2-25-84

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

003660

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No. 10308-1. Review of rat fertility studies and rat and rabbit teratology studies with tetramethrin (neopynamin) and of additional pathology information regarding the significance of testicular adenomas in the rat oncogenicity studies (Vesselinovitch and Ito report) and of a cancer risk assessment for neo-pynamin (Carlborg report).

Tox. Chem. No. 844

TO: T.A. Gardner, PM #17
Registration Division (TS-767)

THRU: William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Background:

Previous review of two rat oncogenicity studies (see J. Doherty review dated April 11, 1983 for EPA Reg. No. 10308-1) indicated that the testes was a target organ for an oncogenic effect of tetramethrin (neopynamin). The registrant has now submitted a detailed analysis of the histopathology of the testes for these oncogenicity studies which included a reexamination of the slides of the testes and a discussion of the biological significance of the tumor type produced. The registrant also presented a cancer risk assessment based on a submitted exposure assessment and these microscopic findings. These two reports are reviewed below. The submitted exposure assessment was referred to Exposure Assessment Branch for review and comment.

The registrant has also submitted teratology studies in rats and rabbits and fertility studies in rats (not multigeneration reproduction studies). These are also reviewed below.

Comments:

1. The rat fertility studies were reviewed and found to be CORE SUPPLEMENTARY. Although the studies were apparently well executed, the protocols were

judged to be inconsistent with the conventional 2-3 generation reproduction study recommended by TB. In particular, the rats on these studies should have been continuously dosed with the test material through the 2-3 generations produced. A copy of the recommended procedures for a multigeneration reproduction study is attached.

2. The teratology studies in rats and rabbits were reviewed and found to be CORE GUIDELINES.

3a. The two reports concerning the rat testes pathology data and the cancer risk assessment were reviewed and TB acknowledges their receipt. Both of these reports are summarized and commented on later in this same review. These reports will again be considered when TB performs the risk assessment for this chemical.

3b. The summary tables presented in the Vesselinovitch and Ito report contain an error concerning the number of control rats in the 1974 Sprague-Dawley study. There were 50 control rats and not 40. Thus the total number of Sprague-Dawley rats for both studies combined is 99 and not 89. This change may adjust the statistical significance of the data toward a slightly more positive effect.

3c. Table 7 of the Vesselinovitch and Ito report concerning historical control data for the spontaneous development of interstitial cell tumors in the rat testes is not precisely clear with respect to the identification of the numerator. ~~For example~~, The registrant should be asked to clarify if the numerator represents the number of rats with tumors or the total number of tumors in the testes of the rats in the group.

This table is also considered to be of limited usefulness because of the low numbers used for the denominators. In order for the information present in Table 7 to be more meaningful, the number of rats at the start of each experiment and the number of rats surviving to the time when the rats were at risk for development of this type of tumor should be provided.

4. TB will conduct a risk assessment based on the neoplastic effect noted in the rat testes.

5. There were several other requests from TB to the registrant made in the memo of April 11, 1983 (EPA Reg. No. 10308-4) which have not yet been submitted by the registrant. This information must be submitted before the rat oncogenicity studies can be upgraded to CORE MINIMUM.

2/14/84

John Doherty
Toxicology Branch
Hazard Evaluation Division
(TS-769)

2/15/84

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Studies and Reports Reviewed

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Study/Report	Result	CORE Classification
Teratology - rats	Not teratogenic at up to and including 1000 mg/kg/day	GUIDELINES
Teratology - rabbit	Not teratogenic at up to and including 500 mg/kg/day	GUIDELINES
Fertility - rats (Prepregnancy, mating and early pregnancy period).	NOEL = 300 mg/kg/day LEL = 1000 mg/kg/day. At this level, an increased latency of copulation is noted. Other systemic effects included decreased pup weight and size.	SUPPLEMENTARY
Fertility - rats (Perinatal and postnatal development).	No effects on pups noted at 1000 mg/kg/day (HDT)	SUPPLEMENTARY
Pathology of the testes report (Vesselinovitch and Ito report).	[see report]	N/A
Cancer risk assessment (Carlborg report)	[see review]	N/A

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A. Reproduction Test of Neopynamin Part 1:

Fertility study in rats

Hamamatsu Seigiken Research, Japan, IT-01-0075
June 14, 1980. EPA Acc. No. 248342, Tab. 1

B. The test material used for this study was Neo-pynamin. It was from lot number 90508 and was supplied by the Sumitomo Chemical Co. The percent purity was not stated. The test material was suspended in 0.5% sodium carboxymethyl celluose.

