OPP-244
ITR-136

TO
N OF:

DATE May 17, 1971

ECT: Residue tolerance requests Carzol, SP

Mr. Drew M. Baker
Pesticides Tolerances Division

PP No. 746 - 4 ppm citrus-temporary

PP No. 961 - 4 ppm lemons, lime and organces

PP No. 989 - 3 ppm apples and pears

PP No. 1141 - 4 ppm grapefruit and tangerines

A review of the toxicology data offered in support of the safety of the requested residue tolerances concluded that the deficiencies did not allow a safety statement for the requested residue tolerances. This opinion, August 19, 1970, by Mr. H. R. Gittes listed the following as the deficiencies that had to be satisfied prior to a safety statement.

1. The toxicological safety of formetanate hydrochloride has not been established.

2. Data with respect to the effect of chronic ingestion of formetanate hydrochloride upon rat blood and brain cholinesterase activity are required.

3. The reduced 5 day survival seen in all dosage groups of the three generation rat study must be explained.

4. The question of possible mutagenicity raised by the increased numbers of resorptions seen in the teratogenicity study must be resolved by suitable investigation.

In the September 30, 1970, conference between the petitioner, the testing laboratory, Industrial Bio-Test, and Mr. Gittes, the following proposed studies were decided upon to satisfy the deficiencies as listed above.

1. A dominent lethal study in mice.

2. A teratology study in rabbits at dose levels of 1.0, 2.5, and 10 mg/kg.

(This would furnish overlap with the study previously done and would give lower levels as well.)

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3. An acute oral $LD_{50}$ on 1, 5 and 14 day old rats using the formetanate hydrochloride, the methyl compound, and the [illegible] compound.

4. An acute oral toxicity study in young adult rats using the same compounds as in 3 above.

5. A tracer study using ring labeled 14C formetanate in rats to determine if formetanate transfers to the pup via the milk.

6. Determination of the no effect and minimum effect dosage level upon rat blood and brain ChE.

7. A 3 to 4 week feeding study in rats using the minimal effect level from 6 above to determine subacute effects upon blood and brain ChE activity.

8. A study in humans to determine minimal and no effect levels upon ChE.

Mutagenic study with Carzol SP in albino mice: IBT No. P9140 Bio-Test Lab., 1971.

Groups of male mice, Charles River strain, 70 to 80 days of age were given 100 mg/kg of methyl methanesulfonate for a positive control and 1 mg/kg of Carzol SP or 2 mg/kg of Carzol SP. The dose was administered intraperitoneally. Twelve males were used for each group.

Each treated male was housed with 3 untreated virgin females per week for 6 consecutive weeks.

Disregarding cage loses of males or females, each group of 12 males were bred to 216 females over this 6th week period.

Observations related to possible mutagenic effects included counting the corpora lutea, implantation sites, resorption sites (early and late differentiated) and number of fetuses. The females were sacrificed approximately one week after removal from the males' cage for examination of the ovaries and uteruses.

RESULTS

The observations did not reveal mutagenic effects as related to corpora lutea, implantation sites and resorption sites.

The positive control treated males resulted in significant resorptions and suppression of the formation of corpora lutea.

This study demonstrates that this compound is non mutagenic as tested in this mouse strain.

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Teratogenic study with Carzol SP in New Zealand albino rats: Bio-Test Laboratories Report No. IBT J9141.

Fifteen females in each group were exposed from gestation day 6 through 18 with daily doses of 0, 1, 2.5 or 10 mg/kg per body weight. Each female was artificially inseminated with pooled semen from proven bucks. On gestation day 29 each doe was weighed, sacrificed and the young were removed by caesarean section.

Observations for teratogenic and reproductive effects included the count of implantation sites, number of resorptions, number of abortions and number of normal and abnormal young.

Possible compound related absorption effects were recorded in the 25 mg/kg group in the 1st rabbit teratology study.

Seventeen (17) resorptions were reported in 7 dams in the 25 mg/kg group. Twelve (12) resorptions were counted in 16 dams in the 10 mg/kg group, compared to 10 resorptions in 4 positive controls and 2 resorptions in 2 negative control females. There were no incidence of possible compound related absorption effects in this second rabbit teratology study. These two studies have demonstrated 10 mg/kg dose levels did not produce an increased number or reabsorptions.

Abortions did not occur in the control group of rabbits of 10 pregnant females. Three does aborted of the 11 pregnant in the test group 1 that were dosed at the rate of 1 mg/kg. Abortions did not occur in the 10 pregnant females in test group that received 2.5 mg/kg. Two does aborted of the 13 pregnant in test group 3 that received 10 mg/kg/day. These abortions are judged to be related to compound ingestion.

Industrial Bio-Test Report No. A9143.

An acute oral $LD_{50}$ study for the parent compound and two metabolites.

Results:
Acute $LD_{50}$ (mg/kg)

| Age of Test Rat | Carzol SP (EP-332 HCl Technical) | Desmethyl-formetanate | m-Formaminophenyl N-methylcarbamate |
|---|---|---|---|
| 1 Day | 4.62 | 160 | 220 |
| 5 Days | 9.0 | 220 | 280 |
| 14 Days | 21.2 | 290 | 305 |
| Young Adults | 20.5 | 267 | 280 |

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Industrial Bio-Test Study No. E9145; the transfer of formetanate to fetal and new born rats through the placenta and maternal milk.

