001204

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

JUL 25 1980

MEMORANDUM

OFFICE OF TOXIC SUBSTANCES

SUBJECT: EPA Reg.#100-540, Terbutryn; 6(a)(2) data
CASWELL#1250; Accession#242568-73

FROM: William Dykstra
Toxicology Branch, HED (TS-769) WHD 6/26/80
CF 7/1/80

TO: Robert Taylor (25)
Registration Division (TS-767)

Recommendations:

1. The memo of January 20, 1978 from John Doherty to Robert Taylor indicates that some studies submitted were performed by I.B.T. and are required to be validated. These studies are I.B.T.#B904, A5458, H5457.

2. The 6-month dog study is acceptable as core-minimum data. The NOEL is 10 mg/kg/day (400 ppm).

3. The two-year mouse oncogenicity study is acceptable as core-minimum data. No oncogenic effects resulted from terbutryn at dietary levels up to 3000 ppm.

4. The two-year rat chronic toxicity oncogenicity study is acceptable as core-minimum data. Statistical analysis of the tumor incidence in the high-dose group, using Chi-Square one-sided test with Bonferroni adjustment, showed statistically significant incidences of thyroid follicular adenomas ($p < .05$), female mammary gland adenomas ($p < .05$) and female liver adenomas ($p < .05$). Male testicular cell adenoma ($p = 0.0752$) and female liver focal cytomegaly ($p = 0.08568$) were of borderline significance.

   These oncogenic effects from terbutryn fed rats trigger an oncogenic RPAR criteria.

5. The 3-generation reproduction study is acceptable as core-minimum data. The NOEL for the study is 300 ppm.

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Review:

1) Six-Month Oral Toxicity Study of Terbutryn Technical in Beagle Dogs (Elars Bioresearch Laboratories, Project No. 1421, Jan. 22, 1980)

Test Material: terbutryn technical, ARS#2080/78, Batch#FL-51552

Sixty purebred Beagle dogs, equally divided by sex and approximately four months of age, were used in this study. The dogs were identified by ear tatoos and individually housed in stainless steel cages in an environmentally controlled room. High Protein® was fed free choice with feed consumption measured daily for a weekly total. Fresh tap water was provided ad libitum . The dogs were dosed orally once a day for at least 180 days consecutively.

Treatment Design

Group	Daily Dose (mg/kg)	Number of Dogs
Control	Control - Sham dosed	8M, 8F
T-I	10 mg/kg	6M, 6F
T-II	25 mg/kg	6M, 6F
T-III	50 mg/kg	8M, 8F

Observations were made daily from seven days predose to the day before the individual dog was necropsied. Each dog had a separate sheet which contained parameters such as appetite, elimination, appearance, behavior and accompanying comments. Food consumption and body weight were measured weekly.

Ophthalmologic examinations were done at predose and at termination.

Specimens for clinical pathology were collected at 10 days pre-dosing and every month for the six month duration of the study. Hematology parameters included: leukocyte (total and differential), erythrocyte and platelet count, hematocrit, hemoglobin, mean corpuscular volume (MCV), retriculocyte count, methemoglobin, Heinz bodies, protime and activated partial thromboplastin time (APTT).

Clinical chemistry determinations included: glucose, BUN, total protein, total bilirubin, direct bilirubin, cholesterol, calcium, potassium, SAP, SGOT, SGPT and LDH.

Urine collection for urinalysis was done 8 days prior to initiation of dosing and then at 2, 4 and 6 months into the study. Urinalysis determinations included: color, specific gravity, pH, protein, glucose, ketones, bilirubin and urobilinogen, nitrite, blood, leukocyte count, erythrocyte count and the presence of epithelium, bacteria and triple phosphate crystals.

For the above tests, the dogs were fasted for 12 hours.

Dogs (AT08) of T-I was necropsied on March 11, 1979, day 24 of the study.

All dogs were fasted for at least 12 hours before sacrifice.

Gross observations were performed and samples of the organs listed below were taken.

