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SRRD/RD **ACTION** CODE/TYPE OF REVIEW 635

MRID #(S) PROTOCOL FOR AVIAN DISCOLORATION STUDIES

DP TYPE 001

PRODUCT MANAGER, NO. WALTER WALDROP 71 ANDREW ERTMAN

PRODUCT NAME(S) CHLOROTHALONIL

TYPE PRODUCT FUNGICIDE

COMPANY NAME ISK BIOTECH

SUBMISSION PURPOSE REVIEW PROTOCOL TO ADDRESS DISCOLORATION OBSERVED IN AVIAN REPRODUCTION STUDIES

COMMON CHEMICAL NAME

REVIEWER: [redacted]

STUDY PROTOCOL REVIEW

1. **Pesticide Name** : Chlorothalonil

2. **Study Type** : Avian Discoloration Study

3. **Pesticide Use** : Fungicide

4. **Study Purpose** : The object of the proposed study is indicated to be to determine the cause of yellow staining of the skin noted in avian reproduction studies with technical Chlorothalonil on mallard ducks and bobwhite quail. In both studies, it was reported that the yellow discoloration of the skin appeared to be a result of dermal contact with the test material and was not considered to have toxicological significance. However, no explanation of this conclusion was provided. Based on the results of these test, coupled with other avian reproduction studies conducted at lower levels, the NOEL was estimated to be 50 ppm, which is below estimated exposure levels. Therefore additional information on the cause or source of the yellowing that was observed is needed to show the cause and whether it is of biological significance.

5. **Study Methods** : Two treatment groups, each containing five male and five female northern bobwhite, will be fed diets containing the test substance at concentrations of 1000 and 5000 ppm. An additional group of five males and five females will be gavaged with the test substance at a dosage approximating the dietary consumption of the 5000 ppm treatment group. A control group containing five males and five females will be maintained concurrently.

The birds will be 16 to 36 weeks old and approaching their first breeding season. The birds will be fed the test diets or gavaged for a twenty week period. During the study, all birds will be monitored for toxicological responses. In addition, each of the treatment groups will be compared to the controls in order to detect significant differences in body weight and feed consumption, change in liver function, and evidence of skin discoloration.

6. **Protocol Evaluation** : The protocol provides limited discussion of the rational for the proposed study approach providing limited insight into whether or not the study will adequately address the stated objectives. We hope the following points will clarify EEB's concerns with the protocol and help define the detail required in protocols submitted to the Agency for review.

The objective of the study is stated to be:

... to evaluate the effects of dietary or oral exposure of a test substance upon adult northern bobwhite (Colinus virginianus) over a 20 week period. Effects on adult health, weight gain and feed consumption will be evaluated. In addition, the effects of adult exposure to the test substance on liver function and possible skin discoloration will be evaluated.

This objective, for the most part, has already been addressed in the previous avian reproduction study which lead to the request for this study. From what is stated it appears that there is some question whether or not skin discoloration occurred, that is , "possible skin discoloration". EEB was under the impression that skin discoloration was observed in previously submitted avian reproduction studies on both mallard ducks and bobwhite quail and the objective of this study was to determine the mechanism of the observed discoloration and whether it is of biological significance. According to the review of these studies, the yellow discoloration was reported to appeared to be stains from dermal exposure rather than the result of a tissue reaction or other physiological process. This, therefore, would seem to be the initial question to be addressed, which from the outline presented on materials and methods appears, at least to some extent, to be what is being addressed, however further clarification is needed.

In the absence of identifying whether the staining is due to dermal exposure or the result of a tissue reaction or other physiological process the monitoring of liver functions seems premature. However, if in fact, information is available which suggest the reported discoloration is due to effects on the liver, this should be presented and the rational why this is going to be monitored should be presented. The protocol is extremely scant on this point, indicating blood samples will be taken, processed as necessary and submitted to a clinical laboratory for liver function tests such as alanine aminotransferase, aspartate aminotransferase, or measurements of bile acid concentrations. It would seem some discussion is warranted on why these are going to be examined and what they would indicate if effected. At the very least a reference should be provided in relation to the named test.

Materials and Methods Section (page 6) indicates the test substance will be administered in the diet at concentrations of 1000 and 5000 ppm because these are the levels which effects were observed at in previous studies. However, the protocol continues and indicates this route of administration was selected because it represents the most likely rout of exposure to avian species in the environment and maximizes the likelihood of dermal exposure. Again, no rational is given, and its not readily apparent why this dietary exposure maximizes the likelihood of dermal

exposure. It would seem that direct application to skin would maximize dermal exposure. In fact it would seem a direct dermal exposure compared to the gavaged exposure would provide a more appropriate comparison to evaluate if the observed discoloration was due to stains from dermal exposure rather than the result of a tissue reaction or other physiological process. What ever method is used some justification would be appropriate.

Little attention is given to sample size except to indicate the test will consist of two dietary exposure groups, a gavaged group and a control group each with five males and five females. This appears to be a relative small sample to provide meaningful results for comparisons between mean body weights, average food consumption, and liver function values of birds in each of the groups as indicated. Attention needs to be given to estimating what sample sizes are needed to detect biological significant differences in these parameters. If however, the test is limited to evaluating if the staining was due to dermal exposure as suggested above smaller sample sizes may be adequate. What ever the objective, what constitutes an adequate sample size needs to be considered and justified in the protocol.

Under the section on necropsy, its indicated that all test birds that die during the course of the study and all adults remaining at the termination of the study will be subject to a gross necropsy. Samples of skin (cranial and breast), liver, and kidney will be collected from each bird at necropsy and submitted for histopathological evaluation. No justification is given for why these tissues will be sampled. Also it would seem appropriate to specify which histopathological evaluation will be used and how they relate to addressing the objective of the study.

7. **Suggested Modifications** : While its somewhat difficult to provide a great deal of guidance on the proposed study, given the scant details given in the protocol, the following suggestions may be useful in revising the study design to help refine the approach to be used:

Limit the initial study to evaluate if the observed skin discoloration observed in previous studies is due to dermal exposure or due to tissue reaction or other physiological processes.

Delete dietary exposure groups and add dermal exposure group for comparison to gavage group. May be appropriate to add additional exposure levels to better define the NOEL.

Study design should incorporate tissue analysis or other techniques to determine, if staining is due to dermal exposure, is chlorothalonil absorbed into the body or limited to the skin surface.

8. **Conclusions** : Due to the limited explanation and absence of details presented in the protocol, a evaluation of the proposed study design to address the state objective is difficult. As indicated above the statedobjective is not completely clear in light of the studies which detected the yellow staining. We'd suggest that the protocol be revised providing greater details and justification of the proposed approach taking into consideration the above comments and suggested modifications. If you have any
questions or needed further clarification in relation to the above suggestion or comments, please let us know.

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