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DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - OPPTS 870.3800, OECD 416.

PC CODE: 099901 **DP BARCODE:** 337621

TEST MATERIAL (PURITY): Acticide ® OIT (2-n-Octyl-4-isothiazolin-3-one) (95.9%)

SYNONYMS: Octhilinone

CITATION: Beekhuijzen, M. E. W. (2006). Two-generation reproduction toxicity study with Acticide ® OIT by dietary administration in Wistar rats. NOTOX B.V, Hambakenweterring 7, 5231, Hertogenbosch, The Netherlands. Laboratory Project Number 397823, November, 9, 2006. MRID 47815801. Unpublished.

SPONSOR: THOR GmbH, Speyer, Germany

EXECUTIVE SUMMARY:

In a two-generation reproduction study (MRID 47815801*)* Acticide ® OIT (2-n-Octyl-4-isothiazolin-3-one) (95.9%) was administered to Wistar rats (24/sex/dose) in diet at dose levels of 0, 300, 800, or 1500 ppm (equivalent to 0, 13-15, 43-51, or 96-120 mg/kg bw/day based on test article recovery).

In the parental P and F_1 generations, no treatment-related clinical signs were observed. No mortalities occurred in the P generation, but in the F_1 parental generation, one animal in the 300 ppm group was found dead and another in the 800 ppm was killed in extremis. These deaths were not considered to be treatment-related. In the first generation, body weights were statistically significantly decreased in dams treated with 1500 ppm test substance on days 7 and 14 of lactation. No treatment-related changes in body weight occurred in the second generation, although the body weights of males and females of the 1500 ppm group were lower during the complete treatment period. Females in the P generation showed decreased absolute spleen weight and increased relative adrenal weight at 1500 ppm. No organ weight changes were noted for the second generation. At the histopathological examination at 1500 ppm , minimal to moderate diffuse hyperplasia/hyperkeratosis of the forestomach was observed in nine males and ten females of the first generation and in eight males and six females of the second generation, all at the 1500 ppm dose level. These observations correlated with the macroscopic observations of irregular surface of the forestomach in four males in the first generation and in four males and one female in the second generation. **The parental systemic LOAEL is 1500 ppm (96-111 mg/kg bw/day in males, 107-120 mg/kg bw/day in females for both generations when corrected for mean value of test article recovery), based on decreased body weight,**

hyperplasia/hyperkeratosis of the forestomach, decreased spleen weight, and increased adrenal weight. The parental systemic NOAEL is 800 ppm (43-45 mg/kg bw/day in males, 48-51 mg/kg bw/day in females for both generations when corrected for the mean value of test article recovery).

The offspring of both generations exhibited decreased body weight gain and decreased spleen weight at the 1500 ppm dose level. **The offspring LOAEL is 1500 ppm (96-111 mg/kg bw/day in males, 107-120 mg/kg bw/day in females for both generations when corrected for mean value of test article recovery), based on decreased body weight gain and decreased spleen weight. The offspring NOAEL is 800 ppm (43-45 mg/kg bw/day in males, 48-51 mg/kg bw/day in females for both generations when corrected for the mean value of test article recovery).**

Measured sperm count, motility, and morphology and estrous cycle regularity were unaffected by treatment in both generations. Reproduction parameters (mating performance, duration of gestation, fertility, number of implantation sites, number of live/dead pups at first litter check) and breeding parameters (postnatal loss, living pups on day 4 post partum, breeding loss, living pups on day 21 post partum, viability index, weaning index) were unaffected by treatment in the first and second generations. **Therefore, the reproductive NOAEL is 1500 ppm (96-111 mg/kg bw/day in males, 107-120 mg/kg bw/day in females for both generations when corrected for the mean value of test article recovery).**

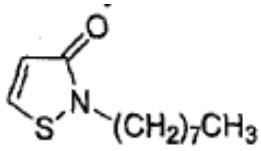
This study is **ACCEPTABLE - GUIDELINE** and satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800; OECD 416) in rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements are provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: 2-n-Octyl-4-isothiazolin-3-one (ACTICIDE® OIT)

Description:	Amber solid to liquid, depending on ambient temperature
Lot/batch #:	K3946511
Purity:	95.9% a.i.
Compound stability:	Stable
CAS # of TGAI:	26530-20-1
Structure:	

2. Negative Control: Standard laboratory animal diet.

2a Vehicle: Test substance mixed in feed without use of vehicle; no positive control

3. Test animals:

Species:	Rat
Strain:	Wistar rats Crl: (WI) BR
Age at study initiation:	(P) 5-6 wks; (F_1) 3 wks
Wt. at study initiation:	(P) Males: 174-177 g; Females: 148-149 g (F_1) Males: 152-186 g; Females: 131-152g
Source:	Charles River Deutschland, Sulzfeld, Germany
Housing:	Pre-mating/after weaning: Housed 4 animals/sex/cage in Macrolon cages with sterilized sawdust as bedding material and paper as cage-enrichment Mating: Females were caged 1:1 with males in suspended stainless steel cages with wire mesh floors Post-mating: Males were individually housed in stainless steel cages with wire mesh floors Post-coitum: Females were individually house in Macrolon cages containing sterilized sawdust and paper Lactation: Offspring were kept with the dam until termination
Diet:	Standard powder laboratory animal diet (Diet VRF-1 from Altromin GmbH, Lage, Germany) *ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	**Temperature:** 17.4-22.7° C (actual range) **Humidity:** 26-100% (actual range) **Air changes:** 15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	5 days prior to start of treatment

B. PROCEDURES AND STUDY DESIGN:

1. Mating procedure: One male and one female in the parental P generation from the same treatment group were housed together. Each morning following pairing, the trays under the cages were checked for ejected copulation plugs. The day on which the copulation plug was found was designated day 0 of gestation. Once mating occurred, the male and

female were separated.

