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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 041701. Dyfonate®. Review of Supplemental Data on Rabbit Developmental Toxicity Study

Tox. Chem. No. 454B
Project No. D177246
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Background and Request:

A developmental toxicity study on dyfonate in rabbits was submitted by ICI Agricultural Products as a generic data submission in support of reregistration (FIFRA '88). The Toxicology Branch (TB-I) reviewed the study and classified it as Core Supplementary Data pending submission of the maternal individual clinical signs data and the complete range-finding study. These additional data were requested in order to clarify the no-effect levels (NOEL's) and the lowest effect levels (LEL's) for both maternal toxicity and developmental toxicity. In the main study, there was an apparent increase in resorptions in the high dose group. This was mainly due to the fact that one doe resorbed her entire litter. The Agency wanted to see the range-finding study in order to verify that the increase in resorptions was not toxicologically significant for this particular chemical. In addition, the Agency wanted to examine the individual clinical signs data, particularly for the one doe which resorbed her litter. Furthermore, the clinical signs seen in the study were not the signs expected for a known cholinesterase inhibitor, and therefore may not be toxicologically significant. The individual clinical signs data were needed in order to clarify this issue.

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ICI Agricultural Products has submitted the requested data and the Toxicology Branch (TB-I) has been asked to review it in order to possibly upgrade the rabbit developmental toxicity study for regulatory purposes.

Toxicology Branch Response:

Conclusions: Dyfonate Technical was tested in a rabbit developmental toxicity study at 0, 0.2, 0.5 and 1.5 mg/kg/day. The study is upgraded to Core Minimum and satisfies the regulatory requirement for a rabbit developmental toxicity study for Dyfonate. The maternal toxicity NOEL is 1.5 mg/kg/day (HDT). TB-I concludes that the does were tested at a sufficiently high dose level because in a range-finding study at dose levels of 0, 0.5, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/kg/day, maternal toxicity was observed at 2.0 mg/kg/day and above. One of 5 does died at 2.0 mg/kg/day, 2/5 does died at 4.0 mg/kg/day and 5/5 does died at 6.0 mg/kg/day and above. In addition, the acute oral $LD_{50}$ for Dyfonate in female rats is 5.8 mg/kg. There were no obvious increases in any clinical signs of toxicity. Since the range-finding study showed no increases in any clinical signs, it is not likely that any of the clinical signs data in the main study are toxicologically significant.

Although there were no data on resorptions submitted with the range-finding study, after careful re-examination of the original study data, TB-I has determined that the NOEL for developmental effects is 1.5 mg/kg/day (HDT). This NOEL is borderline because there was a non-statistically significant increase in the number of resorptions/doe in the high-dose group. It was decided that this increase was not toxicologically significant because it was not statistically significant, it is within the historical control range and because the standard deviation for this measurement was so large.

Review:

TB-I had two major questions concerning the developmental toxicity study with Dyfonate in rabbits. These were:

1. Was the Dyfonate tested at a sufficiently high dose level in the study (i.e., was there any maternal toxicity)?

2. Was the increase in resorptions at the high dose a real or an artifactual response?

The Registrant provided three items: a supplemental discussion and conclusion to the original study which describes the results from all the dose levels tested in the range finding study, individual clinical signs data for the main study and the range-finding study used for selection of dose levels for the

main study. The following review discusses the range-finding study and the individual clinical signs data.

Range-Finding Study:

Protocol:

Dyfonate Technical was tested in a range-finding developmental toxicity study in rabbits at the following dose levels: 0, 0.5, 2, 4, 6, 8 and 10 mg/kg/day by gavage in a volume of 0.5 ml/kg. Dosing was administered from days 7 - 19 of gestation and was based on the most recent body weight measurement. The 0.5 mg/kg/day dose group was added because of excessive maternal toxicity at dose levels of 2 mg/kg/day and above. Five does were selected for each dose level. Mazola® was used as the vehicle control. Semen were collected using an artificial vagina from resident males of the same strain and source. The semen was evaluated for volume, motility and concentration and then diluted with 0.9% physiological saline. Insemination was conducted over a 1 - 3 day period and the day of insemination was designated day "0" of gestation. Each of the females received an intravenous injection of human chorionic gonadotropin (100 U.S.P. units) immediately following insemination.

The protocol stated that does were observed daily for clinical signs of toxicity, moribundity and mortality. Body weights were measured of gestation days 0, 7, 10, 13, 19, 24 and 29. Food consumption was measured daily from days 0 through 29 of gestation. Does were sacrificed on day 29 and the uterus and ovaries were examined. The number of corpora lutea, the location and number of viable and nonviable fetuses, early and late resorptions and the total number of implantation sites were recorded. Each fetus was weighed and the carcass of each pup was discarded. Maternal tissues were saved for histopathological examination. The carcass of each doe was discarded. Uteri which appear nongravid by macroscopic examination were opened and placed in a 10% ammonium sulfide solution for detection of early implantation loss. Females not surviving until the scheduled sacrifice were necropsied and the cause of death was recorded if possible. The number and location of implantation sites and corpora lutea were recorded. Females with evidence of abortion or premature delivery were sacrificed and necropsied. Recognizable fetuses were counted and discarded. Blood samples were taken from the does 24 hours after the last dose on gestation day 19 and at terminal sacrifice and RBC and serum cholinesterase were measured.

Results:

Only one table was provided with the range-finding study. This was entitled "Individual Clinical Observations". At 0.5 mg/kg/day, there was no mortality in the does and only 3 clinical signs were listed: decreased defecation, decreased urination and soft stool. At 2.0 mg/kg/day, 1 of the 5 animals was found dead on gestation day 11 (hyperactivity, red material on nose and brown urogenital matting observed). Six other clinical signs were observed in this dose group, 5 of which were observed in 1 animal per clinical sign. The animal that died displayed 1 of 3 incidences of brown urogenital matting. The mortality increased with increasing dose, while at the same time the clinical signs brown urogenital matting, decreased defecation and decreased urination appeared in fewer animals in the higher doses. At 4.0 mg/kg/day, salivation appeared, which is a clinical sign of toxicity that is often associated with cholinesterase inhibition. Two does died at this dose level, both on gestation day 8 (salivation observed in one and lethargy, labored respiration, salivation, brown urogenital matting observed in the other). At 6.0 mg/kg/day and above, all of the does were either found dead or were sacrificed in moribund condition. The does in the 6.0 mg/kg/day group were found dead on gestation days 8 (4 does) and 10 (1 doe). The only clinical sign observed was salivation (2 does). At 8.0 mg/kg/day, the does were found dead on gestation days 8 (2 does), 9 (2 does) and 10 (1 doe). Clinical signs included lethargy (4 does), salivation (1 doe), hyperactivity (1 doe) and other miscellaneous signs. At 10.0 mg/kg/day, the does were found dead on gestation days 8 (3 does) and 9 (1 doe) and one was sacrificed in moribund condition on gestation day 9. Clinical signs included salivation (1 doe), lethargy (1 doe) and other miscellaneous signs.

None of the other measurements described in the above protocol were provided in the report. The study Directors clarified the Discussion and Conclusion section and repeated a statement that there was mean body weight loss, decreased food consumption during the treatment period and a decrease in intrauterine survival at 2 mg/kg/day and above. Unfortunately, no data were provided to support this statement. The following table summarizes the results from the Individual Clinical Observations table provided in the range-finding study.

| Summary of Clinical Observations | | | | | | | |
|---|---|---|---|---|---|---|---|
| Observation | 0 | 0.5 | 2.0 | 4.0 | 6.0 | 8.0 | 10 |
| Brown urogenital matting | 1 | | 3 | 2 | | 2 | |
| Decreased defecation | | 1 | 4 | | | | 1 |
| Decreased urination | | 2 | 3 | | | | 1 |
| Dry brown urogenital staining | | | 1 | | | | |
| Soft stool | | 2 | 1 | 1 | | | |
| Found dead | | | 1 | 2 | 5 | 5 | 4 |
| Hyperactive | | | 1 | | | 1 | |
| Red material on nose | | | 1 | | | | |
| Hair loss | | | 1 | | | | |
| Salivation | | | | 2 | 2 | 1 | 1 |
| Lethargic | | | | 1 | | 4 | 1 |
| Labored respiration | | | | 1 | | | |
| Sacrificed moribund | | | | | | | 1 |
| Clear nasal discharge | | | | | | 1 | |

Discussion:

Maternal Toxicity:

Although not all the data are available, the results give a clear indication that there is maternal toxicity at dose levels of 2.0 mg/kg/day and above (1/5 deaths increasing to 5/5 deaths at 6.0 mg/kg/day and above with no obvious increase in any clinical signs of toxicity). The data submitted to the Agency suggested that there were few other signs of toxicity associated with these deaths except some clinical signs indicative of cholinergic effects, although the authors stated that there was a mean body weight loss, decreased food consumption during the treatment period and a decrease in intrauterine survival at 2.0 mg/kg/day and above. At 2.0 mg/kg/day, death occurred on the fifth day of dosing. At 4.0 mg/kg/day, two does died on the second day of dosing. For the higher dose levels, the does were dying from the second to the fourth day of dosing. The acute oral $LD_{50}$ for Dyfonate with female rats is 5.8 mg/kg. Therefore,

on the basis of the data submitted above, TB-I has determined that the dose levels selected for the main study (0, 0.2, 0.5 or 1.5 mg/kg/day) were appropriate, although overt maternal toxicity was not observed in the main study. The NOEL for maternal toxicity is 1.5 mg/kg/day (HDT).

Resorption Data:

The data for resorptions was not provided in the range-finding study. However, in the Discussion and Conclusions section of the main study, the authors stated that there was a decrease in intrauterine survival at 2.0 mg/kg/day. Unfortunately, TB-I cannot use this statement without the supporting data. After a careful re-examination of the data from the main study, TB-I has determined that although there was an increase in the number of resorptions/doe in the high-dose group, this dose level will be declared a borderline NOEL because the increase was not statistically significant, it is within the historical control range and because the standard deviation for this measurement was so large. In addition, if the resorption data from the one doe which resorbed her entire litter were eliminated, the number of resorptions/doe (postimplantation loss/doe) in the high dose group would decrease from 1.3 (1.29) to 0.9 (0.92). The following table summarizes the data in question.

| Summary of Resorption Data For Control and High Dose Does | | | | | |
|---|---|---|---|---|---|
| Control Group (0 mg/kg/day) | | | High Dose Group (1.5 mg/kg/day) | | |
| Doe No. | Early Resorptions | Late Resorptions | Doe No. | Early Resorptions | Late Resorptions |
| 1695 | Non Gravid | Non Gravid | 1694 | Aborted | Day 29 |
| 1696 | Non Gravid | Non Gravid | 1697 | 0 | 0 |
| 1701 | 0 | 0 | 1702 | 0 | 0 |
| 1724 | 0 | 0 | 1706 | 1 | 0 |
| 1733 | 0 | 1 | 1707 | Non Gravid | Non Gravid |
| 1738 | 1 | 0 | 1719 | 0 | 0 |
| 1739 | 0 | 0 | 1723 | Gravid | Died Day 21 |
| 1741 | 0 | 0 | 1730 | 0 | 0 |
| 1742 | 0 | 1 | 1734 | 0 | 0 |
| 1749 | Non Gravid | Non Gravid | 1737 | 3 | 0 |
| 1750 | 1 | 0 | 1744 | 1 | 2 |
| 1755 | 0 | 0 | 1753 | Non Gravid | Non Gravid |
| 1756 | 0 | 0 | 1759 | 1 | 0 |
| 1757 | 0 | 0 | 1765 | 1 | 0 |
| 1763 | 0 | 1 | 1766 | 1 | 0 |
| 1769 | 1 | 0 | 1767 | 2 | 0 |
| 1773 | 0 | 1 | 1770 | 0 | 0 |
| 1774 | 0 | 0 | 1775 | 6 | 0 |
| Total | 3 | 4 | Total | 16 | 2 |
| Mean | 0.2 | 0.3 | Mean | 1.1 | 0.1 |
| S.D. | 0.4 | 0.5 | S.D. | 1.7 | 0.5 |
| Total Resorptions/Doe | | 0.5 ± 0.5 | Total Resorptions/Doe | | 1.3 ± 1.7 |
| Postimplantation Loss/Doe | | 0.47 | Postimplantation Loss/Doe | | 1.29 |
| Historical Control: # Postimplantation Losses/Doe: 1.0 (0.3-1.9) | | | | | |

None statistically significant from control at 0.05 level.

Individual Animal Data for Clinical Signs of Toxicity in Main Study:

There were no obvious increases in any clinical signs of toxicity at any dose level. In addition, examination of the individual data for the one doe in the high dose group which resorbed her entire litter showed no obvious connection between maternal toxicity and the resorption.