Adrenals (2)
Esophagus
Heart
Colon
lung with bronchil
mammary gland (female)
muscle
trachea
ovaries or testes
pituitary
mandibular salivary gland (2)
parotid salivary gland (2)
small intestine
spleen
thymus
tissue mass (if present)
urinary bladder + ureters
Any gross lesions (if present)
Aorta
Brain (pons, cerebrum, cerebellum)
Epididymes (2)
gall bladder
Kidneys (2)
Liver
lymph nodes
nerve
pancreas
prostate (male)
skin
spinal cord
stomach
thyroids & parathyroids (2)
uterus
Bone marrow (sternum)
eyes (with optic nerve)

The following organs were trimmed in a uniform manner and weighed: heart, spleen, liver, kidneys (2), adrenals (2), thyroid and parathyroid (2), brain, testes (2), or ovaries (2) and pituitary.

Eight dogs, two males and females from control and T-III were allowed to undergo a recovery period of 29 days. These dogs were treated in exactly the same manner as during the six month study, with the exception that they were no longer given test material.

Results

One dog in TA-II (KR08) and five dogs in T-III (GD08, QU08, HF08, AD08 and FU08) showed symptoms which could be compound related. These dogs exhibited increased salivation, a pronounced response to sharp noises, grinding of teeth, and a marked change in behavior with the dog becoming increasingly apprehensive. There appeared to be no set trend or pattern concerning onset of symptoms which would occur irregularly during the six month period and last from four to six hours.

The compound seemed to have a more pronounced effect on one of the T-III dogs (GD08) than on any of the other dogs. On Feb. 21, 1979 and June 15, 1979, the dog exhibited a mild proprioceptive deficit manifested by an inability to return both knuckled hind feet to a normal position. The placing reflex was slow for both hind legs and the dog appeared dull, apprehensive, and unresponsive.

In conclusion, the dog manifested a mild to moderate posterior ataxia which lasted for approximately 24 hours. There was no significant differences in food consumption between the control and treatment groups. However, there appears to be a trend of reduced weight gain with increasing dose for the two higher levels. There were no lesions consistent through any dose group and no dose-related lesions observed in the ophthalmoscopic examination.

There were no significant differences in urine analysis between the control and treatment groups during the time period being considered. In addition, no trends could be detected.

In hematology, there appears to be a trend in the platelets count although there is not a significant difference. All groups of males show a decrease from predose to six months (termination) with T-III showing the greatest decrease. For females the trend is not as prominent. Significant differences from control values occur for T-I males at three months for reticulocytes and five months for APTT. For T-II males the significant difference occurs at six months for monocytes and T-II females at four months for hemoglobin.

These values, however, are within normal limits for these parameters and the values did not continue to be significantly different from control during suceeding bleeding periods. Significant differences were detected in cholesterol values of T-II females for months one and two, in the calcium values of T-III males for month two, and in direct bilirubin of T-I females for month three. Most of the values that were found to be significantly different were within the normal limits.

No drug related trend was found in electrophoretic scans of total protein. With respect to organ weights, there appears to be no significant difference between the control and treatment groups.

A T-I male dog in a moribund condition, AT08, was necropsied on March 11, 1979, the 24th day of the study. On gross examination, the lungs were dark red to pink in color, firm, congested and edematus. A forthy fluid was visible in the trachea. The mediastinal lymph nodes were swollen and edematous. A firm mass (9 x 13 mm) was attached to the gall bladder.

At necropsy three out of twelve nonrecovery dogs in the T-III group (50 mg/kg) had evidence of edema hemorrhage or mucosal thickening of various segments of the small intestine. Microscopic examination of these areas revealed evidence of hyperemia, and hemorrhage of Peyer's patches, intestinal mucosa and intestinal musculature.

The necropsy and microscopic examination of the four recovery dogs that were in Group T-III did not reveal any evidence of small intestine mucosal or musculature alterations. One dog in the Group III recovery animals (C408) did have gross and microscopic signs of very slight lymphoid hyperplasia in the colon. At necropsy one out of twelve animals in the T-II group (25 mg/kg) had evidence of thickening of the mucosa in segmental areas of the small intestine. Microscopic examination revealed very slight evidence of hyperemia in the Peyer's patches of the ileum, but the remainder of the small intestine samples were judged not remarkable. In the T-I group of animals, one animal was noted at necropsy to have a reddened area of mucosa in the jejunum. Microscopic examination this tissue sample was diagnosed as very slight mucosal congestion. It was also noted that this animal did not exsanguinate well at terminal sacrifice, which could account for the mucosal congestion.

Conclusion:

The low-dose dogs (10 mg/kg/day) in Group T-I are considered the NOEL for the study. The LEL is 25 mg/kg/day (T-II).