2. Study schedule: Parental animals were exposed to test substance in diet for at least 10 weeks prior to mating up to termination. F_1 parental animals were not mated until approximately 13 weeks after allocation from the F_1 litters. Culling of F_1 and F_2 offspring was adjusted on day 4 after birth to yield four males and four females per litter as nearly as possible.

3. Animal assignment: Parental (P) animals were randomized into groups at least 5 days before study start, by computer-generated random algorithm according to body weight, with all animals within ±20% of the sex mean. Pups forming the F_1 generation were selected at random.

TABLE 1. Animal assignment

Test group	Dose in diet [a] (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	24	24	24	24
Low (LDT)	300	24	24	24	24
Mid (MDT)	800	24	24	24	24
High (HDT)	1500	24	24	24	24

[a] Diets were administered from beginning of the study until sacrifice.

4. Dose selection rationale: The dose levels were selected based on the results from a 14-day range finding study and a 90-day toxicity study. In the 14-day study, Wistar rats were exposed to 0, 2000, 6000, or 10000 ppm of test material in the diet. Animals in the 6000 or 10000 ppm dose groups were found dead or killed in extremis on day 6 of the study. Animals at 2000 ppm showed a decreased body weight gain, increased food scatter, and irregular surface of the forestomach. In the 90-day study, Wistar rats were exposed to 0, 100, 300, or 1000 ppm of test material in diet. The high dose for the two-generation reproduction study was chosen to be 1500 ppm based on slight toxicity observed at the 1000 ppm group and too severe toxicity at the 2000 ppm group. The mid dose was selected to be 800 ppm, and the low dose was selected to be 300 ppm.

5. Dosage preparation and analysis: Formulations were prepared bi-weekly by mixing appropriate amounts of test substance with standard powder laboratory diet and were stored at room temperature. Prior to the start of the study, stability of the test substance in diet was evaluated. Homogeneity (top, middle, and bottom) was evaluated at weeks 8, 18, 28, and 38. During the study, random samples of treated food were analyzed at weeks 8, 18, 28, and 38 for accuracy of concentration.

Results:

Homogeneity analysis: Coefficients of variation for the diets of groups 2 and 4 were between 2.1% and 7.9% indicating homogeneity of mixture.

Stability analysis: At least 6 weeks at room temperature for diets with a concentration of 100 and 3200 ppm.

Concentration analysis: The test samples analyzed showed mean recoveries of 59%, 70%, and 80% at the 300, 800, and 1500 ppm diets, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. **Parental animals:** Parental animals were checked for mortality/viability at least twice daily, and any animal exhibiting non-transient distress was sacrificed for humane reasons. Detailed clinical observations were made at least once daily and graded on fixed scales. Body weights were taken on the first day of exposure and weekly thereafter. Mated females were weighed on days 0, 7, 14, and 21 post-coitum and on days 1, 4, 7, 14, and 21 post-partum. Food consumption was measured weekly for males and females except during the mating period. Food consumption of mated females was measured on days 0, 7, 14, and 21 post-coitum and on days 1, 4, 7, 14, and 21 post-partum. Water consumption was not quantitatively measured. Vaginal smears were taken daily 3 weeks prior to pairing and throughout cohabitation in order to assess estrous cycle. Mating date, male pairing, confirmation of pregnancy, and delivery date were recorded.

2. **Litter observations:** According to the report, the following litter observations (X) were made (see Table 2). The day of vaginal opening or balanopreputial separation for several F_1-weanlings selected for mating and for all selected F_2-pups was recorded. Ano-genital distance was measured on day 1 of lactation for all F_2 pups.

TABLE 2. F_1 / F_2 Litter Observations [a]

Observation	Time of observation (lactation day)					
	Day 1	Day 4 [b]	Day 4 [c]	Day 7	Day 14	Day 21
Number of live pups	X	X		X	X	X
Pup weight	X		X	X	X	X
External alterations	X		X	X	X	X
Number of dead pups	X	X				X
Sex of each pup (M/F)	X	X				

[a] Data obtained from page 25 in the study report.
[b] Before standardization (culling).
[c] After standardization (culling).

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were sacrificed and discarded.

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

3. **Postmortem observations:**

   a. **Parental animals:** All surviving parental males were sacrificed as soon as possible after successful mating. Maternal animals were sacrificed on day 21 post-partum or shortly thereafter. For surviving males, sperm motility (all males) and morphology (all P-generation males and ten randomly selected samples from the F_1-generation control and high-dose group males) were assessed from sperm samples taken from the proximal part

of the vas deferens. Enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves were evaluated from one testis and one epididymis, respectively, for 10 randomly selected samples from the control and high-dose groups. The number of implantation sites in each uterus was assessed for all paired females. The uterus of nonpregnant females was stained using the Salweski technique to determine any very early post-implantation losses. Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

The following tissues (X) were prepared for microscopic examination and weighed (XX). Tissues that were only weighed are denoted by (XXX):

X	Cervix	XX	Spleen
X	Coagulation gland	X	Stomach
XX	Epididymis (one)	XX	Testis (one)
XX	Ovaries	XX	Uterus
XX	Prostate gland (weighed when fixed for at least 24 hours)	X	Vagina
XX	Seminal vesicles	X	All gross lesions
XXX	Adrenal glands	XXX	Liver
XXX	Brain	XXX	Pituitary (weighed when fixed for at least 24 hours)
XXX	Kidneys	XXX	Thyroid

b. Offspring: F_1 offspring selected for the next generation were sacrificed via decapitation; all remaining pups were sacrificed using an oxygen/carbon dioxide procedure. The F_1 offspring not selected as parental animals and all F_2 offspring were sacrificed at 21 days of age or shortly thereafter. These animals were subjected to postmortem external examination of the cranium and macroscopic examination of thoracic and abdominal tissues and organs. Gross lesions were preserved for future examination. The weights of brain, spleen, and thymus were taken for one randomly selected F_1 pup and one unselected F_2 pup per sex per litter at day 21 post-partum or shortly thereafter. Offspring found dead before day 14 of lactation were sexed, externally examined, and stomach examined for presence of milk.

