DER #1


9/11/1997
The study reviewer withholds decision. TOXSAC believes that 50 ppm is the NOEL for plasma and RBC GMU findings (500 ppm is NOEL for brain) and 500 ppm is the NOEL for systemic effects. The LOEL is 500 ppm for ChE [plasma and RBC, 6000 ppm for brain] and 6000 ppm for systemic effects consisting of several parameters [e.g., mortality in males, body weight gain decreases in males [3-13%], slight anemia in both sexes, and increased absolute and relative liver weight in both sexes with GGT increases]. Carcinogenic potential is possibly positive at liver & nasopharynx.

DER is unacceptable. I don't agree with some of the author's interpretation of the results [some "effects" are based on marginal findings without statistical significance or are not dose related]. Due to the voluminous size [78 pages plus tables], and the lack of classification of the study by the reviewer as acceptable/unacceptable for either chronic toxicity or carcinogenicity, I consider the DER as unacceptable.

Study is acceptable. Fulfills acceptance criteria. However, ocular examination is questionable. Memo from Dr. Boyes questions the validity of the ocular results.

Although not agreed to in the DER, I believe the dosing was adequate to assess both chronic and carcinogenic potential of malathion [except for ocular exam]. A thorough statistical analysis of tumor data [using Peto's analysis] and latency concerns of testicular tumors are needed by the Cancer SARC. The increased incidence of parathyroid hyperplasia is not dose-related over a 200-fold dose span and is not compound-related. The increases in food consumption [3-7%] are small and animals lost weight at 6000 and 12000 ppm. Nasal turbinates at 50/100 ppm do not need to be examined in both sexes due to absence of compound-related findings at 500 ppm. All of the rats of both sexes were examined in control, 500, 6000, and 12,000 ppm.
EPA Reviewer: Brian Dementi, Ph.D., D.A.B.T.
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Edwin Budd, M.S.
Registration Action Branch II (7509C)

DATA EVALUATION RECORD

Study Type: Combined Chronic/Oncogenicity Study; OPPTS 870.4300 [83-5]

DP BARCODE: D224174
P.C. CODE: 057701

Submission No.: S501871
Tox. Chemical No.: 535

Test Material (purity): Malathion; butanedioic acid, 
[(dimethoxyphosphinothiol) thiol diethyl ester (97.1% a.i.)

Synonym: Mercaptosuccinic acid diethyl ester; S-ester with 0, 0, 
-dimethylthiophosphate

Citation: Ira W. Daly, Ph.D., D.A.B.T., 27 February 1996. A 24- 
Month Oral Toxicity/Oncogenicity Study of Malathion in 
the Rat via Dietary Administration. Huntington Life 
Sciences, East Millstone, NJ, Study No. 90-3641, MRID 
43942901, Unpublished.

Sponsor: Cheminova Agro A/S, P.O. Box 9, DK-7620, Lemvig, 
Denmark

Executive Summary: In a combined chronic toxicity/oncogenicity 
study (MRID 43942901), malathion (97.1% a.i.) was administered to 
90 Fischer 344 rats/sex/dose via the diet for up to 24 months at 
dose levels of 0, 100/50 (100 ppm for first 3 months of study, 50 
ppm for duration of study in both sexes due to finding of 
erthrocyte cholinesterase inhibition in females only at 3 month 
assay) 500, 6,000 or 12,000 ppm [equivalent to respective mean 
values of 0, 4, 29, 359 and 739 mg/kg/day (males) and 0, 5, 35, 
415 and 868 mg/kg/day (females)].

The only clinical sign observed was yellow anogenital 
staining among females at 12000 ppm. Increased mortality was 
seen in females at 12000 ppm and in males at 500, 6000 and 12000 
ppm. All 12000 ppm males died or were sacrificed moribund by 
about 94 weeks. Treatment related decrements in body weight gain 
were observed at 6000 and 12000 ppm in both sexes. Food 
consumption was increased at 100 ppm in males for the first 3 
months (prior to lowering of dose to 50 ppm). At subsequent time 
points for males, and across all time points for females food 
consumption was increased, the LOEL = 6000 ppm and NOEL = 500 
ppm. Among parameters for hematology, erythrocyte count was 
reduced in males at 12000 ppm, mean corpuscular hemoglobin 
concentration was decreased in males at 6000 and 12000 ppm; and
the following were observed in rats of both sexes at 6000 and 12000 ppm: increased platelet count, decreased mean corpuscular volume and mean corpuscular hemoglobin. Hence, for hematologic parameters overall, LOEL = 6000 ppm, NOEL = 500 ppm, both sexes. Among clinical chemistry parameters, erythrocyte cholinesterase inhibition, males, LOEL = 6000 ppm, NOEL = 500 ppm; females, at 3 months, the enzyme was inhibited at all doses, LOEL = 100 ppm. After 3 months, when lowest dose was reduced to 50 ppm, LOEL = 500 ppm, NOEL = 50 ppm. For plasma cholinesterase inhibition, males, LOEL = 500 ppm, NOEL = 50 ppm (100 ppm first 3 months); females, LOEL = 6000 ppm, NOEL = 500 ppm. For brain cholinesterase inhibition, LOEL = 6000 ppm, NOEL = 500 ppm, both sexes. For inhibition of cholinesterase activity, for males the overall NOEL is 50 ppm (4 mg/kg/day) and the LOEL is 500 ppm (29 mg/kg/day) based on inhibition of plasma activity at 24 months. For females the overall (beyond 3 months) NOEL is 50 ppm (5 mg/kg/day) and the LOEL is 500 ppm (35 mg/kg/day) based on inhibition of erythrocyte activity. Decreased aspartate aminotransferase, females, 12000 ppm; decreased alkaline phosphatase, males and females, 6000 and 12000 ppm; elevated blood urea nitrogen, males, 12000 ppm; elevated cholesterol, males and females, 6000 and 12000 ppm; elevated gamma-glutamyl transpeptidase, males and females, 6000 and 12000 ppm. Ocular effects testing inconclusive. Organ weight effects: increased kidney and liver weights, males and females, 6000 and 12000 ppm; thyroid/parathyroid weight increased (males), decreased (females) 6000 and 12000 ppm; increased spleen weight, males, 6000 and 12000 ppm; increased heart weight, males, 6000 ppm (term). In males, increases in liver and thyroid/parathyroid weights may have extended to 500 ppm. Microscopic findings: non-neoplastic: nasal mucosa and nasopharynx (several pathologies), males and females, 6000 and 12000 ppm; bilateral subacute-chronic inflammation/chronic nephropathy (high incidence in all study groups including controls), increased severity, males, 6000 and 12000 ppm, females, 500, 6000 and 12000 ppm; stomach (several pathologies), males and females, 6000 and 12000 ppm; increased incidence parathyroid hyperplasia, males and females, all doses; other findings in various tissues (thyroid, lymph nodés, lungs, liver, spleen, adrenal gland, eyes) as summarized in the review, being more remarkable in males, and often extending across the top three doses in males and top two doses in females; neoplastic: treatment-related increased combined hepatocellular adenomas/carcinomas, females at all doses, incidences: 0/55 (0%), 2/55 (3.6%), 2/55 (3.6%), 3/55 (5.5%) and 6/55 (10.9%) for the 0, 100/50, 500, 6000 and 12000 ppm groups, respectively; rare tumors (one in each of four dose groups) on nasoturbinal slide preparations considered compound related effects: males, carcinoma 12000 ppm, adenoma 6000 ppm; females, squamous cell carcinoma 100/50 and 12000 ppm. Other tumor types observed included testes interstitial cell tumors significant at all doses with possibly decreased latency; significant trend in thyroid follicular cell adenomas and/or carcinomas, males; significant
trend and positive pairwise comparison at 500 ppm for thyroid c-
cell carcinoma, males; significant difference in pair-wise 
comparison, mononuclear cell leukemia, 100/50 ppm, females; 
significant difference in pair-wise comparisons, pituitary pars 
distalis carcinomas, 500 and 6000 ppm, females; significant 
difference in pair-wise comparison, pituitary pars distalis 
adnomas and/or carcinomas combined, 500 ppm, females. 
Tumorigenic responses may have been compromised by high mortality 
in males at 6000 and 12000 ppm and in females at 12000 ppm.

The classification and acceptability of this 
chronic/oncogenicity study in the F344 rat is reserved pending 
review and discussion by the HED Hazid and Carcinogenicity Peer 
Review Committee.

Detailed Summary of Results:

Clinical Signs

There were no clinical signs in the study in either sex at any 
dose level except yellow anogenital staining among females at the 
12000 ppm dose level. This clinical effect was of high incidence 
and consistently expressed throughout the study.

Mortality

Increased mortality among males was observed in a dosing-related 
manner across the 500, 6000 and 12000 ppm dose levels. Among 
females, increased mortality was seen at the 12000 ppm dose level 
only. A principal contributor to the increased mortality 
observed among male rats at 500 ppm was that attributed to 
leukemia. It should be noted that while the incidence of 
leukemia at the 500 dose level was not increased relative to the 
control group incidence, leukemia as a cause of death was 
increased in that group. Evidently, leukemia laden male rats in 
the 500 ppm group, under the added burden of the test material, 
were more likely than controls with the disease to succumb to the 
condition before term.

Body Weight

Treatment-related decrements in body weight gain were observed at 
6000 and 12000 ppm for rats of both sexes.

Food Consumption

Increased food consumption was observed in rats of both sexes. 
Among males at time points prior to the reduction in dosage level 
from 100 ppm to 50 ppm, there were statistically significant 
dosing-related increases of 2-7% in food consumption frequently 
observed across all doses, particularly during weeks 5-12, i.e.,
there was no NOEL. This may be an appetitive (behavioral) effect in males during the first 3 months, prior to reduction of malathion in the diet. Following the reduction in dosage level in group 2 from 100 to 50 ppm (both sexes), there were, over the remainder of the study time, few instances where the low dose group differed from the control group for male rats, and increases in food consumption were variable at the higher doses being generally confined to the top two dose levels. For males, for the bulk of the study period, the LOEL was 6000 ppm and the NOEL was 500 ppm for increased food consumption. For females, the LOEL was 6000 ppm and the NOEL 500 ppm for increased food consumption.

Hematology

The following were small but statistically significant findings: reduced hemoglobin and hematocrit at one or more intervals at 6000 and 12000 ppm in both sexes, where the effect in males was more remarkable in terms of degree of lowering of and endurance in time; erythrocyte count reduction was observed at 12000 ppm at 12 months in males only; platelet count increases at 6000 and 12000 ppm in rats of both sexes; decreases in mean corpuscular volume and mean corpuscular hemoglobin at 6000 and 12000 ppm in rats of both sexes; decreased mean corpuscular hemoglobin concentration in males only at 6000 and 12000 ppm; and increased total leukocyte counts in rats of both sexes at 12000 ppm at the 12 month time point only. Hence, for hematologic parameters overall, LOEL = 6000 ppm for both sexes and NOEL = 500 ppm for both sexes.

Clinical Chemistry

Cholinesterase

At the 3-month time point, erythrocyte cholinesterase was statistically significantly inhibited among females at all dose levels. Inhibitions were 25%, 30%, 58% and 66% at 100, 500, 6000 and 12000 ppm dose levels, respectively. In consideration of the lack of a NOEL at this time point, the dosage level for the low dose group was reduced from 100 ppm to 50 ppm in both sexes. At subsequent time points erythrocyte cholinesterase for females was significantly inhibited in a dose-related manner at 6000 and 12000 ppm at 6 months, and at 500, 6000 and 12000 ppm at 12 months and at term. Inhibitions at term were 27%, 44% and 52% at 500, 6000 and 12000 ppm, respectively. Inhibition at the top three dose levels tended to be less at term than at 3 months suggesting some degree of adaptive recovery. Among males erythrocyte cholinesterase was considered inhibited only at 6000 and 12000 ppm.
In conclusion, erythrocyte cholinesterase inhibition LOEL = 6000 ppm, NOEL = 500 ppm for males. For females, LOEL = 100 ppm. A NOEL was not identified at 3 months. At subsequent time intervals (excepting 6 months), LOEL = 500 ppm, NOEL = 50 ppm for females. The data are not sufficiently definitive to conclude a NOEL for the study for females, given the statistically significant 25% inhibition at 100 ppm on a shallow dose-response curve between 100 and 12000 ppm. Furthermore, even though erythrocyte cholinesterase was not inhibited among females at the 6 month and subsequent time intervals at 50 ppm, there is no certainty the enzyme would not have been inhibited at .50 ppm during the first 3 months of study, particularly in view of the shallow dose response and the propensity for the enzyme to recover for a period from an initial inhibition as it did after 6 months at 500 ppm. Accordingly, the finding establishes the requirement for a 3-month study to define a NOEL for erythrocyte cholinesterase inhibition in the female F344 rat.

Plasma cholinesterase inhibition among male rats was significant at 3, 6 and 12 months at both of the top dose levels. At term it was inhibited at 500 ppm (29%) and at 6000 ppm (64%) (there were no male survivors at 12000 ppm). Among females, the plasma cholinesterase was inhibited across time by 38-61% at 6000 ppm and by 70-89% at 12000 ppm. Hence, for plasma cholinesterase inhibition, LOEL = 500 ppm, NOEL = 50 ppm (100 for first 3 months) (males) and LOEL = 6000 ppm, NOEL = 500 ppm (females).

Brain cholinesterase was significantly inhibited only at 6000 and 12000 ppm in rats of both sexes. At 6000 ppm, inhibition among males across the four time points ranged 11-31% and in females ranged 12-18%. The respective inhibitions at 12000 ppm ranged 15-19% (first 3 time points) for males and 28-67% (28-49% over the first 3 time points) for females. Hence, for brain cholinesterase inhibition, LOEL = 6000 ppm and NOEL = 500 ppm for rats of both sexes.

Additional clinical chemistry parameters considered affected and the lowest dose level at which the effect was observed include: aspartate aminotransferase, decreased among females at 12000 ppm; alkaline phosphatase, decreased among males and females at 6000 ppm; blood urea nitrogen, elevated among males at 12000 ppm; cholesterol, elevated among males and females at 6000 ppm; gamma-glutamyl transpeptidase, elevated among males and females at 6000 ppm.

Urinalysis

Urine pH values tended to decline variably by about one pH unit with increasing dose essentially at the top two dose levels at various assay time intervals in both sexes.
Ocular Testing

The ocular effects component of this study which involved ophthalmoscopy, electroretinography (ERG), fundic photos and histopathology of the retina did not reveal any effects identifiable as compound-related. ERGs were more remarkably impaired in all dose groups than in the controls, but there were no clear dosing-related effects. Variability in ERGs was so great that a dosing-related effect if present could have gone undetected. The F344 rat is considered a poor model for detecting ocular effects by ERG and thus this component of the overall study does not satisfy as a negative ocular effects study.

Pathology

Organ Weights: For the interim (12-month) sacrifice, increases in the weights of the following organs were considered treatment related: males (6000 and 12000 ppm) - kidneys, liver, spleen and thyroid/parathyroid; females (6000 and 12000 ppm) - kidneys and liver.

