UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JAN 10 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Evaluation of the Two Generation Reproduction Study in Rats with CIPC

TO: Robert J. Taylor, PM#25
Registration Division (TS-767c)

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John E Whalan 1-8-85

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R.K. Loche (for Ed Budd) 1/10/85

Tox. Chem. No. 510A 1/10/85

The Toxicology Branch has reviewed the Interim and Final Reports of a Two Generation Reproduction Study in Rats with CIPC. The reproductive system of the rat is only slightly affected by the dietary administration of CIPC over two generations. Most of the toxic effects were observed at the doses of 3000 and 10,000 ppm. The following are the defined doses for this study:

REPRODUCTIVE NOEL $\geq$ 10,000 ppm

SYSTEMIC NOEL = 1000 ppm

SYSTEMIC LEL = 3000 ppm (slow weight gain; microscopic lesions in kidneys, spleen, liver, and marrow; gross splenic lesions; organ weight changes in the ovaries, liver, and spleen).

This study was CORE-GUIDELINE.

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Study Type: Two Generation Rat Reproduction Study

Accession Nos.: 250764, 250765, 250766

Report No.: 81-2573

Sponsor: PPG Industries, Inc.

Contracting Lab: Biodynamics, Inc., East Millstone, NJ

Date: July 5, 1983

Test Material: Technical CIPC (chlorpropham)

Sample Identification and Purity: Lot No. 237-2778, 98% chlorpropham

Homogeneity in Feed: Homogeneity in the feed was judged to be satisfactory.

Thermal Stability: CIPC in feed was stable for >21 days @ room temperature.

Protocol:

CIPC doses of 0, 1000, 3000, and 10000 ppm were administered in the diet to CD® (Sprague-Dawley derived) rats. Dosing, which was based on body weight, began with F_0 weanlings and proceeded throughout the study. The F_0 animals (60 males, 120 females; 15 males, 30 females per group) were dosed for 14 weeks prior to mating and during the mating, gestation, and lactation periods (total of 164-165 days). The F_0 rats were sacrificed after weaning the F_1 generation. From the weanlings, 180 (15 males, 30 females per group) were selected to produce the F_2 generation. Dosing continued at the same levels for approximately 18 weeks prior to mating, and during the mating, gestation, and lactation periods (total of 218-221 days). The F_2 generation was sacrificed at weaning, and the f_1 adults were sacrificed 30 days later.

During the study, all rats were individually housed in stainless steel cages, except during mating (2 females and 1 male per cage) and lactation. Food and water were provided ad libitum. Feed samples were evaluated for homogeneity and stability. During mating, the same 3 rats were housed nightly until there was evidence of mating (maximum of 15 days). Hardwood chip bedding was provided to pregnant females between gestation day 20 and lactation day 14.

Adults were observed twice daily for clinical signs of toxicity, and weekly for signs of local or systemic toxicity (neither individual nor summarized findings were presented in the study report). Food consumption was measured weekly except during mating for the males and except during mating,