D. DATA ANALYSIS:

1. Statistical analyses: If variables could be assumed to follow a normal distribution, the Dunnett-test (many-to-one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex. The Steel-test (many-to-one rank test) was applied if the data could not be assumed to follow a normal distribution. The Fisher Exact test was applied to frequency data.

2. Indices:

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Percentage mating = Number females mated/number females paired x 100

Fertility index = Number of pregnant females/number of females paired x 100
Conception rate = Number of pregnant females/number of females mated x 100
Gestation index = Number of females bearing live pups/number of pregnant females x 100
Duration of gestation = Number of days between confirmation of mating and the beginning of parturition

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

Percentage live males at first litter check = Number of live male pups at first litter check/number of live pups at first litter check x 100

Percentage live females at first litter check = Number of live female pups at first litter check/number of live pups at first litter check x 100

Percentage of postnatal loss days 0-4 post partum = Number of dead pups on day 4 post partum/number of live pups at first litter check x 100

Percentage of breeding loss day 5 until weaning = Number of dead pups between days 5 and 21 post partum/number of live pups on day 4 post partum x 100

Percentage of live males at weaning = Number of live male pups on day 21 post partum/number of live pups on day 21 post partum x 100

Percentage of live females at weaning = Number of live female pups on day 21 post partum/number of live pups on day 21 post partum x 100

Viability index = Number of live pups on day 4 post partum/number of pups born alive x 100

Weaning index = Number of live pups on day 21 post partum/number of live pups on day 4 post partum x 100

3. Historical control data: Provided in Appendix 7 of the study report.

II. RESULTS:

A. PARENTAL ANIMALS:

1. Mortality and clinical signs: No mortality or treatment-related clinical signs were observed in the P parental generation. Incidental findings included alopecia, scabs, wounds, swelling of the genital region, and chromodacryorrheoa; these were not considered to be treatment-related.

In the F_1 parental generation, one female in the 300 ppm treatment group was found dead on day 17 of lactation with no cause of death determined and no clinical signs of toxicity before death. One female in the 800 ppm group was sacrificed on day 1 of lactation due to bad health. This female delivered sixteen dead pups and was observed to have uterine inflammation and retained fetuses upon necropsy. These deaths were not considered

treatment-related. Incidental findings included alopecia of several body parts, hunched posture, chromodacryorrhea, broken or curled tail apex, scabs, swelling of the left flank or abdomen, and scales. A 300-ppm F_1 female showed an absent tail, and a 1500-ppm F_1 male showed a broken apex. None of these findings were considered treatment-related.

2. **Body weight and food consumption:** In the P parental generation, body weight was statistically significantly decreased in dams treated at 1500 ppm on days 7 and 14 of lactation. On several occasions, statistically significantly lower body weights and body weight gains were noted for males of the low- and high-dose groups. These changes were considered to be of no toxicological significance because the data were within the historical control data range. Absolute and relative decreases in food consumption were observed in males and females treated at 1500 ppm from days 1 to 8 of pre-mating. This was not considered to be toxicologically relevant but rather caused by decreased palatability of diet.

   F_1 parental generation body weights were lower during the entire treatment period in the group administered 1500 ppm, and this change was attributed to their lower starting body weight. A slight increase in body weight gain was noted in the males and females of the 800 and 1500 ppm group during pre-mating and not considered an adverse effect of treatment. Females administered 800 ppm test material demonstrated decreased body weight gain during post coitum and lactation period; since no dose-response relationship was observed, this decreased body weight gain was not considered to be a treatment-related effect.

   Reported body weight and selected food consumption results are summarized in Table 4.

TABLE 4. Mean (±SD) Body weight and food consumption - pre-mating, post-coitum, and lactation [a]

Observations/study week	Dose group			
	Control	LDT	MDT	HDT
P Generation males - Pre-mating				
Mean body weight (g)				
Week 1	177 ± 9.3	177 ± 7.1	174 ± 6.9	174 ± 7.7
Week 2	231 ± 15.3	229 ± 11.1	227 ± 10.9	219 ± 10.0**
Week 3	291 ± 18.9	284 ± 13.0	284 ± 15.9	276 ± 12.9**
Week 4	330 ± 22.6	318 ± 15.9	322 ± 22.0	316 ± 19.7*
Week 5	365 ± 29.2	347 ± 19.9*	355 ± 26.0	347± 22.9
Week 6	396 ± 34.0	375 ± 23.0	385 ± 31.7	373 ± 26.4
Week 7	420 ± 36.6	397 ± 24.8*	407 ± 34.4	395 ± 29.4*
Week 8	440 ± 39.7	411 ± 26.6*	426 ± 37.3	413 ± 31.4*
Week 9	462 ± 42.3	430 ± 27.5**	446 ± 42.0	433 ± 33.9*
Week 10	475 ± 44.7	444 ± 30*	460 ± 44.3	449 ± 38.2
Mean weight gain (g)				
Weeks 1-10 (mean of means)	298	267*	286	275
Mean food consumption (g/animal/day)				
Weeks 1-10 (mean of means)	27	26	28	27
P Generation females - pre-mating				
Mean body weight (g)				
Week 1	149 ± 6.2	149 ± 8.0	148 ± 8.4	148 ± 6.7
Week 2	172 ± 12.0	176 ± 9.2	172 ± 13.5	171 ± 8.3
Week 3	193 ± 12.7	194 ± 12.6	191 ± 16.3	190 ± 11.9