At terminal sacrifice, heart weight was increased among males at 6000 ppm; kidney and liver weights were increased in males at 6000 ppm and in females at 6000 and 12000 ppm. There were small nonstatistically significant increases in liver weight by all modes of expression among 500 ppm males. Whether this is a treatment-related effect is uncertain. Also uncertain is a nonstatistically significant increase in testes/epididymides weight at 6000 ppm. Thyroid/parathyroid weight was increased for males at 6000 ppm and considered likely to be increased at 500 ppm, and decreased among females at 12000 ppm and considered likely so at 6000 ppm.

Macroscopic Findings: Increased incidences of irregular surfaces of the kidneys were observed for males at 500, 6000 and 12000 ppm and for females at 12000 ppm. Increased incidences of emaciation were observed for males and females at 6000 and 12000 ppm.

Microscopic Findings:

Nonneoplastic Findings

Both the respiratory and olfactory epithelia of the nasal mucosa exhibited microscopic alterations in both sexes at 6000 and 12000 ppm. Effects seen in both types of epithelia include hyperplasia, subacute (chronic active)/chronic inflammation and dilated glands. In addition, there were findings in the olfactory epithelium of degeneration, epithelial cysts, replacement of the olfactory epithelium by ciliated and
nonciliated columnar epithelial cells and hyperplasia of both of
the latter replacement epithelia. These effects were not
observed in either the 50 or 500 ppm dose groups. A probable
reason for the more remarkable effects observed for olfactory
epithelium than the respiratory epithelium is that the former is
richly endowed with a wide spectrum of metabolic capabilities not
unlike those of the liver, that would potentially activate a
xenobiotic such as malathion.

There were also increased incidences of hyperplasia of the
respiratory epithelium of the nasopharynx in rats of both sexes
at 6000 and 12000 ppm dose levels.

Bilateral subacute-chronic inflammation/chronic nephropathy was
observed at high incidence in all groups in both sexes. However,
the severity of this finding was increased in males and females
at 6000 and 12000 ppm and in females at 500 ppm as well.

Several microscopic compound-related findings were identified in
the stomach. These effects (e.g., congestion, edema, ulcers,
acute/subacute inflammation, subacute (chronic active)/chronic
inflammation; squamous cell hyperplasia, hyperkeratosis) were
observed in males and females at 6000 and 12000 ppm. The bulk of
these were observed in rats that died prior to term, and in the
opinion of the study author were likely due to decreased food
intake in moribund animals.

Congestion was a finding in many tissues (e.g., liver, thyroid,
brain, heart, lung, forestomach, pancreas, pituitary, harderian
gland, lacrimal gland, sternal marrow, femoral marrow) of both
males and females, but were generally more remarkable in males
than in females. In females the effect was primarily in the
12000 ppm group and extending into the 6000 ppm group, while in
males the effect was universal at 6000 and 12000 ppm in the
tissues identified and frequently extended to the 500 ppm group.
These findings were generally observed in decedents and to the
extent congestion was a finding at 500 ppm, it may be a corollary
to increased mortality in that group.

There were certain other non-neoplastic microscopic findings in
various tissues (thyroid, lymph nodes, lungs, liver, spleen,
adrenal gland, eyes) as summarized on p. 49 of this review
generally more remarkably so in males than in females, and often
extending across the top three doses in males and top two doses
in females.

Neoplastic Findings

Neoplastic findings of the liver were identified in this study.
The effects were evidently dosing-related only in the case of
female rats. Among male rats, combined incidences of hepatocellular adenomas and carcinomas were 3/55 (5.5%), 4/55 (7.3%), 4/55 (7.3%), 3/55 (5.5%) and 1/55 (1.8%) at 0, 100/50, 500, 6000 and 12000 ppm dose levels, respectively. The corresponding incidences among females were 0/55 (0%), 2/55 (3.6%), 2/55 (3.6%), 3/55 (5.5%) and 6/55 (10.9%). While there were no statistically significant increases in liver neoplasia in the case of males, high mortality among male rats in the 6000 and 12000 ppm groups may have precluded expression of a tumorigenic response, particularly if late occurring. Since the next lowest dose level tested (500 ppm) was substantially lower than the 6000 ppm dose level (more than 10-fold lower), malathion was not tested for potential carcinogenicity at adequate dose levels for males in this study. Hence, this study does not satisfy as a negative study for liver carcinogenicity among male rats, or for that matter for carcinogenicity at any other anatomic site among male F344 rats. This constitutes a major study deficiency.

In the case of females, increased mortality at 12000 ppm also constitutes a study deficiency. The combined hepatocellular tumorigenic findings for females at all dose levels are considered positive, compound-related findings. At 6000 and 12000 ppm, the findings are statistically significant as claimed in the study report. The findings at 100/50 and 500 ppm are considered positive in view of the effects at the two higher dose levels (suggesting the liver as a target organ) in concert with the facts that hepatocellular adenomas and carcinomas in female F344 rats are rare (i.e. < 1%) as defined by Office of Science and Technology Policy (1985). The most recent NTP (1996) historical control data for the F344 female rat reveals incidences for hepatocellular adenomas of 8/1351 (0.59%) and for carcinomas of 1/1351 (0.07%).

Nasoturbinal tissue tumors that occurred in male rats, i.e., one carcinoma in the 12000 ppm group and one adenoma in the 6000 ppm group, are considered to be positive compound-related tumorigenic effects. Though not identified as statistically significant by any particular method of statistical analysis, the findings are considered positive on the basis of the extremely rare historical incidence and the clear evidence of nasoturbinal tissue being a "target tissue" in this study. There were also identified in the nasoturbinal tissue slides, one incidence each in the 100/50 and 12000 ppm female groups of squamous cell carcinomas of the squamous epithelium lining the alveolus of a tooth. These are also extremely rare tumors, whether viewed as nasal or oral tissues, as documented in the NTP (1996) historical data and thus again for the reason of rarity are likewise considered compound-related findings. Further, in view of the extensive hyperplasia of nasoturbinal tissues in rats of both sexes at the higher doses and the rare tumorigenic findings, there is according to the
FIFRA Guidelines: an outstanding requirement for histopathologic examination of nasoturbinal tissue slides in all study groups not yet examined. Two nasoturbinal sections per rat are considered inadequate to properly evaluate the potential tumorigenic response particularly if rare. The registrant should discuss with the Agency a protocol for adequate histopathologic assessment of possible nasoturbinal tissue effects.

Although similar and high incidences of interstitial cell testicular tumors were observed in all the control and treated male groups in this study, the latency or time to tumor appeared to be decreased in the treated groups. A NOEL for increased interstitial cell testicular tumors at earlier than normal time points (decreased latency) was not identified.

Other tumorigenic findings showing increased incidences as discussed in this review which await independent HED statistical analyses include the following: leukemia (both sexes); thyroid gland follicular cell adenoma and carcinoma in males; thyroid gland C-cell carcinoma in males; pituitary gland, pars distalis, adenomas and carcinomas in females.

Compliance: Signed and dated GLP, quality assurance, data confidentiality and flagging statements were provided.
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Malathion
   Description: clear yellow liquid - stored in steel drums at room temperature.
   Lot Nos.: 11029-01 and 30628-01 (p.19)
   Purity: 97.1% a.i.
   Stability of compound: Stable as analyzed before, during and after the study period.
   CAS. No.: 121-75-5
   Supplier: Cheminova Agro A/S, Lemvig, Denmark

2. Vehicle: None

3. Test Animals:
   Species: Rat
   Strain: CDF (F-344) CrlBR
   Age at study initiation: 48 days
   Mean weight at study initiation: males, 140 grams; females, 103 grams.
   Source: Charles River Laboratories, Kingston, NY
   Housing: Individually in stainless steel cages.
   Diet: Certified Rodent Chow, No. 5002 (meal); Purina Mills Inc., St. Louis, MO. Test diets were prepared weekly by admixture with test material and fed ad libitum.
   Water: Ad libitum; by automated watering system.
   Environmental conditions:
   Temperature: 67-76°F (19-24°C)
   Humidity: 22-84%
   Air Changes: Not provided.
   Photoperiod: 12 hour light/dark cycle
   Acclimation Period: 20 days

B. Study Design

1. In life dates: Start: 12/30/92 End: 1/10/95

2. Animal Assignment

   Animals were assigned into 5 groups of 90 animals per sex (Table 1) by a computerized random sort program so that body weight means for each group were comparable.
### TABLE 1: STUDY DESIGN

#### A: Main Study

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Conc. in Diet (ppm)</th>
<th>Main Study (24 Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>55 Male, 55 Female</td>
</tr>
<tr>
<td>Low (LDT)</td>
<td>50/100</td>
<td>55 Male, 55 Female</td>
</tr>
<tr>
<td>Mid (MDT) 1</td>
<td>500</td>
<td>55 Male, 55 Female</td>
</tr>
<tr>
<td>Mid (MDT) 2</td>
<td>6,000</td>
<td>55 Male, 55 Female</td>
</tr>
<tr>
<td>High (HDT)</td>
<td>12,000</td>
<td>55 Male, 55 Female</td>
</tr>
</tbody>
</table>

#### B: Interim Sacrifices

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Conc. in Diet (ppm)</th>
<th>3 Months</th>
<th>6 Months</th>
<th>12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>10 Male, 10 Female</td>
<td>10 Male, 10 Female</td>
<td>15 Male, 15 Female</td>
</tr>
<tr>
<td>Low (LDT)</td>
<td>50/100</td>
<td>10 Male, 10 Female</td>
<td>10 Male, 10 Female</td>
<td>15 Male, 15 Female</td>
</tr>
<tr>
<td>Mid (MDT) 1</td>
<td>500</td>
<td>10 Male, 10 Female</td>
<td>10 Male, 10 Female</td>
<td>15 Male, 15 Female</td>
</tr>
<tr>
<td>Mid (MDT) 2</td>
<td>6,000</td>
<td>10 Male, 10 Female</td>
<td>10 Male, 10 Female</td>
<td>15 Male, 15 Female</td>
</tr>
<tr>
<td>High (HDT)</td>
<td>12,000</td>
<td>10 Male, 10 Female</td>
<td>10 Male, 10 Female</td>
<td>15 Male, 15 Female</td>
</tr>
</tbody>
</table>

The 3-month and 6-month interim sacrifices (10 rats of each sex per group) were incorporated primarily for ocular tissue pathologic examination. All rats from all dose groups received complete macroscopic examination. All rats from the control and 12000 ppm groups received only ocular tissue microscopic examinations at the 3-month and 6-month interim sacrifices.

3. **Dose Selection Rationale:** Not provided in report.

It should be noted that the original dose chosen for the low dose group, 100 ppm, resulted in
statistically significant erythrocyte cholinesterase inhibition in females when assayed following the first three months of dosing. In pursuit of a NOEL for cholinesterase inhibition, the dietary concentration for the low dose group was reduced to 50 ppm for rats of both sexes for the duration of the study.

4. Diet Preparation and Analysis

Fresh diet was prepared weekly by mixing appropriate amounts of malathion (in three stages: mortar and pestle, Hobart mixer and twin shell mixer) with Certified Rodent Chow No. 5002 (meal) (Purina Mills Inc., St. Louis, Mo.) to yield final designated concentrations. Prior to the initiation of the study, mock batches of treated diets at the 100 ppm (low concentration) and 12000 ppm (high concentration) were prepared and analyzed for achievement of homogeneity. Later in the study, at about Week 20, a 50 ppm batch was similarly prepared for homogeneity analysis. For assessments of homogeneity, nine samples were taken from each diet preparation (three each from the top, middle and bottom portions), and analyzed. As disclosed in Appendix O of the Study Report, "The mean values of each of the three levels were within ± 10.0% of each other, as well as within 15% of nominal. These values indicated that an acceptable mixing procedure for malathion in rodent diet was utilized. (p. 5552 of the Study Report). We concur with the conclusion that satisfactory mixing was achieved. Stability of malathion in the prepared diets was determined in a previous study (Pharmaco LSR study no. 92-3806). The diets were determined to be stable for 14 days at room temperature. Prepared diets were stored in clear polyethylene bags inside of dark plastic buckets with lids, at room temperature.

On 2/23/93, 10/6/93 and 7/7/95, the technical grade malathion was assayed for % purity as compared with analytical grade malathion (p. 5551 and 5554 of the study report). On each occasion two samples were assayed in duplicate. The % purity (mean) on the three respective occasions were 96.4%, 96.8% and 98.2%. The mean of these three assays is 97.1%.
In order to confirm concentration levels of malathion in the diet over the course of the study, all five dietary levels were assayed in duplicate weekly for the first 8 weeks and once every 2 weeks for the following 8 weeks. For the remainder of the study, assays were performed every 4 weeks. The results of the assays, as disclosed in Appendix 0, indicate that diets employed in the study were within 15% of the nominal concentration and duplicate samples were within 10% of each other (pp. 5553-5554 of the Study Report.)

5. Animals Received Fresh Diet [Weekly]: "Test diets were prepared weekly and were offered to the animals ad libitum." (p. 27 of Study Report.)

6. Statistics: The following is a statement regarding statistical methods as it appears in the Study Report:

"Body weight, body weight change, body weight change from Week 0, food consumption, hematology and clinical chemistry parameters, electroretinogram values, terminal organ and body weights and organ/body weight and organ/brain weight ratios, survivorship and time-to-tumor incidences were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables of appendices.

"The time-to-tumor analyses were performed using the Thomas, Breslow and Gart analyses which tests for both tumor incidence (chi-square and Fisher tests) and time-to-tumor (Kaplan-Meier curves, Cox's Tests and the Gehan-Breslow/Kruskal-Wallis Analyses). The chi-square and Fisher Exact Tests consider only simple incidence in a pairwise manner; each treated group is compared to control.

"Cox's test and the Gehan-Breslow/Kruskal-Wallis Analyses are based on incidence and survival. They separately perform a multiple comparison test, a test for trend, and a series of pairwise tests with each treated group compared to control. The Haseman test is a test that divides the study into time segments, tests each segment for tumor
C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality. Detailed examinations (including palpation) were conducted weekly.
2. **Body Weight:**

Animals were weighed three times pretest, weekly for weeks 1-14, once every two weeks during weeks 16-26 and monthly thereafter and at term.

3. **Food Consumption and Compound Intake:**

Food consumption for each animal was determined and mean daily diet consumption was calculated as grams of food/kg body weight/day. Compound intake (mg/kg/day) values were calculated as time-weighted average from the consumption and body weight gain data.

4. **Ophthalmoscopic Examination:**

Performed pretest (all animals) and at 3(35/sex/group), 6(25/sex/group), 12 (all animals) and 24 months (all animals).

5. **Electroretinogram Examinations (ERG):**

From among 35 satellite rats/sex/group, electroretinographic (ERG) evaluations were performed on 7 rats/sex/group at pretest and at months' 3, 6 and 12 (p. 26 of study report). ERG evaluations were also performed on 5 rats/sex/group at 24 months using oncogenicity animals, i.e., chosen from among survivors of the 55 rats/sex/group originally designated for the principal study (p. 24 of the study report). The Materials and Methods section of the study report indicates (p. 35) that methodology and references for the techniques employed are to be found in Appendix A of the study report. In Appendix A (p. 105 of the study report) under the Electroretinographic Parameters the Reference and Description of test procedures says simply: "LKC Technologies, Epic II, Photic Stimulator, Model PS22. Photographs were taken on anesthetized animals." The study report provides no further information as to details of the testing procedure followed.