C. The test animals used in this study were Slc:SD strain rats obtained from a Japanese supplier. Four test dose levels were used: 0, 100, 300 and 1000 mg/kg/day and there were 20 rats of each sex per dose group. [Note: The dose levels were selected based on a preliminary experiment which showed that mature rats of this strain dosed with 1500 mg/kg developed increases in liver (51% males and 20% females) and kidney weight (12%, males only). At 750 mg/kg there was noted an increase in liver weight (39% males and 9% females). The low dose male group (375 mg/kg/day) had liver weight increased by 24%. The test material was administered by stomach tube each day for 9 weeks for males (starting at age 6 weeks) and each day "after mating with females until success or failure of pregnancy was recognized." The females were dosed from 2 weeks before the start of mating at age 11 weeks and continued until the 7th day of gestation.

D. No data confirming that the desired dose levels were achieved was presented.

E. General reactions to treatment. No males or females died as a result of test chemical treatment. Salivation was reported in the males only (no table describing the onset and duration of the behavioral responses was presented). In the male groups, there were no meaningful differences in weight gain noted (the low dose test group was reported as being slightly higher in weight). In females, the high dose test group showed a slightly lower ($P<0.05$) body weight gain at termination (-5%).

F. Organ weight changes in parents.

i. The liver weight of males was increased in all dose groups. Relative liver weights were +9.6%, +16.4% and +44.6% for the low, mid and high dose groups respectively.

ii. The kidney weights for males were also increased as follows (for relative weight): +6.5% (significant), +4.5% (not significant) and +18.3% (significant) for the low, mid and high dose groups respectively.

iii. The spleen weights for males were increased as follows (for relative weight): +6.5% (not significant), +13.3%, and +12.6% for the low, mid and high dose groups.

There were no statistically significant changes in the organ weights of the females (the females were not dosed as frequently as the males).

Of these organ weights changes, the liver at all dose levels (see also the preliminary experiment) and kidney are considered to be affected by the test material. Only the high dose test group is affected by changes in kidney weight.

F. Reproductive performance Pairs of males and females were mated. If pregnancy did not result a second trial with a proven male was made or the male was allowed to mate with an untreated female. The test report claims that "the pregnancy race and reproductive rate of each group were 95 to 100%, and the percentage was not lowered by administration of the test material." It was noted that one female in the mid dose group and one male in each of the mid and high dose groups were not reproductive. Examination of the gonads of these rats did not determine a cause for this sterility. It was also noted that the average number of days required from start of mating until copulation was seven days for the high dose test group but was only 3 or 4 days for each of the other groups. The increase was statistically significant. A NOEL of 300 mg/kg is set for adverse effects on reproduction. At 1000 mg there is an increased latency for copulation.

G. Teratogenic aspects. [Note this aspect of the study cannot be used as an acceptable teratogenic study because the test material was administered only on days 1-7 of pregnancy and not the period of major organogenesis.]

There were 20, 20, 19 and 20 pregnant dams in the control, low, mid and high dose test groups.

The mean number of corpora lutea and implantations, were lower in the high dose group than in the controls. Implanation loss was not affected by treatment and there was no increase in fetal deaths.

There were 274, 280, 270 and 249 fetuses for the control, low, mid and high dose test groups. The sex ratio was near 1 for all groups.

The average body weight (-7%) and body length (-3%) were statistically significantly smaller for the high dose group when compared with the controls.

One-third of fetuses from each mother were examined by Wilson's technique and the remaining two-thirds were examined for skeletal defects. No evidence of dose related teratogenic effects were noted. Signs of delayed ossification were noted in the high dose test group.

Conclusions: This study is CORE SUPPLEMENTARY. Reproductive studies are customarily carried out by feeding the test material in the diet throughout mating, gestation and into the next generation.

As a teratology study, the study is also CORE SUPPLEMENTARY because the dosing was not administered during the major period of organogenesis.

The study does provide useful information in that it is demonstrated that the test material (tetramethrin) has no adverse effects at up to 300 mg/kg, but at 1000 mg/kg some effects on reproductive performance (delayed onset of copulatory behavior) and on fetal development (fetal weight, size and delayed ossification) result.

A. Reproduction Test of Neopynamin Part 4: Perinatal and Postnatal Study in Rats

Hamamatsu Seigiken Research, #IT-01-0078
June 14, 1980, EPA Acc. No. 248342, Tab 4.

B. The test material used for this study was Neopynamin (lot No. 90508) and was supplied by the Sumitomo Chemical Co., Ltd. The exact purity of the test material was not provided in the study report.

C. The test animals used were female Slc:SD rats (SPF) and were purchased from a Japanese supplier. The main aspect of this experiment consisted of 20 pregnant female rats (made pregnant by mating with a male) in each of 4 dose groups. The dosing groups consisted of the 0, 100 mg/kg, 300 mg/kg and 1000 mg/kg of neopynamin dissolved in 0.5% sodium carboxymethyl cellulose which was orally administered by gavage. The rats were dosed with the test material from day 7 of pregnancy till 21 days after delivery. [Note: The high dose level was selected based on a preliminary experiment which showed that 1000 mg/kg could be tolerated by the pregnant rats when dosed on day 17 of pregnancy to 7 days postpartum.] After the pups were weaned, the dams were sacrificed and necropsied. The pups were culled and allowed to mature and subjected to a variety of tests to determine their overall development. For example, two males and two females from each dam were allowed to develop. The other pups were sacrificed at 21 days and assessed for teratogenic effects.