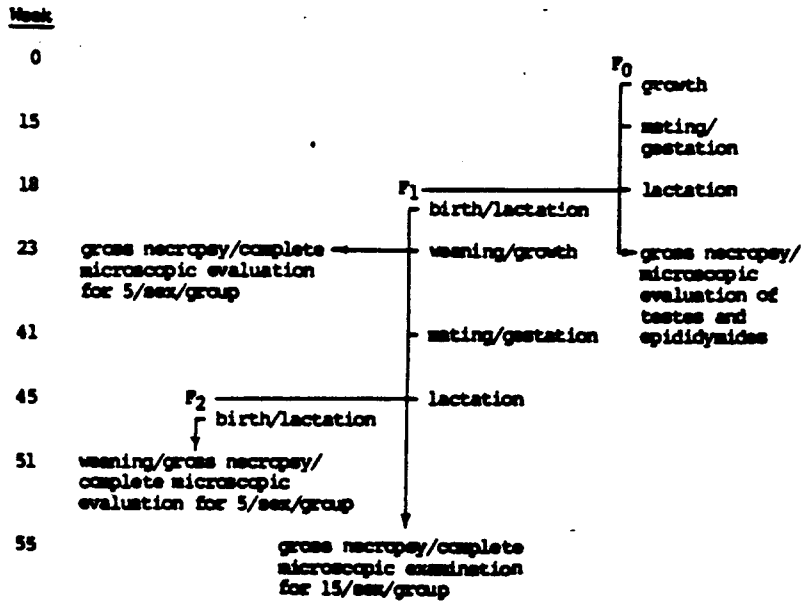
gestation, and lactation periods for the females. Body weights for the F_0 and F_1 rats were measured weekly. In addition, females were weighed on gestation days 0, 6, 15, and 20, and on lactation days 0, 4, 14, and 21. Litters were examined on lactation days 0, 4, 14, and 21 for the number, sex, and weights of pups and the presence of dead pups. Brain, plasma, and erythrocyte levels of cholinesterase in orbital sinus bloods were measured for 10 F_1 adults/sex/group. Dead or stillborn pups were weighed and examined grossly. Those found dead on days 0 to 4 were eviscerated and preserved in 70% ethanol. Those found dead on or after day 5 were discarded unless abnormal tissues were found. Gross necropsies were performed for all rats. The testes and epididymides for all F_0 males were evaluated microscopically. Complete microscopic examinations were performed on tissues from 120 F_1 adults (15/sex/group) and 40 F_1 and 40 F_2 weanlings (5/sex/group). The following tissues were examined histopathologically:

adrenals (2)
bone (sternum)
bone marrow (sternal)
*brain (3 levels)
esophagus
eyes (including optic nerve)
*heart
intestine (cecum, colon, duodenum, and ileum)
*kidneys (2)
*liver (2 lobes)
lungs (with two mainstem bronchi)
lymph nodes
nerve (sciatic)
ovaries (2)
pancreas
pituitary
prostate/seminal vesicles
salivary glands
skeletal muscle (bicep, r.femoris)
skin (with inguinal mammary gland)
*spleen
stomach
*testes (with epididymides)(2)
thymus
thyroid and parathyroid
trachea
urinary bladder
*uterus (body and cervix uteri)
tissue masses
gross lesions (with contiguous normal tissue)

* Asterisked organs were weighed at necropsy (F_1 and F_2 rats).

The oviducts were dissected from the uteri, and the epididymides from the testes.

The following diagram portrays the study chronology:



Results - F_0 Adult Rats:

Clinical observations were similar in the dosed and control groups. They included rales (dry, moist), nasal discharge (red, clear), lacrimation, chromodacryorrhea, soft stools, tissue masses, and stained ano-genital fur. None of these signs were compound-related. Food consumption in the dosed groups resembled the controls. No mortality was observed in any males. One female in group III (3000 ppm) died on gestation day 22 (study day 135). It was found to have 10 dead fetuses and one early resorption. A control female accidentally died during the post-weaning period (study day 153).

The mating and fertility/pregnancy indices of the F_0 mating were as follows:

Group	Dose (ppm)	Mating Indices (%) Males[a]	Mating Indices (%) Females[b]	Male Fertility Indices (%)[c]	Pregnancy Rate (%)[d]
I	0	86.7	86.7	100.0	100.0
II	1000	93.3	90.0	100.0	81.5
III	3000	86.7	76.7	92.3	82.6
IV	10000	100.0	93.3	100.0	82.1

[a] Percentage of males for which mating was confirmed in at least one female.
[b] Percentage off females showing evidence of mating.
[c] Number of males which sired live young / number of males mated.
[d] Percentage of inseminated females that became pregnant.

There was no adverse effect on mating, or on male fertility, but female fertility was reduced in all dosed rats.

The F_0 rats in Group IV had consistently slower body weight gain than the controls during the first 14 weeks of dosing (mean differences of 7.0% for males, and 14.6% for females). The other dose groups resembled the controls in their rates of growth. During gestation, the Group IV females continued to gain weight at a slower rate than the other dosed and control groups. During lactation, the group gained weight at a much faster rate than the other groups, but still remained lighter than the others.

