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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Henry Jacoby, PM#21
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THRU: William L. Burnam, Chief
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SUBJECT: Review of Folpet Pilot and Primary Teratology Studies in Rats. Accession #251659; Tox. Chem.#464
Registration #239-1763

Recommendations:

1. It is recommended that the pilot (rangefinding) teratology study be classified as Supplementary Data. The results of the study suggest that the NOEL for maternal toxicity is 20 mg/kg and that the NOEL for fetotoxicity is 80 mg/kg. Parameters to assess potential teratogenic activity were not monitored in this study.

2. It is recommended that the primary teratology study be classified as Core Guidelines. The results of the study suggest that the NOEL for maternal toxicity is 10 mg/kg and that for fetotoxicity is 60 mg/kg. No embryotoxic or teratogenic effects were observed at doses up to 360 mg/kg (highest dose tested).

Review of Data:

1. Pilot (Rangefinding) Teratology Study in Rats. Conducted by Argus Research Labs, Perkasie, PA. and sponsored by Chevron Chemical Co., Richmond, CA. Final report date 3/7/83. (Argus Project No. 303-011P)

Methods:

Crl:CDBS-CD-(SD)-BR rats, 72 days old, were acclimated for 14 days prior to insemination. Virgin female rats were mated with males from the same source and strain. Females were

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randomly assigned to 5 groups with 8 animals in each group. The groups received gavage doses of 0 (vehicle), 20, 80, 320 and 640 mg/kg/day of Folpet technical (89.5% a.i.; batch no. SX-1388) once daily on days 6-19 of gestation (day 0 was the day of insemination). The vehicle solution consisted of Tween 80 and carboxymethylcellulose suspended in distilled water. Formulations of aqueous suspensions of Folpet technical were analyzed and the purity level confirmed by gas chromatography.

The females were observed for viability twice daily on days 0-28 of gestation, and for physical signs twice daily on days 6-19 of gestation. Body weights were recorded on days 0, 3, and 6-20 of gestation. Food consumption was measured for days 0-6, 6-13, and 13-20 of gestation.

All females were killed with CO_2 on day 20 of gestation. The uterus and ovaries were surgically exposed and the following information recorded: number and location of live and dead fetuses, early and later resorptions, implantations; number of corpora lutea; uterine weight. Fetuses were weighed and examined externally for gross anomalies. All fetuses were discarded. No statistical evaluations were performed in this study because of the limited number of animals tested.

Results:

Findings that occurred in a dose-related manner or only at the highest dose level are summarized in Table 1.

Deaths occurred in 2 females: 1/8 at 20 mg/kg on gestation day 15 possibly due to hydronephrosis (both kidneys were dilated and the urinary bladder distended with white fluid at necropsy), and 1/8 at 640 mg/kg on gestation day 12 due to an intubation error. Both females were pregnant and exhibited implantations in utero (6 at low dose and 13 at high dose) which were normally developed.

Clinical signs observed in females that appeared to be chemical-related included: (a) rales at doses of 80 mg/kg or more; (b) salivation at doses of 320 mg/kg or more; and (c) distension of the GI tract with gas, thin appearance, and soft or liquid feces at the highest dose of 640 mg/kg.

Maternal body weight gain was reduced at 80 mg/kg or more in a dose-related manner on gestation days 6-19 A similar trend in weight gain was noted for dams without intact gravid uteri on gestation days 0-20.

Maternal food consumption was reduced at 320 and 640 mg/kg in a dose-related manner on gestation days 6-13 and 13-20.

All 8 animals were pregnant at each dose level. Litter sizes in all treatment groups were similar to the control group; all fetuses were alive. Average live fetal body weights/litter were lower in the 320 and 640 mg/kg dose groups at day 20 compared to the controls (Table 1).

In this study there were no differences between control and treatment groups with respect to number of corpora lutea, implantations, resorptions (all were early, except for one late each in 20 and 640 mg/kg groups), % dead or resorbed implantations/litter, or % live fetuses/litter. No gross fetal anomalies were observed. Histological examinations of fetuses were not performed.