6. **Hematology and Clinical Chemistry Parameters:**

Blood was collected from 10 rats/sex/group (satellite animals) at months 6 and 12. The month 12 rats were also used for cholinesterase assays. Blood was collected from 10 rats/sex/group (oncogenicity animals) at month 18 and at term.
Blood was collected from these animals via venipuncture of the orbital sinus under light CO₂/O₂ anesthesia. Animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

<table>
<thead>
<tr>
<th>a. Hematology</th>
</tr>
</thead>
<tbody>
<tr>
<td>x Hematocrit (HCT)</td>
</tr>
<tr>
<td>x Hemoglobin (HGB)</td>
</tr>
<tr>
<td>x Leukocyte count (WBC)</td>
</tr>
<tr>
<td>x Erythrocyte count (RBC)</td>
</tr>
<tr>
<td>x Platelet count</td>
</tr>
</tbody>
</table>

Blood clotting measurements
- Thromboplastin
- Thromboplastin
- Clotting
- Prothrombin

* Minimum required for carcinogenicity studies (only on Cont. and HDT unless effects are observed based on Subdivision F Guidelines)
b. Clinical Chemistry*

<table>
<thead>
<tr>
<th>ELECTROLYTES</th>
<th>OTHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>x Calcium</td>
<td>x Albumin</td>
</tr>
<tr>
<td>x Chloride</td>
<td>x Blood creatinine</td>
</tr>
<tr>
<td>x Magnesium</td>
<td>x Blood urea nitrogen</td>
</tr>
<tr>
<td>x Phosphorus</td>
<td>x Total cholesterol</td>
</tr>
<tr>
<td>x Potassium</td>
<td>x globulin/(calculated)</td>
</tr>
<tr>
<td>x Sodium</td>
<td>x A/G ratio</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>x Alkaline phosphatase (ALK)</td>
<td>x Glucose (fasting)</td>
</tr>
<tr>
<td>Cholinesterase (ChE)*</td>
<td>x Total bilirubin</td>
</tr>
<tr>
<td>x Creatine phosphokinase</td>
<td>x Direct bilirubin</td>
</tr>
<tr>
<td>Lactic acid dehydrogenase (LDH)</td>
<td>x Total serum protein (TP)</td>
</tr>
<tr>
<td>x Serum alanine aminotransferase (also SGPT)</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>x Serum aspartate aminotransferase (also SGOT)</td>
<td>Serum protein electrophoresis</td>
</tr>
<tr>
<td>x Gamma glutamyl transferase (GGT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See statement below on cholinesterase.

Cholinesterase assays were performed on 10 rats/sex/dose at 3, 6 and 12 months (satellite animals) and at 24 months (oncogenicity animals). In addition, erythrocyte assays were performed on selected animals in the control and low dose (100/50 ppm) groups six weeks after the dose level was reduced from 100 to 50 ppm. These animals were not fasted prior to blood collection. Plasma, erythrocyte and brain cholinesterase activity was determined on a Hitachi 717 Boehringer Mannheim Diagnostics Automatic Analyzer using a modified Ellman method (kinetic).
7. **Urinalysis**

Urine was collected from fasted, water-deprived animals (approx. 2 hours) at months 6, 12, 18 and term. The checked (X) parameters were examined.

<table>
<thead>
<tr>
<th></th>
<th>x</th>
<th></th>
<th>x</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Appearance</td>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volume</td>
<td>Ketones</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specific gravity</td>
<td>Bilirubin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sediment (microscopic)</td>
<td>Nitrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>Urobilinogen</td>
<td></td>
</tr>
</tbody>
</table>

*Not required for carcinogenicity studies based on Subdivision F Guidelines.*

Also, 16-hour urine volume samples were collected from animals not deprived of food or water, also at months 6, 12, 18 and term.
8. **Sacrifice and Pathology:**

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition were weighed.

<table>
<thead>
<tr>
<th>DIGESTIVE SYSTEM</th>
<th>CARDIOVASC./HEMAT.</th>
<th>NEUROLOGIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>Aorta*</td>
<td>Brain*</td>
</tr>
<tr>
<td>Salivary glands*</td>
<td>Heart*</td>
<td>Periph. nerve*</td>
</tr>
<tr>
<td>Esophagus*</td>
<td>Bone marrow*</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Stomach*</td>
<td>Lymph nodes*</td>
<td>(3 levels)*</td>
</tr>
<tr>
<td>Duodenum*</td>
<td>Spleen*</td>
<td>Pituitary*</td>
</tr>
<tr>
<td>Jejunum*</td>
<td>Thymus*</td>
<td>Eyes (optic n.)</td>
</tr>
<tr>
<td>Ileum*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon<em>Rectum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver*+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gall bladder*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESPIRATORY</th>
<th>UROGENITAL</th>
<th>GLANDULAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea*</td>
<td>Kidneys**+</td>
<td>Adrenal glands*</td>
</tr>
<tr>
<td>Lung</td>
<td>Urinary bladder*</td>
<td>Lachrimal gland</td>
</tr>
<tr>
<td>Nose (2 levels)</td>
<td>Testes**</td>
<td>Mammary gland*</td>
</tr>
<tr>
<td>Pharynx</td>
<td>Epididymides</td>
<td>Parathyroids**+</td>
</tr>
<tr>
<td>Larynx</td>
<td>Prostate</td>
<td>Thyroids**+</td>
</tr>
<tr>
<td></td>
<td>Seminal vesicle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovaries**+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uterus*</td>
<td></td>
</tr>
</tbody>
</table>

| OTHER |
### SACRIFICE AND PATHOLOGY

<table>
<thead>
<tr>
<th>RESPIRATORY (cont.)</th>
<th>UROGENITAL (cont.)</th>
<th>Bone</th>
<th>Skeletal muscle</th>
<th>Skin</th>
<th>All gross lesions and masses</th>
</tr>
</thead>
</table>

* Required for carcinogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.

In addition to the above, the following tissues were specifically identified as examined histopathologically: harderian glands, oviducts, retina, vagina and zymbal's gland.

## II. RESULTS

### A. OBSERVATIONS:

#### 1. Clinical Signs of Toxicity:

An examination of Appendix C "Physical Observations" (pp. 141-217 of the study report) reveals little evidence of dosing-related clinical signs of toxicity except yellow anogenital staining most notably among females at the 12000 ppm dose level and less so among males at the same dose level. At times this sign extended in a less remarkable manner to females of the 6000 ppm group. There is the absence of the spectrum of cholinergic signs, though mortality was extensive among males and females at 12000 and among males at 6000 ppm as well.

#### 2. Mortality:

Percent survivorship is reproduced below as it appears on p. 50 of the study report.
<table>
<thead>
<tr>
<th>Group</th>
<th>Dose Level (ppm)</th>
<th>Month 12</th>
<th>Month 18</th>
<th>Termination (Month 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>II</td>
<td>100/50</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>500</td>
<td>100</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>IV</td>
<td>6000</td>
<td>100</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>V</td>
<td>12000</td>
<td>96</td>
<td>98</td>
<td>71</td>
</tr>
</tbody>
</table>

Based upon statistical analyses of survivorship data as performed and reported in the study report, mortality was concluded in the study report to be significantly increased at the $p = .01$ level at 6000 ppm and 12000 ppm among males and at 12000 ppm among females.

A matter of concern is whether the 53% survivorship for males at 500 ppm relative to 67% in the control group constitutes evidence of enhanced mortality at this dose level. While there were 90 rats/sex/group at the beginning of the study, interim sacrifices by year one reduced the number in each group by 35 rats, leaving, by design, 55 rats/sex/group to continue on for the second year. However, 1 rat in group 5 died spontaneously prior to the one year time point (see p. 119. of the study report, copy included here in Exhibit 1). So the number of male rats per group post year one were 55 for all but group 5 for which the number at risk post year one was 54. Among these animals, the number of unscheduled deaths during year two were 18 (33%), 14 (25%), 26 (47%), 41 (74.5%) and 54 (100%) for groups 1 through 5 in the respective order. An independent statistical treatment of this data yields $p = 0.0000$ for Cochran-Armitage Trend Test and Fisher’s exact p values versus control of 0.2646, 0.0846; 0.0000 and 0.0000 for groups 2, 3, 4 and 5, respectively. In view of these findings which include a remarkably significant trend test and Fisher’s exact comparisons for the two high dose groups, while the p value for the 500 ppm dose group did not achieve statistical significance at the $p < 0.05$ level, the level of significance that was observed, $p = 0.0864$ is sufficient to indicate as likely an adverse effect on survival at 500 ppm.
Furthermore, according to Haseman et al (1990), "normal 2-year survival rates in NTP carcinogenicity studies are approximately 66% for untreated male F344 rats and 73% for untreated female F344 rats." (p. 556). Thus, control survival rate in this study is consistent with historical control survival data for the F344 rat. The unscheduled deaths in the 500 ppm male group is close to being 50% in excess of that of the contemporaneous and historical control values. For these reasons it is considered appropriate to identify 500 ppm was an effect level for increased mortality among male F344 rats. For increased mortality, LOEL = 500 ppm, NOEL = 50 ppm (males); LOEL = 12000 ppm, NOEL = 6,000 ppm (females).

The study report says that in males, the early deaths were beginning around day 400 (month 14) for the 12000 ppm dose group and around day 600 (month 20) for the 6000 ppm dose group. Independent inspection of the data shows that extra deaths in 500 ppm male group occurred late in the study. In the case of females, deaths were toward the end of the animal's normal lifespan (p. 53 of study report).

According to the study report (p. 50), chronic nephropathy and mononuclear cell leukemia were the two most common causes of moribundity and/or death among males and females at 6000 and 12000 ppm dose levels. This assessment requires some clarification and comment. Tabulated below (p. 25) are the incidences of leukemia and nephropathy for the various dose groups during the second year of the study as compiled from tables of data from various pages of the study report, specifically from pages 2734, 2775, 2810 and 2858. An examination of this table shows that for Group 5 males, leukemia was not a meaningful contribution to moribundity or mortality, although nephropathy was the major cause of death, i.e., death among 47 of 56 unscheduled deaths was attributed to nephropathy. In Group 4 males, both leukemia and nephropathy were major contributors to unscheduled deaths, where together they accounted for 36 of the 41 unscheduled deaths. For all groups of male rats, nephropathy was observed in essentially all animals, but it was only in Groups 4 and 5 that nephropathy was claimed in large number as the cause of death.

Examination of page 2810 of the study report (Exhibit 1) where unscheduled deaths is concerned, the frequency and severity of nephropathy for males tended to increase with increasing dose across Groups 3-5 relative to Groups 1 and 2; and for females across Groups 4 and 5 relative to Groups 1-3. Among terminal sacrificed animals (p. 2734 of the study report, Exhibit 1) interpretation is more difficult.
because of high mortality in groups 4 and 5 and elevated mortality in group 3 males. It is also difficult to interpret for females due to high mortality in Group 5, but there appears to be a dose-related trend toward proportionally higher grade of nephropathy across Groups 3-5 relative to Groups 1 and 2. In the hope of clarifying this somewhat, a table which consolidates the data for nephropathy for terminal sacrifices and unscheduled death animals has been prepared and included in Exhibit 1.

The combined data for females suggest that while nephropathy across dose groups affects nearly all animals, a trend (dosing-related) toward higher graded nephropathy is evident for Groups 3-5 relative to Groups 1-2. In other words, there appears to be a malathion related increase in severity of nephropathy for Group 3 (500 ppm) and becoming progressively more severe in Groups 4 (6000 ppm) and 5 (12000 ppm). The study report claims severity among females increased only at 6000 and 12000 ppm.

Among females, leukemia incidence (p. 21) does not appear to be affected by dosing, although the incidence in Group 5 may have been higher had mortality not been increased in that group, i.e., animals in that group would have been at greater risk of developing leukemia had they lived longer. Mortality from leukemia was essentially the same in all groups (female) and cannot be said to have contributed disproportionately to the increased mortality in Group 5 females.

The problem of competing toxicity is more evident among males, particularly where Groups 3 and 4 are concerned. Leukemia incidence was low in Group 5 probably due to premature mortality from nephropathy. One cannot say what effect malathion would have had on leukemia incidence and leukemia related mortality in Group 5 had there not been the fatal kidney effects. The same can be said of group 4 where the leukemia incidence (18/55) may also have been compromised by increased mortality. Male Group 3 poses a particular challenge for interpretation. Leukemia incidence was not increased relative to the control group, but it was an increased cause of death, i.e., death was attributed to leukemia in 14 group 3 males as opposed to 7 males in the control group.

Furthermore, in consideration of the percentage of male rats in each group diagnosed with leukemia that died of the condition, namely, 7/23 (30%), 7/16 (44%) 14/24 (58%), 13/18 (72%) and 1/1 (100%) for groups 1 through 5, respectively, there is evidence of dosing-related increased
mortality from leukemia among those rats harboring that condition. Rats with leukemia are more likely to die of leukemia as the result of a competing dosing related toxicologic burden of the test material. This effect appears to be evident at 500 ppm and 6000 ppm and perhaps so at 50 ppm, and constitutes supporting evidence of chronic toxicity of the test material at these lower doses.

In summary, for males chronic nephropathy (not leukemia) was the most common cause of death in the 12000 ppm group. At 6000 ppm, chronic nephropathy and leukemia were the two most common causes of death (and presumably moribundity) among males. Also, among males, 500 ppm apparently was an effect level in terms of increased mortality (unscheduled deaths), with leukemia (and not chronic nephropathy) as an increased cause of unscheduled deaths. The incidence of leukemia among males of the 500 ppm group was not increased. It is uncertain whether the incidence of leukemia (18/55) in the 6000 ppm male group would be the expected incidence for that group when adjusted for the increased mortality and the resulting decreased time of exposure. The fact that greater percentages of male rats with leukemia died of the condition in groups 3 and 4 (and possibly group 2 as well) is supporting evidence for a malathion related chronic toxicity effect at these doses.