Most of the gross lesions observed in the F_0 adults were due to ear-tagging and fighting. Microscopic findings of testicular and epididymal lesions were sporadic and not biologically significant.

Results - F_1 Generation:

The mean length of gestation and survival indices of the F_1 generation for days 0 and 21 were as follows:

Group	Dose (ppm)	X Gestation Length (days)	Survival Indices (%)[a] Day 0	Day 21
I	0	22.0	96.7	93.3
II	1000	22.1	96.6	87.8
III	3000	22.0	93.4	87.0
IV	10000	22.2	97.3	90.9

[a] Mean number of live pups / mean litter size.

The mean length of gestation, mean litter sizes, and the F_1 pup survival indices at parturition and during lactation were similar for control and dosed rats. Survivability was similar in the males and females. Sex distribution was normal. One control female rat appeared to have shortened forelimbs. No other abnormalities were seen in this study.

The body weights of the F_1 pups on lactation days 0, 4, 14, and 21 were as follows:

Group	Dose (ppm)	- Mean Pup Weight (grams) - Lactation Day 0	4	14	21
I	0	5.9	9.2	26.0	41.0
II	1000	5.9	9.2	25.7	39.7
III	3000	5.9	8.9	24.9	38.5
IV	10000	6.1	8.9	23.8	35.4

A mild decrease in the rate of growth was seen in the lactating Group IV pups. Compared to the controls, mean weight gain in the weaned F_1 rats during the growth period was slower in the males (-8.9% in Group III, -16.1% in Group IV) and the females (-12.9% in Group III, -14.3% in Group IV). During gestation, all groups gained weight at equivalent rates, but during lactation, Group III and IV gained weight at a faster rate than Groups I and II. Mean weight gain during the post-weaning period was slow in the Group III and IV males and females.

The F_1 pups selected for necropsy on lactation day 21 had a variety of organ weight changes. A disproportionate number of changes in the males occurred in Group II, and were attributed to this group having the highest mean body weight. The female pups had dose-related decreases in absolute and relative ovary weights in all dosed groups. In addition, mild decreases in absolute and organ/brain ratios were seen for liver (Group III and IV) and spleen (Group IV). No test article-related gross or histopathologic lesions were observed in the F_1 pups sacrificed on lactation day 21.

The culled F_1 pups which were not selected for histopathologic evaluation were sacrificed approximately one week after the selected pups. Groups III and IV had frequent dose-related occurrences of dark red spleens. Although nearly all of the Group IV culled pups has this lesion, none of the selected F_1 pups were reported to have splenic lesions. There were no compound related histopathologic lesions.

Clinical observations in the F_1 adults were similar in the dosed and control groups. They included rales (dry, moist), nasal discharge (red, clear), lacrimation, chromodacryorrhea, soft stools, tissue masses, and stained anogenital fur. None of these signs were considered compound-related. Food consumption was normal.

No mortality was observed in any weaned males. One control female died during the post-weaning period (study day 175). Although it mated, it did not deliver a litter.

The mating and fertility/pregnancy rates of the F_1 mating were as follows:

Group	Dose (ppm)	Mating Indices (%)		Male Fertility Indices (%)[c]	Pregnancy Rate (%)[d]
		Males[a]	Females[b]		
I	0	100.0	93.3	80.0	67.9
II	1000	93.3	86.7	92.9	88.5
III	3000	100.0	96.7	100.0	93.1
IV	10000	86.7	90.0	92.3	81.5

[a] Percentage of males for which mating was confirmed in at least one female.
[b] Percentage off females showing evidence of mating.
[c] Number of males which sired live young / number of males mated.
[d] Percentage of inseminated females that became pregnant.

In order to maximize the number of pregnancies, the mating period was extended by five days and females were housed with sexually active males. The mating indices for dosed males and females were reduced slightly, but the fertility indices remained unaffected.