Results:

D. General reactions in the treated dams. No dams died and no signs of toxicity (behavioral) were reported as occurring. No dose related changes in net body weight were reported.

E. Gross necropsy and organ weight changes in the treated dams. The only organ which showed evidence of being affected was the liver. The liver showed signs of swelling and was increased in relative weight at 300 mg/kg (+7%) and 1000 mg/kg/(+14.4%). Changes in liver weight would be an expected result of dosing with neopynamin.

F. Intrauterine data in the treated dams. There was no effect noted on the gestation period, number of implantations per liter, number of live pups, rate of birth, sex ratio, body weight (the high dose group was 4% lower than the control), number of stillbirths, or survival rate.

G. Nursing period (postpartum to weaning). There was some evidence of reduced weight gain in the rats dosed with 100 and 1000 mg/kg, but the differences were small and no dose response was evident.

H. Growth and sensory function tests. Assessments were made on the number of days required for development of auricle, covering of abdominal part with hair, emergence of incisors and opening eyes and development of the visible

genital organs (descent of testes and opening of vagina). No adverse effects of the insecticide were noted on any of these parameters.

I. Sensory function. Sight was assessed by the visual placing response, hearing was assessed by Preyer's reflex (involuntary ear movement in response to a noise), the sense of pain was assessed using the pain reflex. Also the surface righting reflex and the air righting reflex was used to assess motility. The test report maintains that there were no effects of the test material. TB reviewer notes that vision, hearing, pain sense and motility were affected in more animals in the mid dose group than in the other groups. However, because of a lack of a dose response and because only about 5-6% of the pups were affected (except for motility), this observation is considered incidental.

J. Teratogenic assessment of the pups.

There were 243, 227, 237 and 252 pups available for external examination for the control, low, mid and high dose test groups. After taking pups out for development there were 116, 114, 116, and 117 pups available for both internal assessment and skeletal assessment for the control, low, mid and high dose test groups. No signs of dose related teratogenic affects were noted. The weights of the main organs were assessed at 3 and 8 weeks but no signs of consistent dose related effects were noted.

K. Tests run to assess the emotional state (open field test), motor coordination (rota rod test) and learning ability (water filled T-maze test) were conducted but no dose related adverse effects were noted.

L. Reproductive ability test consisted of mating males with females in the same dose groups (avoiding brother and sister pairings). Only a single female in the treated groups (300 mg/kg) and one in the control group were found to be sterile (the cause could not be determined). Thus, neopynamin did not affect the reproductive capacity of offspring of dams dosed with up to and including 1000 mg/kg of neopynamin.

M. The rats that were made pregnant in the reproductive test were allowed to carry their pups which were delivered by Caesarian section on day 20. There were no adverse effects on the intrauterine data or appearance or size of the pups.

CONCLUSION: As a reproductive study this study must be classified as CORE SUPPLEMENTARY - the test material was not administered in the diet through 2 (or more) successive generations.

This study does provide interesting and useful information in that it demonstrates that tetramethrin does not have effects on the dams or the pups when administered from day 7 of pregnancy to day 21 postpartum at doses up to and including 1000 mg/kg.

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A. Reproduction Test of Neopynamin Part 2: Teratology Study in Rats

Hamamatsu Seigiken Research, Japan, #IT-01-0076
June 14, 1980. EPA Acc. No. 248342, Tab. 2

B. The test material for this study was neopynamin and was from lot #90508. The purity of the lot was not stated.

C. The test animals used were male and female Slc:SD rats (SPF) and were obtained from a Japanese supplier. The females were mated with a male and copulation was confirmed by checking for the presence of a vaginal plug. The main study consisted of four groups of 30 pregnant rats. The dose levels used were 0, 100, 300 and 1000 mg/kg/day. Preliminary investigation revealed that the highest test dose level technically possible was 1500 mg/kg/day. At this level, although no rats died, there was evident an increase in liver weights. The test material was suspended in 0.5% carboxymethyl cellulose and administerd by stomach tube for an 11 day period from day 7 to 17 of pregnancy. [Note: The dose level administered was based on the day 7 body weight].

In this study 20 of the 30 dams were sacrificed at day 20 of pregnancy and the remaining 10 were allowed to deliver naturally. The 10 dams were allowed to live for 2 additional weeks. The pups from the groups that were allowed to deliver naturally were subjected to a series of tests to determine any adverse effects of in utero exposure (see below).

No positive control group was run concurrently.