Table 1. Summary of Compound-Induced Changes in Pilot Teratology Study in Rats

	Dose of Folpet (mg/kg)					
	0	20	80	320	640	
Maternal deaths	0/8	1/8	0/8	0/8	1/8	(dosing error)
Clinical Signs:						
Rales	0/8	0/8	2/8	6/8	7/8	
Salivation	0/8	0/8	0/8	5/8	5/8	
GI tract distension	0/8	0/8	0/8	0/8	4/8	
Thin appearance	0/8	0/8	0/8	0/8	4/8	
Soft/liquid feces	0/8	0/8	0/8	0/8	6/8	
Maternal weight gain (gm)						
Days 6-19 (uteri intact)	+91	+90	+82	+67	+41	
Days 0-20 (minus uteri)	+65	+69	+56	+35	+21	
Maternal feed consumption (gm)						
Days 6-13	+139	+153	+141	+121	+97	
Days 13-20	+161	+162	+159	+132	+110	
Live fetal body weights per litter (gm)	3.5	3.4	3.4	3.1	2.8	

Conclusions: Folpet produced both maternal toxicity and fetal toxicity. Maternal toxicity was characterized by several clinical toxic effects (see table 1) and reduced maternal weight gain and feed consumption: the NOEL for maternal toxicity was 20 mg/kg. Fetal toxicity was manifested as a reduction in live fetal body weights per litter: the NOEL was 80 mg/kg.

Core Classification: The study should be classified as Supplementary Data. Because it was intended only as a pilot study, few animals were used at each dose level and complete fetal examinations were not performed.

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2. Teratology Study in Rats. Conducted by Argus Research Labs, Perkasie, PA. and sponsored by Chevron Chemical Co., Richmond, CA. Final report date 8/23/83. (Agrus Project No. 303-001).

Methods:

Crl:COBS-CD-(SD)-BR rats, 72 days old, were acclimated for 4 weeks prior to insemination. Virgin female rats were mated with males from the same source and strain. Females were randomly assigned to 4 groups with 25 animals in each group. The groups received gavage doses of 0 (vehicle), 10, 60 and 360 mg/kg/day of Folpet technical (89.5% a.i., batch no. SX-1388) once daily on days 6-19 of gestation (day 0 was the day of insemination). The vehicle solution consisted of Tween 80 and carboxymethylcellulose suspended in deionized water. Formulations of aqueous suspensions of Folpet technical were analyzed and the purity level confirmed by gas chromatography.

The females were observed for viability and clinical signs of toxicity twice daily on days 6-19 of gestation. Body weights were recorded on days 0, 3, and 6-20 of gestation. Food consumption was measured for days 0-6, 6-13, and 13-20 of gestation.

All females were killed with CO_2 on day 20 of gestation. The uterus and ovaries were surgically exposed and the following information recorded: number and location of live and dead fetuses, early and later resorptions, implantations; number of corpora lutea; uterine weight. Fetuses were individually weighed and examined for gross external anomalies. All live fetuses were killed. In each litter, approximately 1/2 of the fetuses were fixed in Bouin's solution and examined for soft tissue anomalies, and 1/2 of the fetuses were cleared, stained using Alizarin red-S, and subsequently examined for skeletal defects. In this study, data were evaluated using various statistical tests; the criterion for significance was $P < 0.05$.

Results:

Findings that occurred in a statistically significant manner are summarized in Table 2.

Deaths occurred in 3 high dose (360 mg/kg) females on gestation days 12, 17 and 20. Two of these females died due to intubation errors; both were pregnant and exhibited implantation in utero (7 in one dam and 13 in the other dam) which were normally developed. The third female died from unknown causes, and was not pregnant.

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Clinical signs in females that appeared to be chemical-related included several effects at the highest dose of 360 mg/kg; i.e., rales, salivation, chromorrhinorhea, decreased motor activity, and soft or liquid feces. No compound-related postmortem maternal gross lesions were observed.

