) One avian acute single dose oral study using mallard duck or bobwhite quail.

b) Two sub-acute avian dietary studies; one with the mallard duck (waterfowl) and the second with bobwhite quail or ringneck pheasant (upland game bird).

The following acute oral toxicity test is acceptable for use in a hazard assessment:

Species	T.M.	LD_{50} mg/kg	Author	Date	ID No.	Fulfills Requirement
Bobwhite	82%	>2000	Hinken	1986	409745-25	Yes

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The guideline requirement (71-1) for an avian acute oral study has been satisfied.

The acceptable sub-acute dietary toxicity studies for use a hazard assessment are listed below:

Species	T.M.	LC_{50} (PPM)	Author	Date	ID NO.	Fulfills Requirement
Bobwhite	82%	>4270	Hinken	1986	409745-26	Yes
Mallard	82%	>3978	Hinken	1986	409745-27	Yes

The guideline requirements (71-2) for avian subacute dietary toxicity have been satisfied only up to the LC50 values listed above. In the future new use patterns or increased rates may dictate the need to repeat the 71-2 studies due to solubility limits in the above referenced tests. There is sufficient information on technical clethodim to classify it as slightly to practically non-toxic to birds on an acute basis.

Avian reproduction tests are required for an end use pesticide when birds may be exposed to continuous residues through persistence, bioaccumulation, or multiple applications, or if tests indicate reproductive hazards.

The following bird reproduction studies were submitted to the Agency:

Species	T.M.	NOEL (ppm)	Author	Date	ID No.	Fulfills Requirement
Mallard	83%	1000	Beavers	1988	410302-05	Yes*
Bobwhite	83%	300	Beavers	1988	410302-06	No**

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* If the need for such a test arises, this study would fulfill the waterfowl guideline requirement.

** This study is [redacted] and would not fulfill the upland game-bird guideline requirement if the need arises.

Supplemental (ICP)

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Avian reproduction tests (71-4) are not required at this time.

The following 6 week pilot bird production studies were submitted to the Agency but were not reviewed.

Mallard	83%	Beavers	1987	410302-03
Bobwhite	83%	"	"	"

Precautionary Label

Based on available avian toxicity data, no precautionary label statements for birds are required.

Effects on Fish

Two studies in two documents were evaluated under this topic. The two studies were acceptable for use in a hazard assessment.

Author	Date	MRID No.
Swigert	1986	409745-28
Swigert	1986	409745-29

The minimum data requirements for establishing the acute toxicity to fish are the results from two 96-hour studies with the technical grade material. The studies are to be performed on one coldwater species (preferably rainbow trout) and one warmwater species (preferably bluegill sunfish).

The acceptable acute toxicity studies are presented below:

Species	T.M.	LC_{50} (ppm)	Author	Date	MRID No.	Fulfills Requirement
Rainbow Trout (Salmo gairdneri)	83%	18	Swigert	1986	409745-28	Yes
Bluegill-sunfish (Lepomis macrochirus)	83%	33	Swigert	1986	409745-29	Yes

The guideline requirement for fish acute toxicity testing

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using technical clethodim (72-1) has been satisfied. In the future, new use patterns or increased rates may dictate the need to repeat the 72-1 warmwater species test due to solubility limits in the above referenced test. There is sufficient information on technical clethodim to classify it as slightly toxic to cold and warm water fish species.

Chronic Testing

A fish early life stage study is required if an active ingredient is persistent in water, or chronic exposure is otherwise expected. The fish early life stage test will be held in reserve until the registrant can show that the active ingredient does not persist in water and that multiple treatments will not be recommended on Section 3 labeling.

Field Testing

Field testing (72-7) may be requested for pesticides expected to transport to and persist in the aquatic environment at hazardous levels. Based on available data it is unlikely that aquatic field testing (to determine effects on fish) will be required.

Precautionary Labeling

Based on available toxicity data, no precautionary statements for fish are required.

Effects on Freshwater Invertebrates

One study in one document was evaluated. This study was invalid, and not acceptable for use in a hazard assessment.