A increase in absolute and relative spleen weights was seen in the adult Group IV males (62.6%; 93.5%, respectively) and Group III and IV females (28.7-99.0; 43.7-128.5%, respectively). Other mild changes in organ/body weight ratios were due to decreased body weight gain in the higher dose groups and were not biologically significant. Absolute testis and ovary weights remained fairly constant in all groups. No compound-related gross lesions were found.

Brown pigmented granules were observed in the reticuloendothelial cells of the spleen, the reticuloendothelial Kupffer cells of the liver, and the convoluted tubular epithelial cells of the kidneys. The liver and kidney lesions were seen in all groups including controls, but the frequency of occurence increased with dosage. In the high dose group, all animals had liver lesions and most had kidney lesions. Splenic lesions were seen in all F_1 adults. Severity increased with dosage. Groups III and IV for both sexes were most severely affected. Sternal marrow hypercellularity was seen in all groups, but severity and frequency of occurence increased with dosage, Groups III and IV being the most severely affected.

Measurements of cholinesterase levels in the brain, plasma, and erythrocytes of the F_1 rats did not reveal any significant changes.

Results - F_2 Generation:

The mean length of gestation and survival indices of the F_2 generation for days 0 and 21 were as follows:

Group	Dose (ppm)	$\bar{X}$ Gestation Length (days)	- Survival Indices [a] - Day 0	Day 21
I	0	22.4	91.5	73.1
II	1000	22.3	94.0	87.0
III	3000	22.1	97.5	86.1
IV	10000	22.1	97.5	87.9

[a] Mean number of live pups/mean litter size.

The length of gestation and mean litter sizes in the dosed and control groups were comparable. There was no adverse effect on fertility, gestation length, or survivability. Sex distribution was normal. F_2 pup survival in the dose groups slightly exceeded the control survival indices.

The body weights of the F_2 pups on lactation days 0, 4, 14, and 21 were as follows:

Group	Dose (ppm)	- Mean Pup Weight (grams) - Lactation Day 0	4	14	21
I	0	6.0	8.6	24.5	39.1
II	1000	6.0	9.4	25.2	39.5
III	3000	5.8	8.3	24.1	36.9
IV	10000	6.0	8.3	21.5	30.8

A moderate decrease in the rate of growth was seen in the Group IV pups.

Organ body weight changes in the males occurred mostly in Group IV and were attributed to the fact that these rats had mean terminal weights far below those of the other groups. Absolute and relative splenic weights were moderately decreased, however (-46.2 and 29.4%, respectively). Among the females, mild to moderate absolute and relative organ weight decreases were measured for ovaries in Groups III (-25.7, -23.7%, respectively) and IV (-36.0, -24.4, respectively) and for spleens in Group IV (-31.5, -18.1% respectively). Other absolute organ weight decreases seen in Group IV rats were attributed to low terminal body weights relative to the controls. No compound-related gross or histopathologic lesions were found.

Discussion:

Clinical signs observed in the F_0 and F_1 adult rats included rales (dry, moist) nasal discharge (red, clear), lacrimation, chromodacryorrhea, soft stools, tissue masses, and stained ano-genital fur. Since these signs were reported to have occurred in both the dosed and control groups, they were probably not induced by CIPC. A female F_0 rat in Group III died during the post-weaning period. Although it mated, it did not deliver a litter.

Mating indices in the F_0 rats were comparable in the dosed and control groups, and male fertility was unaffected by CIPC. Female fertility was slightly reduced in all the treated groups, relative to the controls. In contrast, fertility indices in the F_1 adults were unaffected, but the mating indices were reduced slightly. There were no clear effects on reproduction.

The test article had no effect on the length of gestation, mean litter sizes, or on pup survival indices at parturition or during lactation in the F_1 and F_2 generations. An F_1 female control pup appeared to have shortened forelimbs, but no other abnormalities were observed.