Maternal body weight gain was reduced over gestation days 0-20 at 60 mg/kg in dams without intact gravid uteri, and at 360 mg/kg both in dams with and without intact gravid uteri). Maternal feed consumption was also reduced over days 6-13 and 13-20 at 360 mg/kg. The decreases in food consumption were about 15% at each time interval.

The following caesarean delivery data and litter data parameters were not significantly altered by chemical administration: % pregnant animals, no. of corpora lutea, no. of implantations, no. of resorptions (early or late), mean litter size or no. of live fetuses (no fetuses were dead), live fetal body weights/litter (males and females combined or each sex separately), % dead or resorbed implantations/litter, and fetal sex ratios.

Examinations for gross-external anomalies: No significant effects for the % of fetuses with any variation observed, % fetuses with any variation observed/litter, or % of litters with fetuses with any variation observed. A finding seen only at the 60 mg/kg dose level was [illegible] (1/204 fetuses; 0.4%), which presented as a shortened, fused mandible at skeletal examination. The appearance of a thread-like tail occurred in 1/239 (0.4%: litter incidence 4.8%) fetuses at 360/kg, but no caudal vertebrae were ossified upon skeletal examination. These incidences were not statistically significant and not considered compound related.

Examinations for soft tissue anomalies: A finding seen only at the 60 mg/kg dose levels was malpositioned cardiac vessels plus ectopic accessory liver lobes surrounding the stomach in the same fetus (1/115; 0.9%: litter incidence 4.8%). A finding seen only at the 360 mg/kg dose level was slight dilation of the brain lateral ventricles (1/115; 0.9%: litter incidence 5.3%). These incidences were not statistically significant and not considered compound related.

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Examinations for skeletal malformations and average numbers of ossification sites: Observations at the high dose included one shortened rib, maligned thoracic vertebrae, and nonossified caudal vertebrae (1/124 or 0.8% fetuses each; litter incidence 5.3%). Incomplete ossification of one or both pubes and/or eschia were observed in 3 fetuses of one control litter (3/39 or 2.2%; litter incidence 4.8%) and in 1 fetus in each of 3 high dose litters (3/124 or 2.4%; litter incidence 15.8%). These incidences were not statistically significant but could be considered suggestive of a mild fetotoxic effect. No other compound related changes in fetal ossification site averages were apparent.

Table 2. Summary of Compound-Induced Changes in Primary Teratology Study in Rats

	Dose of Folpet (mg/kg)				
	0	10	60	360	
Maternal deaths	0/25	0/25	0/25	3/25*	(2 were dosing errors)
Clinical Signs:					
Rales	0/25	0/25	3/25	9/25*	
Salivation	0/25	0/25	0/25	6/25*	
Diarrhea/blk. stool	0/25	0/25	0/25	3/25*	
Motor Activity	0/25	0/25	0/25	3/25*	
Soft/liquid feces	0/25	0/25	0/25	3/25*	
Maternal weight gain (gm)					
Days 0-20 (uteri intact)	+132	+121	+122	+111**	
Days 0-20 (minus uteri)	+65	+53	+55*	+47**	
Maternal feed consumption (gm)					
Days 7-13	153	154	151	136**	
Days 13-20	116	102	102	101**	

*p < 0.05 compared to vehicle control
**p < 0.01 compared to vehicle control

Conclusions: Folpet produced maternal toxicity characterized by several clinical signs of toxicity (see table 2), reduced maternal weight gain, and reduced maternal feed consumption. The NOEL for maternal toxicity was 10 mg/kg and for fetotoxicity 60 mg/kg. No teratogenic effects were observed at doses up to 360 mg/kg.

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Core Classification: It is recommended that this study be classified as Core Guideline. The study was adequately conducted and reported, and in several instances the procedures in the study actually exceeded those prescribed in our Guidelines (e.g., they used more animals/dose group than were required, administered the chemical on gestation days 6-19 rather than days 6-15, adjusted dosages on a daily basis rather than on the basis of day 6 weight alone, obtained maternal weights at more frequent intervals than we require, etc.).

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