Author	Date	MRID No.
Forbis	1986	409745-30

The minimum requirement to establish the acute toxicity to freshwater invertebrates is a 48-hr aquatic study with the technical material. The preferred test species is Daphnia magna. The submitted study is presented below.

Species	T.M.	EC_{50}	Author	Date	MRID No.	Fulfills Requirmts
Daphnia magna	83%	--	Forbis	1986	409745-30	No

The submitted test does not fulfill the guideline requirement (72-2) for acute testing with aquatic

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invertebrates.

In correspondence submitted to the Agency, 10/16/89, the registrant agreed to repeat the Daphnia magna study using the TEP.

Chronic Testing

Data from a freshwater aquatic life-cycle test are required if an active ingredient is persistent in water or chronic exposure is otherwise expected. Chronic freshwater aquatic life-cycle testing will be held in reserve until receipt of a valid acute Daphnia magna study.

Field Testing

Field testing (72-7) may be required for pesticides expected to transport to and persist in the aquatic environment at hazardous levels. The requirement for 72-7 testing cannot be determined at this time and will be held in reserve.

Precautionary Labeling

The need for precautionary labeling to protect freshwater invertebrates cannot be determined at this time.

Effects on Estuarine Organisms

No documents are available for evaluation. The minimum data requirements to determine the toxicity of a pesticide to estuarine organisms are acute studies with an estuarine fish species, a shrimp and a mollusc. The proposed use patterns for clethodim (cotton and soybeans) trigger EEB estuarine data requirements. However, based on fish LC50 values for the technical and "inert" chemicals; and the low aquatic EEC value, estuarine studies are not required.

Chronic Testing

Chronic estuarine studies will be held in reserve until the registrant can show that the active ingredient does not persist in water or sediment and that multiple treatments will not result in a significant accumulation of residue.

Field Testing

Field Testing (72-7) may be required for pesticides expected to transport to and persist in the estuarine environment at hazardous levels. The need for field testing on estuarine organisms cannot be determined at this time.

Precautionary Labeling

No precautionary statements to protect estuarine organisms are required at this time.

Effects on Beneficial Insects

One study in one document was evaluated under this topic:

Author	Date	MRID No.
Atkins	1986	409745-32

The following test was considered acceptable, and fulfills the requirements for testing with beneficial insects.

Species	T.M.	LD_{50}	Date	MRID No.	Fulfills Requirement
Honeybee	88%	>100 ug/bee	1986	409745-32	Yes

This test indicates that clethodim technical is practically non-toxic to honeybees.

Precautionary Labeling

The need for precautionary labeling to protect beneficial insects cannot be determined at this time.

Effects on Non-Target Plants

No documents are available for evaluation. The potential exists for herbicides to move from the site of application through drift, volatilization and runoff. If applied aerially or if volatility or water solubility are sufficiently high, non-target plant studies may be requested. Based on available use pattern information, the following non-target plant tests are required prior to Section 3 registration using the TEP.

1) 123-1 Seed germination/seedling emergence

2) 123-1 Vegetative vigor

3) 123-2 Aquatic plant growth using:
   Selenastrum capricornutum
   Lemna gibba
   Skeletonema costatum
   Anabaena flos-aquae
   A freshwater diatom

4) 201-1 Drift

5) 201-2 Drift

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Clethodim Ecological Effects

Disciplinary Review

I. Ecological Effects Profile

A. Manufacturing Use

1. Avian Studies

The avian acute oral LD_{50} of >2000 mg/kg (bobwhite, 409745-25) and the avian dietary LC_{50}'s of >4270 ppm, (bobwhite, 409745-26) and >3978 ppm, (Mallard, 409745-27) indicate that Clethodim technical is slightly to practically non-toxic to birds. The avian reproduction NOEL for mallard is 1000 ppm (410302-05).

2. Aquatic Studies

The fish LC_{50}'s for rainbow trout and bluegill are 18 ppm and 33 ppm respectively (409745-28 and 409745-29 respectively). Clethodim technical is classified as slightly toxic to cold and warm water fish species.