Exposure Scheme

Two pair of females were orally dosed during gestation and sacrificed 24 hours later. Whole body hemogenates of the pups, the placenta and whole blood were radio assayed. The second pair of females were orally dosed just before delivery and were sacrificed 96 hours later. One to two pups were removed from the mothers prior to nursing, other pups were removed at 12 hour intervals over a 96 hour period. Stomach contents, liver, kidneys were radio assayed. The third pair of pregnant mice were given oral doses immediately after delivery and were sacrificed 96 hours later. Pups from these females were sacrificed at 12 hour intervals over a 96 hour period and the stomach contents, liver and kidneys were radio assayed.

The radio assay of the six parent females included liver, kidney, blood, and other tissues (excluding fat, muscle and skin). The expired air, urine and feces of group 1 and 2 were radio assayed at two periods, during the first 12 hours and during the 12 hours following. Group 3 females expired air, urine and feces were radio assayed at 12 hour intervals over a 96 hour period.

RESULTS

These studies demonstrated that placental transfer of 14C material occurred. A total of 0.24 and 0.23% of the 14C administered to the maternal animal was recovered from the fetuses. Milk transfer of 14C material was demonstrated in the animals dosed immediately following delivery. Absorption of 14C from the stomach of young rats occurred as indicated by detection of radio activi in liver and kidney tissues.

Formetanate was rapidly excreted by the female animals with the bulk of the radio activity appearing in the urine during the first 12 hours post dose administration.

Urinary recoveries ranged from 67 to 92% with fecal excretion accounting for from 4 to 20.. Very little tissue storage was detected.

Industrial Bio-Test Study No. A9144.

Acute oral cholinesterase activity study with Carzol in female albino rats and a 28 day subacute oral cholinesterase activity study with Carzol in female rats. 4

Single oral doses of Carzol at 0.5, 2.0 or 10.0 mg/kg demonstrated that the 0.5 mg/kg was without effect in regards to cholinesterase inhibition in the tested animal. The 2 and 10 mg/kg single dose resulted in depression of

cholinesterase activity in blood and brain of the tested animal. Recovery of activity was evident within 3 to 6 hours after dose administration. Daily oral administration of 0.5 mg/kg for 28 consecutive days to a group of 20 young female albino rats, Charles River strain, did not effect cholinesterase activity in blood and brain. Measurements of cholinesterase activity in plasma, red cells, and brain were done at weekly intervals beginning at the 7th exposure day.

The above described Industrial Bio-Test Laboratory studies Nos. P9140, J9141, A9143, E9145, and A9144 satisfy the studies numbered 1 through 7 of the September 30, 1970 conference.

The number 8 item, a study in humans to determine minimal and no effects levels upon cholinesterase, is not contained in the recently submitted data. This is not a requirement for the support of the safety of the residue tolerances and it would have been an elective study by the petitioner, if done.

The September 30, 1970 conference memorandum relates to a question that had been asked with respect to the increased 5 day pup mortality in the submitted three generation rat reproduction study. Mean values of 5 day survival indexes of all six litters were 71.3, 75.5, and 75.0 at levels of 10, 30, and 100 ppm respectively.

The Industrial Bio-Test Laboratory agreed to furnish data on control groups from other studies in support of their opinion that the noticed increase mortality was not unusual and not related to compound ingestion.

They submitted 5 day survival indexes of control groups from 16 different rat reproduction studies. The 5 day survival indexes varied and ranged from 44 to 97 for these numerous control rat litters. This submitted tabulation of the control groups in the numerous reproduction studies done in this laboratory substaniates their opinion that the 5 day survival index in this study for this compound is within the limits of experimental variations.

Toxicity data summary:

Rat long term - (19 Aug., 1970 memo)

NEL 100 ppm 2 years
NEL 200 ppm 14 months

Dog long term (19 Aug.,1970 memo)

NEL 100 ppm 0-308 days followed by 200 ppm from 309-730 days

Reproduction Studies (19 Aug., 1970 memo)

Rats - 3 generation - 2 litters each generation

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NEL 10-30-100 ppm

Dogs - 1 generation

NEL - as for the dog long term studies

Mouse dominent lethal test-negative for mutagenic activity.

Rabbit teratology - negative with oral exposure of 1, 2.5, and 10 mg/kg.

Acute Toxicity:

$LD_{50}$ - Carzol

| | |
|---|---|
| 1 day old rats | 4.62 mg/kg |
| 5 days old rats | 9.0 mg/kg |
| 14 days old rats | 21.2 mg/kg |
| Young adults | 20.5 mg/kg |

$LD_{50}$ - desmethyl formetanate

| | |
|---|---|
| 1 day old rats | 160 mg/kg |
| 5 days old rats | 220 mg/kg |
| 14 days old rats | 290 mg/kg |
| Young adults | 267 mg/kg |

$LD_{50}$ - formaminophenyl-n-methylcarbamate

| | |
|---|---|
| 1 day old rats | 220 mg/kg |
| 5 days old rats | 280 mg/kg |
| 14 days old rats | 305 mg/kg |
| Young adults | 280 mg/kg |

Carzol

0.5 mg/kg female albino rats
Single dose - No ChE effect.

0.5 mg/kg female albino rats
28 day - daily dosing - No ChE effect.

CONCLUSION

Submitted Carzol toxicity data support the safety of the requested residue tolerances of Petition Nos. 746, 961, 989, and 1141.

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cc:
OGFitzhugh
JCCummings
RD/EPA
Perrine Br.
Atlanta Br. (CLewis)
PP Nos. 746, 961,
989, and 1141

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