Classification: Core-Minimum Data

2) Two-Year Carcinogenicity Study in Mice (IRDC Report#382-005; April 21, 1980)

Test Material: terbutryn technical; ARS No. 2046/76; Batch No. FL-761552, 64 lbs.; white powder.

Two-hundred forty male and 240 female Charles River CD-1 mice, weighing from 23 to 32 grams and 18 to 24 grams, males and females respectively, were initiated in this study. The mice were housed individually in suspended wire-mesh cages and maintained in a temperature-, humidity- and light-controlled room. Water and the control and test diets were available ad libitum.

The mice were ear punched to identify the dosage-level groups. Ear punches were verified at each cage change and before necropsy. Terbutryn technical was offered daily in the diet at dosage levels of 3, 1000, and 3000 ppm. Sixty male and 60 female mice were used at each dosage level and in a control group. The control group received basal laboratory diet only, on the same regimens as treated mice.

The mice were observed three times daily (Monday-Friday) or twice daily (weekends and holidays) for signs of overt toxicity, moribundity and mortality. Detailed observations were recorded weekly. Individual body weights and sex-group food consumptions were recorded every four weeks.

All surviving mice were sacrificed and necropsied at termination of the study. Necropsy examination consisted of examination of the external body surface and body orifices. The mouse was then opened and the contents of the body cavities were examined in situ, removed and again examined. All organs, tissues and remaining eviscerated carcass were then collected and mixed in phosphate buffered 10% formalin. Mice which died or were sacrificed in extremis during the course of the study were necropsied as above.

Hematoxylin and eosin stained paraffin sections of the following tissues were prepared by standard histologic methods and examined microscopically from all mice from the control group and the 3000 ppm dietary group. Microscopic examination of tissues from the 3 and 1000 ppm dietary groups was limited to gross lesions observed at necropsy.

Brain
spinal cord (3 sections)
sciatic nerve
eye
optis nerve
pituitary
spleen
liver
kidney
urinary bladder
testes or ovaries
prostate or uterus
mammary gland (females)
salivary gland
esophagus
skin
thyroid
parathyroids
adrenals
trachea
lung
heart
aorta
stomach
small intestine
large intestine
pancreas
lymph nodes
bone marrow (sterum)
skeletal muscle
any other tissue with gross lesions

Results

No changes considered to be related to compound were observed in general behavior and appearance. No difference considered to be related to compound were observed in body weight changes of treated mice as compared with controls. No differences considered to be related to compound were observed in the food consumption values of treated mice as compared with controls.

Survival at week 104 was as follows:

Dosage Level ppm	Number Surviving/ Number Initiated	
	Male	Female
0 (control)	29/60	35/60
3	29/60	34/60
1000	28/59*	30/60
3000	34/60	38/60

*one mouse missing

No gross pathologic lesions which were considered related to Terbutryn technical were observed in any mice from the experimental groups. No microscopic pathologic lesions which were attributable to Terbutryn technical feeding were observed in any tissues examined from any mice from the experimental groups.

Conclusion:

Terbutryn technical was not oncogenic to mice at dietary levels up to 3000 ppm.

Classification: Core-Minimum Data

3) 2-Year Chronic Oral Toxicity Study in Rats (IRDC Report No. 382-008; March 27, 1980)

Test Material: Terbutryn technical, ARS No. 2046/76, Batch No. FL-761552, 65 lbs.

Two-hundred sixty male (weighing from 128 to 225 gms) and 260 female (weighing from 115 to 181 gms) weanling Charles River CD rats were initiated in this chronic oral toxicity study. Five animals of each sex from the control a d 3000 ppm groups were sacrificed and necropsied after 12 months of study. At that same time, a second 5 rats/sex in each of these groups were placed on a control diet for 4 weeks before being sacrificed and necropsied. The rats were housed individually in hanging wire-mesh cages and maintained in a temperature-, humidity- and light-controlled room. Metal ear tags were placed on the rats approximately 3 months after study initiation.

The rats were randomly distributed into groups and offered the appropriate diets as follows:

Dosage Level ppm	No. of Rats/Group	
	Male	Female
0 (control)	70	70
2	60	60
300	60	60
3000	70	70

The rats were observed twice daily for signs of overt toxicity and mortality. Detailed observations were recorded weekly. Individual body weights and food consumption (10 rats/sex/group) were recorded weekly for the first 3 months and monthly thereafter.