TABLE 4. Mean (±SD) Body weight and food consumption - pre-mating, post-coitum, and lactation [a]

Observations/study week	Dose group			
	Control	LDT	MDT	HDT
Week 4	211 ± 15.6	214 ± 15.9	207 ± 18.7	205 ± 13.3
Week 5	224 ± 17.4	230 ± 17.5	223 ± 19.7	219 ± 14.0
Week 6	236 ± 18.2	242 ± 16.1	235 ± 22.2	231 ± 16.4
Week 7	248 ± 18.2	249 ± 16.3	244 ± 22.8	241 ± 17.6
Week 8	255 ± 18.4	258 ± 14.3	248 ± 22.9	246 ± 18.8
Week 9	270 ± 20.5	272 ± 18.4	266 ± 23.8	263 ± 20.0
Week 10	278 ± 24.5	277 ± 16.9	274 ± 27.7	269 ± 19
Mean weight gain (g) Weeks 1-10 (mean of means)	129	128	125	121
Mean food consumption (g/animal/day) Week 1-10 (mean of means)	19	20	20	19
F1 Generation males - Pre-mating				
Mean body weight (g) Week 1	186 ± 25.9	189 ± 17.9	182 ± 14.5	152 ± 19.0**
Week 2	245 ± 27.3	247 ± 18.2	239 ± 17.5	200 ± 22.1**
Week 3	298 ± 29.0	298 ± 18.4	296 ± 23.0	252 ± 22.9**
Week 4	348 ± 29.5	350 ± 20.2	346 ± 26.8	304 ± 23.3**
Week 5	388 ± 30.5	386 ± 22.2	379 ± 31.1	337 ± 23.5**
Week 6	419 ± 32.3	413 ± 24.1	408 ± 37.0	366 ± 23.9**
Week 7	441 ± 32.9	435 ± 26.7	428 ± 38.2	390 ± 26.0**
Week 8	454 ± 33.8	447 ± 27.7	440 ± 40.6	402 ± 25.8**
Week 9	480 ± 37.1	469 ± 28.3	465 ± 43.6	425 ± 28.5**
Week 10	498 ± 38.5	488 ± 30.2	482 ± 46.3	442 ± 29.6**
Mean weight gain (g) Weeks 1-10 (mean of means)	312	299	300	290
Mean food consumption (g/animal/day) Weeks 1-10 (mean of means)	30	31	31	30
F1 Generation females - pre-mating				
Mean body weight (g) Week 1	146 ± 17.0	152 ± 10.2	149 ± 11.1	131 ± 13.9**
Week 2	176 ± 16.1	183 ± 11.5	179 ± 14.3	161 ± 13.8**
Week 3	196 ± 14.3	202 ± 12.7	201 ± 15.7	183 ± 15.9*
Week 4	214 ± 15.8	222 ± 15.3	216 ± 18.3	201 ± 16.3*
Week 5	231 ± 15.1	237 ± 15.5	232 ± 20.4	217 ± 15.5*
Week 6	241 ± 16.4	248 ± 15.8	242 ± 21.9	227 ± 16.5*
Week 7	246 ± 15.5	252 ± 17.0	249 ± 22.3	235 ± 16.4
Week 8	253 ± 14.2	261 ± 17.1	255 ± 25.4	242 ± 16.8
Week 9	259 ± 15.7	267 ± 17.8	264 ± 23.4	252 ± 17.5
Week 10	265 ± 18.9	274 ±20	274 ± 27.1	260 ± 17.4
Mean weight gain (g) Weeks 1-10 (mean of means)	119	122	125	129
Mean food consumption (g/animal/day) Weeks 1-10 (mean of means)	20	21	21	21
P Generation females – post coitum and lactation				
Mean body weight (g) Post coitum Day 0	275 ± 19.8	278 ± 16.7	275 ± 24.7	267 ± 20.1

TABLE 4. Mean (±SD) Body weight and food consumption - pre-mating, post-coitum, and lactation [a]