D. Effects on the dams. No rats died and no clinical toxic signs were reported to result in the animals dosed at any dose level. Only slightly lower body weight changes were noted (they were reported as being statistically significantly lower in the high dose test group). There were tendencies for reduced food consumption and increased water consumption during administration of the test material.

After Caesarian section or terminal sacrifice, the weights of the heart, lung, spleen, liver, kidney and ovary were determined for both sets of dams. Of these organs, the liver, kidney and ovary were found to be increased in weight for the group that was sacrificed at the time of Caesarian section. The weight of these organs were reported as equivalent to the controls for the group sacrificed 2 weeks after delivery.

The relative liver weights were 4% higher for the mid and 12% higher for the high dose group.

The kidney weight was 7.3% higher for the high dose test group.

The ovary weight was 9.3% higher for the high dose test group.

E. Intrauterine Data. There were no statistically significant differences noted with regard to the number of the corpora lutea or implantations (although the number of implantations was slightly higher in the high dose test group). There were no dose related differences noted with respect to fetal deaths.

F. Fetal data: There were 242, 248, 251, and 260 live fetuses for the controls, low, mid and high dose test groups. There is noted a slightly higher number of fetuses in the high dose test group. The sex ratio was 0.86 for the control group but 1.32 for the high dose test group. The low and mid dose test groups were 1.02 and 0.86 respectively. This increase in the sex ratio is considered only as a curiosity of the study. The fetal body weights, lengths, and tail lengths were all reported to be equivalent to the controls.

There were no pups found to have external abnormalities.

The internal abnormalities of the viscera included "remnant of the azygos vein," "esophagectasis" and "serpiginous aorta" in the low dose test group. Three rat pups affected came from two different litters. In the mid dose group there were single incidences of "remnant of azygos vein" and "diaphragmatic hernia." Each rat was from a different litter. There were no internal abnormalities reported in the control or high dose test group. There were 79, 81, 82 and 84 fetuses, reported to be examined for the control, low, mid and high dose test groups.

There were no skeletal abnormalities reported after examining 163 control, 167 low dose, 169 mid dose and 176 high dose group fetuses. Skeletal variation was not affected by the test material. No effects were noted in the degree of ossification.

G. Effects in pups allowed to develop. 10 dams from each dose group were allowed to deliver and nurse their pups. The pups were then tested for any adverse effects of the in utero exposure.

There were no compound related effects noted with respect to period of pregnancy, delivery data such as number of pups, pup body weight, and body weight gain, nursing or body weight gain after nursing.

The special inspections of the pups allowed to develop included separation of auricle, emergence of abdominal hair, eruption of lower incisors, separation of the eyelid, descent of testes, opening of vagina, vision, hearing, pain sense and motility. None of these (except motility) appeared to be affected by the test material. Motility was higher in the controls (8 incidences in 91 offspring) compared to the high dose test group (0 incidences in 100 offspring).

External, internal, and skeletal examinations were made on the pups (some were allowed to continue developing) at the 3rd week postpartum. No effects

of the test material were noted. At three weeks, however, there were noted increases on kidney (+9.7% for males and +22.6% females) and testes weights (29.8%).

At 8 weeks of age only the kidney of males showed some signs of persistent weight difference (+17%) that was statistically significant for absolute weight but not relative weight.

A series of tests were conducted to assess motor coordination, emotionality and learning ability. Emotionality was assessed by an open field test where the distance walked, number rearing, grooming, defecating and urinating in 3 minutes were determined. Motor coordination was assessed using a rota rod test. Learning ability was assessed when the pups were six weeks old by using a water filled multiple T-maze. These studies resulted in a statistically significant increase in the number of face washings in the high dose test group only. No other indications of abnormal effects were reported.

The selected pups from each group were also reared until they were 11 weeks of age and fertility/teratology assessments carried out. There was a single group which did not mate in the high dose test group. Body weight gain was reported to be highest in the high dose test group. None of the other parameters related to a fertility/teratology study were affected except that there were reported to be higher numbers of corpora lutea, implantations and surviving fetuses in the high dose group.

CONCLUSION. This study in CORE GUIDELINES. No positive control was run concurrently. Tetramethrin was shown not to be teratogenic at doses up to and including 1000 mg/kg/day. Some signs of toxicity were noted in the dams which included body weight and organ weight changes (liver, kidney and uterus). No signs of fetotoxicity developed. The special aspect of this study which studied the development of the fetuses through adulthood (one generation) also did not indicate adverse effects of tetramethrin.

An item of interest in this study is that for both the main phase and second phase (growth of the pups) there were noted <u>increases</u> in viable pups and implantations. This phenomena was also noted in the rabbit study.

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A. Reproduction Test of Neopynamin Part 3: Teratology Study in Rabbits

Hamamatsu Seigiken Research, Japan, #IT-01-0077, June 14, 1980, EPA Acc. No. 248342, Tab 3.

