(5-10-96)

MRID No.: 439426-04

**DATA EVALUATION RECORD**
**AVIAN EGG-SPRAY STUDY**

1. **CHEMICAL:** Paraquat dichloride (061601)

2. **TEST MATERIAL:** Gramoxone Super; 17.3% w/w

3. **CITATION:**

| | |
|---|---|
| Author: | Hakin, B. and D.O. Chanter |
| Title: | The effect of paraquat on the hatchability of fertile mallard duck eggs |
| Date: | 1988 |
| Laboratory: | Huntingdon Research Centre, Ltd., Cambridgeshire, England |
| Lab. Report ID: | ISN 170/881711 |
| Sponsor: | ICI Agrochemicals |
| MRID No.: | 439426-04 |

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: W. Erickson
Date: 4/29/96

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: Harry Craven
Date: 5/10/96

6. **CONCLUSIONS:** The study is scientifically sound. Mallard eggs in subgroups of 10 were sprayed on one of six spray occasions (day 0, 2, 4, 10, 14, or 20) during incubation. At a test concentration equivalent to 2 lb ai/acre, Gramoxone Super (17.3% paraquat dichloride) adversely affected embryonic deaths, the number of chicks hatched, chick survival, and chick body-weight gain on one or more of the six spray occasions. At 1 and 2 lb ai/acre, liver weight of chicks examined *post-mortem* was significantly less than that of the control for eggs treated on day 0 of the incubation period.

7. **STUDY CLASSIFICATION:** Supplemental (not a guideline requirement).

8. **MATERIALS AND METHODS:**

**Test Organism:**

| Criteria | Reported Information |
|---|---|
| **Species** | Mallard Duck<br>*Anas platyrhynchos* |
| **Egg supplier** | The County Game Farms, Hothfield, Ashford, Kent |
| **Chick food** | standard HRC chick meal |

**Test System:**

| Criteria | Reported Information |
|---|---|
| **Incubator:**<br>**type**<br>**temp. (°C)**<br>**rel. humidity (%)** | Bretagne incubator<br>37.6 ± 0.03<br>54 |
| **Chick housing** | floor pens (1.5 x 1.2 m) constructed from galvanized steel and wire mesh |
| **Hatcher** | PH 150 hatcher |
| **Spray equipment** | manually operated box sprayer with a 1-m boom pressurized by carbon dioxide through a single even flat spray jet (Tee jet SS8002E/2) |
| **Spray method** | replicates (10 eggs each) were placed on paper plates (to imitate a clutch arrangement) and sprayed 1 repl. at a time |

**Test Design:**

| Criteria | Reported Information |
|---|---|
| **Treatment doses** | 0, 0.5, 1, and 2 lb ai/acre |
| **No. subgroups (spray occasions) per dose** | 6 |



a: egg weights a: day -2

| Criteria | Reported Information |
|---|---|
| **Day of spray treatment for each subgroup** | a - day 0<br>b - day 2<br>c - day 4<br>d - day 10<br>e - day 14<br>f - day 20 |
| **No. replicates per subgroup** | 4 |
| **No. eggs per replicate** | 10 |
| **Control** | sprayed with tap water |
| **Measurement endpoints** | infertile eggs;<br>embryonic deaths;<br>no. dead in shell;<br>chick mortality;<br>chick bodyweight |
| **Observation period:** | incubation: 27-29 days<br>chicks: 28 days |

9. **REPORTED RESULTS:**

| Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | yes |
| **Egg observations:**<br>**a: egg weights**<br>**b: infertile eggs and early embryonic deaths**<br>**c: late embryonic deaths**<br>**d: dead in shell** | a: day -2<br>b: day 13 (candling)<br>c: day 19 (candling)<br>d: at days 31 and 32 (eggs that did not hatch) |
| **Chick observations:**<br>**a: no. hatched**<br>**b: clinical obs.**<br>**c: individual bodyweights**<br>**d: mortalities** | a: days 27-29<br>b: daily<br>c: on hatching and at 28 days<br>d: daily |

| Criteria | Reported Information |
|---|---|
| **Post-mortem exams** | all dead chicks plus 6 ♂ and 6 ♀ from each subgroup examined for:<br>- body length<br>- gross macroscopic abnormalities<br>- liver weight<br>- position, no., and abnormalities of gonads<br>- length of vestigial right Mullerian duct<br>- absence of oviduct in males |
| **Raw data included?** | yes |

**Eggs:**

| Appl. rate (lb ai/A) | Infertile eggs | | Early embryonic deaths (day 13) | | Late embryonic deaths (day 19) | | Dead in shell (day 31) | |
|---|---|---|---|---|---|---|---|---|
| | no. | %[1] | no. | %[2] | no. | %[2] | no. | %[2] |
| Control | 12 | 5 | 12 | 5.3 | 2 | 0.9 | 37 | 16.2 |
| 0.5 | 8 | 3.3 | 7 | 3.0 | 4 | 1.7 | 34 | 14.7 |
| 1 | 9 | 3.8 | 10 | 4.3 | 4 | 1.7 | 33 | 14.3 |
| 2 | 9 | 3.8 | 45 | 19.5* | 34 | 14.7* | 61 | 26.4* |

[1] % of eggs set
[2] % of fertile eggs
* significantly different from the control on one or more of the 6 spray occasions (ANOVA and Williams test)

to start in March 1993, and the final report is to be submitted in December 1995. The studies will be conducted at ICI Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK.