3. Mammal Studies

Information from a Toxicology Branch one-liner for technical clethodim gives a rat LD_{50} of 1360 mg/kg (007222) and a mouse LD_{50} of 2,430 mg/kg (007222).

B. Formulated Product

1. Aquatic Studies

The registrant has agreed to repeat the Daphnia magna freshwater aquatic invertebrate study using the TEP due to solubility problems with the technical chemical.This study has not been submitted for review to date.

2. Mammal Studies

The formulated Clethodim product, Select 2EC, gave a rat LD_{50} of 2,920 mg/kg (007222).

C. Degradate Testing

No degradate testing needs have been identified to

date.

II. Ecological Effects Hazard Assessment

A. Uses

Clethodim (Select) herbicide is a selective postemergence herbicide for control of annual and perennial grass weeds in soybeans or cotton. Select contains 2# clethodim active ingredient per gallon.

B. Environmental Fate

No one-liners were available from EFGWB for this review.

C. Manufacturing Use

N/A

D. End-Use Product

For the purposes of this risk assessment, the use rates from the proposed Section 3 label for cotton and soybeans are 0.5# ai/A/yr for one application, or two applications per year at 0.25 #ai/A each. Ground or aerial methods of application are recommended; a minimum of 3 gallons spray solution per acre for aerial.

1. Terrestrial

If clethodim is applied at 0.5 #ai per acre, the following maximum residues would be expected on various food items:

0.5 # ai/A	Plant	Immediately After Application (ppm)
	Short grass	120 ppm
	Long grass	55
	Leafy crops	63
	Forage crops	29
	Legumes pod crops	6
	Tree Fruits	3.5

The maximum expected residue levels are substantially lower than the avian LC_{50} values of >4270 for bobwhite, and >3978 for mallard and below the avian reproductive NOEL of 1000 ppm for mallard.

If the mammalian LD_{50} is used to estimate a one day LC_{50}, the result is approximately 13,600 ppm*. The estimated residues on foliage are substantially lower than this value. Mammalian reproductive values were not available from Toxicology Branch at the time of this review. Since Clethodim is applied once per season at 0.50 #ai/A or twice at 0.25 #ai/A each early in the growing season, long term exposure is not expected. If additional treatments are proposed, or if clethodim is found to be persistent in the environment, these conclusions will require reevaluation.

2. Aquatic

Using a maximum application rate of 0.5 #ai/Acre, the following exposure estimates are given:

10 Acre treated field drains into a 1 acre pond 6' deep
5 percent runs off into adjacent aquatic habitat

10 Acre x 0.5 #ai/A x 5% (0.05) x 61 ppb = 15.25 ppb

This estimated terrestrial field runoff value is less than 1.8 ppm (1,800 ppb) (1/10 18 ppm fish LC_{50}). Adverse acute effects to warm water and coldwater fish are not expected from the proposed use pattern. EEC values cannot be calculated at this time for aquatic invertebrates or estuarine organisms.

* Assuming a young rat weighs 0.10 kg and consumes 10% of it's body weight per day, the formula for a 1 day

$$LC_{50} \text{ in ppm} = \frac{LD_{50} \text{ (mg/kg) x wt. (Grams)}}{\text{consumption/day (grams)}}$$

The potential for chronic effects on freshwater fish cannot be evaluated without a more complete environmental fate data base.

Degradates and their effects are not known at this time.

3. Endangered Species

The endangered species triggers are:

Group	Rep. NOEL	LC_{50}
Birds	2750 ppm	398 ppm (3978/10)
Mammals	No data	1,360 ppm (13,600/10)*
Fish	18 ppm	0.9 ppm (18/20)
Aquatic Invertebrate	No data	No data
Estuarine Organisms	No data	No data

Terrestrial

If clethodim is applied at 0.5 #ai/A (MAXIMUM LABEL RATE), the following residues (ppm) would be expected on various food items:

Short grass	-	120 ppm
Long grass	-	55 ppm
Leafy crops	-	63 ppm
Forage crops	-	29 ppm
Legumes, pod crops	-	6 ppm
Tree fruits	-	3.5 ppm

* The extrapolated 1-day LC_{50} for mammals, assuming the organisms consumes 10% of its body wt./day.

