6-20-86

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUN 20 1986

MEMORANDUM

SUBJECT: Ecological Effects Branch Review of Avian Reproduction Studies With Whip (fenoxaprop-ethyl)

FROM: Larry Turner, Biologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

THRU: Norman Cook, Head-Section 2
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

THRU: Michael W. Slimak, Chief
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

TO: Richard Mountfort, PM 23
Fungicide-Herbicide Branch
Registration Division (TS-767C)

Ecological Effects Branch (EEB) has reviewed the subject avian reproduction tests on bobwhite and mallard. Both studies are considered to be scientifically valid and would fulfill Guidelines requirements for these types of studies. EEB agrees with the study investigators that the NOEL for mallard ducks is 180 parts per million (ppm), but not > 180 ppm as reported in the hazard evaluation. EEB does not agree that the reduced hatchability at 180 ppm in the bobwhite study is not "of any biological significance." EEB agrees that this effect is not likely to be of substantial biological consequence, particularly in view of the overall results, but the NOEL for the bobwhite study is concluded to be 30 ppm.

DER's for the two studies are attached.

DATA EVALUATION RECORD

1. Chemical: Fenoxaprop-ethyl, Shaughnessy No. 128701

2. Test Material: HOE 033171, 95.5% ai

3. Study Type: Avian reproduction

   Species Tested: Mallard, Anas platyrhynchos

4. Study ID: Roberts, N.L.; Phillips, C.N.K.; Anderson, A.; Dawe, I.S. and Chanter, D.O. (1985) The Effects of Dietary Inclusion of HOE 033171 - Active Ingredient Technical (Code: HOE 033171 OH Z096 0001) on Reproduction in the Mallard Duck. (Unpublished Study Conducted by Huntingdon Research Centre, Cambridgeshire, England; Submitted by American Hoechst Corporation, Somerville, NJ, Accession No. 073953).

5. Reviewed By: Larry Turner
   Biologist
   EEB/HED
   Signature: Larry Turner
   Date: 6/16/86

6. Approved By: Norman Cook
   Head-Section 2
   EEB/HED
   Signature: Norman Cook
   Date: 6.19.86

7. Conclusions:

The study is scientifically valid and would fulfill a Guideline requirement for an avian reproduction study with waterfowl. With no significant effects up to 180 parts per million (ppm) is the diet, fenoxaprop-ethyl is considered not to impair mallard reproduction up to this dietary level.

8. Recommendations: N/A.

9. Background: N/A.

10. Discussion of Individual Test: N/A.

11. Materials and Methods:

a. Test Animals: Test animals were mallard ducks, Anas platyrhynchos, obtained from John Coles, County Game Farms, Ashford, Kent. Test birds were 7 months old when obtained and 9 months old at the start of the study. Mean weight was 977 g at the beginning of the 7-day acclimation period and 975 g at the beginning of treatment.

b. Test System: Birds were housed in groups of two males and five females per pen. Pens were "floor pens" measuring 0.7 x 1.4 meters, constructed of galvanized steel with solid sides and wire mesh floors. Each pen contained an automatic cup drinker and a food hopper. During the egg production period, the floors were covered with plastic padding to minimize the risk of cracked eggs.

Mean maximum and minimum temperatures were 22 °C and 18 °C, respectively. Relative humidity was 73 ± 16 percent. Ventilation rate was 7.5 to 15 air changes per hour. Photoperiod was 7L/17D from time of arrival through week 8 of the study. From week 9 until the end, photoperiod was 16L/8D.

Basal diet was quail layer diet, containing no antibiotics or other growth promoters. Test substance concentrations were analyzed on weeks 1, 13, and 23. Water was available at all times. Diets were prepared weekly, starting first with a premix at 5000 ppm. Prepared diets were also analyzed for stability and homogeneity.

Egg collection for the purposes of analysis was begun on week 11 and all eggs were collected for the 12-week production period. Some eggs were recorded as early as week 9, but were not incubated, in accordance with the study plan. The first egg(s) laid in each replicate during weeks 11, 13, 15, 17, 19, and 21 were examined for shell thickness. After eggs were broken, washed out, and allowed to dry for 48 hours, thickness was measured at 4 points around the circumference.

Eggs were incubated at 37.7 °C and 55 percent RH, with automatic turning. They were transferred to a hatcher on day 23, with the hatcher being at 37.5 °C. Hatching chicks were kept separate by replicate and identified individually. They were housed in floor pens with concrete floors covered by wood shavings. A supplemental heat lamp was used. Minimum and maximum mean temperatures were 30 °C and 27 °C, respectively, with relative humidity at 69 ± 9 percent. Chicks were fed

standard HRC chick diet and were held until 2 weeks of age.