Observations/study week	Dose group			
	Control	**LDT**	**MDT**	**HDT**
Day 7	301 ± 24.8	305 ± 17.5	302 ± 25.5	295 ± 19.8
Day 14	333 ± 26.5	336 ± 20.0	331 ± 26.0	323 ± 21.5
Day 21	436 ± 35.4	435 ± 34.6	424 ± 35.1	416 ± 30.2
Lactation Day 1	319 ± 29.4	322 ± 21.3	312 ± 28.5	304 ± 20.5
Day 4	326 ± 28.8	328 ± 21.0	318 ± 26.7	309 ± 21.6
Day 7	333 ± 25.6	336 ± 18.1	327 ± 33.2	314 ± 21.7*****
Day 14	347 ± 25.5	351 ± 18.5	333 ± 27.0	323 ± 24.9******
Day 21	330 ± 23.7	332 ± 18.8	324 ± 26.7	318 ± 25.0
F_1 Generation females – post coitum and lactation				
Mean body weight (g) Post coitum Day 0	276 ± 24.3	284 ± 20.9	278 ± 26.6	261 ± 18.8
Day 7	304 ± 25.4	310 ± 21.4	306 ± 29.0	285 ± 20.0*****
Day 14	338 ± 27.6	341 ± 24.6	335 ± 31.3	316 ± 20.5*****
Day 21	436 ± 34.6	435 ± 24.8	423 ± 38.3	399 ± 29.6******
Lactation Day 1	320 ± 25.4	322 ± 23.0	318 ± 29.4	299 ± 19.5*****
Day 4	329 ± 28.4	333 ± 24.0	326 ± 31.0	304 ± 20.4******
Day 7	338 ± 29.7	339 ± 25.1	333 ± 33.3	312 ± 19.8******
Day 14	348 ± 33.5	345 ± 23.8	336 ± 30.6	315 ± 18.2******
Day 21	331 ± 32.0	322 ± 19.1	316 ± 24.4	302 ± 17.1******

[a] Data obtained from pages 57-60, 65, 66, 340-343, 348, and 349 in the study report.

***** Statistically different from control, $p<0.05$ (Dunnett's test based on pooled variance).

****** Statistically different from control, $p<0.01$ (Dunnett's test based on pooled variance).

3. **Test substance intake:** Based on food consumption, body weight, and test material recovery in diet, the doses expressed as mean daily mg test substance/kg body weight during the 10-week pre-mating period are presented in Table 5. The values for the P and F_1 generation are similar and considered to be representative of the test substance intake for the entire study.

TABLE 5. Mean test substance intake during premating (mg/kg body weight/day) corrected for mean value of recovery [a]

	Male			Female		
	LDT (177 ppm recovered)	MDT (560 ppm recovered)	HDT (1275 ppm recovered)	LDT (177 ppm recovered)	MDT (560 ppm recovered)	HDT (1275 ppm recovered)
P	13	43	96	15	48	107
F_1	14	45	111	15	51	120

[a] Data obtained from pages 33 and 38 in the study report.

4. Reproductive function:

a. Estrous cycle length and periodicity: Results from the evaluation of vaginal smears indicated no treatment-related effects.

b. Sperm measures: Results from the evaluation of sperm parameters revealed no treatment-related effects on sperm count, motility, or morphology.

5. Reproductive performance: In both generations, mating performance, duration of gestation, fertility parameters, number of implantation sites, and number of dead and living pups at first litter check were similar for the control and treated groups. Breeding parameters were unaffected by treatment up to 1500 ppm. In the P generation, eight males and eight females were suspected of infertility across dosing groups (2 males and 2 females in the control group, 5 males and 5 females in 300 ppm group, 1 male and 1 female in the 800 ppm group), and four males and four females were suspected of infertility in the F_1 parental generation. These observations were not considered to be treatment-related. Postnatal loss between days 0-4 post partum, living pups on day 4 post partum, breeding loss between days 5-21 post partum, living pups on day 21 post partum, viability index and weaning index were similar for the control and treated groups for both generations. Results for the parental animals are summarized from the report in Table 6.

TABLE 6. Reproductive performance and fertility parameters[a]

Observation	Dose group (ppm)			
	Control	300	800	1500
P Generation				
Mean (±SD) precoital interval (days)	3.2	3.0	2.8	2.7
MALES				
Number mated	24	24	24	24
Number fertile	22	19	23	24
Suspected infertile	2	5	1	0
Intercurrent deaths	0	0	0	0
FEMALES				
Number mated	24	24	24	24
Number fertile	22	19	23	24
Suspected infertile	2	5	1	0
Intercurrent deaths	0	0	0	0
Mean (±SD) gestation interval (days)	21.3 ± 0.6	21.6 ± 0.6	21.5 ± 0.6	21.2 ± 0.7
Number of litters	22	19	23	24
Percentage mating	100	100	100	100
Fertility index	95.8	83.3	95.8	100

TABLE 6. Reproductive performance and fertility parameters[a]				
Observation	**Dose group (ppm)**			
	Control	**300**	**800**	**1500**
Conception rate	95.8	83.3	95.8	100
Gestation index	95.7	95.0	95.7	100
F_1 Generation				
Mean (±SD) precoital interval (days)	4.0	3.1	2.9	2.0
MALES				
Number mated	24	24	24	24
Number fertile	22	24	24	22
Suspected infertile	2	0	0	2
Intercurrent deaths	0	0	0	0
FEMALES				
Number mated	24	24	24	24
Number fertile	22	24	24	22
Suspected infertile	2	0	0	2
Intercurrent deaths	0	1	1	0
Mean (±SD) gestation interval (days)	21.5 ± 0.7	21.4 ± 0.7	21.3 ± 0.6	21.2 ± 0.4
Number of litters	22	24	24	22
Percentage mating	100	100	100	100
Fertility index	91.7	100	100	91.7
Conception rate	91.7	100	100	91.7
Gestation index	100	100	95.8	100

[a] Data obtained from pages 35, 40, 81, 82, 365, 366, 741-745 in the study report.

6. Parental postmortem results:

Organ weights: The report noted decreased absolute spleen weight and increased relative adrenals weight in females treated with 1500 ppm test material in the P generation. Additionally, there was a decrease in absolute and relative ovary weight at 1500 ppm. This change, however, was not considered related to treatment because there were no corresponding pathological or histopathological changes, and female fertility was not affected.