The maximum clethodim residues on foliage do not exceed the acute endangered bird or mammal species triggers. Use of clethodim, as proposed, is unlikely to adversely affect terrestrial species.

A 10 acre treated field drains into a 1 acre pond 6 feet deep; 5% of the applied pesticide moves into the pond via runoff = 15.25 ppb in adjacent aquatic habitat.

[10A x 0.5 #ai/A x 5% x 61 ppb = 15.25 ppb]

This value is less than the endangered species value of 900 ppb for freshwater fish species.

EEC values for endangered aquatic invertebrate and estuarine species cannot be calculated at this time.

Plants

No data are available for analysis. The proposed Clethodim label recommends use by aircraft using low volume sprays thus increasing potential for exposure to plants.

Endangered Species Summary

The available information for clethodim technical indicates that use of clethodim on cotton and soybeans, as proposed, is not expected to adversely affect endangered birds, beneficial insects, mammals, or freshwater fish species. However, a final determination of potential effects on beneficial insect species cannot be made until formulation testing is complete.

The potential impacts of clethodim use on endangered aquatic invertebrates and plant species cannot be determined due to the absence of data.

III. Precautionary Labeling

A. Manufacturing-Use Product

The following statement is required on manufacturing use product labeling:

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or public water unless this product is specifically identified and addressed in an NPDES permit. Do not discharge effluent containing this product into sewer systems without previously notifying the sewage treatment plant authority. For guidance contact your State Water Board or Regional EPA office."

B. End-Use Product for Terrestrial Food Crop

Precautionary label statements for end use clethodim products will be reserved pending receipt of all required studies.

IV. Data Requirements

Outstanding Data Requirements

Outstanding data requirements for ecological effects are as follows:

1) 72-7 Acute honeybee LD50 with formulated product (TEP).

2) 72-2 Acute Aquatic invertebrate testing on *Daphnia magna* using formulated product.

3) 123-1 Non-target effects on terrestrial plants - seed germination/seedling emergence using the formulated product (TEP).

   123-1 Non-target effects on terrestrial plants - vegetative vigor using the formulated product (TEP).

   123-2 Non-target effect on the following aquatic plants using the formulated product (TEP).
   Selenastrum capricornutum
   Lemna gibba
   Skeletonema costatum
   Anabaena flos-aquae
   A freshwater diatom

   201-1 Drift (using formulated product).

   201-2 Drift (using formulated product).

V. Studies not used in risk assessment

One study in one document was not used for avian reproductive effects:

Author	MRID No.
Beavers	410302-06

One study in one document was not used for effects on aquatic invertebrates:

Author	MRID No.
Forbis	409745-30

File - 129009

MRID No. 410302-06

DATA EVALUATION RECORD

1. CHEMICAL: Clethodim. Shaughnessey Number: Not available.

2. TEST MATERIAL: RE-45601 Technical (Select); (E,E)-(±)-2-[1-[((3-chloro-2-propenyl)oxy)imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; Lot No. SX-1688; 83.3% purity; an amber liquid.

3. STUDY TYPE: Avian Reproduction Study.
Species Tested: Bobwhite quail (Colinus virginianus).

4. CITATION: Beavers, J.B. 1988. RE-45601 Technical: A One-Generation Reproduction Study with the Bobwhite (Colinus virginianus). Prepared by Wildlife International Ltd., Easton, Maryland. Laboratory Project No. 162-183. Submitted by Chevron Chemical Company, Richmond, California. Chevron Project No. S-2836. MRID Number: 410302-06.

5. REVIEWED BY:

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date:

6. APPROVED BY:

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date: A.C. Kosalwat 4/05/90

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven

Date: 4/05/90

7. CONCLUSIONS: Mean measured concentrations of RE-45601 Technical at 100, 250, and 833 ppm as test material had no effects upon egg shell thickness in adult bobwhite quail during the 22-week exposure period. The NOEC was 250 ppm, based upon reduced embryo viability and 14-day-old survivors of eggs set. The study is scientifically sound but does not fulfill the requirements for an avian reproductive test, since a high rate of adult mortality was not adequately explained. The high rate of mortality in adults and chicks (due to incubator failure) contributes to a level of variation high enough to prevent statistical accuracy.

8. RECOMMENDATIONS: N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The birds used in the test were pen-reared, unmated bobwhite quail and were purchased from Sand Prairie Quail Farm, Maquoketa, Iowa. All birds were acclimated to the facilities for 9 weeks prior to initiation of the test. Birds that did not appear healthy at test initiation were discarded. The birds were 25 weeks of age at test initiation.

B. Dose/Diet Preparation/Food Consumption: Test diets were prepared by mixing RE-45601 Technical into a pre-mix which was used for weekly preparation of the final diet. Control diet and three test concentrations (120, 300, and 1000 ppm) were prepared weekly. Portions of the freshly prepared diet were presented to the birds on Friday of each week, and the remainder was stored frozen. On Monday of each week, diets in all treatment groups were replaced with fresh frozen diet. On Wednesday of each week, diets in the 120-ppm group were again replaced with fresh frozen diet. When necessary, additional feed was prepared. Dietary concentrations were not adjusted for purity of the test substance. The control diet contained an amount of the carrier (corn oil) and solvent (acetone) equal to that in the treated diets.

Adults were fed a game bird ration formulated for breeding birds. All offspring received a game bird ration formulated for young growing birds. The test substance was not mixed into the diet of the offspring. Food and water were supplied ad libitum during acclimation and during the test. Samples of the control diet and each of the test diets were taken weekly after mixing, and immediately after removal from the freezer, and used for analysis of the active ingredient.

Food consumption in each pen was determined once each week throughout the study.

C. Design: The birds were randomly distributed into four groups as follows:

RE-45601 Technical

Nominal Concentration	Number of Pens	Birds Per Pen	
		Males	Females
Control (0 ppm)	16	1	1
120 ppm	16	1	1
300 ppm	16	1	1
1000 ppm	16	1	1

"Treatment levels were based upon known toxicity data and consultation with the client." Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

1. Acclimation - 9 weeks.
2. Pre-photostimulation - 7 weeks.
3. Pre-egg laying (with photostimulation) - 3 weeks.
4. Egg laying - 12 weeks.
5. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 6 weeks.

D. Pen Facilities: Adult birds were housed indoors in pens constructed of wire grid and sheeting. Pens measured approximately 30 cm x 51 cm. The pens had sloping floors which resulted in a ceiling height ranging from 21 to 26 cm. The average temperature in the adult study room was 20.2°C ± 3.0°C (SD) with an average relative humidity of 42%.

The photoperiod during the first 7 weeks of the study was 8 hours of light per day. The photoperiod was increased to 17 hours of light per day during Week 8, and was maintained at that length until sacrifice of adult birds. The birds received approximately 130 lux of illumination throughout the study.

E. Adult Observations/Gross Pathology: All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, at the end of weeks 2, 4, 6, 8, and at study termination.

F. Eggs/Eggshell Thickness: Eggs were collected daily from all pens, marked according to pen of origin, and fumigated to prevent pathogen contamination. The eggs were then stored at 10.5°C ± 1.4°C (SD) and 75% relative

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humidity until incubated. Eggs were removed from the storage room weekly and candled. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal or used for egg shell thickness measurements were placed in an incubator at 37.4°C ± 0.1°C (SD) and 56% relative humidity. Eggs were candled again on day 11 of incubation to determine embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in the incubator and placed in a hatcher on incubation day 21. Temperature in the hatcher was 37.1°C ± 0.8°C (SD) with a relative humidity of 73%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were used for egg shell thickness measurements. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the waist of the egg using a micrometer.

G. Hatchlings: All hatchlings and unhatched eggs were removed from the hatcher on day 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were leg-banded for identification by pen of origin and then placed in brooding pens until 14 days of age. Each brooding pen measured 72 cm x 90 cm x 23 cm high, and was constructed of galvanized wire mesh and sheeting. Brooder temperatures were maintained at approximately 38°C. The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age, the average body weight by parental pen of all survivors was determined.

H. Statistics: Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data.

Each of the following parameters was analyzed statistically:

Adult Body Weight
Adult Feed Consumption
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of Viable Embryos
Hatchlings of 3-Week Embryos
Hatchlings of Eggs Set
Offspring's Body Weight
Hatchlings of Maximum Set
14-Day Old Survivors of Maximum Set
14-Day Old Survivors of Eggs Set
14-Day Old Survivors of of Hatchlings
Egg Shell Thickness

12. REPORTED RESULTS

A. Diet Analysis: The test material was analyzed by Chevron's Analytical Services Laboratory. The results of the analysis were presented as an addendum report (MRID # 410302-06, Vol. 2 of 2). Since the diet formulation, test chemical, and dose levels were the same as in a simultaneous study using mallards (MRID No. 410302-05), and since the same diet preparations were administered in both studies, a single series of chemical analyses were conducted for both studies. The mean measured concentrations for freshly prepared diets were 87.5%, 90.4%, and 94.5% of the nominal concentrations (adjusted for active ingredient) of 100, 250, and 833 ppm, respectively.

B. Mortality and Behavioral Reactions: "There were no treatment related mortalities during the study. However, incidental mortalities occurred in the control group and all treatment groups. In all instances, mortalities appeared to be related to either head or foot lesions, resulting from cannibalism or self-inflicted injury. Birds from this particular lot (hatch) seemed prone to flushing and injuring themselves." The deaths of three females were recorded in the control group, and occurred during weeks 4 and 9. Six females died in the 120-ppm group, during weeks 10, 12, 13, and 16. Three females and one male died during weeks 9, 13, 17, and 22 in the 300-ppm group. Six females and one male died in the 1000-ppm group, during weeks 12, 13, and 15.

No overt signs of toxicity were observed at any concentration.

All birds that died during the study and all survivors were necropsied. All lesions observed were considered to be unrelated to treatment. Results of the necropsies are reported in Appendix IV (attached).

C. Adult Body Weight and Food Consumption: No significant differences in body weights between the control and any treatment group were noted at any body weight interval.

"Due to excessive wastage by some birds, feed consumption was variable between pens. There was no apparent treatment related effect upon feed consumption among birds at any concentration tested." When compared to the control group, at 120 ppm there were significant decreases in food consumption during weeks 1 and 22, and significant increases during weeks 4 and 12. At 300 ppm, there was a significant decrease in food consumption during week 16. These differences were considered to be unrelated to treatment. There were no significant differences between the control and 1000-ppm group during the study. Mean feed consumption and levels of significance are shown in Table 2 (attached).

D. Reproduction: When compared to the control group, there were no significant differences in reproductive parameters at any concentration tested. "While not statistically significant, at the 1000 ppm concentration there appeared to be a slight treatment related reduction in the percentage of viable embryos of eggs set"(Tables 3 & 3A, attached). The effect upon viability was also reflected in the numbers of hatchlings and 14-day survivors expressed as percentages of eggs set.

"A brooder failure during rearing of hatchlings from the fifth set of eggs resulted in incidental mortalities of offspring. One other incidental mortality, a hatchling euthanized because of a broken leg, also occurred during the course of the study. There were 44 incidental mortalities from the control group, 25 from the 120 ppm treatment group and 32 incidental mortalities from the 300 ppm treatment group. Incidental mortalities were not utilized when computing the percentage of 14 day old survivors of hatchlings or of eggs set."

E. Egg Shell Thickness: When compared to the control group, there were no significant differences in egg shell thickness at any concentration.