Eggs were candled prior to incubation for cracks, on day 14 for fertility and early embryonic death, and on day 21 for late embryonic death. Adult birds and chicks were observed daily. Body weights of adults were recorded every 14 days throughout the study; for chicks, bodyweights were measured at hatching and on day 14. All adult birds were examined postmortem, as were chicks that died during the 14-day observation period (except for one group that was inadvertently deprived of water). Tissue samples of liver, kidney, muscle, and fat were taken from three male and three females of each treatment group for residue analysis.

c. Dose: Dietary concentrations were 0 (control), 5, 30, and 180 ppm. Analyzed concentrations were at least 92 percent of nominal.

d. Design: Six replicate pens were used for each dietary concentration. Each replicate consisted of two males and five females housed together, for a total of 12 males and 30 females for each concentration.

e. Statistics: The following parameters were analyzed statistically:

1. Adult food consumption.
2. Adult mortality and body weight.
3. Number of eggs laid and proportion damaged.
4. Egg weight.
5. Egg shell thickness
6. Numbers of infertilities, embryonic deaths, and hatchings.
7. Numbers of 14-day-old surviving chicks.
8. Chick bodyweights at hatching and 14 days later.

Analysis was by Williams Test (Biometrics 27:103-117, 1971 and Biometrics 28:519-531, 1972), with angular transformations for proportions. ANOVA was used for food consumption analysis.

12. Reported Results:

At dietary concentrations of 5, 30, and 180 ppm HOE 033171, general behavior, health conditions, body weights, and food consumption remained unaffected and were not impaired by treatment. Mortalities attributed to treatment did not occur, and necropsies revealed no treatment-related effect. Reproductive parameters analyzed were number of eggs laid, broken and cracked eggs, egg weights, shell

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thickness, number of infertile eggs, early and late embryonic death, hatching, chick health and weights, and number of 14-day-old survivors. No indication of any reproductive impairment was observed.

13. Study Author's Conclusions/QA Measures:

Under the conditions of this test, there was no evidence that dietary administration of HOE 033171 technical up to 180 ppm had any adverse effects on the reproduction of the mallard duck. The high dose level of 180 ppm is equivalent to an estimated intake of approximately 30 mg/kg/day.

The study was conducted in compliance with the FDA GLP's as well as GLP's for OECD and Japan. The report was audited and signed as "an accurate description" by the HRC Quality Assurance Unit.

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

a. Test Procedures: Test procedures were scientifically sound and in general accordance with acceptable protocols. The following deviations from typical protocols were noted:

1. No information on crippled hatchlings.
2. No rationale for selection of concentrations.
3. No information reported on history of rearing practices.
4. The three treatment levels and six replicates exceeded minimum requirements.
5. Illumination intensity not reported.

b. Statistical Analysis: The statistical methodology is acceptable. Examination of the results indicated no treatment-related effects. Analysis of ANOVA/Duncan was conducted on cracked and broken eggs, a parameter showing one of the larger differences between treated and control groups. The analysis showed no statistically significant differences, even at the $\alpha = 0.25$ level, which is consistent with the authors' analysis.

c. Results/Discussion: The following results were tabulated by the reviewer, based on data included in the study authors' report:

Parameter	Treatment Level (ppm) 0	5	30	180
Eggs laid (n)	933	889	945	899
Eggs laid/hen ($\bar{x}$)	31.1	29.6	31.5	30.0
Eggs cracked or broken (n)	58	88	98	82
Eggs cracked or broken (% of eggs laid)	6	10	10	9
Egg weights (g)	61	59	60	61
Eggshell thickness (mm)	0.315	0.305	0.313	0.309
Eggs set (n)	812	741	784	746
Fertile eggs (n)	737	695	745	692
Fertile eggs (% of eggs set)	90.8	93.8	95.0	92.8
Early embryonic death (% of fertile eggs)	1.8	4.0	4.8	5.2
Late embryonic death (% of fertile eggs)	7.5	8.3	10.2	8.8
Eggs hatched (n)	476	491	488	495
Eggs hatched (% of fertile eggs)	64.6	70.6	65.5	71.5
14-Day survivors (n)	431	424	442	417
14-Day survivors (% of hatched)	90.5	86.4	90.6	84.2
14-Day survivors/hen ($\bar{x}$)	14.4	14.1	14.7	13.9

Although no statistically significant differences between controls and treatment groups were reported by the authors for any of the above parameters, there appears to be some difference for cracked/broken eggs and for early embryonic death. Only the latter has the appearance of a dose-response relationship. When the early and late embryonic deaths are combined, the totals exceed typical values for both controls and treatment groups. However, the percentage of fertile eggs that hatched is well above the minimum end of typical for this parameter, which includes embryonic deaths. This reviewer concludes that while there may be slight effects on certain parameters, these effects are not statistically significant, do not result in differences in surviving chicks per hen, and are quite unlikely to affect field populations.

d. Adequacy of Study:

1. Classification: Core.

2. Rationale: Valid study with only minor deviations from typical protocols.

3. Repairability: N/A.

15. Completion of One-Liner:

One-liner form completed June 4, 1986.