B. The test material used in this study was neopynamin and was from lot no. 90508. The purity of the test material was not stated.

C. The test animals used were white rabbits described as being "Japanese white rabbits." In the main aspect of the experiment 4 groups of 10 pregnant rabbits were dosed with either 0, 50, 150, or 500 mg/kg/day of test material on days 6 to 18. Dosing was by oral adminstration with a catheter placed into the stomach. The test material was dissolved in 0.5% carboxymethyl cellulose. No positive control was run concurrently.

The selection of the dose levels for the main study was based on a preliminary study which showed that rabbits dosed at 1500 mg/kg/day (the technically maximum dose) resulted in body weight and liver weight changes. Liver weight changes were also noted in the group receiving 500 mg/kg/day for the preliminary study.

The rabbits in the main study were sacrificed on the 29th day (by injection of air into the auricular vein), and the pups were delivered by Caesarian section.

D. Effects in the dams

1. No obvious signs of toxicity were noted in the dams with regard to survival; body weight and food consumption (only minor changes in the high dose group at best); behavior; necropsy; or organ weight changes. The increases in liver weight at 500 mg/kg noted in the preliminary study were not noted in the definitive study, but is excusable because of the time difference with regard to the last test dose and time of sacrifice.

2. Uterine data. There were no differences in the number of corpora lutea but the number of implantations when expressed as the mean were statistically significantly higher in the high dose test group when compared to the controls. For example these were 7.1, 7.5, 8.4 and 8.7 for the controls, low, mid and high dose test groups. The uterine data also revealed that there were slightly more uterine early deaths (for example there were 1/71, 0/75, 2/84 and 3/87 early deaths per implantation for the control, low, mid and high dose test groups). There was also a very slight increase in the high dose test group of "placental remnants" (2 incidences in the high dose group but 0 or 1 in the other groups). Overall there were 1.4, 4.0, 5.4 and 6.0 fetal deaths per implantation implying only a hint of an effect in the high dose test group. The conclusion is confounded because the exact period of early deaths was not stated, the period could have included deaths in the predosing period (prior to day 6).

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TB concludes that the NOEL for effects on the dams is 150 mg/kg/day. There is slight and transitory effect noted on body weight gain and food consumption in the group receiving 500 mg/kg/day.

3. Fetal data: There were 70, 73, 80 and 82 live fetuses in the control, low, mid and high dose test groups meaning there were more fetuses in the mid and high dose test groups. The sex ratio for the fetuses was 1.41, 0.95, 0.86, and 0.95 for the control, low, mid and high dose test groups. There was noted a decrease in pup weight for the high dose test group. The body weights, body lengths and placental weights all appeared smaller in the high dose test group as follows.

	Body Weight	Body Length	Placental Weight
Control	39.76 ± 4.29	9.5 ± .69	4.62 ± .87
Low	38.02 ± 6.08	9.6 ± .68	4.06 ± .75
Mid	39.06 ± 4.73	9.4 ± .53	4.14 ± .41
High	33.04*± 6.42	8.9 ± .91	3.89 ± 0.84

*stat. sig $p < .05$

Of these only the body weight was statistically significantly lower.

Following delivery, the fetuses were kept in an incubator for 24 hours and their survival rate was noted. There was a slight decrease in survival noted for the high dose test group. For example, survival at 24 hours was 97.1% for the controls, 89.5% for the low dose group, 98.9% for the mid dose group and 84.2% for the high dose group.

The live fetuses were examined for external abnormalities. A single fetus in the high dose test group had an umbilical hernia.

There were no internal (visceral) abnormalities noted.

The skeletal system was examined by a modified Dawson's method (staining with Alizarin red S). There were incidences of abnormalities found in the mid and high dose test groups which were not also found in the control or low dose group. These included reports of a single case of "nodular rib" in the mid dose group; two incidences of "abnormality in the sternebrae" and one case of "fusion of rib and thoracic vertebral arch" in the high dose group. Information on the progress of ossification was reported in a separate table and no test chemical effects on ossification were noted.

CONCLUSION: This study is CORE GUIDELINES. No positive control was run concurrently. This study demonstrates that tetramethrin was not teratogenic at dose levels up to and including 500 mg/kg. At 500 mg/kg there were some effects in the dams (transitory body weight loss) and on the pups (smaller pup weight). The effect on pup weight may have been a result of there being more pups in the high dose test group.

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Histologic Evaluation and Interpretation of NEO-PYNAMIN Bioassay Studies Carried Out on Sprague-Dawley (1974-1981) and Long-Evans (1981) Rats by Hazleton Laboratories, Inc. for Sumitomo Chemical Company Ltd.

