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MRID 148992

DATA EVALUATION RECORD

1. Chemical: Endosulfan

2. Test Material: Technical, 97.2% ai

7-1-4

3. Study Type: Avian Reproduction

   Species tested: Bobwhite quail (Colinus virginianus)

4. Study ID: Roberts, N.L., R.H. Almond, I.S. Dawe, D.O. Chanter, S.S. Cook. (1984) The Effects of Dietary Inclusion of Endosulfan-technical (Code: HOE-002671 OIZD970003) on Reproduction in the Bobwhite Quail. Performed by Huntingdon Research Centre, England; submitted by American Hoechst Corp., Somerville, NJ. Registration Number 8340-13; Accession No. 256129.

5. Reviewed by: John J. Bascietto
   Wildlife Biologist
   EEB/HED

   Signature: John J. Bascietto
   Date: 13 Sept. '85

6. Approved by: Dave Coppage
   Supervisory Biologist
   EEB/HED

   Signature: Dave Coppage
   Date: 13 Sept 85

7. Conclusions:

The study is not scientifically sound. There are excessive number of nontreatment-related mortalities in all groups, including controls. This may be a "bad" lot of birds or the handling and husbandry are completely unacceptable. There could have been contaminated food and/or water, but this was not checked. The authors offered no explanation other than "stress." Although spare replicates were used, they could not explain the over 25 percent overall population mortality (which also occurred in spare replicates). The study does not fulfill the requirement of the Pesticide Assessment Guidelines for a reproduction study on upland game species.

"INVALID"

8. Recommendations:

The results may not be used in hazard assessment. The study must be reperformed.

9. Background:

The study was submitted in response to the Registration Standard, but was 2 years late.

10. Discussion of Individual Test:

N/A

11. Materials and Methods
(Definitive test)

a. Test Animals - Eighty male and 80 female bobwhite quail (Colinus virginianus) with additional 17 male and 10 female for "spares"; obtained from Dr. D. Wise, Monkfield, Bourne, Cambridge, England; 7 months old on arrival at test facility; 12 months old at beginning of test. They were maintained on an 8-hour photoperiod from age 8 weeks to 7 months.

b. Dose - Dietary inclusion of toxicant to treatment groups. Toxicant was dispersed and mixed in basal bird diets by acetone (to dissolve) and corn oil (for dispersal). Mixture of corn oil, acetone and toxicant were added to 500 g of diet to make a (2%) premix. This was stirred at 40 °C to evaporate acetone, then more basal diet added to complete the premix and shaken in polyethene bags for 3 minutes. Aliquots of premix were used to prepare final diets, which were made in 40 kg batches in large blenders. Diet samples were taken for analysis at various times during the study.

Birds were fed control or test diets for 12 weeks prior to start of egg production, then for an additional 12 weeks during egg production. Because of apparent effect at high levels of toxicant, a 3-week withdrawal period (all birds fed control diet only) was added.

c. Design - Seven days before start of test birds were randomly distributed to cages, one male and one female in each cage. There were a total of 3 treatment groups, i.e., 30, 60, and 120 ppm of endosulfan in diets, and 1 control group, 0 ppm (basal diets). Groups were: a) 0 ppm; b) 30 ppm; c) 60 ppm; d) 120 ppm. Each group was replicated by 20 replicates (20 pairs). "Spare"

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replicates were assigned designators at beginning of study - "Spare replicates for Groups a and b had a male-female pair, plus two extra males; for Groups c and d three male-female pairs plus one extra male (2 in Group D).

All birds were individually identified by means of numbered metal wing tags. Replicates were allocated to cages in five batteries using a "latin square" randomization. Spares were housed in additional batteries - when possible replacements were made from same tier of the battery as the original replicate. Tiered battery cages were of polyethene-coated steel wire, each 31.5 cm x 38.5 cm x 24 cm. Each had a nipple drinker and an external attached stainless steel food hopper; sloped floors with a lo[redacted] cm. egg catcher. Maximum and minimum temperature and relative humidity were recorded daily.

Summary of Design

Group	Treatment	# of Replicates	Birds per Replicate		Birds per Treatmen	
			M	F	M	F
A	Control	20	1	1	20	20
B	Endosulfan 30 ppm	20	1	1	20	20
C	Endosulfan 60 ppm	20	1	1	20	20
D	Endosulfan 120 ppm	20	1	1	20	20

Inducement of egg-laying was brought about by gradual increase in the daily lighting pattern from 8 hours per day prior to study to 14 hrs per day by week 7 of the study.

d. Statistics - The following were analyzed by ANOVA:

- Adult food consumption
- Adult mortality and bodyweight
- Number of eggs laid and proportion damaged
- Egg weight
- Egg shell thickness
- Numbers of infertilities, embryonic deaths and hatchlings
- Numbers of 14-day old surviving chicks
- Chick bodyweights at hatching and 14 days later

12. Reported Results

The authors reported the following:

- At all toxicant levels, behavior, health, bodyweight, and food consumption were not affected.

- Mortalities attributed to treatment did not occur.

- Postmortems of birds dead on test and those sacrificed at termination showed no marked treatment-related effects.

- At 30 and 60 ppm levels, results of all reproductive parameters gave no indication of any reproductive impairment.

- At 120 ppm, there was some evidence of increased infertility in comparison with the control. No other parameters showed any apparent significant deviations from the control for the 120 ppm group.