For females, chronic nephropathy was the principal cause of the increased deaths in the 12000 ppm group. Neither the incidence of leukemia nor deaths due to leukemia was elevated in that group. Yet, for females, 500 ppm appears to be an effect level in terms of increased severity (not incidence) of chronic nephropathy.
## Carcinogenicity Study 83-5

### Incidences (mortality) of Leukemia and Nephropathy

<table>
<thead>
<tr>
<th>Dose Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Examined</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>56</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td><strong>a) Terminal Sacrifice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>41</td>
<td>29</td>
<td>14</td>
<td>0</td>
<td>38</td>
<td>41</td>
<td>41</td>
<td>34</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>b) Unscheduled Deaths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$# 18$</td>
<td>14</td>
<td>26</td>
<td>41</td>
<td>56</td>
<td>17</td>
<td>14</td>
<td>14</td>
<td>21</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td><strong>No. with Leukemia (Death Due To)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23(7)</td>
<td>16(7)</td>
<td>24(14)</td>
<td>18(13)</td>
<td>1(1)</td>
<td>9(5)</td>
<td>18(4)</td>
<td>15(6)</td>
<td>12(5)</td>
<td>10(7)</td>
<td></td>
</tr>
<tr>
<td><strong>No. with Chronic Nephropathy (Death Due To)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54(2)</td>
<td>54(2)</td>
<td>54(4)</td>
<td>55(23)</td>
<td>55(47)</td>
<td>49(0)</td>
<td>53(1)</td>
<td>52(1)</td>
<td>54(2)</td>
<td>53(20)</td>
<td></td>
</tr>
<tr>
<td><strong>No. of Deaths Due to Leukemia or Chronic Nephropathy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>18</td>
<td>36</td>
<td>48</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

*For ranking of severity of chronic nephropathy, see Exhibit 1, a) terminal sacrificed animals, p. 2734 of Study Report and b) unscheduled deaths, p. 2810 of Study Report.*
B. BODY WEIGHT:

An inspection of mean body weight change data as presented in Appendix A (pp. 399-445 of the study report) discloses a generally consistent decrement of body weight gain ranging upward in time for males of about 3-13% at 6000 and 12-32% at 12000 ppm dose levels. Among females, there were consistent decrements of about 10-15% at 12000 ppm throughout the bulk of the study period with numerical inhibitions of about 4-5% observed at 6000 ppm which were at certain time intervals (e.g. 0-10 and 0-20 weeks) statistically significant. See table below of selected body weight gain data as taken from the study report. The study report says "The mean body weights and body weight gains of male and female animals at 6000 and 12000 ppm dose levels were reduced compared to the controls throughout most of the study. Differences from controls were statistically significant in the males and females at 12000 ppm throughout the treatment period. At 6000 ppm, differences from controls were statistically significant in the males from week 13 through termination and in females from week 3 through week 50." (p. 62 of study report). It is reasonable therefore to conclude that for body weight effects, LOEL (M,F) = 6000 ppm; NOEL (M,F) = 500 ppm.
<table>
<thead>
<tr>
<th>Weeks</th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5/100</td>
<td>500</td>
<td>6000</td>
<td>12000</td>
<td></td>
<td>0</td>
<td>5/100</td>
<td>500</td>
</tr>
<tr>
<td>0-5</td>
<td>94.3</td>
<td>93.1</td>
<td>94.9</td>
<td>92.6</td>
<td>82.4**</td>
<td>43.3</td>
<td>44.5</td>
<td>45.2</td>
<td>42.1</td>
</tr>
<tr>
<td>0-10</td>
<td>134.2</td>
<td>135.1</td>
<td>137.6</td>
<td>130.0</td>
<td>118.3**</td>
<td>59.7</td>
<td>60.5</td>
<td>62.0</td>
<td>56.3*</td>
</tr>
<tr>
<td>0-20</td>
<td>174.8</td>
<td>177.3</td>
<td>179.3</td>
<td>166.3**</td>
<td>155.7*</td>
<td>77.3</td>
<td>79.8</td>
<td>79.9</td>
<td>73.4*</td>
</tr>
<tr>
<td>0-30</td>
<td>201.6</td>
<td>202.7</td>
<td>205.8</td>
<td>192.1**</td>
<td>175.6**</td>
<td>88.9</td>
<td>91.1</td>
<td>93.0</td>
<td>85.0</td>
</tr>
<tr>
<td>0-50</td>
<td>228.5</td>
<td>225.6</td>
<td>227.9</td>
<td>211.3*</td>
<td>193.8*</td>
<td>106.9</td>
<td>109.5</td>
<td>110.5</td>
<td>101.8</td>
</tr>
<tr>
<td>0-82</td>
<td>237.8</td>
<td>231.0</td>
<td>233.5</td>
<td>206.7**</td>
<td>162.0**</td>
<td>137.2</td>
<td>141.7</td>
<td>144.2</td>
<td>131.8</td>
</tr>
</tbody>
</table>

* p ≤ 0.05 Dunnett's Test
** p ≤ 0.01
C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. Food consumption:

Inspection of mean food consumption data as expressed in g/kg/day (pp. 446-457 of the study report reproduced here as Exhibit 2) discloses the following:

**Males:** Possible early treatment-related increases in food consumption at all doses. For example, by week 4, there were statistically significant increases in food consumption of 2.6% at 6000 and 3.3% at 12000 ppm dose levels. On weeks 5 and 7 through 12 there were statistically significant increases in food consumption of approximately 2-7% in all dose groups, except the increase for week 12 at 500 ppm, which did not achieve statistical significance. It is noteworthy that the table for mean percent differences in food consumption presented on p. 63 of the study report (p. 63 included in Exhibit 2) used in support of the Registrant’s arguments, begins with data as late as week 14, and does not disclose the earlier time point effects or food consumption. The low dose (group II) effects mentioned above preceded the reduction in dosage level from 100 to 50 ppm, which took place after week 16. The increased food consumption during the earlier period of the study tended to be a dosing-related effect. Throughout the bulk of the study, beyond week 18, food intake was statistically significantly elevated in the high dose group, and somewhat later in the study, weeks 74 through 86, significant increases were again frequently evident at 6000 ppm. Hence, for males there was no NOEL, though an effect was not observed in the low dose group following the adjustment in dosage concentration from 100 to 50 ppm. One cannot be certain that effects on food intake would not have been evident early in the study had 50 ppm been the initial low dose concentration.

**Females:** Food consumption at 12000 ppm was generally increased, often with statistical significance at various weekly intervals. From week 78 to the end of the study the effect was most pronounced, where increases were 12-26% at various weekly intervals relative to control values. Food consumption at lower doses was not so remarkably affected except in the 6000 ppm group late in the study, post week about 78, where increases were of sufficient magnitude and statistically significant often enough to conclude that increased food consumption was a treatment-related
effect. However, during the first five weeks of study, food consumption among females of the 100 ppm group was elevated by 3-5%, and was statistically significant at all but one of the six weekly-time point assessments involved. Similar elevations of no greater magnitude were seen in other dose groups during these early weeks. Between weeks 6 and 16, before dosing was reduced in the low dose group to 50 ppm, there were no clear dosing related effects at any dose level, although group 4 tended to be lower at times after week 18. Hence, for males and females, LOEL = 6000 ppm; NOEL = 500 ppm for the bulk of the study period, however, a NOEL cannot be established for either sex during the earlier periods before the dose level change was made.

2: Compound Intake:

Mean test substance intake, based upon food consumption data, body weight and nominal dose levels, as tabulated in the study report (p. 64) is reproduced below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose Level (ppm)</th>
<th>Weeks</th>
<th>Male</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>1-102</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>1-16</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>18-102</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>100/50</td>
<td>1-102</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>500</td>
<td>1-102</td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td>IV</td>
<td>6000</td>
<td>1-102</td>
<td>359</td>
<td>415</td>
</tr>
<tr>
<td>V</td>
<td>12000</td>
<td>1-102</td>
<td>739</td>
<td>868</td>
</tr>
</tbody>
</table>

D: OPHTHALMOSCOPIC EXAMINATION:

An examination of results as presented in Appendix D (pp. 212-312 of the study report) does not disclose any treatment related effects of malathion. Furthermore, this was the conclusion of the study pathologist for ophthalmoscopic effects, Dr. Lionel Rubin. It is interesting that retinal degeneration (see summary incidence for males, p. 226 of the study report) as
assessed ophthalmoscopically occurred with greater incidence among rats examined after 52 weeks than after 104 weeks. For example, among Group II male rats, there were 8 incidences of retinal degeneration, at 52 weeks, yet after 104 weeks there was but one rat with retinal degeneration even though 7 of 8 rats observed with retinal degeneration at week 52 were among those examined at 104 weeks. Evidently, 7 rats with retinal degeneration at week 52 no longer had the condition at week 104 as assessed ophthalmoscopically. It appears therefore that retinal degeneration observed after 52 weeks was essentially reversed during the second year. To the extent that the retina is considered to be a neural tissue, restoration of degeneration with continued dosing is somewhat puzzling and unexpected.

E. ELECTRORETINOGRAPHIC EXAMINATION:

The results of ERG testing are summarized as follows. Mean values for a- and b-amplitudes (uV) and latencies (msec) for all study groups as evaluated at pretest (P) and months 3, 6, 12 and 24 are appended as Exhibit 3 (pp. 325-326 of the study report). In discussing this data, the study author evaluated changes in these parameters over the first 12 months by pairing mean data at each time point (3, 6 and 12 months) with that of the previous time point for each study group.

Since ERG data taken at term (24 months) were on different animals for which there were no earlier time point or pretest data, comparisons were made with mean values for all other pretest animals assessed. The particular discussion in question by the study author is located on page 55-61 and 314-322 (Exhibit 4) of the study report.

A fundamental hypothesis of the study author is that in the course of time, and particularly in the ageing F344 rat there is a progressive drop-out or decline in the number of retinal neurons that are responsive to a flash of light as used in ERG testing. If there were a test material enhancement of this decline in retinal neurons, there would be superimposed on the age-related decline a dose-related decline as well. Such an effect of the test material is not apparent in this study. For example, at the 12-month time point, b-wave and a-wave amplitudes expressed as percentages of pretest values, as tabulated in the study report (p. 56) are reproduced below. The percentages presented in this table have been confirmed from data elsewhere in the
study as presented in Exhibit 4. Inspection of these tables confirms the study authors' conclusion that after 12 months there is no dosing-related effect on a- or b-wave amplitudes. Similarly reproduced below are tables of percentage changes in a- and b-wave at 24 months, where mean pretest values for all animals tested were used for comparison. Again, there is no evidence of a dosing-related effect.

<table>
<thead>
<tr>
<th>12 month b-wave amplitudes expressed as a percent of pretest values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12 month a-wave amplitudes expressed as a percent of pretest values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
</tbody>
</table>
### 24 month mean b-wave amplitudes expressed as a percent of mean pretest values for the entire pretest population

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose Level (ppm)</th>
<th>Male (189.4 uV*)</th>
<th>Female (135.4 uV*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>62</td>
<td>87</td>
</tr>
<tr>
<td>II</td>
<td>100/50</td>
<td>49</td>
<td>22</td>
</tr>
<tr>
<td>III</td>
<td>500</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>IV</td>
<td>6000</td>
<td>52</td>
<td>.62</td>
</tr>
<tr>
<td>V</td>
<td>12000</td>
<td></td>
<td>51</td>
</tr>
</tbody>
</table>

* pretest mean

### 24 month mean a-wave amplitudes expressed as a percent of mean pretest values for the entire pretest population

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose Level (ppm)</th>
<th>Male (189.4 uV*)</th>
<th>Female (87.4 uV*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>37</td>
<td>71</td>
</tr>
<tr>
<td>II</td>
<td>100/50</td>
<td>51</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>500</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>6000</td>
<td>35</td>
<td>44</td>
</tr>
<tr>
<td>V</td>
<td>12000</td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>

* pretest mean

It is noteworthy that while there is no evidence of a progressive compromise of a- or b-wave response with increasing dose, the responses for all dose groups appear more remarkably impaired than the control. One would have to conclude at least that variability in a- and b-wave testing results is too great for the test model to detect what could be meaningful compromises in ERG responsiveness that may have resulted from dosing. The study author concludes "The lack of dose response and the high degree of variability of the data make a compound related effect unlikely." (p. 57 of study report). An alternative conclusion is that variability was so remarkable that a dosing-related effect is rendered indeterminable.
Additional independent comparisons made between ERG responses of various dose groups and control values for a- and b-wave amplitude and latency at various time intervals taken from mean electroretinogram values as presented in the study report (pp. 325-326). (Exhibit 3) serves to confirm the basic conclusion that there are no progressive dosing related effects. However, there is also confirmation of the finding that dose groups as a whole were less responsive, and the data too variable to be useful in detecting meaningful effects of the test material, if occurring.

In addition to ERG a- and b-wave amplitude and latency data, the incidence of abnormal electroretinograms was discussed in the study report. Among rats at 24-months (termination), for example, the tabulation of incidences of abnormal ERGs presented in the study report (p. 58) is reproduced below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose Level (ppm)</th>
<th>Total Incidence</th>
<th>Male Incidence</th>
<th>Female Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>1/9</td>
<td>1/4</td>
<td>0/5</td>
</tr>
<tr>
<td>II</td>
<td>100/50</td>
<td>4/8</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>III</td>
<td>500</td>
<td>7/9</td>
<td>3/4</td>
<td>4/5</td>
</tr>
<tr>
<td>IV</td>
<td>6000</td>
<td>3/8</td>
<td>1/4</td>
<td>2/4</td>
</tr>
<tr>
<td>V</td>
<td>12000</td>
<td>2/4</td>
<td>-</td>
<td>2/4</td>
</tr>
</tbody>
</table>

As discussed by the study author (p. 320), the above incidences exclude animals in which unilateral disease indicates the animal is not capable of providing a response to stimulation with white light as employed in ERG testing. These data also do not reveal any dose-related increases in abnormal ERGs in rats of either sex, but in terms of total incidences, all dose groups had higher incidences of abnormal ERGs. The study report affirms (p. 58) that the number of abnormal or nonrecordable ERGs of treated rats is greater in each treated group than in the control. Again, the high variability in abnormal ERGs would be expected to mask meaningful compound-related effects.

In reference to those rats that received ERG testing at termination, the study report claims the following: "There was no significant difference in the histologic appearance of the retina between untreated (Group I)
animals and high-dose (Group V females and Group IV males) at termination (24 months). The histology of the fellow eyes (i.e., the left eye rather than the ERG-tested right eye) was compared in high-dose and control animals. Most (4/5) Group I males had a peripheral retinal degeneration of a slight to moderate degree. Four of five Group IV males had peripheral retinal degeneration and one rat had generalized retinal degeneration." (p. 61 of the study report). The male rats in question as identified in the report of Dr. Lionel Rubin were untreated (Group I): 1010, 1012, 1015, 1024 and 1043, and Group IV: 4013, 4020, 4029, 4039 and 4050. (pp. 352-353 of the study report) An independent reading of the individual histopathology sheets for the identified rats discloses that in Group I, rats 1012 and 1015 were negative for retinal degeneration while rats 1010, 1024 and 1043 exhibited retinal "degeneration/atrophy" of a slight to minimal degree that was "focal" for rat 1024 and "multifocal" for rats 1010 and 1043. For Group IV rats, rat 4013 exhibited "marked" retinal degeneration/atrophy while the other four rats in this group exhibited slight to minimal, focal or multifocal retinal degeneration/atrophy. Thus among controls there were 3/5 (not 4/5) with slight to minimal retina degeneration (the term peripheral does not appear on the pathology sheets) while among Group IV rats 5/5 were affected, one with "marked" retinal degeneration/atrophy. The term "generalized" retinal degeneration does not appear.

It is noteworthy that during ERG testing, rat # 4013 was "incapable of responding to stimulation with light due to complete cataract and unilateral retinopathy", according to the report of Dr. Rubin. (p. 353 of the study report) The other rats in Group IV yielded ERG data. So when comparing histopathology of the retinas for the very male animals that received ERGs at term, there is some level of concern that the dosed group is more remarkably affected. However, this is not reflected in a comparison of retinal degeneration/atrophy for male Groups I versus IV in the study-wide histopathology assessment as disclosed in the overall summaries of microscopic postmortem findings, pp. 2924-25 of the study report.