During the 4-week withdrawal period, individual body weights and food consumption were determined weekly for the recovery rats. At 3, 6, 12, 18 and 24 months, blood for hematologic and biochemical studies and urine for urinalysis were collected from 10 rats/sex from the control and high-dose groups. At 12 and 18 months of study, rats from the

low- and mid-dose groups were also selected for testing. Hematologic examinations were done for the recovery rats after they had completed 4 weeks of compound withdrawal.

Microscopic examinations were conducting upon the following tissues, preserved in buffered neutral 10% formalin, taken from the 12-month interim sacrifice, all surviving animals in the control and high-dose groups at the 24 month sacrifice, as well as from all rats in these groups which died on study. These were prepared by standard histologic method using hematoxylin and eosin.

stained parafin sections	pancreas	aorta
adrenal gland	bone marrow	sciatic nerve
parathyroid	pituitary	cecum
brain	colon	skin
salivary gland	small intestine	eye
esophagus	spinal cord	harderian gland
gonads	heart	sterum
spleen	stomach	liver
kidney	thyroid	lymph node
lung	urinary bladder	mammary gland
trachea	muscle	optic nerve
uterus		

The following tissues were taken from all low- and mid-dose animals at 24-month sacrifice:

testes	thyroid
liver	mammary gland

Significant gross lesions were also examined microscopically. All tissues from the 12-month sacrifice and rats which died on study up until 12-months were processed and examined microscopically at IRDC. Tissues from all other animals were processed and examined by Experimental Pathology Laboratories (Dr. J.F. Ferrell, D.V.M.). Statistical analysis of the data was performed.

Results

There were no signs of overt toxicity noted for the treated rats in the regular and recovery groups. No compound-related effects were observed on the survival rate of the treated rats.

Survival at 104 weeks of study was as shown below:

Dosage Level (ppm)	No. Survivors/No. Initiated	
	Male	Female
0 (control)	38/60	35/60
2	41/60	32/60
300	47/60	40/60
3000	44/60	38/60

Survival for the 4-week recovery period was 100%.

The high-dose males and females showed a large decrease in body weight throughout the study [week 104: males)-21.7%) and females (-30.0%)]. Decreases were noted in the food consumption values over the 2-year study for both male and female rats at the high-dosage level. No changes indicative of a compound effect were noted in the hematologic profile or urinalysis of the groups tested.

Blood biochemical values showed some apparent compound-related effects in the high-dose females.

Significant changes were seen in the fasting blood sugar, SAP, SGOT and cholesterol values at various intervals. These effects indicate that the test material caused physiological significant changes.

There were occasional statistically significant variations in organ weight but the biological significance of the variations noted in the organ weights is unknown.

The histopathological evaluation of non-neoplastic lesions indicated that, with the exception of focal cytomegaly, non-neoplastic lesions were randomly distributed between the control and 3000 ppm groups.

The focal pneumonitis was considered to be of dubious significance.

Statistical analyses of the tumor incidence demonstrated significant tumor incidences in the high-dose group. Thyroid follicular adenoma in males was statistically significant.

In females, mammary gland adenoma and liver adenoma were statistically significant. Borderline statistical significance occurred in testicular interstitial cell adenoma in males and liver focal cytomegaly in females. These results are summarized in Table I.

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TABLE I: Tumor Incidence

Tumor Type	Control	Low Dose	Mid Dose	High Dose	Chi Square	P of 2 Side test	P of 1 Side test	With Conferroni's Adjustment
Males								
Thyroid follicular adenoma	1/65	0/59	0/60	7/65	4.795	.02930	.01465	.04395*
Males								B
Testicular interstitial cell adenoma	13/65	11/60	14/60	23/65	3.841	.05001	.02501	.07502
Females								
Mammary gland adenomas	6/64	9/57	8/58	15/64	4.628	.03145	.01573	.04718*
Females								B
Liver focal cytomegaly	9/64	13/59	9/59	18/65	3.619	.05712	.02856	.08568
Females								
Liver adenoma	3/64	2/59	3/59	12/6[illegible]	5.954	.01468	.00734	.02202*