Report prepared jointly by Dr. Stan P. Vesselinovitch (Univ. of Chicago) and Dr. Nobuyuki Ito (currently with Nagoya University, Japan). Date of report August 13, 1982.
EPA Acc. No. 248341, Tab 1

This report represents a reevaluation of the original histological diagnosis of the testes for all rats in both of the previous two rat oncogenesis studies which showed that there were increased incidences of testicular adenomas in Sprague-Dawley rats (2 studies) and Long-Evans rats (one study). The pathologist responsible for the initial phase of the reassessment (Dr. Stan D. Vesselinovitch) reassessed all of the testes tissues including some tissues that were not originally assessed (additionally sectioned testes tissues). Those tissues which were thought to show the presence of either hyperplasia or neoplasia were examined a second time under a two-headed microscope jointly by Dr. Vesselinovitch and Dr. Ito at the Hazleton Laboratories. In addition to the testicular tissue, all available lung tissues were also "read jointly." The lung tissue was read because identification of distant metastases to the lungs would help to assess malignancy of the primary lesion (in this case malignancy of the testes).

In the reexamination of the tissue, the testicular interstitial lesions were classified into one of these categories: (1) interstitial, Leydig cell, diffuse hyperplasia, (2) interstitial, Leydig cell, nodular hyperplasia, and (3) interstitial, Leydig cell, adenoma. The report contains the criteria for classifying the microscopic observations into each of these three categories. Both the diffuse hyperplasia and nodular hyperplasia were not regarded as primary neoplasms.

The results of the reanalysis of the testes as determined by Drs. Vesselinovitch and Ito are shown in Tables 1-6 (Table 5 presents the original tabulation of neoplasms in testes by Hazleton pathologists) attached. It should be noted that neither of these tables depicts the incidence of diffuse hyperplasia.

Comparison of Table 4 and 5 shows that there were 21 more adenoma bearing rats recognized by the original pathologist (Table 5) than by the reanalysis (Table 4). This discrepancy was attributed to the inclusion by Hazleton pathologists of hyperplastic nodules among the lesions described as adenomas. In the opinion of Drs. Vesselinovitch and Ito the difference is due to "over diagnosing," and some of the lesions originally described as adenoma were redesignated as hyperplastic nodules in the revised report. Of the 21 changes in diagnosis made, 11 were in the high dose group and 6 were in the controls. Thus, the original report would give the impression of more rats with an oncogenic effect in the high dose test group.

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The incidence of "hyperplastic nodules" in Sprague-Dawley rats is summarized for both studies as there being 4/99 (4.0%), 2/50 (4.0%), 2/89 (2.2%), 2/40 (5.0%) and 6/88 (6.8%) for the controls, low, mid dose levels and high dose groups respectively. There was a possibility of there being increased incidence of hyperplastic nodules in the Long-Evans strain as follows: 1/49 (2.0%), 1/50 (2.0%), 1/49 (2.0%) and 5/43 (10%). These data did not reach statistical significance by the method used by the authors of this report (the test used was not indicated). TB also evaluated these data from the study with the Long-Evans rats using Fisher's Exact test (TB computer program) and it was shown that the high dose group P statistic was 0.102, indicating that statistical significance of $P<.05$ was not attained.- [redacted]

Tables 2, 3, 4 and 6 show that the high dose test group is associated with higher incidences of adenomas (tables 2, 3 and 4) and combined nodular hyperplasia plus adenomas (table 6) in the testes of rats dosed with neopynamin.

The report prepared by Drs. Vesselinovitch and Ito also contains historical control data for the spontaneous development of interstitial cell tumors in Sprague-Dawley rats (Table 7). Of the 20 studies presented (conducted between 1976-1980) the tumor incidence ranged from 0 to 62.5%). TB notes that the high percentage rate may be misleading because of the low numbers used for denominators. In order for this information to be more meaningful, the number of rats at the start of the study and the number of rats surviving to the time when the rats were at risk for development of this type of tumor should be provided. Also, it is not clear as to whether the number of tumors or the number of rats with tumors was reported. The registrant should be asked to state clearly which is correct.

The observed range (0-62.5%) reported in Table 7 for Sprague-Dawley rats exceeds the range of 0-20% reported by Sher for the Charles-River CD rat (Toxicology Letters 11, 103-110, 1982). The total percentage for the control rats in the 1974 and 1981 studies with the Sprague-Dawley rat was 6.0%. This does not compare favorably with the 29.5% found in the high dose groups. The 29.5% is also in excess of the 0-20% range reported by Sher.