16. CBI Appendix: N/A.

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Mallard Reproduction : Fenoxaprop-ethyl 95.5%
response: broken and cracked eggs

THE FOLLOWING RESULTS ARE FOR:
TRT$ = a 0 ppm

TOTAL OBSERVATIONS: 6

RESPONSE

N OF CASES	6
MEAN	9.667
STANDARD DEV	4.633

THE FOLLOWING RESULTS ARE FOR:
TRT$ = b 5 ppm

TOTAL OBSERVATIONS: 6

RESPONSE

N OF CASES	6
MEAN	14.667
STANDARD DEV	6.683

THE FOLLOWING RESULTS ARE FOR:
TRT$ = c 30 ppm

TOTAL OBSERVATIONS: 6

RESPONSE

N OF CASES	6
MEAN	16.167
STANDARD DEV	12.090

THE FOLLOWING RESULTS ARE FOR:
TRT$ = d 180 ppm

TOTAL OBSERVATIONS: 6

RESPONSE

N OF CASES	6
MEAN	13.667
STANDARD DEV	7.763

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 4.685
APPROXIMATE F = 1.445 DF = 3, 720 PROBABILITY = .227

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	139.125	3	46.375	.681	.574
WITHIN GROUPS	1362.833	20	68.142		

DUNCAN MULTIPLE RANGE TESTS
ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	9.947
3	10.437
4	10.751

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

DATA EVALUATION RECORD

1. Chemical: Fenoxaprop-ethyl, Shaughnessy No. 128701

2. Test Material: HOE 033171, 95.5% ai

3. Study Type: Avian reproduction

   Species Tested: Northern bobwhite, *Colinus virginianus*

4. Study ID: Roberts, N.L.; Phillips, C.N.K.; Anderson, A.; Dawe, I.S. and Chanter, D.O., Cook, S.C., Haynes, S.M. (1985) The Effects of Dietary Inclusion of HOE 033171 - Active Ingredient Technical (Code: HOE 033171 OH Z096 0001) on Reproduction in the Bobwhite Quail. (Unpublished Study Conducted by Huntingdon Research Centre, Cambridgeshire, England; Submitted by American Hoechst Corporation, Somerville, NJ, Accession No. 073953).

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   Signature: Norman Cook
   Date: 6.19.86

7. Conclusions:

The study is scientifically valid and would fulfill a Guideline requirement for an avian reproduction study with upland game birds. A slight effect on hatching at 180 parts per million (ppm) is considered to be of minor biological significance and is unlikely to affect populations. The NOEL is considered to be 30 ppm, and fenoxaprop-ethyl does not appear to impair bobwhite reproduction up to this level.

8. Recommendations: N/A.

9. Background: N/A.

10. Discussion of Individual Test: N/A.

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11. Materials and Methods:

a. Test Animals: Test animals were Northern bobwhite, Colinus virginianus, obtained from Dr. D. Wise, Monkfield, Bourne, Cambridgeshire, UK. Test birds were 7 months old when obtained and 9 months old at the start of the study. Mean weights were 198 g at the beginning of the 7-day acclimation period and 195 g at the beginning of treatment.

b. Test System: Birds were housed in replicates consisting of one male and one female per pen. Pens were 31.5 x 38.5 x 24 cm and were constructed of polyethylene-coated steel wire. Cages were "tiered" (presumably meaning stacked batteries). Each cage had a nipple drinker and an external food hopper. Cages had sloping floors with a 10 cm egg catcher.

Mean maximum and minimum temperatures were 23 ± 3 °C and 19 ± 4 °C, respectively. Relative humidity was 71 ± 12 percent. Photoperiod was 7L/17d from the beginning of acclimation through week 8, and was 16L/8D from week 9 through week 25. Ventilation rates were not reported.

Basal diet was "quail layer diet" containing no antibiotics or other growth promoters. Test substance concentrations were analyzed on weeks 1, 13, and 23. Water was available at all times. Diets were prepared weekly, starting first with a premix at 5000 ppm. Treated diets were also analyzed for stability and homogeneity.

