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DATE: 1/24/78

SUBJECT: EPA Reg. No. 192-RET, Benomyl

FROM: R.B.Jaeger
TB

TO: John Lee
PM 22

The following data, summarized below, has been previously reviewed and supports the registration of EPA Reg. No. 192-RET:

AO LD50 (rabbit, 50%WP) > 10,000 mg/kg

AD LD50 (rat, 50% WP) > 10,000 mg/kg

AI LC50 (rat, 50%WP) > 2.0 mg/l

Subacute Inhalation (rat, 21 day, 53.5%WP) NEL 200 mg/kg/day

Eye Irritation (rabbit, 53%WP) mild

Dermal Irritation (guinea pig, 50%WP) mild

Dermal Sensitization (guinea pig, 50%) mild

Teratology (rat, 53.5%WP) Negative at 5000 ppm

(Reference: reviews of EPA Reg. No. 352-EXP-73G, 1/17/73 and 2/23/73 by R.D.Coberly; and memos of DR. M.L.Quaife dated 3/25/70 [PF0F0906, OG0936], 5/3/71 [0F0906, 0F1000, 1F1010, 1F1033, 1F1045], and 1/3/72 [1F1145, 2F1192, 2G1197].

Precautionary labeling is adequate as is.

Robert B. Jaeger, Physiologist
Toxicology Branch

RBJ for WHB 1/25/78

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REPORT ON THE STATUS OF TERATOGENICITY STUDIES WITH BENOMYL
April 23, 1979

The teratogenic potential of benomyl has been previously assessed by both DuPont (Haskell Laboratory Report No. 286-70 [1970] and Hazelton Laboratories Report No. MRO-1079 [1968])and by an independent laboratory (Shtenberg and Torchinsky, Vestnik Akademii Nauk SSR 3(27):39-46 [1972]). The DuPont studies involved dietary administration during the period of organogenesis at levels up to 5000 ppm in Charles River CD rats (approximately 370 mg/kg/day) and 500 ppm in New Zealand rabbits (approximately 125 mg/kg/day) and concluded that there were no adverse effects at any dose level on the development of these species. /The Shtenberg study involved oral intubation of benomyl as a suspension in vegetable oil to Wistar rats at doses up to 500 mg/kg/day on days 7-15 of gestation. A summary of the data is provided in Table 1. Benomyl was embryocidal and teratogenic at doses of 125 mg/kg/day and greater. Brain hernias, hydrocephaly and microphthalmia were listed as the predominant types of malformations. This study, however, has received criticism from several sources due to the small number of litters involved, the lack of detail in reporting the incidence of terata, and, most importantly, because of the apparent lack of concurrent controls. After reviewing the available teratogenicity studies, we decided that the Shtenberg study required replication under more controlled circumstances. A study following the Shtenberg protocol as closely as possible was therefore initiated at Midwest Research Institute (MRI). Upon notification by MRI that their preliminary data was confirming the results of the Shtenberg study, we examined the effects of benomyl in a teratogenic screening system being developed at HERL/RTP.

MRI RAT TERATOLOGY STUDY

oral intub

This study was designed to use the route of administration, dosing vehicle and strain of rat used in the Shtenberg study. Changes to the Shtenberg protocol included the extension of the dosing period to days 6-15 of gestation and the utilization of concurrent controls and of appropriate sample sizes. These modifications were made in order that the study conform to EPA's Pesticide Testing Guidelines. Benomyl was supplied by DuPont and was identified as technical grade with a purity of >98%. No lot number was supplied with the sample. Wistar rats were obtained from Charles River Breeding Laboratory. Females were between 60 and 90 days of age when mated to proven male breeders. The day that a sperm positive vaginal smear was obtained was identified as day 0 of gestation. The inseminated females were divided into five groups and treated with 0, 62.5, 125, 250 or 500 mg/kg/day from days 6-15 of gestation. The benomyl was administered as a suspension in corn oil at a rate of 5 ml/kg. Maternal weight on day 6 was used for calculation of doses. Dosing solutions were prepared fresh daily. As of this date, the sacrifice of the dams on day 20 of gestation has been completed. No necropsy or skeletal analysis of the fetuses has been initiated as yet. Preliminary data are presented in Table 2. No females in any dose died or showed clinical signs of toxicity. Doses of 125 mg/kg/day and greater resulted in a dose-related increase in embryonic death. Doses of

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62.5 mg/kg/day (the lowest dose tested) and greater resulted in a dose-related decrease in fetal weight and a dose-related increase in fetal malformations. The anomalies observed to date consist primarily of herniations of the central nervous system and reduction defects of the extremities.

HERL/RTP MOUSE TERATOLOGY SCREEN

The protocol used in this study is the result of an attempt by our laboratory to develop a rapid, inexpensive screening technique for potential teratogens. Nominally it involves treating pregnant CD-1 mice on days 8-12 of gestation (day 1 defined as the day on which a sperm plug was identified) with a maximally tolerated dose of the substance. The dams are then allowed to deliver and the growth and survival of the pups are monitored over a 72-hour period. Embryonic and fetal death along with gross terata are detected by a decreased litter size on day 1 postpartum (grossly malformed pups are known to be cannabalized by the dam). In some instances, malformed pups can be removed from the dam prior to cannabilization and examined for anomalies. More subtle forms of developmental toxicity are detected by decreased birth weights and by a failure to survive or grow at normal rates in the first 72 hours postpartum. Preliminary results from this system indicate that it is sensitive in detecting as positive known teratogens and insensitive to detecting as positive known non-teratogens.