Other historical data provided in the report indicated that as rats increase in age the likelihood of developing testicular tumors increases (Table 8). The authors (Vesselinovitch and Ito) present a discussion on the comparison with historical control data and factors influencing the development of testicular interstitial tumors. Their conclusion of this study is indicated as follows (as copied from the report, pp. 13-14):

"CONCLUSIONS

Under the conditions of the bioassay and based on the pairwise comparison between the untreated controls and Neo-Pynamin exposed rats, there was a statistically significant increase in the incidence of the benign, spontaneously developing, interstitial (Leydig cell) adenomas in males exposed to the highest dose level used (5000 ppm). This incidence, however, did not exceed the maximal incidence observed in the historic controls. The incidence of no

other tumors was affected by the Neo-Pynamin treatment. The statistical indication of Neo-Pynamin tumorigenicity is biologically questionable because the tumor involved is hormonally dependent, occurred only at a single site, in a single sex, in a single species, and because the response to the highest dose was within the incidence range observed in the historic controls. Since the treatment with Neo-Pynamin did not influence the development of malignant tumors at any site and because the interstitial (Leydig cell) adenomas represent a morphologic endpoint which is not associated with the malignancy, it has been concluded that conducted bioassays did not show carcinogenic potential of Neo-Pynamin."

TOXICOLOGY BRANCH (TB) comments on the report.

TB acknowledges receipt of this report but maintains that the conclusions made in the previous review (see J. Doherty review dated April 11, 1983) are not changed by this report. Although there were some quantitative differences noted with regard to total count of rats with neoplasms because of the classification of some lesions as neoplastic nodules, it is still TB's conclusion that both studies with the Sprague-Dawley rats and the study with Long-Evans rats show statistically significant increases in the development of testicular adenomas, and that the "biologic interpretation" offered by this report is insufficient and/or too speculative to discount this finding. The purpose of conducting oncogenicity studies is to assess the oncogenic potential of the test material. Based on the results of the three sets of data with male rats, tetramethrin has been demonstrated to exhibit a potential to produce a neoplastic effect in rats.

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003660

Cancer Risk Assessment for Neo-Pynamin

Report prepared by Frank W. Carlborg, September 8, 1982. EPA Acc. No. 248341, Tab. 3.

This report is a "risk assessment" prepared at the request of the registrant by a consulting statistician. The risk assessment was based on "(1) the results of three experiments with rats exposed by ingestion over a lifetime, (2) the estimated human exposures according to the anticipated uses, and (3) the assumption that Neo-Pynamin is a human carcinogen."

The statistician's opinion was "the estimated human cancer risk from an exposure to Neo-Pynamin is essentially zero regardless of how one performs the risk assessment."

TB notes the opinions expressed in this document but declines from commenting on the acceptability of the conclusions. One fault of the procedures was that information was used on exposure that has not been verified by Hazard Evaluation Division's Exposure Assessment Branch.

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HED/TOX:DCR-44870:J.Doherty:tar:efs:Raven:557-2226:CBI-11:2/9/84:Del.2/15/84

003669

Zeroed from the study report.

TABLE 1

INTERSTITIAL HYPERPLASTIC NODULES

Dose (ppm)	1974 S.D. Ratio	1974 S.D. Percent	1981 S.D. Ratio	1981 S.D. Percent	Total Ratio	Total Percent	Statistical significance
0	2/~~40~~ 50 *	~~5.0~~ 4% **	2/49	4.0	4/~~89~~ 99 ***	~~4.5~~ 4% ****	....
200	....	...	2/50	4.0	2/50	4.0	N.S.
1000	1/40	2.5	1/49	2.0	2/89	2.2	N.S.
3000	2/40	5.0	....	...	2/40	5.0	N.S.
5000	3/40	7.5	3/48	6.0	6/88	6.8	N.S.

S.D. = Sprague-Dawley, Charles River.

* should be 50
** should be 4%
*** should be 99
**** should be 4%

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Zeroxed from the study report

TABLE 2

INTERSTITIAL ADENOMA

Dose (ppm)	1974 S.D.		1981 S.D.		Total		Statistical significance
	Ratio	Percent	Ratio	Percent	Ratio	Percent	
0	1/40	~~2.5~~	5/49	10.2	6/89	6.7	....
200	....	...	7/50	14.0	7/50	14.0	N.S.
1000	3/40	7.5	2/49	4.1	5/89	5.6	N.S.
3000	8/40	20.0	....	....	8/40	20.0	N.S.
5000	12/40	30.0	14/48	29.2	26/88	29.5	P < 0.001

S.D. = Sprague-Dawley, Charles River.

X should be 5
XX should be 7.