* = significant
p < .05
B = borderline significant

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Conclusion

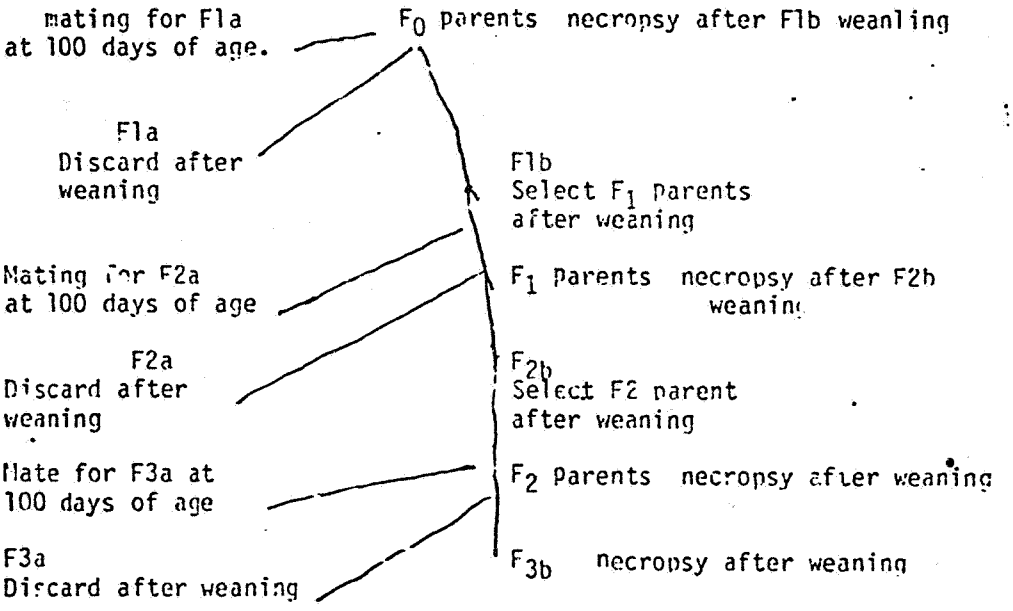
Terbutryn technical was oncogenic at the high-dose in both males and females.

Classification: Core-Minimum Data

4) Three-Generation Reproduction Study in Rats (IRDC Report No. 382-011, Oct. 8, 1979)

Test Material: terbutryn technical, ARS No. 2046/76, Batch No. FL-761552, 65 lbs., white powder

In this three generation reproduction study in Charles River-CD rats, the test material was administered in the diet at dosage levels of 2, 300 and 3000 ppm. Forty male and 80 female rats were evenly distributed among the three treatment groups and one control group. Each generation was mated twice to produce two litters. The matings scheme is shown below:



Results

No changes considered to be related to treatment of test material were seen in relation to the general behavior, appearance or survival of the treated parental rats when compared to the controls. Dam#41958AA in the F_2 generation (3000 ppm) died and was replaced by dam#41974BC on study week 65.

Moderately to severely reduced mean body weights were seen in the 3000 ppm groups during the entire generation of the F_0 parents, with significant differences mean body weights reported at study weeks 9 and 34 when compared to the control groups. In the F_1 generation at the 3000 ppm level, severely reduced mean body weights in the males and moderately to severely reduced mean body weights in the females were seen as compared to control values with significant differences in mean body weights at the $p < .01$ level at all points of analysis (weeks 33, 42 and 64).

In the F_2 males, slightly to moderately reduced body weights were seen throughout the entire generation in the 2 ppm and 300 ppm dosage groups; but, there were no statistically significant differences in these changes as compared to control values. Severely reduced mean body weights were observed in the 3000 ppm group, with a significant difference in mean body weight at the $p < 0.01$ level when compared to the control group at all points of the analysis (weeks 63, 73 and 94).

At the 3000 ppm level, moderate to severe decreases in mean body weight were also seen in the F_2 females during the entire generation, with significant differences in mean body weights at the $p < 0.01$ level when compared to the control groups at all points of analysis (weeks 63, 73 and 94). All other treatment groups were comparable to the control groups in respect to parental body weights.

Based on the average of mean food consumption values of parental rats, a slight decrease in mean daily food consumption was observed in the F_0 males in the 3000 ppm dosage group as compared to controls.

The F_0 females exhibited a slight decrease in mean daily food consumption values in the 300 ppm group and a moderate decrease in the 3000 ppm group, as compared to the control group. All other treatment groups showed no meaningful or biological differences when compared to the control group in mean daily food consumption.