Egg collection for the purpose of analysis was begun on week 14 and all eggs were collected for the 12-week production period ending with week 25. Some eggs were produced as early as week 12, but were not incubated, in accordance with the study plan. The first eggs laid in each replicate during weeks 15, 17, 19, 21, 23, and 25 were examined for shell thickness. After eggs were broken, washed out, and dried for at least 48 hours, thickness was measured at 4 points around the circumference.

Eggs were incubated at 37.7 °C and 55 percent relative humidity, with automatic turning. They were transferred to hatchers on day 21, where the temperature was 37.5 °C. Hatched chicks were individually marked and housed in wooden pens with concrete floors covered by wood shavings. A supplemental heat lamp was used. Minimum and maximum mean temperatures were 29 °C and 23 °C, respectively, with relative humidity at 51 ± 6 percent. Lighting was continuous. Chicks were fed standard HRC chick diet,

with no antibiotics or other growth promoters. Chicks were held for 14 days after hatching.

Eggs were candled prior to incubation for cracks, on day 11 for fertility and early embryonic death, and on day 18 for late embryonic deaths. Adult birds and chicks were observed daily. Body weights of adults were recorded every 14 days throughout the study. Bodyweights of chicks were taken within 24 hours of hatching and on day 14. All adult birds were examined postmortem, as were chicks that died during the 14-day observation period. Tissue samples of liver, kidney, muscle, and fat were taken for residue analysis from three males and three females from each treatment group.

c. Dose: Dietary concentrations were 0 (control), 5, 30, and 180 ppm. Analyzed concentrations were at least 92 percent of nominal.

d. Design: Twenty replicate pens, each containing one male and one female, were used for each of the three treated levels and the control.

e. Statistics: The following parameters were analyzed statistically:

1. Adult food consumption.
2. Adult body weights.
3. Number of eggs laid and proportion damaged.
4. Egg weight.
5. Egg shell thickness
6. Numbers of infertilities, embryonic deaths, and hatchings.
7. Numbers of 14-day-old surviving chicks.
8. Chick bodyweights at hatching and 14 days later.

Analysis was by Williams Test (Biometrics 27:103-117, 1971; and Biometrics 28:519-531, 1972), with angular transformations for proportions. ANOVA was used for food consumption analysis.

12. Reported Results:

The findings in the study can be summarized as follows.

At all dietary concentrations general behavior, health, bodyweights and food consumption remained unaffected and were not impaired by treatment with HOE 033171 technical. Mortalities attributed to treatment did not occur and postmortem examinations performed on birds which died during the study and birds sacrificed at termination of the study revealed no marked treatment-related effects.

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After feeding at 5 ppm, 30 ppm, and 180 ppm the results of all reproductive parameters, including number of eggs laid, broken and cracked eggs, egg weights, egg shell thickness, number of infertile eggs, early and late embryonic death, hatching, chick health, chick bodyweights and number of 14-day survivors, gave no indication of any reproductive impairment.

The slightly reduced hatchability of eggs set after day 18 candling at 30 ppm and 180 ppm and also at 180 ppm in terms of the overall numbers of fertile eggs in comparison with the concurrent control was not considered to be of any biological significance as the numbers of chicks hatched overall were within the limits of normal variation. In addition, there was no evidence of any reproductive impairment in any of the other parameters recorded.

13. Study Author's Conclusions/QA Measures:

"Under the conditions of this test, and taking the results as a whole, it was concluded that the dietary level of 180 ppm of HOE 033171 technical, equivalent to an estimated intake of approximately 18 mg/kg/day, represented the "no toxic effect level" for reproductive impairment in the bobwhite quail. The slightly reduced hatchability of eggs set after Day 18 candling at 30 ppm and 180 ppm and also at 180 ppm in terms of the overall numbers of fertile eggs in comparison with the concurrent control was not considered to be of any biological significance as the numbers of chicks hatched overall where within the limits of normal variation. In addition, there was no evidence of any reproductive impairment in any of the other parameters recorded."

The study was conducted in compliance with the FDA GLP's, as well as GLP's for OECD and Japan. The report was audited and signed as "an accurate description" by the HRC Quality Assurance Unit.

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

a. Test Procedures: Test procedures were scientifically sound and in general accordance with acceptable protocols. The following deviations from typical protocols were noted:

1. No rationale for selection of concentrations.
2. No information reported on history of rearing practices.
3. Three treatment levels and 20 replicates exceeded the minimum requirements.
4. Illumination intensity not reported.