- N.B. - Forty-two birds died on test but were replaced. They initiated the test with 160 birds, added 42 replacements to total 202 birds used. Forty-two dead/202 = 20 percent population mortality. Most of the deaths occurred before the laying period, during the first 9 weeks of the study. Most mortalities were preceded by bodyweight loss over a period of time. The authors stated that the reason for the mortality was unknown, but speculated that birds were stressed by the randomization process and by routine handling during bodyweight determinations. Thirty-five of the original 160 birds died during weeks 1 to 12 (pre-egg-laying period); 21.8 percent of the original birds.

  When birds died on test - initially if a bird died, the entire replicate was replaced by a spare replicate. When there were no further complete spare replicates, individual birds wre replaced by other spare individuals.

  Most of the 42 mortalities, on test, were found to have "atrophy of muscles" and no other symptoms other than one female (20 ppm) which had white fluid in oviduct and one male (120 ppm) which had been pecked. One female (30 ppm) without atrophied muscles had a pale swollen liver covered with a jelly-like material. The muscle atrophy was not considered treatment related.

  Upon termination of the test (after a 3-week withdrawal period) all remaining birds were sacrificed. A large number of birds had undeveloped or under-developed reproductive organs, particularly female birds at 120 ppm.

  At the start of egg-laying period (week 13) the groups consisted of different numbers of replicates, i.e., Group a - 17 reps.; Group b - 20 reps.; Group c - 17 reps.; Group d - 20 reps.

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Seven (7) birds died during the laying period - five females, two males (5 birds at 30 ppm, 1 each of 60 and 120 ppm; nondose related).

13. Study Authors' Conclusions/QA Measures

The study authors concluded: "Under the conditions of this test, and taking the results as a whole, it was concluded that the dietary level of 60 ppm of Endosulfan technical equivalent to an estimated intake of approximately 6 mg/kg/day represented the no-observed-effect level for reproductive impairment in the bobwhite quail."

The "Compliance with Good Laboratory Practice Standards" statement appears after authors signature pages, and is signed by Nicholas L. Roberts, one of the authors. The "Quality Assurance Audit Statement" and a report of dates of QA Unit audits appear after the Good Lab Practice Compliance Statement. Both are signed by Kenneth G. Shillam, Director of Q.A. for the test facility.

14. Reviewer's Discussion and Interpretation of the Study

a. Test Procedures: The reported operations during this study as well as the general protocol are in accordance with the Pesticide Assessment Guidelines recommendations for this study. However, the dose levels were based on a preliminary feeding study, not on expected environmental concentrations although the levels tested would be expected in a limited number of uses.

b. Statistical Analysis: The results of the statistical analysis were not validated because the study is invalid (see c and d below).

C. Discussion/Results: The study is not considered scientifically sound because there are an unacceptable number of unexplainable mortalities in all groups tested, including the controls (20%). The authors cite "stress due to handling" as a "possible" cause. Even if this is the case, the excessive mortality is not acceptable. This indicates either poor husbandry, unacceptable operational procedures, or poor quality control.

The authors did not present an explanation of what, if any, tests, procedures, precautions, etc., were taken to investigate the possibilities of microorganism contamination of the laboratory or toxic contamination of the food or water supply. (N.B.- We note that a mallard duck reproduction study, undertaken at or about the same time, had to be terminated for "physiological and

management problems." Circumstantially then, the "stress handling" theory proposed for the bobwhite study is dubious.)

Substitution of spare replicates, while allowing for statistical calculations, is not an adequate substitute for very poor test conditions as evidenced by the mortality observed in treatments and controls (the mortality was not treatment related).

This study may not be used in hazard assessment as the results are considered invalid. It is particularly important that a good study be submitted because adverse effects at all levels tested were observed in the mallard duck study performed at a later date (Roberts, et al., 1985). The requirement of the Pesticide Assessment Guidelines for a reproduction study of an upland game-bird is not fulfilled. The registrant is required to submit another study.

d. Adequacy of Study:

1. Classification: Invalid
2. Rationale: Excessive (20%) unexplainable mortality, not treatment related, in controls and all treatment levels.
3. Repair: None possible

15. Completion of One-liner

One-liner form completed September 6, 1985.

16. CBI Appendix

"Results" of study attached.

Endosulfan OECD 6

Page_____ is not included in this copy.

Pages 7 through 23 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.

Meeting August 16, 1985

Subject: Endosulfan - Field Dissipation Studies

Attending: Vic Dorr, Hoescht
Joseph O'Grodnick, Hoescht
Robert Holst, HED, EPA
John Bascietto, HED, EPA
John Jordan, HED, EPA
Richard Newkirk, RD, EPA

Mr. Dorr explained that his company held that the data submitted in response to EPA's request for field dissipation studies were both timely and met the requirements.

These were some studies reviewed in the standard

Mr. Dorr stated that his company held that the four field dissipation studies submitted to EPA were both appropriate and submitted within the 14 months specified by EPA.

Mr. Bascietto stated that the studies were not adequate to meet the needs of the Agency.

Mr. Dorr suggested that because use on watercress and most forest uses are no longer accepted, some of the data required on the standard may no longer be needed.

Dr. Holst discussed with blackboard diagrams possible protocol approaches that may be used in development of field dissipation studies.

Dr. O'Grodnick said he would like copies of data identifying fish kills resulting from the use of endosulfan.

Hoescht representatives indicated that they will prepare a protocol for use spring of 1986 using both 50WP and 3EC formulations and submit it to EPA for approval. Crops will be cotton and tomatoes.

Mr. Dorr indicated he would like to give a presentation to EPA explaining their specific protocol.

HED representatives would like to know the status of the use of endosulfan on watercress and forest uses. The PM Assistant (Dr. Pilitt) will provide HED with this information.