Body weight gain in the F_0 Group IV females was consistently slower than in the other groups during the growth and gestation periods. These rats gained weight quickly during the lactation period but never attained the weights of the other groups. The F_1 lactating pups in Group IV has a slower rate of weight gain than the other groups. Weight gain during the growth period and during the post-weaning periods remained slow in Groups III and IV. In the F_2 pups a moderate decrease in body weight gain was seen in the Group IV rats on days 14 and 21. Food consumption in the F_0 and F_1 adults was normal.

No biologically significant gross or histopathologic lesions were observed in the testes and epididymides of the F_0 males. Although fluctuations in organ weights were frequently observed, most were caused by body weight fluctuations. Test article induced changes observed in the F_1 pups on lactation day 21 included dose-related decreased absolute and relative ovary weights in all dosed groups, and mild decreases in absolute and organ/brain weight ratios for liver (Groups III and IV) and spleen (Group IV). Since no compound-related gross or microscopic lesions were observed in these pups, these changes in organ weights are of dubious significance. It should be noted, however, that dark red spleens were observed in many of the Group III and IV F_1 pups not selected for use as parents or for histopathologic evaluation. These pups were

necropsied one week after the previously mentioned F_1 pups. This suggests that splenic lesions may develop approximately one month after parturition.

Severe increases in absolute and relative spleen weights were seen in the adult F_1 males (Group IV) and female (Group III and IV). No compound-related gross lesions were seen, although several rats had splenic cysts. Dose-related histopathologic findings included brown pigment granules in the reticuloendothelial cells of the spleen and liver, the convoluted tubular epithelial cells of the kidney; and sternal marrow hypercellularity. Group III and IV were most affected.

Mild to moderate absolute and relative organ weight decreases were seen in the F_2 pups on lactation day 21 in the F_2 pups for ovaries (Groups III and IV) and spleen (Group IV). No compound-related gross or histopathologic lesions were found.

Measurements of cholinesterase levels in the brain, plasma, and erythrocytes of the F_1 rats did not reveal any significant changes.

Conclusions:

The dietary administration of CICP to CD® rats at doses of 1000, 3000, and 10000 ppm caused little toxicity, and virtually no effect on reproduction. Mating and fertility indices in the F_0 and F_1 rats were not significantly reduced. Similarly, there was no effect on the length of gestation, mean litter size, and survival for the F_1 and F_2 pups. There were no compound-related abnormalities.

Body weight gain was slowed in rats dosed at 3000 and 10000 ppm, but no effect was seen at the 1000 ppm dose level.

No compound-related gross lesions were seen in any rats, except the culled F_1 adults. They included dose-related histopathologic findings of brown pigment granules in the reticuloendothelial cells of the spleen and liver and the convoluted tubular epithelial cells of the kidney, and marrow hypercellularity. Rats dosed at 3000 and 10000 ppm were most affected.

Organ weight changes in the F_1 pups (lactation day 21) included mild dose-related decreases in absolute and relative ovary weights in all dosed groups, and mild decreases in absolute and organ/brain weight ratios for liver (3000 and 10000 ppm) and spleen (10000 ppm). In the F_1 adults, a severe increase in absolute and relative spleen weights was seen in males (10000 ppm) and females (3000 and 10000 ppm). The F_2 pups had mild to moderate absolute and relative organ weight decreases for ovaries (3000 and 10000 ppm) and spleens (10000 ppm).

Measurements of cholinesterase levels in the brain, plasma, and erythrocytes of the F_1 rats did not reveal any significant changes.

Nearly all indications of toxicity were observed at the two highest dose levels. The only compound-related effect seen in the 1000 ppm dose group was a mild decrease in mean ovary weights in the F_1 pups. Since no ovarian lesions were observed grossly or microscopically in these pups and the F_1 adults had normal ovarian morphology, the decreased ovarian weights were probably just an indication of slight organ development delay.

REPRODUCTIVE NOEL $\geq$ 10,000 ppm

SYSTEMIC NOEL = 1000 ppm

SYSTEMIC LEL = 3000 ppm (slow weight gain; microscopic lesions in kidneys, spleen, liver, and marrow; gross splenic lesions; organ weight changes in the ovaries, liver, and spleen).

Classification: CORE-GUIDELINE

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