The report noted that no toxicologically significant changes were present in organ weights and organ:body weight ratios in the F_1 generation. Males of the 1500 ppm dose group showed statistically significantly decreased terminal body weights at necropsy, decreased absolute weights of the brain, liver, kidneys, spleen and epididymides, and increased relative weights of the brain, pituitary and kidneys. Females of the 1500 dose group showed statistically significantly decreased terminal body weights at necropsy, decreased absolute weights of the brain and uterus and decreased absolute and relative weights of the thyroids and ovaries. These changes were most likely associated with decreased body weights in this group across this generation (including decreased terminal body weights). In addition, no microscopic correlate was observed for the changes in the reproductive organs (epididymides, uterus and ovaries). Therefore, the changes were not considered a sign of toxicity.

Selected absolute and relative (to body weight) weight values for ovaries, spleen, and

adrenals of females in the P generation are presented in Table 7 below.

TABLE 7. Selected organ weights for P-generation females[a]

Organ (weight measurement)	Dose group (ppm)			
	Control	300	800	1500
Ovaries (absolute, grams)	0.183 ± 0.033	0.183 ± 0.022	0.172 ± 0.028	0.139 ± 0.022**
Spleen (absolute, grams)	0.813 ± 0.137	0.783 ± 0.148	0.722 ± 0.094	0.693 ± 0.161*
Ovaries (relative, %)	0.056 ± 0.009	0.056 ± 0.007	0.054 ± 0.009	0.045 ± 0.006**
Adrenals (relative, %)	0.027 ± 0.003	0.026 ± 0.003	0.027 ± 0.003	0.030 ± 0.003**

[a] Data extracted from pages 77 and 78 of the study report.

* Statistically different from control, $p<0.05$ (Dunnett's test based on pooled variance).

** Statistically different from control, $p<0.01$(Dunnett's test based on pooled variance).

b. Pathology:

1. **Macroscopic examination:** In the P parental generation, irregular surface of the forestomach was observed in four males administered 1500 ppm test diet. This was the only observation considered related to test-substance administration. In the F_1 generation, four males and one female of the 1500 ppm dose group also exhibited an irregular surface of the forestomach. Other findings in either generation were not considered treatment-related and were incidental.

2. **Microscopic examination:** The report noted the following observations in the P parental generation. Diffuse hyperplasia/hyperkeratosis was observed in the forestomach of nine males and ten females treated with 1500 ppm test substance. The severity was minimal to moderate and correlated with the gross observations in the stomach. Testicular tubular atrophy was observed in one mid-dose male. These observations were not considered to be treatment-related findings.

   In the F_1 parental generation, diffuse hyperplasia/hyperkeratosis of minimal to moderate severity was observed in the forestomach of eight males and six females in the 1500 ppm group, and correlated with the macroscopic observations of irregular surface of the forestomach. No other treatment-related findings were noted. Incidental findings included fetal loss/uterine inflammation, mammary adenoma in one control female, and hemopoietic foci in the spleen of one control female.

B. OFFSPRING:

1. Viability and clinical signs: The following findings were reported:

For F_1 pups, no treatment-related clinical signs of toxicity were observed at any dose. Incidental findings that were not considered to be treatment-related were little or no milk, small, pale, weak or cold appearance, exopthalmus, scabs, and reduced size of the left eye. One pup in the 300 ppm group had no tail. One pup in the 800 ppm group had a wounded

right ear that was later reduced in size, and two pups in the 1500 ppm group showed scabs on the tail apex, absence of nails, and a broken and/or curled tail apex. These observations were considered to be within normal biological variation.

F_2 pups did not exhibit any treatment-related clinical signs of toxicity at any dose. Symptoms that were not treatment-related included weak or cold appearance, no milk, alopecia, thickened area of the shoulder, eye discharge and opacity, missing tail, and reduced testes size. Additionally, one pup of the 300 ppm showed an absent tail and one pup of the 800 ppm group showed an absent right eye. These findings were considered incidental and were not considered to be caused by treatment.

Mean litter size and viability (survival) results from pups during lactation are summarized from the report in Table 8.

TABLE 8. Litter parameters for F_1 and F_2 generations [a]

Observation	Dose group (ppm)			
	Control	300	800	1500
F_1 Generation				
Mean implantation sites	15.1	12.7	14.0	15.4
Number born live	326	266	313	335
Number born dead	3	2	7	0
Sex ratio Day 0 (%)	53M / 47F	53M / 47F	53M / 47F	56M / 44F
# Deaths Days 0-4 (%)	3 (0.9)	6 (2.3)	3 (1.0)	5 (1.5)
# Deaths Days 4-21 (%)	2 (1.1)	0 (0)	2 (1.1)	2 (1.1)
Mean litter size Day 0	14.8	14.0	13.6	14.0
Day 4 [b]	14.7	13.7	13.5	13.8
Day 4 [c]	8.0	7.9	7.7	7.9
Day 7	NR	NR	NR	NR
Day 14	NR	NR	NR	NR
Day 21	8.0	7.9	7.6	7.8
Birth index [d]	90.9	87.9	95.0	90.8
Live birth index [e]	99.0	99.3	97.8	100
Viability index [f]	99.1	97.7	99.0	98.5
Lactation index [g]	98.9	100	98.9	98.9
F_2 Generation				
Mean implantation sites	14.8	16.4	15.5	14.9
Number born live	322	350	321	298
Number born dead	2	2	19	2
Sex ratio Day 0 (%)	48M / 52F	46M / 54F	50M / 50F	48M / 52F
# Deaths Days 0-4 (%)	9 (2.8)	6 (1.7)	1 (0.3)*	1 (0.3)*
# Deaths Days 4-21 (%)	2 (0.9)	10 (4.2)**	0 (0)	1 (0.5)
Mean litter size Day 0	14.6	14.6	13.4	13.6
Day 4 [b]	14.2	14.3	13.3	13.5
Day 4 [c]	9.8	10	9.5	9.6