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b. Statistical Analysis: The statistical methodology is acceptable. A visual examination of the results (tabulated below under "c") showed no dose-related effects, nor any apparent statistically significant effects. The authors did report that the proportions of eggs hatching was significantly reduced at 30 and 180 ppm when based on the number of eggs set at day 18, and significantly reduced at 180 ppm based on eggs set at day 0. However, neither treatment level was significantly different from controls when based upon fertile eggs. In addition, it should be noted that hatchability was at least 82 percent in each of the groups, which values are at the upper end of the range of "typical values."

Because the standard parameters appeared to have no dose-related effects, only a "spot-check" statistical analysis was performed, using the number of 14-day-old survivors per hen as the parameter. The EEB ANOVA/Duncan test showed no statistically significant differences.

c. Results/Discussion: The following results were tabulated by the reviewer, based on data included in the study authors' report:

Parameter	Treatment Level (ppm) 0	5	30	180
Eggs laid (n)	1036	909	920	1044
Eggs laid/hen ($\bar{x}$)	51.8	45.5	46.0	52.2
Eggs cracked or broken (n)	186	156	150	152
Eggs cracked or broken (% of eggs laid)	18.0	17.2	16.3	14.6
Egg weights (g)	10.5	10.5	10.7	10.8
Eggshell thickness (mm)	0.18	0.17	0.17	0.19
Eggs set (n)	770	661	685	797
Fertile eggs (n)	708	552	667	688
Fertile eggs (% of eggs set)	91.9	83.5	97.4	86.3
Early embryonic death (% of fertile eggs)	2.1	2.0	1.6	2.2
Late embryonic death (% of fertile eggs)	0.8	0.5	1.3	0.6
Eggs hatched (n)	619	470	553	571
Eggs hatched (% of fertile eggs)	87.4	85.1	82.9	83.0
14-Day survivors (n)	388	334	373	361
14-Day survivors (% of hatched)	62.7	71.1	67.5	63.2
14-Day survivors/hen ($\bar{x}$)	19.4	16.7	18.6	18.0

The results are fairly clear. Although the statistical analysis revealed a slight effect on hatching at the highest doses, relative to control groups, the hatching percentage was quite high even in the high-dose groups.

This reviewer is unable to concur with the study authors' conclusion of no biological significance of these differences. However, it does appear that the

biological significance is small and quite unlikely to result in a population effect.

The number of eggs cracked or broken was quite high when compared to "normal values," even though this was found for control groups as well as treated groups. An increase in cracked and broken eggs has been found previously where sloped pens have been used (R.L. Cochrane, Cochrane, In Press). It should also be noted that egg production was very high, exceeding normal values even when cracked and broken eggs are subtracted. The high egg production may have contributed to the number of cracked eggs because the available calcium and phosphorous would be diluted among the high egg numbers. Although it is suggested that HRC investigate the cause of the high proportion of cracked eggs, it is this reviewer's opinion that his parameter does not negate the validity and usefulness of the test.

d. Adequacy of Study:

1. Classification: Core.

2. Rationale: Valid study with only minor deviations from typical protocols.

3. Repairability: N/A.

15. Completion of One-Liner:

One-liner form completed June 9, 1986.

16. CBI Appendix: N/A.

Bobwhite Reproduction
Fenoxaprop-ethyl 95.5%
response: 14-day old survivors/hen

THE FOLLOWING RESULTS ARE FOR:
TRT$ = a 0 ppm

TOTAL OBSERVATIONS: 20

RESPONSE

N OF CASES	20
MEAN	19.400
STANDARD DEV	13.272

THE FOLLOWING RESULTS ARE FOR:
TRT$ = b 5 ppm

TOTAL OBSERVATIONS: 20

RESPONSE

N OF CASES	20
MEAN	16.700
STANDARD DEV	13.845

THE FOLLOWING RESULTS ARE FOR:
TRT$ = c 30 ppm

TOTAL OBSERVATIONS: 20

RESPONSE

N OF CASES	20
MEAN	18.650
STANDARD DEV	13.554

THE FOLLOWING RESULTS ARE FOR:
TRT$ = d 160 ppm

TOTAL OBSERVATIONS: 20

RESPONSE

N OF CASES	20
MEAN	18.150
STANDARD DEV	13.052

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = .075
APPROXIMATE F = .024 DF = 3, 10396 PROBABILITY = .990

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	77.850	3	25.950	.144	.933
WITHIN GROUPS	13716.100	76	180.475		

DUNCAN MULTIPLE RANGE TESTS
ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	8.464
3	8.904
4	9.196

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL