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OFFICE OF  
PREVENTION, PESTICIDES, AND  
TOXIC SUBSTANCES

**SUBJECT:** Tebuconazole (Folicur Technical) - Review of 6(a)(2) Submission of Oral Developmental Toxicity Studies in Rabbits and Mice (§83-3)

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**Registrant:** Bayer Corporation, Kansas City, MO 64120-0013

**Action Requested:** Evaluate the submission of 6(a)(2) data to the Agency by the Registrant, Bayer Corporation. These data include the following two developmental toxicity studies which were conducted with tebuconazole (Folicur Technical):

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1. Becker, H. and K. Biedermann (1995) HWG 1608 technical (c.n. tebuconazole), embryotoxicity study (including teratogenicity) and supplemental investigations on the maternal toxicity in pregnant rabbits. Research and Consulting Company Ltd., Itingen, Switzerland. Study Nos. T 9040771 and T 9050301, May 31, 1995. MRID No. 43776201. Unpublished.
2. Becker, H. and K. Biedermann (1995) HWG 1608 technical (c.n. tebuconazole), embryotoxicity study (including teratogenicity) and supplemental embryotoxicity study (including teratogenicity) in the mouse. Research and Consulting Company Ltd., Itingen, Switzerland. Study Nos. T 0040772 and T 0050302, May 31, 1995. MRID No. 43776202. Unpublished.

The initial Agency screen of these data indicated that the 6(a)(2) status of the rabbit study was questionable, while the mouse study met 6(a)(2) criteria and a 90-day expedited review was justified. Confirmation of this analysis was requested.

**Summary of Study Findings:** The following executive summaries are based upon a cursory review of the study reports and do not represent an in-depth review of the data.

1. Developmental toxicity study in rabbits: Tebuconazole (HWG 1608 Technical, 96.3-96.8%) was administered by gavage at doses of 0, 10, 30, or 100 mg/kg/day to pregnant Chinchilla rabbits (16/group) on gestation days 6-18 (with the day of mating defined as gestation Day 0). The rabbits were observed for signs of toxicity; body weight and food consumption values were recorded. Cesarean section was performed on Day 28 of gestation; the does were necropsied and uterine contents were examined. Fetal specimens were evaluated for external, visceral, and skeletal abnormalities by standard methodologies. In a supplementary study conducted with an identical treatment regimen, blood was collected on gestation days 6, 12, and 19 from 5 rabbits/group for hematology and clinical chemistry evaluations. At necropsy of these does (gestation day 19), liver samples were collected for clinical biochemical investigations (cytochrome P-450, N- and O-demethylase, and triglycerides) and the adrenals, kidneys, liver, and spleen were weighed. All weighed tissues were examined histopathologically.

In the main study, treatment-related decreases in maternal body weight and food consumption were noted for gestation days 6-11 at 100 mg/kg/day. Histopathological examination of tissues from the supplementary study revealed single cell necrosis (minimal severity) in control and treated groups, with increased incidence in tebuconazole-treated groups. Necrosis of the liver was noted in one rabbit each at 30 and 100 mg/kg/day. These findings, although observed at low incidences in an inadequate sample size (5 rabbits/group), were conservatively interpreted as an indication of hepatic toxicity at doses of 10 mg/kg/day and higher.

**Maternal LOEL = 10 mg/kg/day, based upon single cell necrosis in the liver**  
**Maternal NOEL = < 10 mg/kg/day (not determined)**

At 100 mg/kg/day, postimplantation loss was increased and mean fetal weight was slightly reduced (6.8%). A slight increase in the incidence of fetuses with ossification sites that were incomplete or non-ossified was correlated to individual fetuses of low weight. At both 30 and 100 mg/kg/day, the percent fetuses with external and/or visceral abnormalities was slightly, but significantly, increased. The findings included runting, hemidiaphragm, limb abnormalities, and neural tube defects (e.g., meningocele and spina bifida); the study author attributed fetal anomalies observed at 100 mg/kg/day to treatment with tebuconazole, but considered findings at 30 mg/kg/day to be incidental.

**Developmental LOEL = 100 mg/kg/day, based upon increased resorptions, decreased fetal weight (supported by reductions in ossification), and increased percent fetuses with external abnormalities**

**Developmental NOEL = 30 mg/kg/day**

- Developmental toxicity study in mice: Tebuconazole (HWG 1608 Technical, 96.3-96.8%) was administered by gavage at doses of 0, 10, 30, or 100 mg/kg/day to pregnant NMRI mice (35/group) on gestation days 6-15 (with the day of mating defined as gestation Day 0). The mice were observed for signs of toxicity; body weight and food consumption values were recorded. Cesarean section was performed on Day 18 of gestation; the dams were necropsied; liver, spleen, kidney, and adrenal weights were recorded; and uterine contents were examined. Fetal specimens were evaluated for external, visceral, and skeletal abnormalities by standard methodologies. In a concurrently-conducted subgroup, with an identical treatment regimen, blood was collected prior to sacrifice on gestation day 16 from 10 mice/group for hematology and clinical chemistry evaluations and for liver biochemical investigations (cytochrome P-450, N- and O-demethylase, and triglycerides). The adrenals, kidneys, liver, and spleen were weighed, and all weighed tissues were examined histopathologically. A supplementary study was also conducted, in which 30 pregnant NMRI mice/group were administered tebuconazole by gavage at doses of 0, 1, or 3 mg/kg/day on gestation days 6-15 and sacrificed with cesarean section at GD 18; all maternal and fetal parameters examined at the higher dose levels were evaluated.

Marginal treatment-related decreases were noted in maternal body weight gain at 100 mg/kg/day on GD 6-16 and in maternal food consumption at 30 and 100 mg/kg/day on GD 6-11. At 100 mg/kg/day, increased absolute and relative reticulocyte counts and a shift in the reticulocyte fluorescence ratios were observed; increased AST and ALT were also noted at that dose. Absolute and relative spleen and liver weights were increased at 100 mg/kg/day, and relative liver weights were significantly increased at 30 mg/kg/day. Dose-dependant increases in cytochrome P-450 and N-demethylase activity were observed in liver homogenates at 10 mg/kg/day and above. At 30 and 100 mg/kg/day, dose-dependant increases in O-demethylase activity and liver triglyceride content, and marginal increases in ALP were observed. Histopathological examination revealed higher average degrees of vacuolization in maternal livers in dams treated at 10 mg/kg/day and higher, and at 30 and 100 mg/kg/day, a treatment-related increase in the

average degree of hepatic lipid storage was noted.

**Maternal LOEL = 10 mg/kg/day, based upon hepatic enzyme induction and vacuolization**

**Maternal NOEL = 3 mg/kg/day**

At 100 mg/kg/day, postimplantation loss was significantly increased, with a resulting decrease in the mean number of fetuses per dam; a marginal increase in postimplantation loss was also observed at 30 mg/kg/day. Mean fetal bodyweight was significantly decreased at 100 mg/kg/day. Increased incidences in abnormal external fetal findings and in retardation of skeletal development were noted at 100 mg/kg/day, and marginally at 30 mg/kg/day. The external fetal anomalies consisted primarily of runting, palatoschisis, exencephaly, and the presence of a "small wart-like growth" on a foredigit.

**Developmental LOEL = 30 mg/kg/day, based upon marginal increases in postimplantation loss, the incidence of abnormal external fetal findings, and retardation of skeletal development**

**Developmental NOEL = 10 mg/kg/day**

Other Pertinent Information:

1. In a previously conducted (1987) oral developmental toxicity study in Chinchilla rabbits (MRID No. 40700945; HED Doc. No. 007200), tebuconazole was administered by gavage at dose levels of 0, 10, 30, or 100 mg/kg/day. A maternal LOEL of 100 mg/kg/day was based upon reductions in body weight gain and food consumption during dosing. The developmental LOEL of 100 mg/kg/day was based upon significant increases in fetal resorptions and upon the presence of malformations (primarily of the limbs) which were not present in concurrent controls or occurred at low frequency in the historical controls. The maternal and developmental NOELs were determined to be 30 mg/kg/day.

The findings of the 1995 developmental toxicity study in rabbits do not conflict with those of the previous 1987 study. The developmental LOEL and NOEL are identical in both studies, based upon increased fetal death and malformations (presuming that the fetal findings at 30 mg/kg/day are confirmed as incidental following more extensive Agency review of the data). However, the new study identifies, through examination of additional parameters, the suggestion of maternal hepatic toxicity at oral dose levels as low as 10 mg/kg/day. The liver has been identified as a target organ in other species (rat and dog), but the 1995 oral developmental toxicity study presents the first evidence that hepatic toxicity also occurs in the rabbit.

2. In a previously conducted (1988) oral developmental toxicity study in NMRI/ORIG Kisslegg mice (MRID No. 40821501; HED Doc. No. 007200), tebuconazole was administered by gavage at levels of 0, 10, 20, 30, or 100 mg/kg/day. At 100

mg/kg/day, significant decreases in hematocrit and mean corpuscular volume, and significant increases in hepatic triglycerides, pale lobular liver, and severity of hepatic vacuoles and lipidosis were observed. Further examination of a subset of maternal animals revealed similar effects at dose levels as low as 10 mg/kg/day, suggesting the presence of hepatic toxicity at dose levels less than 100 mg/kg/day. Additionally, decreased hematocrit and mean corpuscular volume were observed at 20 mg/kg/day, thus defining the maternal LOEL. The developmental LOEL of 30 mg/kg/day was based upon a dose-dependant, statistically significant increase in the number of runts per litter. At the 100 mg/kg/day dose level, increased fetal and litter incidences of malformations, primarily in the skull, brain, and spinal column, were observed. The maternal NOEL was determined to be 10 mg/kg/day, while the developmental NOEL was 20 mg/kg/day.

The 1995 study submitted to the Agency presents a similar toxicological profile in mice; however, in the 1995 study, distinct maternal hepatic findings were noted at 10 mg/kg/day, whereas similar toxicity was merely suggested at 10 mg/kg/day in the 1988 study. Additionally, in the 1995 study, an incidental significant increase in the incidence of abnormal fetuses was observed at 10 mg/kg/day. The Registrant pointed out that in the previous (1988) study, the developmental LOEL, based upon similar fetal findings, was 30 mg/kg/day; however, since the 1995 study concluded that the incidence of abnormal fetuses at 10 mg/kg/day was within the range of spontaneous variation for this strain of mice, this finding is not considered to be an issue of immediate concern.

3. A Reproductive and Developmental Peer Review of tebuconazole was conducted on May 7, 1992. The committee concluded that developmental toxicity was induced in mice, rats, and rabbits via the oral route of administration, with the lowest NOEL observed in mice (10 mg/kg/day). Dermal exposure at 1000 mg/kg/day in the rat and mouse did not induce developmental effects, although additional pharmacokinetic data was suggested in order to verify the adequacy of the dermal study design. It was recommended that the NOEL for developmental toxicity in the mouse be used for the assessment of acute dietary risk, and that the developmental risk associated with occupational exposure should be assessed based upon the NOEL of 1000 mg/kg/day in the dermal developmental toxicity study in the mouse.
4. At an RfD Peer Review Committee meeting on tebuconazole (March 5, 1991), the data package was reviewed in its entirety and a reference dose determined (0.01 mg/kg/day, based on the NOEL of 1 mg/kg/day in the chronic toxicity study in dogs, with an uncertainty factor of 100). The critical effects were lenticular and corneal opacity and hepatic toxicity.

The NOELs established by the 1995 developmental toxicity studies in rabbits and mice will not alter the previous RfD determination.

5. A Less-Than-Lifetime Risk Assessment peer review has not yet been conducted on tebuconazole.

6. Neither the developmental toxicity study in rabbits nor in mice meet the criterium specified in 40 CFR 158.34 for flagging a study for potential adverse effects under FIFRA 6(a)(2) ["When compared with concurrent controls, treated animals show a dose-related increased in malformations (or deaths) on a litter basis in the absence of significant maternal toxicity at the same dose levels."] The flagging statements contained within each study report correctly state that the applicable criteria are neither met nor exceeded.

Recommendations:

1. For the reasons detailed above, the two studies submitted by the Registrant as 6(a)(2) data (MRID Nos. 43776201 and 43776202) do not meet 6(a)(2) criteria; therefore, it is recommended that they not receive expedited (90-day) review.
2. It is recommended that a Less-Than-Lifetime Risk Assessment review of tebuconazole be conducted as soon as feasible; it is not considered critical to hold the LTL Assessment pending completion of the DERs for the 1995 studies. Nevertheless, although the 1995 rabbit and mouse developmental toxicity studies do not identify an acute or short-term developmental endpoint of concern at lower doses than in developmental toxicity studies conducted in 1987-88 (and previously reviewed by the Agency), maternal endpoints may need to be reconsidered following DER completion.