Lower female fertility indices were seen for the F_0 females at the 3000 ppm dosage level in both the F1a and F1b litters, with a statistically significant difference at the $p < .05$ level reported in this high dose group in the F1b litter when compared to the control group.

In the F3a litters, a lower male fertility index was observed at the 3000 ppm level; but there was no statistically significant decrease observed in the 5 day survival index at the 3000 ppm level ($p < 0.1$) in

the F1a litters when compared to their respective control. A statistically significant decrease was also observed in the number of live pups at day 0 of lactation at the 300 ppm level in the F1a litters when compared to control values. At the 2 ppm level in the F1b litters, a statistically significant increase was reported in the number of live pups at day 0. However, this difference was attributed to random occurrence and was not considered a result of treatment.

No other differences were observed with respect to male and female fertility, the length of the gestation periods and the viability and survival of the pups through weaning.

The mean pup body weights at lactation day 21 of each of the six litters produced (F1a, F1b, F2a, F2b, F3a, F3b) at the 3000 ppm treatment level were statistically significantly lower ($p < 0.01$) than the mean weights of the control pups. Also, the mean pup weights of both sexes in the F2a at the 300 ppm treatment level were statistically lower ($p < 0.05$) when compared to the control values. No significant gross lesions were observed in the F_0 and F_1 parents. In the F_2 parental females, two animals each of group II and III and three animals of group IV out of 10 females in each group had hydrometra. In the F3b generation weanlings, two males and three females of group IV were smaller in size. The animals dying on study showed mainly autolysis. One animal in the 2 ppm F2 parental group had protrutary enlargement.

No histopathological change of significance was observed in F_0 and F_1 parents. In three out of 10 animals in group IV had atrophy of the endometrium with dilatation of the uterine lumen to a moderate degree. In the F3b generation weanlings belonging to group IV, two males and two females of 10 of each sex had very slight to slight lymphoid hyperplasia in the colon.

Statistically significant variations in the absolute/relative weights of organs of the parental rats were seen as follows:

F_0 parents

Organ	Dosage Level (ppm)	Sex	Weight	Change	p<
liver	3000	M	relative	increase	0.05
	3000	F	relative	increase	0.01
kidneys	3000	F	relative	increase	0.01
gonads	3000	M	relative	increase	0.05
heart	3000	M	absolute	increase	0.01
	3000	M	relative	increase	0.01
	3000	F	relative	increase	0.05
brain	3000	M	relative	increase	0.01
	3000	F	relative	increase	0.01

F_1 Parents

Organ	Dosage Level (ppm)	Sex	Weight	Change	p<
liver	300	M	absolute/relative	increase	0.05,0.01
	3000	M	relative	increase	0.01
	3000	F	relative	increase	0.01
kidneys	2	M	absolute	increase	0.05
	300	M	absolute	increase	0.01,0.05
	3000	M	relative/relative	increase	0.01
	3000	F	absolute	decrease	0.05
spleen	3000	F	absolute	decrease	0.05
gonads	2	F	absolute/relative	increase	0.05,0.01
	3000	M	relative	increase	0.01
brain	3000	M	relative	increase	0.05

F_2 Parents

Organ	Dosage Level (ppm)	Sex	Weight	Change	p<
liver	2	M	absolute/relative	decrease	0.05
	3000	M	absolute	decrease	0.01
	3000	F	absolute	decrease	0.01
kidneys	3000	M	absolute/relative	decrease	0.05
	3000	F	absolute/relative	decrease	0.05
gonads	3000	M	relative	increase	0.01
	3000	F	relative	increase	0.05
heart	3000	M	absolute/relative	decrease/ increase	0.01
	3000	F	absolute	decrease	0.01
brain	3000	M	relative	increase	0.01
	3000	F	absolute/relative	decrease/ increase	0.05

Conclusion

The NOEL for the study is considered to be 300 ppm. Although, effects on number born alive occurred in F1a and a significant decrease in pup body weight occurred in F2a, these effects did not occur in any other generation at this dose level and are therefore not considered biologically meaningful. The significance of the change in organ weights is unknown since no histopathology was observed. The lowest effect level (LEL) is 3000 ppm (the high dose group).

Classification: Core-Minimum Data

11pp for WLB per

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