Caswell file

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: CGA-64250 Fungicide; PP#1G2530 (Pecans); 100-EUP-70; Accession #072661 (Submitted 5/23/84); Review of Additional Information on Rat and Rabbit Teratology Studies.

Caswell # 323EE

TO: Henry Jacoby
Product Manager (21)
Registration Division (TS-767C)

THRU: Robert Zendzian 2/28/85
Acting Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769C)

FROM: Alan Katz 2/26/85
Toxicologist, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Additional information on the rat and rabbit teratology studies with CGA-64250 Technical, including the DER submitted by Dynamac Corporation (EPA #68-01-6561; Task 58; 8/29/84) pertaining to the specific issues discussed below, has been evaluated by W. Dykstra (Toxicology Branch) and this reviewer.

Four issues were raised by the Toxicology Branch. These issues, and the Toxicology Branch assessment of each, are as follows:

A. Teratology Study in Rabbits (Ciba-Geigy Ltd., Project No. 790009; 9/10/79; Accession No.244272)

1) "In order to evaluate whether a high enough dose was utilized, more information is needed addressing the lack of a decrease in maternal weight gain in the high dose group in face of a 35% reduction in food consumption."

Assessment:
Maternal toxicity or significantly reduced palatability was evident in the high dose group, based on: (1) reduced body weight; (2) reduced body weight gain; and (3) reduced food consumption. It is noted that, although group mean body weights were comparable at termination, the high dose group did exhibit a statistically significant ($p<0.05$) reduction in body weight gain during the dosing period.

2) "The results for frequency of delayed ossification in pups are poorly reported. Additional information must be submitted delineating this effect by litter."

Assessment:
The required statistical analyses were performed (chi-square, Fisher Exact). Delayed ossification was not statistically significantly increased in the litters of CGA-64250-treated rabbits.

B. Teratology Study in Rats (Ciba-Geigy Ltd., Report No. 790011; 9/10/79; Accession No. 244272)

1) "The reporting of maternal weights and fetal ossification data were not presented individually or separated as to litter. The lack of this information precludes the performance of an adequate statistical analysis."

Assessment:

Maternal body weight data submitted were sufficient to resolve this question. Mean maternal weights in the high dose group were statistically significantly less than control values during the dosing period.

2) "Additional information by litter reporting the skeletal disorders among pups is needed. There is some suggestion of possible effects in the mid-dose group."

Assessment:

Additional information on skeletal disorders, by litter, was submitted. As stated by Ciba-Geigy in the Summary (EPA Accession #072661, p.4), "Differences were noted for frequency of no ossification of the hindlimb phalangeal nuclei presented by litter for the low and intermediate dose as well as the high-dose but the response was not observed in the lower doses when evaluated by frequency noted in the fetuses examined."
The data for frequency of no ossification of hindlimb phalangeal nuclei were as follows:

No Ossification: Hindlimb Proximal Phalanges, Digit 5

Frequency by:	Dose Level (mg/kg) 0	30	100	300
Number of fetuses	46/190 (24.2%)	52/200 (26.0%)	67/206 (32.5%)	109/158** (69.0%)
Litters affected	11/24 (45.8%)	18/24* (75.0%)	21/24** (87.5%)	19/19** (100%)

* Different from control ($p \leq 0.05$), Contingency Chi-Square Analysis.
** Different from control ($p \leq 0.01$), Contingency Chi-Square Analysis.

Analysis by litter suggests a dose-related trend. This reviewer concludes that, based on the data presented above, the lack of ossification of hindlimb phalangeal nuclei was toxicologically significant at the mid and high doses, but not at the low dose. Because of the relatively high natural frequency of incomplete ossification, the fiduciary limit where $p \leq 0.01$ appears to be the most appropriate statistical measurement of significance for this effect.

Additional Concerns:

In its review, Dynamac Corporation expressed additional concerns regarding the rabbit teratology study. These concerns are shared by this reviewer, and re-stated as follows:

1) Four animals were removed from the study due to "spontaneous litters." This reviewer finds this euphemistic expression to be a misnomer for a potentially serious breach of good animal husbandry practices. These "spontaneous litters" are described in the final report as being "due to erroneous mating of dams before the initiation of the experiment (i.e., before being supplied or during the period of acclimation)." In fact, based on examination of the day of delivery, it appears likely that these animals were impregnated during the acclimation period. It is clear that animals which are individually housed should not become pregnant. The applicant should specify, if possible, whether impregnation during the acclimation period period was attributable to (1) failure to observe or record copulation during the planned mating period, or (2) uncontrolled circumstances (e.g., escaped animals or inadvertent multiple housing).

2) A cleft palate was found in 1 of 123 fetuses in the high dose (180 mg/kg) group. This lesion was not found in any of the other groups. According to the study report (p.4), "this malformation is considered of a spontaneous origin. Missing palatal closure was recorded to occur spontaneously in rabbits of control populations at a rather high incidence." A contradiction to this rationale is noted in the EPA Toxicology Branch review (W. Dykstra, 4/30/81, p. 12), which stated "although this malformation may not be considered compound-related; the historical control data has no fetuses with cleft palate of 928 examined."

3) The number of pregnant rabbits in each group at day 28 of gestation was low. These data are presented below:

	Dose Level (mg/kg)			
	0	30	90	180
# of Pregnant Females (Day 28)	10	6	8	11

Conclusions:

CGA-64250 was not teratogenic in rats or rabbits as doses up to 300 and 180 mg/kg, respectively. Based on the additional information on skeletal disorders, including analyses by litter, the LEL for fetotoxicity for the rat teratology study is revised downward to 100 mg/kg. The NOEL with respect to fetotoxicity for this study is likewise changed from 100 to 30 mg/kg.

The rat and rabbit teratology studies are considered CORE-Minimum. Due to the issues expressed above (i.e., "Additional Concerns"), the rabbit study is only marginally acceptable as CORE-Minimum data.