The registrant proposes to use paraquat $^{14}$C-labeled on the 2,2',6, and 6' carbons only (see Figure 1), with a specific activity of 1.76 GBq/mmol. This is not acceptable. The test substance must be uniformly radiolabeled on each carbon of the pyridinyl rings (carbons 2,2',3,3',4,4',5,5',6,6'). This provides the maximum opportunity to detect and isolate metabolic products, including any from degradation of the pyridinyl rings.

For the preplant soil treatment, carrot and lettuce seeds will be sown at a depth of 2.5 cm in 30 cm X 30 cm pots filled with sandy-loam soil. Immediately after sowing, the pots will be treated with $^{14}$C-pyridinyl labelled paraquat, formulated as Gramozone Extra with $^{14}$C-paraquat diluted with unlabeled paraquat to give a specific activity of about 2000 Bq/µg, at a rate of 12.5 lbs. a.i./acre. The registrant claims that this rate is 10X the label rate. Both the Gramoxone® Extra (10182-280) label and the Gramoxone® Super (10182-103) label specify a maximum preplant treatment rate for vegetables of 0.938 lb. a.i./acre. Thus, a 10X rate would be 9.4 lbs. a.i./acre. The 12.5 lbs. a.i./acre rate may have resulted from considering the active ingredient to be paraquat dichloride, rather than the paraquat ion. The $^{14}$C-paraquat will be applied with a tlc sprayer. Plastic sheeting will be used to prevent spray drift. The pots will be maintained in a greenhouse. It appears that one pot each of carrot and lettuce will be treated and one pot each of carrot and lettuce will serve as controls (4 pots total). This may not provide adequate treated sample for extraction and residue identification.

At maturity, carrot roots and lettuce heads will be harvested. Soil will be brushed away, and carrots will be water washed. Samples will be stored at -15°C ± 5°C. Residue levels will be determined in lettuce leaves and carrot roots by combustion/lsc. Where residues exceed 0.01 ppm, samples will be extracted, and the extracts will be characterized/identified (tlc, hplc). The reference compounds available include 1,1'-dimethyl-4,4'-bipyridilium ion (paraquat), 1-methyl-4,4'-bipyridilium ion, and 4-carboxy-1-methyl pyridilium ion.

For desiccant treatment, seed potatoes and soy bean seeds will be grown in 30 cm X 30 cm pots filled with sandy-loam soil and maintained in a greenhouse. The soy beans will be planted at a depth of about 2.5 cm. The seed potatoes will be planted at a depth of 40 cm. The plants will be grown to maturity. In July or August, one soybean plant pot and one potato plant pot will be treated with $^{14}$C-pyridinyl labelled paraquat, formulated as Gramozone Extra with $^{14}$C-paraquat diluted with unlabeled paraquat to give a specific activity of about 2000 Bq/µg, at a rate of 7.5 lbs. a.i./acre. This is a 10X rate only if the registrant is using

paraquat dichloride as the active ingredient. The maximum label use rate for desiccation of potatoes and soybeans is 0.562 lb. a.i./acre for potatoes on the Gramoxone® Extra (10182-280) label. Thus, a 10X rate would be 5.6 lbs. paraquat ion per acre or 7.76 lbs. paraquat dichloride per acre. The test substance will be applied to the foliage with a tlc sprayer, and plastic sheeting will be used to avoid spray drift. One potato plant pot and one soy bean plant pot will serve as controls. The treatment of only one pot each of potatoes and soybeans may not provide adequate sample for extraction and identification of residues.

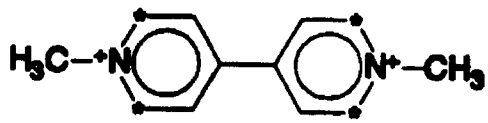
After spraying, the plants will be kept in direct sunlight during the day and returned to the greenhouse at night or during rain. The crops, potato tubers and soybeans, will be harvested 3 days after treatment. Tubers will be brushed and water washed to remove soil. The beans will be separated from the pods and will be blasted with air to remove any dry pod before analysis. Foliage will not be analyzed. This is not acceptable for soybeans, because soybean foliage (forage) may be an animal feed item. The 15 day grazing restriction on the paraquat labels does not negate the need for analysis of the soybean foliage.

The tubers will be chopped, and subsamples will be placed in plastic pots. The beans will be mixed, and subsamples will be placed in plastic pots. Samples will be stored at -15°C ± 5°C.

Soybean residues will be determined by combustion and lsc. The tubers will be extracted with acetonitrile and the radiolabeled residue determined separately for the extract and solid residue. Where residues exceed 0.01 ppm, samples will be extracted, and the extracts will be characterized/identified (tlc, hplc). The reference compounds available include 1,1'-dimethyl-4,4'-bipyridilium ion (paraquat), 1-methyl-4,4'-bipyridilium ion, and 4-carboxy-1-methyl pyridilium ion.

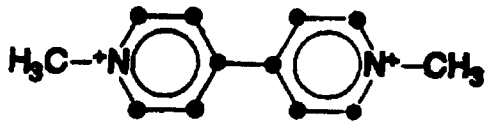
Storage stability determinations are not included for any of the four studies.

Figure 1: Structure of Paraquat and Registrant's Proposed Radiolabeling



* - position of $^{14}C$-radiolabel

Figure 2: $^{14}C$-Radiolabeling Required by CBRS



* - position of $^{14}C$-radiolabel

cc: Paraquat Dichloride Registration Standard File, Subject File, RF, circ., S. Funk.

RDI: A. Rathman:01/26/93:M. Metzger:01/28/92:E. Zeger:01/29/93:

H7509C:CBRS:S.Funk:305-5430:CM#2:RM803:SF(0193.9):01/25/93.