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Zeroxed from the study report

TABLE 3

INTERSTITIAL HYPERPLASTIC NODULES AND ADENOMAS
OBSERVED IN LONG-EVANS RATS (1981)

Dose (ppm)	Hyperplastic nodules			Adenomas		
	Ratio	Percent	Statistical significance	Ratio	Percent	Statistical significance
0	1/49	2.0	....	1/49	2.0	....
200	1/50	2.0	N.S.	2/50	4.0	N.S.
1000	1/49	2.0	N.S.	3/49	6.1	N.S.
3000	....	...	....	....	...	....
5000	5/49	10.0	N.S.	15/49	30.6	P < 0.001

TABLE 4

EXPERIMENTAL RESULTS OF FEEDING NEO-PYNAMIN TO RATS IN THREE DIFFERENT STUDIES

(Evaluated by Drs. Vesselinovitch and Ito)

Dose (ppm)	Sprague-Dawley (1974)		Sprague-Dawley (1981)		Long Evans (1981)		Pooled	
	Ratio	Percent	Ratio	Percent	Ratio	Percent	Ratio	Percent
0	1/44	2.27	5/41	12.19	1/43	2.32	7/128	5.47
200	....	....	7/45	15.55	2/44	4.54	9/89	10.11
1000	3/30	10.00	2/41	4.87	3/43	6.97	8/114	7.02
3000	8/36	22.22	....	.....	....	.....	8/36	22.22
5000	12/37	32.43	14/40	35.00	15/47	31.91	41/124	33.06

Number of rats bearing interstitial tumor(s)/number of rats at risk after 80 weeks.*

* Animals dying prior to 80 weeks are not included because the first tumors were noted after 80 weeks and only "at risk" rats are included.

Xeroxed from the study report

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TABLE 5

EXPERIMENTAL RESULTS OF FEEDING NEO-PYNAMIN TO RATS IN THREE DIFFERENT STUDIES

(Evaluated by Hazleton Pathologists)

Dose (ppm)	Sprague-Dawley (1974)		Sprague-Dawley (1981)		Long Evans (1981)		Pooled	
	Ratio	Percent	Ratio	Percent	Ratio	Percent	Ratio	Percent
0	2/44	4.54	7/41	17.07	4/43	9.30	13/128	10.15
200	....	....	7/45	15.55	3/44	6.82	10/89	11.23
1000	3/30	10.00	3/41	7.31	4/43	9.30	10/114	8.77
3000	9/36	25.00	....	.....	....	....	9/36	25.00
5000	14/37	37.84	16/40	40.00	22/47	46.80	52/124	41.93

Number of rats bearing inters titial tumor(s)/number of rats at risk after 80 weeks.

Removed from the study report

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Zeroxed from the study report

TABLE 6

INCIDENCE OF INTERSTITIAL HYPERPLASTIC NODULES AND ADENOMAS IN SPRAGUE-DAWLEY RATS

Dose (ppm)	Ratio	Percent	Statistical significance
0	10/89	11.2	....
200	9/50	18 0	N.S.
1000	7/89	7.9	N.S.
3000	10/40	25.0	N.S.
5000	32/88	36.4	P < 0.001

Zeroxed from the study report

TABLE 7

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HISTORICAL CONTROL DATA FROM HAZLETON LABORATORIES

Chronic Rodent Studies with Sprague-Dawley Rats from Charles River Breeding Laboratories, Inc.

Incidence of Interstitial Cell Tumors of the Testes

Group	Project No.	Year	Duration (weeks)	No. of interstitial cell tumors noted				
				One testis		Both testes		
				Ratio	Percent	Ratio	Percent	
1	417-351 Grp.1-Control	1976	104	0/27	0.0	4/7	57.1	4/34
2	417-351 Grp.2-Control	1976	104	0/25	0.0	5/8	62.5	5/33
3	610-119	1976	104	0/30	0.0	1/3	33.3	1/33
4	785-300	1976	104	....	...	0/3	0.0	
5	165-1-9	1977	104	0/10	0.0	1/4	25.0	1/14
6	174-123	1977	104	0/8	0.0	0/2	0.0	0/10
7	174-122	1977	104	...	...	3/31	9.6	
8	141-263	1977	104	...	...	2/20	10.0	
9	132-134	1978	104	6/35	17.1	0/6	0.0	6/41
10	132-132	1978	104	0/29	0.0	2/6	33.3	2/35
11	132-137	1978	104	2/17	11.8	7/16	43.7	9/33
12	958-102	1978	104	0/12	0.0	5/8	62.5	5/20
13	798-177	1979	104	....	....	1/23	4.3	
14	947-103	1979	104	....	....	7/31	22.6	
15	798-181	1979	104	....	....	2/28	27.1	
16	417-383	1980	104	....	....	2/22	9.1	
17	444-215 Grp.1-Control	1980	130	....	....	8/13	61.5	
18	444-215 Grp.2-Control	1980	130	....	....	10/23	43.5	
19	444-214 Grp.1-Control	1980	130	....	....	5/19	26.3	
20	444-214 Grp.2-Control	1980	130	....	....	3/16	18.7	

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TABLE 8

AGE DEPENDENT DEVELOPMENT OF INTERSTITIAL CELL TUMORS OF THE TESTIS

Age in weeks	52	104	130
Ratio	2/76	30/123	26/71
Percent incidence	2.6	24.4	36.6