In the benomyl study, sufficient time was not available to establish a maximally tolerated dose, and therefore two doses similar to those producing effects in the MRI rat study were used (200 and 400 mg/kg/day). The sample of benomyl was identical to that used by MRI and was administered by oral intubation as a suspension in corn oil. Dosing volume was 0.5 ml/day. Maternal weight on day 7 of gestation was used as the basis for preparing dosing solutions. The data are presented in Table 3. Doses of 400 mg/kg/day resulted in the complete resorption of 63% of the inseminated females and only 2 of 24 females delivered pups at this dose level. At 200 mg/kg/day, 8% of the inseminated females completely resorbed their litters. Of the remaining dams in this group, 15 delivered live litters. Litter size at birth was only 59% of the control value and the weights of surviving pups were diminished by 11%. By day 3 postpartum, the average litter size was only 48% of the control value, while pup weights were now similar to that of controls. In malformed pups obtained from three litters in 200 mg/kg/day group, herniations of the central nervous system and reduction anomalies of the extremities were apparent.

RESEARCH PROSPECTUS

The three teratology studies utilizing oral intubation as the route of administration are unanimous in identifying benomyl as a potent teratogen. However, these studies did not establish a no effect level for teratogenic action nor did they attempt to establish why the results of oral intubation studies and those of the dietary administration studies should be at variance. In fact, data from the CD-1 mouse study tends to diminish the most plausible reason for those differences (an unusual sensitivity of the Wistar rat to benomyl-induced teratogenesis).

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From a regulatory standpoint, it is essential that a no effect level be established for benomyl-induced teratogenesis. This minimal effort would require approximately 3 months and could be accomplished at either HERL/RTP, MRI or the Haskell Laboratory. This minimum effort, however, would not examine the reasons for the differences in response to benomyl between the oral and dietary routes of administration, a point that could be important to the risk assessment portion of the RPAR. Furthermore, because the no effect level for the inhibition of testicular function may be lower than that for teratogenic effects, and because inhibition of testicular function during critical periods of sexual maturation may produce permanent effects on behavior and reproductive physiology, we are prepared to initiate studies at HERL/RTP to address the deficiencies in the benomyl data base. This research would include teratology studies in the rat by the dietary and oral routes, combined with plasma concentrations of benomyl and its metabolites at several times during the period of exposure; studies of the age-related effects of benomyl on reproductive functions in the rat; and studies on the effects of benomyl on sexual development in the hamster (model systems for these latter two studies are available at RTP). These studies would require approximately 6 months to complete following their initiation. It is estimated that this research could be underway within 45 days. Final decision of the course of future action must await the impact of the studies reported here on the regulatory options for benomyl.

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Table 1. Summary of data from the Shtenberg study

Dose (mg/kg/day)	No. litters treated	No. implants resorbed	% Females with abnormal fetuses	% Live fetuses with abnormalities
500	11	86	100.	100
250	9	42	100	80
125	10	14	55	31
62.5	8	4	0	0
0	21	4	0	0

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Table 2. Preliminary results of the MRI Rat Teratology Study

Observations	Dose (mg/kg/day) 0	62.5	125	250	500
Maternal					
No. inseminated	24	23	24	26	26
% completely resorbed	0	0	30	30	58
% pregnant (term)	88	87	67	54	38
% females with abnormal fetuses	0	20	38	57	90
Weight change during pregnancy (g)	123	110	104	69	69
Fetal					
No. implantations	13.0 ± 0.9[1]	12.8 ± 0.6	13.3 ± 0.4	12.2 ± 0.7	12.6 ± 0.7
No. live fetuses	12.2 ± 0.8	11.0 ± 0.8	8.0 ± 1.2[2]	6.5 ± 1.3[2]	4.0 ± 1.2[2]
Average weight (g)	3.89 ± 0.06	3.50 ± 0.12[2]	3.30 ± 0.12[2]	2.89 ± 0.15[2]	2.59 ± 0.15[2]

[1] Mean ± Standard Error
[2] Significantly different from control, $p < 0.05$, Mann-Whitney U-test

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Table 3. Results of HERL/RTP Mouse Teratology Screen

Dose (mg/kg/day)	No. females treated	No. completely resorbed	No. pregnant (term)	D1 live	D1 weight	D3 live	D3 weight
0	24	0	16	11.6 ± 0.5[1]	1.53 ± 0.03	10.6 ± 0.6	1.89 ± 0.07
200	24	2	15	6.9 ± 1.3[2]	1.36 ± 0.04[2]	5.1 ± 1.3[2]	1.80 ± 0.13
400	24	15	2	9.5	1.28	8.0	1.41

[1] Mean ± Standard Error

[2] Significantly different from control value, $p < 0.05$, ANOVA