F. Offspring Body Weight: There were no significant differences between the control and any treatment group in body weights of offspring at hatching or at 14 days of age.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

"Dietary concentrations of RE-45601 Technical at 120, 300, or 1000 ppm did not result in treatment related mortality, overt signs of toxicity, or effects upon body weight or feed consumption among adult bobwhite during the 22 week exposure period. No treatment related effects upon reproductive performance were noted at 120 or 300 ppm. While not statistically significant, there appeared to be a slight reduction in the percentage of viable embryos of eggs set at the 1000 ppm concentration. The no-observed-effect concentration for bobwhite in this study was 300 ppm."

The report stated that study was conducted in conformance with Good Laboratory Practice regulations. The data were inspected and the final report signed by Quality Assurance representatives of Chevron Chemical Company and Wildlife International, Ltd.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The test procedures were in accordance with the SEP and Subdivision E guidelines except for the following deviations:

Eggs were stored at a temperature of approximately 11°C and a relative humidity of approximately 75%; 16°C and 65% are recommended.

Eggs were candled on day 21 to determine embryo survival; day 18 is recommended.

Observations on food palatability were not reported.

Behavioral observations of offspring were not reported.

B. Statistical Analysis: Statistical procedures differed from recommended methods. Specifically, there is no basis for transforming the number of eggs laid and the number of hatchlings to percentile values of the maximum number of eggs laid or set in any test group.

Analyses of reproductive parameters were verified (attached) and found to match those reported by the author, except in the ratio of 14-day-old survivors/eggs

set. The analysis of this parameter, using the author's "adjusted" data (see below) indicated that the 1000-ppm group was significantly ($p = 0.042$) lower than the control group, contrary to the author's conclusion of no significant difference.

C. Discussion/Results: The reduced food consumption does not appear to be related to treatment.
Using SAS egg shell thickness data were evaluated. No significant differences from the controls were found at any treatment level.

The observed mortality among adult bobwhites is of concern. Twenty birds (2 males, 18 females) died during the study. The author stated that all mortalities appeared to be related to either head or foot lesions that resulted from cannibalism or injury, and further stated that birds from this particular lot (hatch) seemed prone to flushing and injuring themselves. This explanation, however, does not adequately explain why 18 of 20 mortalities were females.

The necropsy reports (Appendix IV, attached) of the 20 mortalities appear to be incomplete. The pathological observations of birds that died during the study do not include categories for the ovaries, egg yolk peritonitis, or liver. It is unclear whether these categories are included under the broad category of "internal" on page 2 of Appendix IV. Since the effects of toxic chemicals are often seen in the liver and reproductive tract, the absence of these categories seems odd. The inclusion of categories for egg yolk peritonitis and regressing ovary in birds which were sacrificed at study termination, while excluding the same categories for birds that died during the study is also peculiar.

The deaths of 101 hatchlings due to a brooder malfunction are also of concern. The author's exclusion of these mortalities from the analyses of 14 day survivors/eggs set and 14 day survivors/hatchlings is not acceptable statistical procedure. The absence of survival data associated with these hatchlings, combined with the absence of data from 20 other pens due to adult mortalities, seriously reduces the sample size available for analyses of these two parameters.

Mortality in 14 day old birds across all pens was 31%; and 19% in the controls. The total number of pens at the study conclusion were reduced from 16 to 12; a level considered unacceptable by ASTM standards. Mortalities were as follows:

Treatment	Mortalities
control	3
120 ppm	6
300 ppm	4
1000 ppm	7

Brooder failure occurred resulting in the death of 101 chicks as follows:

Treatment	Mortalities
control	44
120 ppm	25
300 ppm	32

A consideration of the problems discussed above generates serious doubts about the validity of the test. It is unclear whether the adult mortalities were due to the test chemical, poor husbandry, random error, or a combination of these factors. This, combined with unclear necropsy results and a large number of incidental mortalities of hatchlings, prevents a satisfactory risk assessment of the test substance. The study, therefore, while scientifically sound in its design, does not fullfill the requirements for an avian reproductive test.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The high mortality of adults and chicks plus unclear necropsy results, prevent a determination of whether the deaths were related to treatment.

(3) Repairability: The study can be upgraded only if the registrant can show that the adult mortalities were not treatment-related.

15. COMPLETION OF ONE-LINER: Yes; February 15, 1990.