TABLE 8. Litter parameters for F_1 and F_2 generations [a]

Observation	Dose group (ppm)			
	Control	300	800	1500
Day 7	NR	NR	NR	NR
Day 14	NR	NR	NR	NR
Day 21	9.7	9.5	9.5	9.5
Birth index [d]	91.5	89.6	90.7	84.0
Live birth index [e]	99.4	99.4	94.4	99.3
Viability index [f]	97.2	98.3	99.7*	99.7*
Lactation index [g]	98.6	95.8	100	99.5

[a] Data obtained from pages 82, 189, 366, and 475 in the study report.
[b] Before standardization (culling); calculated by adding the number of pups post-cull on day 4 plus the number of culled pups then dividing by the number of litters.
[c] After standardization (culling).
[d] Birth index = (total number of pups born [live and dead])/number of implantation scars.
[e] Live birth index = (# pups born alive/ # pups born) x 100.
[f] Viability index = (# pups alive on PND 4, pre-cull/ # pups born alive) x 100.
[g] Lactation index = (# pups alive on PND 21/ # pups alive on PND 4, post-cull) x 100.
NR=Not reported
* Statistically different from control, $p<0.05$ Fisher's exact test.
** Statistically different from control, $p<0.01$ Fisher's exact test.

2. **<u>Body weight</u>:** Offspring body weights were statistically significantly lower in both male and female F_1 pups in the 1500 ppm group from day 14 of lactation onwards. In the F_2 pups, body weights were statistically significantly lower in the 1500 ppm group from day 21 of lactation onwards. Theses changes were attributed to pups beginning to switch to feed and therefore increasing compound intake. Selected mean pup body weight data are presented in Table 9.

TABLE 9. Mean (±SD) Litter and pup weights (g) [a]

Lactation day	Dose group (ppm)							
	0	300	800	1500	0	300	800	1500
	F_1 Litters				F_2 Litters			
1	6.4 ± 0.4	6.6 ± 0.5	6.6 ± 0.5	6.4 ± 0.7	6.3 ±0.6	6.5 ± 0.5	6.5 ± 0.4	6.4 ± 0.6
4 [b]	NR	NR	NR	NR	NR	NR	NR	NR
4 [c]	9.4 ± 0.8	10.0 ± 1.2	9.8 ± 1.0	9.4 ± 1.4	9.3 ± 1.0	9.7 ± 1.0	9.8 ± 0.9	9.3 ± 1.2
7	15.1 ± 1.4	16.1 ± 1.6	15.7 ± 1.7	14.6 ± 2.1	14.3 ± 1.6	15.5 ± 1.5*	15.3 ± 1.3	14.2 ± 1.6
14	33.9 ± 2.1	35.5 ± 2.1	33.8 ± 3.1	30.8 ± 2.9**	29.9 ± 2.9	32.2 ± 2.4*	31.1 ± 2.4	28.1 ± 2.9
21	57.6 ± 3.5	59.7 ± 3.6	54.8 ± 5.1	47.1 ± 4.4**	48.0 ± 5.6	52.2 ± 4.1*	48.6 ± 4.9	41.7 ± 4.5**
	F_1 Pups – male				F_2 Pups – male			
1	6.5 ± 0.4	6.7 ± 0.5	6.8 ± 0.5	6.6 ± 0.7	6.5 ± 0.6	6.7 ± 0.5	6.7 ± 0.5	6.5 ± 0.6
4 [b]	NR	NR	NR	NR	NR	NR	NR	NR
4 [c]	9.6 ± 0.8	10.2 ± 1.2	10.1 ± 1.1	9.7 ± 1.5	9.5 ± 1.0	10.0 ± 1.0	10.0 ± 0.9	9.6 ± 1.2
7	15.5 ±1.4	16.4 ± 1.7	16.2 ± 1.6	15.0 ± 2.2	14.6 ± 1.6	16.0 ± 1.5**	15.7 ± 1.5	14.6 ± 1.7
21	59.0 ± 3.6	61.2 ± 3.9	56.4 ± 5.3	48.1 ± 4.7**	48.8 ± 5.8	53.6 ± 4.4**	49.6 ± 5.8	42.5 ± 4.9**
	F_1 Pups – female				F_2 Pups – female			
1	6.2 ± 0.4	6.4 ± 0.6	6.4 ± 0.5	6.2 ± 0.6	6.2 ±0.6	6.4 ± 0.5	6.4 ± 0.4	6.2 ± 0.6
4 [b]	NR	NR	NR	NR	NR	NR	NR	NR
4 [c]	9.1 ± 0.9	9.7 ± 1.3	9.6 ± 1.0	9.1 ± 1.3	9.1 ± 1.0	9.4 ± 1.0	9.6 ± 0.9	9.1 ± 1.2
7	14.8 ± 1.6	15.8 ± 1.8	15.3 ± 1.9	14.1 ± 2.1	14.0 ± 1.6	15.0 ± 1.5	15.0 ± 1.4	13.9 ±1.7
21	56.1 ± 4.0	58.2 ± 3.7	53.3 ± 5.1	45.9 ± 4.3**	47.1 ± 5.7	50.9 ± 3.9*	47.9 ± 4.7	41.0 ± 4.4**

[a] Data obtained from pages 83 and 367 in the study report.
[b] Before standardization (culling)
[c] After standardization (culling)
NR= Not reported
* Statistically different from control, $p<0.05$ (Dunnett-test based on pooled variance).
** Statistically different from control, $p<0.01$ (Dunnett-test based on pooled variance).

3. **Sexual maturation (F_1):** Sexual maturation was not affected by treatment, and no effects were seen in ano-genital distance or vaginal opening. The occurrence of balanopreputial separation (prepuce opening) was slightly delayed in males treated at 1500 ppm. This was considered to be caused by a slight delay in development due to lower body weights and was not considered to be treatment-related.

4. **Offspring postmortem results:**

   a. **Organ weights:** Absolute and relative spleen weights (male and male pups) for F_1 pups were significantly decreased at the high dose; this effect was considered treatment-related. Female pups at the 800-ppm dose level also exhibited decreased absolute and relative spleen weight; however, this effect was not considered treatment-related since the

values were within the historical control range; the magnitude of the change was slight; changes were not observed across generations; and changes were not consistent with males. Significantly decreased absolute and relative thymus weight for 1500-ppm females was not considered toxicologically significant since this effect was not observed in the second generation.

TABLE 10. Selected organ weights for F_1-generation (males and female pups) [a]

Organ (weight measurement)	Dose group (ppm)			
	Control	300	800	1500
MALES				
Spleen (absolute, grams)	0.340 ± 0.0529	0.362 ± 0.0691	0.317 ± 0.0599	0.244 ± 0.0481**
Spleen (relative, %)	0.59 ± 0.076	0.60 ± 0.087	0.55 ± 0.077	0.51 ± 0.078**
FEMALES				
Spleen (absolute, grams)	0.349 ± 0.0487	0.355 ± 0.0608	0.304 ± 0.0413**	0.246 ± 0.0480**
Spleen (relative, %)	0.61 ± 0.072	0.60 ± 0.084	0.56 ± 0.070*	0.54 ± 0.084**
Thymus (absolute, grams)	0.264 ± 0.0572	0.231 ± 0.0376*	0.236 ± 0.0415	0.183 ± 0.0292**
Thymus (relative, %)	0.46 ± 0.081	0.39 ± 0.053**	0.44 ± 0.060	0.40 ± 0.060**

[a] Data extracted from pages 86-87 of the study report.

* Statistically different from control, $p<0.05$ (Dunnett's test based on pooled variance).

** Statistically different from control, $p<0.01$(Dunnett's test based on pooled variance).

Absolute and relative spleen weights (male and male pups) for F_2 pups were significantly decreased at the high dose; this effect was considered treatment-related. All other changes in organ weights were not considered treatment-related.

TABLE 11. Selected organ weights for F_2-generation male pups [a]

Organ (weight measurement)	Dose group (ppm)			
	Control	300	800	1500
Spleen (absolute, grams)	0.276 ± 0.0892	0.269 ± 0.0356	0.240 ± 0.0597	0.194 ± 0.0390**
Spleen (relative, %)	0.55 ± 0.126	0.51 ± 0.090	0.49 ± 0.074 *	0.47 ± 0.078**

[a] Data extracted from pages 371-372 of the study report.

* Statistically different from control, $p<0.05$ (Dunnett's test based on pooled variance).

** Statistically different from control, $p<0.01$(Dunnett's test based on pooled variance).

b. Pathology:

1. **Macroscopic examination:** No significant findings were noted in F_1 or F_2 pups during macroscopic examination. Incidental findings that were not found to be dose-related or were within normal biological variation included small appearance, no milk, pelvic dilation of the kidneys, absent right eye, alopecia, reduced size testes

and/or epididymides, constricted spleen and cannibalism.

2) **Microscopic examination:** Microscopic examination data were not presented for the offspring.

III. DISCUSSION AND CONCLUSIONS:

A. CONCLUSIONS: The investigators concluded that the parental systemic LOAEL is 1500 ppm (96-111 mg/kg bw/day in males, 107-120 mg/kg bw/day in females for both generations when corrected for mean value of test article recovery), based on decreased body weight, hyperplasia/hyperkeratosis of the forestomach, decreased spleen weight, and increased adrenal weight. The parental systemic NOAEL is 800 ppm (43-45 mg/kg bw/day in males, 48-51 mg/kg bw/day in females for both generations when corrected for mean value of test article recovery).

The offspring LOAEL is 1500 ppm (96-111 mg/kg bw/day in males, 107-120 mg/kg bw/day in females for both generations when corrected for mean value of test article recovery), based on decreased body weight gain and decreased spleen weight. The offspring NOAEL is 800 ppm (43-45 mg/kg bw/day in males, 48-51 mg/kg bw/day in females for both generations when corrected for mean value of test article recovery). Reproduction parameters (mating performance, duration of gestation, fertility, number of implantation sites, number of live/dead pups at first litter check) and breeding parameters (postnatal loss, living pups on day 4 post partum, breeding loss, living pups on day 21 post partum, viability index, weaning index) were unaffected by treatment in the first and second generations. Therefore, the reproductive NOAEL is 1500 ppm (96-111 mg/kg bw/day in males, 107-120 mg/kg bw/day in females for both generations when corrected for mean value of test article recovery). Adverse findings which did not exhibit a dose-related effect or did not have histopathological correlations with macroscopic findings were not considered to be caused by treatment.

B. STUDY DEFICIENCIES: No deficiencies in the study were noted.

C. STUDY CLASSIFICATION: This study is **ACCEPTABLE - GUIDELINE** and satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800; OECD 416) in the rat.

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