With regard to females examined by ERG at term, the study report says: "In the untested eyes of female rats subjected to electroretinography, all Group I animals (5/5) had peripheral retinal degeneration histologically. In four of five Group V female rats
the preparation of the retinas was good; in the fifth rat there were sufficient fracture artifacts of the tissue to make interpretation difficult. Two of the four Group V female rats had peripheral retinal degeneration; the other two had no significant retinal degeneration." (p. 61 of the study report).

The histopathology data in concert with ERG data support a conclusion that the test model is inadequate to detect meaningful chronic effects of the test material on visual parameters assayed.

Data from this study were provided to Dr. William Boyes for evaluation. His conclusions are appended (Exhibit 5).

F. BLOOD WORK:

1. Hematology:

Hematology parameters which exhibited statistically significant alterations considered treatment related are tabulated in the Results and Discussion Section of the study report (pp. 65-66) and reproduced here as Exhibit 6, appended. An inspection of the tabulations of data in conjunction with the mean findings for all hematologic parameters as presented in the study report (pp. 494-541) confirms the adequacy of the reproduced table to convey positive findings. Actually, the only hematologic parameter assayed that did not respond to treatment was reticulocyte count. From Exhibit 6, it can be seen that the following were small but statistically significant findings: reduced hemoglobin and hematocrit at one or more time intervals at 6000 and 12000 ppm in both sexes, where the effect in males was more remarkable in terms of degree of lowering of and endurance in time; erythrocyte count reduction was observed at 12000 ppm at 12 months in males only; platelet count increases at 6000 and 12000 ppm in rats of both sexes; decreases in mean corpuscular volume and mean corpuscular hemoglobin at 6000 and 12000 ppm in rats of both sexes; decreased mean corpuscular hemoglobin concentration in males only at 6000 and 12000 ppm; and increased total leukocyte counts in rats of both sexes at 12000 ppm at the 12 month time point only. Hence, for hematologic parameters overall, LOEL = 6000 ppm (M,F); NOEL = 500 ppm (M,F).
2. Clinical Chemistry:

a. Cholinesterase:

Cholinesterase data (plasma, erythrocyte, brain) are adequately tabulated in summary form in the Results and Discussion Section of the study report (p. 69) and is reproduced here as Exhibit 7 for reference purposes. Examination of mean cholinesterase data as presented in Appendix J of the study report (pp. 566-714) supports the tabulation of cholinesterase data reproduced here as Exhibit 7.

Cholinesterase data as summarized in Exhibit 7 discloses the following:

1) Erythrocyte Cholinesterase: at the 3-month time point statistically significant inhibition was observed among females at all dose levels. The inhibition for females relative to mean values of the contemporaneous control were: 25% at 100 ppm, 30% at 500 ppm, 58% at 6000 ppm and 66% at 12000 ppm. The inhibition of erythrocyte cholinesterase among females was not only statistically significant at each dose level, but exhibited a dose-response across all doses. In consideration of the finding of erythrocyte cholinesterase inhibition among females at 100 ppm, the lowest test dose, the Registrant elected to reduce the concentration of malathion in the diet at the low dose level to 50 ppm for dosing beyond month 3. This reduction was effected for both sexes even though the enzyme among males was inhibited only at 6000 and 12000 ppm dose level at 3 months. At the subsequent time points erythrocyte cholinesterase for females was significantly inhibited in a dose related manner at 6000 ppm and 12000 ppm dose levels at 6 months and at 500, 6000 and 12000 ppm dose levels at 12 months and term. Inhibitions at term were 27% at 500 ppm, 44% at 6000 ppm and 52% at 12000 ppm. Inhibition at the top three doses tends to be less at term than at 3 months, indicating some degree of adaptive recovery.

Among males, erythrocyte cholinesterase was inhibited only at 6000 and 12000 ppm, but consistently so at these dose levels. For example, at the 3-month time point, the enzyme was inhibited 48% at both 6000 and 12000 ppm and at the 12-month time point, was inhibited 45% at 6000 ppm and 58% at 12000 ppm.
Hence, for erythrocyte cholinesterase inhibition LOEL = 6000 ppm, NOEL = 500 ppm for males.

For females, LOEL = 100 ppm. A NOEL was not identified at 3 months. At subsequent time intervals (excepting 8 months), LOEL = 500 ppm, NOEL = 50 ppm for females. The data are not sufficiently definitive to conclude a NOEL for the study for females, given the statistically significant 27% inhibition at 100 ppm on a shallow dose-response curve between 100 and 12000 ppm.

Furthermore, even though erythrocyte cholinesterase was not inhibited among females at the 6 month and subsequent time intervals at 50 ppm, there is no certainty the enzyme would not have been inhibited at 50 ppm during the first 3 months of study, particularly in view of the shallow dose response and the propensity for the enzyme to recover for a period from an initial inhibition as it did after 6 months at 500 ppm.

Accordingly, the finding identifies the need for a 3-month study, employing a larger number of animals, to define with more certainty the NOEL for erythrocyte cholinesterase inhibition in the female F344 rat.

2) **Plasma Cholinesterase**: In reference to Exhibit 7, at the 3, 6 and 12 month time points, among males, plasma cholinesterase was inhibited only at the 6000 and 12000 ppm dose level, where inhibition ranged 17-27% at 6000 ppm and 43-53% at 12000 ppm. However, at terminal sacrifice, the enzyme was inhibited 29% at 500 ppm and by 64% at 6000 ppm. Among females, the enzyme was not inhibited at doses up to and including 500 ppm. The enzyme was statistically significantly inhibited at 6000 ppm by 38-61% across the four time intervals and at 12000 ppm by 70-89% across the four time points. Hence, for plasma cholinesterase inhibition, LOEL = 500 ppm, NOEL = 50/100 ppm for males and LOEL = 6000 ppm; NOEL = 500 ppm for females.

3) **Brain Cholinesterase**: Brain cholinesterase was statistically significantly inhibited only at 6000 and 12000 ppm in rats of both sexes. At 6000 ppm, inhibition among males across the four time points ranged 11-31% and in females ranged 12-18%. The respective inhibitions at 12000 ppm ranged 15-19% (first 3 time points) for males and 28-67% (28-49% over the first 3 time points) for females. Hence, for brain cholinesterase inhibition, LOEL = 6000 ppm and NOEL = 500 ppm for rats of both sexes.
Other Clinical Parameters:

From among the various clinical parameters assayed, the study report consolidated in tabular form those for which there may be treatment related effects (pp. 75-76), reproduced here as Exhibit 8. An examination of mean values for all clinical chemistry parameters as presented in Appendix J of the study report (pp. 566-714) confirms the tabulated findings to represent essentially all of the possibly treatment related effects. In reference to Exhibit 8, the following were noteworthy effects of dosing with malathion:

Aspartate Aminotransferase:

Among females there were statistically significant reductions in enzyme activity at 500, 6000 and 12000 ppm at the 12 month time point. The respective magnitudes of reduction at the three dose levels were 41%, 40% and 50%. These effects might be due simply to an inordinately high value for the control group (132 IU/L) at week 12. Control values at other time points were uniform and considerably lower. The enzyme was also depressed 42% (statistically significant) and 28% (non-statistically significant) at 18 months in the 12000 and 6000 ppm groups, respectively. There were no remarkable effects for the enzyme among male rats at the various time points and dose levels.

Alanine Amino Transferase:

Among females at 12 months there were statistically significant decreases in this enzyme of about 36% in all three of the top dose levels. The lack of a dose response and the inordinately high control group value of 86 IU/L at 12 months as contrasted with the other time points renders questionable the significant findings at the top three dose levels. There were no noteworthy effects for this enzyme among male rats.

Alkaline Phosphatase:

Responses in males and females were remarkably similar. At the 6 months time point, the enzyme was decreased by 24-28% at 6000 ppm and by 29-36% at 12000 ppm; at 12 months, the same respective
dose group inhibitions were 25-29% and 27-45%. At the 18 months time point the enzyme was significantly inhibited in males and females by 30-40% in the 12000 ppm dose group. For reduced alkaline phosphatase activity, LOEL = 6000 ppm; NOEL = 500 ppm (M, F).

Blood Urea Nitrogen (BUN):

The findings for this parameter are somewhat difficult to interpret. Among males, the small but statistically significant reductions of 12-15% at 6000 and 12000 ppm during the first 12 months are of uncertain interpretation. However, the statistically significant 90% increase in this parameter at month 18 in the 12000 ppm group is likely a manifestation of deterioration of the health of the group of rats that resulted in their total mortality prior to term. Among females, the similar minor decrease of 11-14% during the first 12 months are considered to be of questionable importance. For increased BUN, LOEL = 12000 ppm, NOEL= 6000 ppm (M); LOEL > 12000 ppm; NOEL = 12000 ppm (F).

Cholesterol:

This parameter was elevated in a dose-related manner among rats of both sexes at the 6000 and 12000 ppm dose levels. Among males at 6, 12, 18 and 24 months (term), the parameter was increased, respectively, by 65%, 43%, 46% and 139%, at 6000 ppm, where all but the 18 month increases were reportedly statistically significant. Increases in the same respective order at 12000 ppm were 109%, 124%, 227% and N/A. All increases were reported to be statistically significant. Among females, the increases at 6, 12, 18 and 24 months (term) for the 6000 ppm dose group were 22%, 20%, 83% and 30%, respectively, where only the latter increase was not reported to be statistically significant. The same respective increases for the 12000 ppm group were 48%, 32%, 127% and 63%, all of which values were statistically significant. Hence, for increased cholesterol levels, LOEL = 6000 ppm (M, F); NOEL = 500 ppm (M, F).
Gamma-Glutamyl Transferase:

An inspection of the tabulated data discloses highly variable, and often times remarkable, increases in the enzyme at the various time points in both the 6000 and 12000 ppm groups of both sexes. Hence, LOEL = 6000 ppm, NOEL = 500 ppm (M,F).

The following are additional findings identified in Appendix J not presented in the Table (Exhibit 8) which were perhaps more equivocal in nature; reduced albumin in males at 6000 and 12000 ppm at 12 and 18 months; reduced albumin/globulin ratio in males at 6000 and 12000 ppm at 18 months; increased globulin and decreased albumin/globulin ratio in females at 6000 and 12000 ppm at 18 months; increased creatine kinase in males at 500 and 6000 ppm at term; decreased total protein in males at 6000 ppm at term; decreased total protein and albumin in females at 6000 and 12000 ppm at term and decreased albumin/globulin ratio in females at 12000 ppm at term. All of these effects were essentially limited to the two high dose levels except increased creatine kinase in males which also occurred at 500 ppm.

G. URINALYSIS:

An inspection of individual urinalysis data presented in Appendix K (pp. 715-754 of the study report) reveals few remarkable findings of dosing-related effects. Following is a tabulation of mean urine pH values as computed from the individual data.

<table>
<thead>
<tr>
<th>Dose Group (ppm)</th>
<th>6 Mo.</th>
<th></th>
<th>12 Mo.</th>
<th></th>
<th>18 Mo.</th>
<th></th>
<th>24 Mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td></td>
<td>M</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.5</td>
<td>7.75</td>
<td>6.85</td>
<td>6.91</td>
<td>7.30</td>
<td>7.20</td>
<td>7.05</td>
</tr>
<tr>
<td>50</td>
<td>7.25</td>
<td>7.28</td>
<td>6.83</td>
<td>6.85</td>
<td>7.00</td>
<td>7.00</td>
<td>7.15</td>
</tr>
<tr>
<td>500</td>
<td>7.15</td>
<td>7.28</td>
<td>6.65</td>
<td>7.15</td>
<td>7.20</td>
<td>6.75</td>
<td>7.15</td>
</tr>
<tr>
<td>6000</td>
<td>6.8</td>
<td>7.35</td>
<td>6.75</td>
<td>6.70</td>
<td>6.90</td>
<td>6.60</td>
<td>6.0</td>
</tr>
<tr>
<td>12000</td>
<td>6.25</td>
<td>6.65</td>
<td>6.80</td>
<td>6.00</td>
<td>6.80</td>
<td>7.20</td>
<td>-</td>
</tr>
</tbody>
</table>
There is an indication in the data of a drop in pH with increasing dose for rats of both sexes, particularly males, at the 6 month time point. Whether this finding early in the study is related to dosing or is a random finding is uncertain. Malathion is a diethyl ester of a dicarboxylic acid, which upon hydrolysis yields carboxyl groups which in turn could explain decreased pH. The data also indicate an increase of cloudiness of urine in rats of both sexes, particularly at the highest dose level. Beyond these observations, the study report claims that there were no treatment related urinalysis findings, an observation supported by independent inspection of the data as presented.

H. SACRIFICE AND PATHOLOGY:

1. Organ Weights:

For reference purposes, mean absolute organ weight and relative organ weight values for the interim (12-month) and terminal sacrifices are reproduced here (Exhibit 9) from pp. 756-771 of Appendix L of the study report.

Noteworthy findings relative to control values (i.e., percentage changes) are as follows:

a. Interim Sacrifice:

Brain: Statistically significantly increased on a body weight basis by 11% in males and 9% in females at 12000 ppm. These increases in both sexes are considered the consequence of reduced body weight. Adrenals: Statistically significantly increased on a body weight basis by 17% in males and a non-statistically significant increase of 10% in females at 12000 ppm. These effects are considered consequences of body weight decline in both sexes. Heart: Statistically significantly increased on a body weight basis by 7% at 6000 ppm and 10% at 12000 ppm among males and 7% at 12000 ppm among females. Kidneys: Absolute organ weight was statistically significantly increased by 14% at 6000 ppm and 29% at 12000 ppm among males and by 9% and 13% among females, respectively. Relative to body weight, the respective kidney weight increases were 19% and 45% among males and 12% and 24% among females. Similarly on a brain weight basis, kidney weights were increased by 13% and 31% among males and by
9% and 15% among females. Liver: Liver weights were statistically significantly increased for males on all three bases of assessment at the two highest doses. These respective increases at 6000 ppm and 12000 ppm were: absolute, 22% and 36%; relative to body weight, 23% and 53%; relative to brain weight, 23% and 37%. For females, liver weight was statistically significantly increased on all three bases at 12000 ppm. These increases were 16% (absolute), 28% (relative to body weight) and 18% (relative to brain weight). At 6000 ppm, increases occurred on all three bases of expression, but achieved statistical significance only on the relative to body weight basis. These increases were 8% (absolute), 11% (relative to body weight) and 8% (relative to brain weight). Spleen: Among male rats, spleen weight was statistically significantly increased at 12000 ppm by 23% (absolute weight), 38% (relative to body weight) and 24% (relative to brain weight). The respective increases for males at 6000 ppm were 9%, 14% and 9%, where only the increases on the relative to body weight basis was reportedly statistically significant. Spleen weight for females was not remarkably altered on any basis of expression. Testes/Epididymides, there were no remarkable effects on absolute or relative to brain weight modes of expression. Statistically significant increases on a body weight basis of 7% and 13% at the 6000 ppm and 12000 ppm dose levels are considered expressions of body weight reduction. Ovaries: There were no remarkable effects. Thyroid/Parathyroid: Among male rats, statistically significant increases in all three modes of expression were found at the top two dose levels. At 12000 ppm, the increases were 25% (absolute), 40% (relative to body weight) and 27% (relative to brain weight). The respective increases at 6000 ppm were 15%, 20% and 15%. Among females, there were no remarkable effects on absolute and relative to brain weight modes of expression. Increases of 12% (non-significant) at 12000 ppm and 14% at 6000 ppm were observed on the relative to body weight basis.

In conclusion, for the interim (12-month) sacrifice, the following organ weight increases are considered treatment related. Males: (6000 ppm and 12000 ppm), kidneys, liver, spleen and
thyroid/parathyroid; **females**: (6000 ppm and 12000 ppm), kidneys and liver.

b. **Terminal Sacrifice**:

**Brain**: There were no remarkable effects on brain weight for male rats in the 6000 ppm dose group. (recalling there were no surviving male rats of the 12000 ppm group). Among females, there was a statistically significant 24% increase relative to body weight at 12000 ppm, considered to be a reflection of the remarkable decline in body weight at that dose level. Brain weight among females was not remarkably affected at 6000 ppm.

**Adrenals**: There were no remarkable effects for rats of either sex. There was a large variability in adrenal weight data, making it more difficult to detect any effect of treatment that may have occurred. **Heart**: Among male rats of the 6000 ppm dose group there were statistically significant increases in heart weight of 17% (relative to body weight) and 10% (relative to brain weight) and a non-statistically significant increase in absolute heart weight of 6%. The concerted effects of a 6% increase in absolute weight and the decline of body weight and a modest brain weight decline explains the significant relative heart weight increases. The effect on heart weight is here considered an effect of treatment at 6000 ppm. Among females, there were no remarkable treatment related effect on heart weight. **Kidneys**: Among males, kidney weight was statistically significantly increased at 6000 ppm by all three modes of expression, 11% (absolute), 22% (relative to body weight and 15% (relative to brain weight). Among females, kidney weight was statistically significantly increased at the top two doses by all three modes of expression. The respective increases at 6000 and 12000 ppm were 22% and 37% (absolute), 28 and 72% (relative to body weight) and 23% and 41% (relative to brain weight).

**Liver**: Among male rats, liver weight was statistically significantly increased at 6000 ppm, 34% (absolute), 47% (relative to body weight) and 38% (relative to brain weight). Although not statistically significant, the respective increases at the 500 ppm dose level were 8%, 10% and 7%. For females, liver weight was statistically significantly increased at 6000 ppm and 12000 ppm, the respective increases being 30%
and 31% (absolute), 35% and 61% (relative to body weight) and 31% and 35% (relative to brain weight). Non-statistically significant increases at 500 ppm were 7% (absolute), 4% (relative to body weight) and 7% (relative to brain weight). Spleen: There were no remarkable effects of treatment at any dose level for rats of either sex. Testes/Epididymides: At 6000 ppm there were non-statistically significant weight increases of 11% (absolute), 21% (relative to body weight) and 15% (relative to brain weight). Ovaries: Very remarkable decline in ovary weight at all doses. suggests control values were excessively high (note high standard deviation for the control). Thyroid/parathyroid: Among males there were statistically significant increases at 6000 ppm of 19% (absolute), 29% (relative to body weight) and 22% (relative to brain weight). Respective non-statistically significant increases at 500 ppm were 98%, 82% and 77% (note the high standard deviation for the absolute weight mean value). For females, respective decreases in weight at 12000 ppm were 26%, 8% and 24%. Of these values, only the change relative to body weight (i.e., 8%) was statistically significant. At 6000 ppm respective decreases were 8%, 6% and 8%, all of which were statistically significant. There were non-statistically significant decreases at 50 ppm and 500 ppm, which may be indicative of inordinately high control weight (standard deviation for the control group was considerably greater than for the other groups).

In conclusion, at terminal sacrifice, heart weight was increased among males at 6000 ppm. Kidney weight and liver weight were increased in males at 6000 ppm and in females at 6000 ppm and 12000 ppm. There was a small non-statistically significant increase in liver weight among 500 ppm males by all three modes of expression. Whether this is treatment related is uncertain. Also uncertain is a non-statistically significant increase in testes/epididymides at 6000 ppm. Thyroid/parathyroid weight was increased for males at 6000 ppm, and likely so at 500 ppm, and decreased among females at 12000 ppm and likely so at 6000 ppm.
2. **Gross Pathology:**

a. **Three-Month Interim Sacrifice:**

Ten rats/sex/group were sacrificed at the three month time point into the study with primary intent to address ocular effects findings. However, each rat received a complete postmortem macroscopic examination. Inspection of Appendix M, Table 1A (pp. 2604-2608 of the study report) does not reveal any noteworthy macroscopic findings except perhaps 2 of 10 females with ovarian cyst in the 12000 ppm group and 1 of 10 in the 6000 ppm group, with none in lower dose groups or control.

b. **Six-Month Interim Sacrifice:**

Ten rats/sex/group were sacrificed at the six-month interim sacrifice, again with primary intent directed toward the visual system. Inspection of Appendix M, Table 1B (pp. 2609-2613 of the study report) does not reveal any noteworthy findings.

c. **Twelve-Month Interim Sacrifice:**

Fifteen rats/sex/group were sacrificed at the twelve month time point. An examination of macroscopic findings as presented in Appendix M, Table 1C (pp. 2614-2619 of the study report) does not disclose any clear treatment related findings. The following are noted: There were a number of abnormalities of the eyes, but were random in nature and not evidently treatment related. One in 14 male rats of the 12000 ppm group exhibited irregular surface of the kidneys. One in 15 females in both the 6000 ppm and 12000 ppm groups had liver "module(s)/mass(es)."

d. **Terminal (24-month) Sacrifice:**

Rats surviving to term were sacrificed. An examination of macroscopic findings as presented in Appendix M, Table 1D (pp. 2620-2627 of the study report) reveals the following. Irregular surfaces of the kidneys where the following incidences were found: 1/37 (3%); 2/41 (5%); 4/29 (14%) and 4/14 (29%) among males of the respective 0, 100/50, 500 and 6000 ppm dose groups and among females 0/38 (0%); 0/41 (0%); 3/41 (7%); 2/34 (6%)
of the same respective dose groups plus 2/20 (10%) of the 12000 ppm dose group. Enlarged liver was observed among 0/37 (0%); 1/41 (2%); 2/29 (7%) and 3/14 (21%) male rats of the 0, 50/100, 500 and 6000 ppm dose groups. Liver enlargement was not a reported finding among females of any group. Macroscopic findings were not reported for the nose/turbinate of any animal in any dose group.

e. Unscheduled Deaths:

The following are noteworthy findings derived from an inspection of Appendix M, Table 1E (pp 2628-2636 of the study report). Incidences for irregular surfaces of the kidneys were for male rats: 2/18 (11%); 3/14 (21%); 2/26 (8%); 19/41 (46%) and 23/56 (41%) for the 0, 50/100, 500, 6000 and 12000 ppm groups, respectively. For females, the respective incidences were 1/17 (6%); 0/15 (0%); 1/14 (7%); 2/21 (10%) and 13/35 (37%). Rats described as emaciated, presented in the same order, males: 0/18 (0%); 3/14 (21%); 4/26 (15%); 7/41 (17%); and 23/56 (41%), females: 2/17 (12%); 5/15 (33%); 5/14 (36%); 8/21 (38%) and 14/35 (40%).

f. Overall Macroscopic Postmortem Findings:

The following are noteworthy findings from inspection of Appendix M, Table 1F (pp. 2637-2646 of the study report). Incidences for irregular surfaces of the kidneys, males: 3/90 (3%); 5/90 (6%); 6/90 (7%); 23/90 (26%) and 24/90 (21%) at the 0, 50/100, 500, 6000 and 12000 ppm dose levels. The respective incidences for females: 1/90 (1%); 0/90 (0%); 4/90 (4%); 4/90 (4%) and 15/90 (17%). Emaciated incidences in the same respective order, males: 0/90, 4/90, 4/90, 7/90 and 23/90, females: 2/90, 5/90, 5/90, 8/90 and 15/90.

In conclusion, with respect to macroscopic findings, the following appear to be treatment related. Increased incidences of irregular surfaces of the kidneys for males at the 500, 6000 and 12000 ppm dose groups and for females at 12000 ppm. Increased incidence of emaciation were observed for males and females at 6000 and 12000.
3. **Microscopic Findings:**

a. **Three-Month Interim Sacrifice**

Ten rats/sex/group were sacrificed at the three-month time point with the specific purpose of evaluating ocular tissues. Generally, such tissues were examined microscopically in the control and 12000 ppm dose groups. Inspection of Appendix M, Table II A (pp. 2648-2653 of the study report) did not reveal any remarkable microscopic findings.

b. **Six-Month Interim Sacrifice**

Ten rats/sex/group of the control and 12000 ppm groups sacrificed at the six-month time point were examined microscopically for ocular tissue effects. Inspection of Appendix M, Table III B (pp. 2654-2660 of the study report) yielded the following noteworthy findings: one female rat in the 12000 ppm group exhibited lens degeneration and one exhibited retinal degeneration/atrophy. Individual animal data sheets disclose the same rat accounted for both of the findings.

c. ** Twelve-Month Interim Sacrifice**

Fifteen rats/sex/group were sacrificed at the twelve-month time point. All rats in the control and 12000 ppm groups were examined microscopically for effects in all tissues. Lower dose level groups were also examined sequentially as considered needed. The following are noteworthy findings: plasma cell hyperplasia of the mesenteric lymph node in three of the 15 high dose group females versus none in the control. Subacute-chronic inflammation/chronic nephropathy was observed in 6 of 15 high dose group females versus none in the control. While in males the effect was found in all males of the high dose group versus 11/15 in the control. The high dose group male findings were of higher order of severity than in the control. These findings support that tissues in the lower dose groups of both sexes should have been
examined for the assessment of possible dosing-related early onset of this effect. Testicular maturation arrest/degeneration/atrophy of the germinal epithelium (unilateral) was observed in 3 of 14 high dose group males versus 1 of 15 in the control group. The one observed in the control group and one of the three in the high dose group were classified as "minimal," the lowest rating in terms of severity. However, the remaining two in the high dose group were of higher order of severity. Also, in both of the rats, oligospermia was noted. An effect not observed in any of the controls or in any other high dose group of rats examined. As it turns out, after terminal (2-year) sacrifice, testicular degeneration/atrophy was observed in virtually all rats of all study groups, control included. This finding at 12 months may indicate an earlier onset of this condition in dosed animals. Hence, rats at the lower dose group levels should have been examined for this lesion at the 12-month time point. Retinal degeneration/atrophy (unilateral) was observed in 3 of 14 male rats of the high dose group versus 1 of 15 in the control group. One of the three in the high dose group was rated severe (the highest rating). This particular rat exhibited degeneration of the lens, also noted as severe. However, among females, there was one rat in the control with this lesion that was rated moderate while none was observed in the high dose group.

Microscopic examination of nasal turbinate sections revealed many dosing-related findings such that rats in the 500 and 6000 ppm dose groups were examined in addition to those of the 12000 ppm and control groups. There are several findings that appear elevated in incidence in rats of both sexes in both dose groups 4 and 5. These are delineated as follows: nasal turbinate, Section 2: nasal mucosa (respiratory)-hyperplasia; nasal mucosa (olfactory)-epithelium degeneration. Nasal turbinate, Section 4: nasal mucosa (olfactory)-epithelial degeneration, epithelial
hyperplasia, epithelial cysts and olfactory epithelium replaced by ciliated and non-ciliated epithelial cells. In addition, in nasal turbinates Section 2, among male rats only of groups 4 and 5 there were increased incidences of subacute (chronic active)/chronic inflammation of the nasal mucosa (olfactory).

The effects observed as described above were clearly evident in both groups 4 and 5, but appear to be confined to these two dose levels in both sexes, as characterized.

d. Terminal Sacrifice

Non-neoplastic Findings:

Findings which the study author considers related to malathion treatment include those of nasal turbinates and nasopharyngeal tissues, the kidneys and stomach (p. 84 of the study report). Those particular findings will be evaluated first.

1) Nasal Tissues:

For purposes of facilitating discussion of nasal tissue effects, appended as Exhibit 10 is a copy of the "Consolidated Nasoturbinal Tissue Findings", as reproduced from pp. 86-88 of the study report. An inspection of the "Expanded Incidence Summary Data for nasal tissue effects (pp. 2932-2943 of the study report) reveals that data were obtained for two sectionings of the nasal passages (sections 2 and 4), and these data from the two sectionings are combined to form the table of consolidated findings. One must refer to individual animal data to confirm the consolidated findings.

Both the respiratory and olfactory epithelia of the nasal mucosa were adversely affected in rats of both sexes at the two high dose levels. Effects seen in both types of epithelia include hyperplasia, subacute (chronic active)/chronic inflammation and dilated glands. However, there are other endpoints for which there were increased
incidences among both sexes at the top two dose levels that involve only the olfactory epithelium. These include degeneration of the olfactory epithelium, epithelial cysts (considered as an aspect of the degenerative process), the olfactory epithelium being replaced by ciliated and nonciliated columnar epithelial cells, and hyperplasia of the ciliated and nonciliated columnar epithelial cells which replaced the olfactory epithelium. This is interpreted to mean that in the case of the nasal mucosa, the olfactory epithelium (a neural tissue) is being compromised and replaced by a nonneural tissue as a result of test material at the top two dose levels.

For these particular effects there is no clear evidence to indicate that the nasal mucosa was affected in rats of either sex at the doses of 500 or 100/50 ppm, i.e., NOEL = 500 ppm.

Since anatomically the olfactory epithelium is located essentially posterior to the respiratory epithelium in the nasal passages, section 4 discloses more effects on the olfactory epithelium, while section 2 is more expressive of effects occurring in the respiratory epithelium. According to Boorman et al (1990), a publication cited on p. 93 of the study report, it is customary at NTP to take three sections of the nasal passages, while according to these same authors, some laboratories take four sections. Given that remarkable nasal tissue effects were found in this study of malathion, an outstanding question is whether two sections should be considered adequate. More will be said about this in the discussion of neoplastic effects.

With regard to the nasal tissue findings, we are unable to say whether the effects are the result of systemic exposure via blood supply to nasal tissues or local, i.e., resulting from inhalation of compound laden food particles during feeding, or the concerted effects of both types of exposure. We are more inclined to conclude the effects are due to systemic exposure. Bogdanffy et
al (1987) cite information indicating that nasal passages are highly vascularized, receiving up to 0.9% of cardiac output. Also, Morgan (1994) says that the nose has a rich and complex vascular system and that for noninhaled materials, and possibly certain inhaled gases or vapors, vascular delivery of toxic chemicals is probably a significant cause of nasal lesions. He also says that local blood perfusion and/or regional metabolism combine to produce patterns of tissue damage.

There are possible reasons why the olfactory epithelium may be more vulnerable than the respiratory epithelium to compound induced degeneration as observed in this study of malathion. The olfactory epithelium is a neuronal tissue consisting of sustentacular, sensory and basal cells. The sensory cells are bipolar neurons interposed between the sustentacular cells. The basal cells are considered to be the stem cells for the regenerating olfactory neuroepithelium. Olfactory neurons in contrast to other neurons, are capable of regeneration. Also Bowman’s glands are located in the olfactory epithelium. [Boorman et al (1990)]. There are publications which show that the respiratory tract epithelium as a whole contains cytochrome P-450 activity, which is particularly rich in the olfactory epithelium. [Reed et al (1993)] As studied in the dog, Dahl et al (1982) reports activity of cytochrome P-450 in the olfactory epithelium comparable to that of the liver. They suggest that high activity in the olfactory epithelium may play a role in the removal of odorants from the olfactory tissue and may be important in chemical-induced tumorigenicity.

Bogdanffy et al (1987) say that preferential degeneration of the olfactory epithelium, as opposed to the respiratory epithelium, by certain types of compounds may be due to high levels of carboxylesterases located in the olfactory epithelium. (Apparently such activity in the olfactory region is localized in Bowman’s gland and sustentacular tissue as
opposed to neurons.) Specifically, they say, for example, that acute inhalation exposure of rats or mice (of either sex in either species) to certain esters results in selective degeneration of the olfactory epithelium characterized by necrosis of the neuronal cell layer. They view the effect to be systemic even though exposure was via inhalation. The rationale for the selective degeneration of the olfactory epithelium is the effect of acids generated via carboxylesterase hydrolysis of the esters. Olfactory neurons they say may be "exquisitely" sensitive to acids. Also, Trela et al (1992) claim, based upon their own studies on the nasal tissue effects of dimethyl adipate in concert with the published findings of others on dimethyl glutarate and dimethyl succinate, that esters of such diacids exert a selective degenerative effect on the olfactory epithelium (as opposed to the respiratory epithelium), attributable to the location of carboxylesterases within the olfactory epithelium. These findings are particularly relevant in the review of this study on malathion, since this agent is a diethylster of a dibasic acid. Malathion is a diethyl phosphorodithioate derivative of succinic acid. It is well known that carboxylesterases located in the liver and plasma of the rat will catalyze hydrolysis of the malathion ester groups to yield mono- and dibasic acids that no longer inhibit acetylcholinesterase, i.e., this is the mechanism of detoxification of malathion in the cholinergic sense. Thus from these publications there are reasons to explain the more selective effect observed in the malathion study in terms of degeneration of the olfactory epithelium, i.e., possible metabolism by cytochrome P-450 and hydrolysis by carboxylesterase, effects like those expected to occur in the liver.

2) Nasopharyngeal Hyperplasia:

The tabulated incidences of nasopharyngeal hyperplasia of the respiratory epithelium as presented in the study report (p. 89) are reproduced here as Exhibit 11. There were
increased incidences in both males and females at 6000 ppm and among females at 12000 ppm. High mortality among males of the 12000 ppm group serves to explain the lack of a finding among that group. The NOEL for this finding is 500 ppm in rats of both sexes.

3) Kidneys:

Incidences of bilateral subacute-chronic inflammation/chronic nephropathy as summarized in the study report (p. 90) are reproduced as Exhibit 12. This particular nephropathy was of high incidence in all groups, control included. In terms of incidence there appears to be no particular treatment related effect in rats of either sex. However, upon consideration of the aspect of severity of the findings, there was evident a treatment related increased effect, for which the NOEL was 500 ppm in male rats and 100/50 ppm for female rats. The study author says that "... treatment with malathion appeared to have exacerbated the severity", but considers the severity to have increased only at the top two dose levels. The study author also says "This finding correlated with the surface irregularities of the kidneys which were noted macroscopically and with the increased kidney weight observed at necropsy" (p. 90 of the study report).

4) Stomach:

According to the study report "When examined by light microscopy, squamous cell hyperplasia and hyperkeratosis of the epithelium covering the forestomach (non-glandular portion) were seen in numerous decedents from the 6000 ppm and 12000 ppm dose levels and in a small number of those from the 0, 100/50, and 500 dose levels. Congestion, edema, erosions/ulcers and acute to chronic inflammation were also seen in one or more of the affected animals" (p. 91 of the study report). An inspection of the Expanded Incidence Summary for Microscopic Findings for the Stomach, as reproduced here from page 2885-2889 of the study report,
Exhibit 13, discloses the following treatment related effects at 6000 and 12000 ppm:

Congestion of the forestomach (M,F), forestomach edema (M,F), erosion(s)/ulcer(s)/necrosis of the forestomach (M,F), acute/subacute inflammation of the forestomach (M,F), subacute (chronic active)/chronic inflammation of the forestomach (M,F), basal cell hyperplasia of the forestomach (M,F) (F at 12000 ppm only), squamous cell hyperplasia of the forestomach (M,F), hyperkeratosis of the forestomach (M,F). The bulk of the findings were in rats that did not survive to terminal sacrifice. The study report attributes these findings to likely decreased food intake in moribund animals in concert with continued proliferation of the squamous epithelium covering the forestomach, such that the forestomach becomes prominent and cornified. Regardless of the explanation for these changes in the forestomach, the effect appears to be limited to the 6000 and 12000 ppm groups for both sexes. Gastric erosions and ulcers in the rats of the higher dose groups are attributed by the study report to stress (p. 92 of the study report). The NOEL is 500 ppm (M,F) for these effects on the forestomach.

Further inspection of the "expanded incidence summary of microscopic postmortem findings" (pp. 2867-2954) for additional non-neoplastic findings discloses the following (dose groups in which findings were identified are numbered 2, 3, 4, 5, representing the 100/50, 500, 6000 and 12000 ppm dose levels, respectively):

Congestion: This histopathologic end point was a reported finding in all study groups for many tissues including brain, thyroid gland, thymus gland, salivary gland, mediastinal lymph node, liver, kidney, forestomach, pituitary gland; adrenal gland, hardenian gland, sternal marrow, femoral marrow, nasal mucosa and possibly others. Among certain of these tissues, histopathology was performed on all animals
in all groups in accordance with Guideline requirements. From among those listed examples include thyroid, lung, liver, forestomach. In other cases only the control and high dose group animals were examined and then sequentially the next lower dose groups as evidently considered necessary by the study pathologist in search of a NOEL. As examples, such tissues include brain, heart, pancreas, pituitary, harderian gland, lacrimal gland. In certain cases where we were not satisfied that a NOEL had been secured, we estimated (conservatively) what the incidences of congestion might have been at a lower dose(s) by combining incidences obtained for unscheduled deaths (which the guidelines require for all such animals) with assumed zero incidences of congestion for all terminally sacrificed animals.

The overall results of the assessment of data supports the conclusion that congestion was a positive (or predicted positive) finding for the tissues listed below for the dose groups indicated.

<table>
<thead>
<tr>
<th>Tissue Congestion relative to Controls</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4, 5</td>
<td>5</td>
</tr>
<tr>
<td>Kidneys</td>
<td>4, 5</td>
<td>4, 5</td>
</tr>
<tr>
<td>Thyroid</td>
<td>3, 4, 5</td>
<td>5</td>
</tr>
<tr>
<td>Brain</td>
<td>3, 4, 5</td>
<td>5</td>
</tr>
<tr>
<td>Heart</td>
<td>3, 4, 5</td>
<td>4, 5</td>
</tr>
<tr>
<td>Lung</td>
<td>4, 5</td>
<td>4, 5</td>
</tr>
<tr>
<td>Forestomach</td>
<td>4, 5</td>
<td>4, 5</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3, 4, 5</td>
<td>-</td>
</tr>
<tr>
<td>Pituitary</td>
<td>4, 5</td>
<td>5</td>
</tr>
<tr>
<td>Harderian Gland</td>
<td>3, 4, 5</td>
<td>4, 5</td>
</tr>
<tr>
<td>Lacrimal Gland</td>
<td>3, 4, 5</td>
<td>4, 5</td>
</tr>
<tr>
<td>Sternal Marrow</td>
<td>3, 4, 5</td>
<td>4, 5</td>
</tr>
</tbody>
</table>
These findings show increased incidences of congestion in certain tissues in the top three dose groups (males) and top two dose groups (females), which are probably correlates of increased mortality in these groups evident in the mortality data. The findings in the case of group 3 males is of particular interest as it is consistent with the conclusion of increased mortality in that group.

Continuation of findings: thyroid (follicular cyst) 5 (M,F); parathyroid (hyperplasia) 2, 3, 4, 5 (M,F) mediastinal lymph node (lymphoid cell depletion/atrophy) 3, 4, 5 (M); lungs alveolar collapse 4, 5 (M); liver (spongiosis hepati) 4, 5 (M), 5 (F); spleen (lymphoid cell depletion/atrophy) 4, 5 (M,F); mesenteric lymph node (lymphoid cell depletion/atrophy) 4, 5 (M,F); adrenal glands (bilateral cortex zona glomerulose - vesiculated/vacuolated) 4, 5 (M); eyes (cornea, mineral deposits) 3, 4, 5 (M), 5 (F); eyes (bilateral-cornea, neutrophilic infiltrate) 4, 5 (M), 5 (F).

In commenting on these various findings the study report claims: "Numerous tissues and organs from the decedents were congested. These animals were either not exsanguinated (found dead or accidental death) or were incompletely exsanguinated (sacrificed in extremis) prior to postmortem examination. In the decedents from the treatment groups, this finding was not considered to be related to the dietary administration of malathion." (p. 92 of study report). It appears reasonable that rats in the moribund condition would be expected to have multiple and varied tissue evidence of congestion. However, to the extent that premature mortality itself occurred in this study particularly as evidenced at the two highest dose levels, the finding of congested tissues would not be unanticipated and, hence, at least an indirect effect of treatment with malathion. To the extent that increased incidences of congested tissues is indicative of animals in extremis as a result of treatment, while the bulk of the findings
were in the 6000 and 12000 ppm groups, congestion was of such frequency in the 500 ppm (group 3), particularly in males, to support a conclusion that 500 ppm is an effect level. This also supports increased mortality as an effect level at 500 ppm in males.

The study report also advises that "Also considered to be stress associated was the lymphoid cell depletion/atrophy in the thymus and spleen and the mediastinal and mesenteric lymph nodes in a number of the animals which were killed in extremis or were found dead," (p. 92 of study report).

It is to be noted there was no NOEL for parathyroid gland hyperplasia. (Exhibit 14) In rats of both sexes the incidence was essentially constant across all doses, i.e., no dose response, but high mortality at 12000 ppm in males and females and at 6000 ppm in males as well may have preempted a dose response. Parathyroid hyperplasia may be a corollate of kidney chronic nephropathy which was a principal cause of mortality in this study. Parathyroid hormone increases the movement of calcium from bone into extracellular fluid in the regulation of extracellular calcium concentration. It also increases renal tubular calcium resorption.

b. Neoplastic Findings:

1) Liver: According to the study report, "Hepatocellular adenomas and carcinomas were seen in a small number of males and females from the treatment and/or control groups." (Note: these were not seen in the control female group.) "Among the females, the incidence was statistically significantly increased for both neoplasms at the 12000 ppm dose level and for the incidence of hepatocellular adenoma at the 6000 ppm dose level. At these dose levels, the increased incidences of both neoplasms were considered to be attributed to the dietary administration of malathion."
(p. 94 of the study report). A tabulation of the findings as presented on p. 95 of the study report is reproduced below.

<table>
<thead>
<tr>
<th>Dose Levels (ppm)</th>
<th>0</th>
<th>100/50</th>
<th>500</th>
<th>6000</th>
<th>12000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MALES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular Adenoma</td>
<td>2/70</td>
<td>2/55</td>
<td>3/55</td>
<td>2/55</td>
<td>1/70</td>
</tr>
<tr>
<td></td>
<td>(2.9%)</td>
<td>(3.6%)</td>
<td>5.5%</td>
<td>(3.6%)</td>
<td>(1.4%)</td>
</tr>
<tr>
<td>Hepatocellular Carcinoma</td>
<td>1/70</td>
<td>2/55</td>
<td>1/55</td>
<td>1/55</td>
<td>0/70</td>
</tr>
<tr>
<td></td>
<td>(1.4%)</td>
<td>(3.6%)</td>
<td>(1.8%)</td>
<td>(1.8%)</td>
<td>(0%)</td>
</tr>
<tr>
<td><strong>FEMALES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular Adenoma</td>
<td>0/70</td>
<td>1/55</td>
<td>1/55</td>
<td>3/55xc</td>
<td>3/70x</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(1.8%)</td>
<td>(1.8%)</td>
<td>(5.5%)</td>
<td>(4.3%)</td>
</tr>
<tr>
<td>Hepatocellular Carcinoma</td>
<td>0/70</td>
<td>1/55</td>
<td>1/55</td>
<td>0/55</td>
<td>3/70x</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(1.8%)</td>
<td>(1.8%)</td>
<td>(0%)</td>
<td>(4.3%)</td>
</tr>
</tbody>
</table>

a. \( p \leq 0.05 \) (Fisher Exact Test, Haseman Test, and Cox's Test).
b. \( p \leq 0.05 \) (Haseman Test, Cox's Test and Gehan-Breslow).
c. \( p \leq 0.01 \) (Gehan-Breslow).

There is no apparent increased tumorigenic response among males. It is important to note, however, that among males there were no survivors at term in the 12000 ppm group, and survival was reduced to 26% at term in the 6000 ppm group as compared with 67% survival in the control group and 75% survival in the low dose (100/50 ppm) male group. Hence, while not discussed in the report, it is fair to say that male rats, particularly in the 6000 and 12000 ppm groups were not as at risk as control and lower dose groups for the full exposure period, due to competing toxicity of the test material. Therefore it cannot be affirmed that males were adequately evaluated at the 6000 and 12000 ppm dose levels. Among females, there was clearly a tumorigenic response of the liver at 6000 and 12000 ppm. At 12000 ppm, the combined incidences of adenomas and carcinoma was 8.6% versus 0% in the control group, a finding, which is clearly statistically significant. It would actually be more appropriate to express all incidences on a 55 rat/group basis since 15 rats each in the control and high dose groups were
actually eliminated at the 12 month interim sacrifice. Under that circumstance, the combined incidence at 12000 ppm would be 10.9% versus 0% in the control, an even more remarkable finding. It is also noteworthy, that survival at terminal sacrifice in the 12000 ppm female group was compromised relative to the control group, and hence, the potential for a tumorigenic response in the group was also compromised by reduced survivorship. There was no evidence from liver histopathology in terms of non-neoplastic findings (e.g., hepatocellular hypertrophy or hyperplasia) for female rats to suggest that the 12000 ppm was an excessive dose with respect to the liver. However, liver weight was increased at 6000 and 12000 ppm for female rats.

From the standpoint of historical control data, the study report claims that: "In the NTP historical control data, the incidence of hepatocellular adenoma in 1979 comparable, untreated F344-females was 2.3% with a range of 0-10%; the incidence of hepatocellular carcinoma was 0.2% with a range of 0-2%. In this laboratory, in six previous studies (254 control females), the incidence of hepatocellular adenoma was 1.6% with a range of 0-5.4%; the incidence of hepatocellular carcinomas was 1.1% with a range of 0-2.4%." (p. 94 of the study report) These findings are tabulated below as reproduced from p. 95 of the study report. No historical data were provided on combined incidence. NOTE: In Appendix P (p. 5596 of the study report) presenting HLS historical data, study dates for Study Code Nos. 8, 58 and 59 require editing.

<table>
<thead>
<tr>
<th>Historical Control Data (Hepatocellular Tumors in Female F344 Rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of Tumor</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hepatocellular Adenoma</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Hepatocellular Carcinoma</td>
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<td></td>
</tr>
</tbody>
</table>
The source of the NTP historical information mentioned above is given in the study report as Haseman et al (1990). An independent inspection of that publication reveals that for control female F344 rats in feeding studies, the incidence of "neoplastic nodules" (taken to mean adenomas) of the liver is 45/1979 (2.3%) and for "carcinomas" of the liver is 3/1979 (0.15%). In a more recent publication of the NTP historical data, National Institute of Environmental Health Sciences (1996), the historical incidence in oral feeding studies among female F344 rats is "hepatocellular adenoma" 8/1351 (0.59%) and "hepatocellular carcinoma" 1/1351 (0.07%). It is noteworthy that in this particular publication of the NTP data base, the single incidence of carcinoma in 1351 feeding study control females was actually the only incidence of hepatocellular carcinoma recorded in a total of 3621 control female F344 rats when other types of chronic studies, i.e., gavage (corn oil and water vehicle), oral (water vehicle), inhalation (air), are included. It is reasonable to conclude that spontaneous hepatocellular carcinomas of the liver of female F344 rats is extremely rare in the NTP data base. A spokesman at NIEHS who worked on the 1996 NIEHS publication advised (oral communication) that the 1996 publication provides a more contemporary window of historical control data than does Haseman et al (1990). Dr. Joseph Haseman rendered via personal communication the opinion that the more recent NTP data is more appropriate than the 1990 data for use with a study as recent as 1995. He also advised that the term neoplastic nodules is not strictly adenomas only. The more recent (1996) data is limited to hepatocellular adenomas and is of lower incidence.

In conclusion, for males there was no clear evidence of a treatment related tumorigenic effect on the liver. However, high mortality in the 6000 and 12000 ppm groups may have compromised the risk factor of developing such tumors, particularly if late occurring.

Among females, there was a treatment related statistically significant carcinogenic effect at 6000 and 12000 ppm, the expression of which may also have been somewhat compromised at 12000 ppm due to increased
unscheduled deaths in that group. The increased incidences of adenomas and carcinomas, 3.6% combined, in both of the lower dose groups are also considered to be results of treatment. This is rationalized on the grounds that 1) The zero incidence (particularly of carcinoma) in the control group is not unexpectedly low; 2) further increased findings were observed at the higher doses, i.e., 5.5% at 6000 ppm and 10.9% at 12000 ppm; and 3) the hepatocellular tumor incidences (particularly carcinoma) in female F344 rats are very rare. In support of the conclusion is the following comment on the interpretation of rare tumors from the Office of Sciences and Technology Policy (1985).

"The spontaneous tumor incidence can be of considerable importance in the interpretation of results from carcinogenicity studies. If, for example, the effect of a chemical is to double or triple the background tumor incidences, tissue sites with low spontaneous tumor rates are more likely to yield false-negative results than are sites with high spontaneous tumor rates. For example, if a tumor is a rare tumor even a slight increase in incidence may be biologically significant and may be considered adequate evidence of carcinogenicity (53). This factor must be taken into account in the overall evaluation of the data. For such tumors (e.g., with spontaneous tumor rates of 1% or less) the utilization of historical control data may be particularly useful in increasing the sensitivity of the study for detecting carcinogenic effects." (P. 10418)

It is noteworthy that microscopic examination of the liver did not disclose any attendant dosing related increased hypertrophy, hyperplasia, necrosis or inflammation that might provide a corollary to neoplasia. Just how important such findings might be to the interpretation of increased incidences of rare tumors is perhaps open to discussion.

2) Nasoturbinal Tissues: The study report says the following: "Neoplasms which were considered to be related to treatment with malathion were seen in the nasoturbinal tissues and liver. In the nasoturbinal
tissues, an adenoma was observed in one male (animal number 4033) from the 6000 ppm dose level and a carcinoma was observed in one male (animal number 5040) from the 12000 ppm dose level. Spontaneous neoplasms of the nasoaurinal tissues are rare in F344 rats. In untreated dietary and corn oil control animals from eight recent NTP studies only six were identified from nearly 4000 control males and none occurred in a similar number of control females (citing Boorman et al, 1990). None have been observed in this laboratory in six previous studies (238 control males and 241 control females." (p. 93 of the study report) The historical NTP data discussed in Boorman, et al (1990) is published in Haseeman et al (1990), mentioned previously.

An inspection of the summary table of microscopic findings in the study report confirms the finding of the two tumors in male rats. However, both tumors occurred in the olfactory region of the nasal mucosa. (individual animal data, pp. 3805 and 4100 of the study report) An independent reading of Boorman et al (1990) confirms nasal tumors as rare among NTP historical control. However, the claim of some six tumors among nearly 4000 control males is with reference to the respiratory epithelium (confirmed by personal communication with the principal author and inspection of Haseeman, et al (1990). Boorman et al (1990) and Haseeman, et al (1990) claim/identify zero incidence of tumors of the olfactory epithelium from among nearly 4000 control male rats, and none among a similar number of control females. In fact, Boorman et al (1990) says "Neoplasms of the olfactory epithelium have occurred in F344 rats exposed to certain carcinogens, but have not been observed in controls." (p. 332) So the finding in this study of two such tumors of the olfactory epithelium is exceedingly rare indeed, and heretofore unique to carcinogens.

Since the histopathology sheets for the two nasal tumor bearing rats in question say no more than the tumors are located in the olfactory epithelium, it would be important for the registrant to say just what kinds of tumors (i.e.,
of what tissue) these are. For example, are they of the neurons or other tissues of the olfactory epithelium.

In support of a conclusion that the neoplastic findings in question are treatment-related, we would cite here the quotation of the Office of Science and Technology Policy (1985) p. 10418 reproduced on p. 54 of this review.

The above cited background incidence for nasoturbinal tissues among male rats (6/4000) is about 0.15% (or 0% in the case of tumors of the nasal olfactory epithelium) while the incidence in this study was 1/55 or 1.8% in each of two dose groups, specifically the 6000 and 12000 ppm dose groups.

Thus, the finding of the two rare nasal passage tumors in this study viewed in light of the position of OSTP (1985) on the assessment of rare tumors serve to support the study author’s conclusion that these are test material related responses. A question to be posed at this point is whether additional nasal sections should be examined to help confirm the absence of such effects at lower doses. More on this will follow.

Further inspection of the expanded incidence summary of microscopic findings in the study report discloses two additional rats (female in this case) with malignant tumors identified in nasal turbinate Section 4 (p. 2943 of the study report). These are described as "squamous-cell carcinoma arising from the squamous epithelium lining the alveolus of a tooth." One was seen in a female rat, No. 5503, from the high dose group and the other in a female rat, No. 2546, of the low dose group. It is noteworthy that only fourteen rats (unscheduled deaths) were examined in the low dose group, so the incidence for this tumor is one in fourteen. In the high dose group, 65 rats were examined (includes early sacrifice animals). Such tumors are also evidently rare tumors. According to the NTP data base, again as reported in Haseman et al (1990), the incidence of squamous cell carcinoma of the oral mucosa (any site) of the F344 rat is one in nearly 4000 untreated or corn oil gavage female F344 control rats. The likelihood that that particular
finding was of the alveolus of the root of a tooth is probably low. Furthermore, these squamous cell carcinomas being located in the alveolus of the root of a tooth, as identified in the nasoturbinal sections, places the tumors in very close proximity to the nasal passages (more will be presented on this subsequently). To the extent that these tumors may possibly have a nasal tissue etiology or involvement the same NTP data shows no incidence of squamous cell carcinomas of the nasal passages among nearly 4000 female F344 rats. So whether these tumors be classified as oral or nasal, the incidence is exceedingly rare. The registrant is invited to submit any additional information that might be used to further define the historical incidence of the carcinomas in question.

A few comments are indicated to facilitate an appreciation of the anatomic closeness of the alveolar tissue of the root of the tooth to the nasal tissues. The study revealed a high incidence of periodontal disease among rats of both sexes in all study groups. There are published works which indicate that the periodontal disease process may violate separations between nasal and oral cavities perhaps making it more difficult to say how tumors in the alveolus may arise. For example, Morgan (1991) in discussing approaches to the identification and reading of nasal lesions in toxicology studies says: "Nasal lesions may result from 'spontaneous' or treatment-related dental problems. The roots of the incisor teeth lie very close to the lateral recess of the lateral meatus (45), and periodontal lesions in this region can readily involve the adjacent nasal tissues. Lesions of the molar teeth, buccal cavity, and even the skin may also lead to nasal involvement, at which point it is critical to identify the primary target site. Thus, awareness of the potential role of aging-related nasal lesions as well as changes in adjacent tissues can be of considerable value to the toxicologic pathologist during interpretation of toxic responses." (p. 341)

Further along, this author also says: "Feron and Woutersen (21) reported that periodontal inflammation
can be associated with nasal lesions in aging rats. In our laboratory, periodontal disease has been observed around the roots of the incisor teeth in aging rats and mice. In some cases, these lesions were severe, and led to the development of rhinitis and granulomas that partially obstructed the nasal airway in the regions adjacent to the lateral recess of the lateral meatus. The close physical relationship between the incisor roots and this lateral recess (45) provides a ready means of progression from periodontitis to rhinitis. The fine membranes of bone separating the lateral recess of the lateral meatus from the periodontal ligament are frequently structurally altered during this process, with localized bone dissolution permitting passage of the inflammatory process into the adjacent nasal tissue." (p. 343)

This discussion is introduced here to illustrate the close proximity of the nasal cavity and that of the alveoli of roots of teeth. It is therefore difficult for this reviewer to appreciate the level of distinction that may exist between the etiology of neoplasms of the two regions, particularly when periodontal diseases may be a complicating variable. It is necessary for pathologists to provide more descriptive information on the location of tumors of the alveolus of the teeth and the conditions of surrounding tissue.

We believe that to the extent the two neoplasms of the nasal olfactory epithelium are to be considered compound related because of infrequent incidence among historical controls, the two squamous cell carcinomas of the alveolus of roots of teeth should also be considered compound related. These latter findings should also have been discussed in the text of the study report.

Of added concern is the fact that all rats in the low dose group were not examined histopathologically for nasal tissue effects. The FIFRA Guidelines for carcinogenicity testing (83-2) reads under the histopathology section, "The following histopathology should be performed": "Target organs in all animals" [paragraph (e)(11)(i)(C)] and "If a significant difference is observed in hyperplastic, preneoplastic or neoplastic lesions between
the highest dose and control groups, microscopic examination should be made on that particular organ or tissue of all (emphasis added) animals in the study" [paragraph (e)(11)(iii)]. In this particular study hyperplasia of the nasal respiratory and olfactory epithelia was observed at the top two dose levels. In addition, there was an adenoma of the olfactory epithelium of a male rat at the penultimate dose level and a carcinoma of the olfactory epithelium of a male rat of the highest dose group. Add to this the finding of two squamous cell carcinomas of the alveolus of the roots of teeth, one in a low dose group female and one in a high dose group female, we must conclude there was an incumbency on the performing laboratory to have examined the nasoturbinal slides (2 and 4) not only of the 14 male and 14 female rats of the low dose groups that died early, but for all animals in that group.

Inspection of individual animal histopathology data sheets for the low dose female group shows that of the 14 rats to be examined for nasal tissue effects, one data sheet (#2503) claims the nasal tissue to be missing, three (#s 2525, 2545, 2552) claim "extensive postmortem autolysis" and one (2541) claims moderate postmortem autolysis". It could therefore be argued that of 14 rats (unscheduled deaths) in question, only 9 could be expected to provide fully adequate histopathologic information, one of which did show the squamous cell carcinoma of the alveolus of the tooth.

The fact that nasal tissue sections for all rats in the low dose group were not similarly examined is considered a deficiency.

Further to these particular findings more than two sections of the nasal mucosa should be examined. For instance, Morgan (1991) says that if nasal lesions are expected, it is recommended that a series of 6-8 section levels from selected regions be prepared for laboratory rats. The registrant should discuss with EPA examining additional segments of the nasal epithelium.
3) Testes: Testicular interstitial cell tumor incidence was extensive in all groups. The appended Exhibit 15 contains selected pages from the Expanded Incidence Summary of the study report which incorporate incidences of unilateral and bilateral interstitial cell hyperplasia and interstitial cell tumors for (a) the 12-month interim sacrifice, (b) unscheduled deaths, (c) terminal sacrifice and (d) consolidated findings.

At the 12-month time point, 15 rats in both the control and high dose groups were examined histopathologically. Incidences of hyperplasia were essentially equivalent in control and high dose groups. The combined incidences for unilateral and bilateral interstitial cell tumors were 2 (13%) in the control and 5 (33%) in the high dose groups. Among unscheduled deaths, incidences of hyperplasia appeared to be unaffected at any dose level. The combined incidences of unilateral and bilateral interstitial cell tumors were 83%, 79%, 92%, 98% and 95% for groups 1 through 5 in the given order. So, among rats dying early in the study after year one, there were numerical increases in groups 3 through 5 versus groups 1 and 2. At terminal sacrifice, incidences of rats with unilateral or bilateral tumors were 100% in groups 1 through 4. Of course, no male rats in group 5 survived to terminal sacrifice.

The tabulation of consolidated incidence data for rats exhibiting combined unilateral and bilateral interstitial cell tumors from among the approximately 55 rats per group at risk post-year one were 54/55 (98%), 52/55 (94.5%), 53/55 (96%), 54/55 (98%) and 47/55 (85%).

The overall consolidated incidences of combined tumors do not illustrate a compound-related effect. However, among unscheduled deaths there appear to be increased incidences in groups 3 through 5 even though rats in groups 4 and 5 died at earlier time points. There is a possibility that the test material provokes an earlier onset of this type of tumor at the high dosages.
Proliferative lesions of the interstitial cells in the testis as disclosed in Boorman et al (1990) for the F344 rat is reproduced below.

<table>
<thead>
<tr>
<th>Sacrifice Interval</th>
<th>(%)*</th>
<th>3 Mo.</th>
<th>9 Mo.</th>
<th>15 Mo.</th>
<th>24 Mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia</td>
<td></td>
<td>0</td>
<td>3</td>
<td>33</td>
<td>54</td>
</tr>
<tr>
<td>Adenoma</td>
<td></td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>83°</td>
</tr>
</tbody>
</table>

a. Rats were 6 weeks old at start of study.
b. Percentage of animals with a lesion in one or both testes; n = 30 per group except at 24 months, when n = 100.
c. Represents both early death and terminal sacrifice animals.

In comparing the above data with that of the malathion study, there is an obviously greater incidence of hyperplasia and a lesser incidence of adenoma in the historical data at the 24 month time point than in the study under review. It remains possible, that the incidence of tumors in the current study was increased in groups 3 through 5 as evaluated on the basis of unscheduled deaths. The study author claims that "statistically, the dietary administration of malathion was considered to be associated with increased incidence of this tumor." (p. 95 of the study report) However, he goes on to say that this is a very common tumor in the F344 rat and that nearly all will develop this tumor if allowed to complete their natural life span. We should note, however, that in this study that particular condition was not met and many male rats did not live out their normal life spans, but nonetheless developed the tumors, suggesting decreased latency, a critical parameter in defining carcinogenicity. The study report also cites the high historical control incidence for the tumor, but again that historical data is for animals having normal survival and may not be appropriate to employ in this case as a reason to say there was no real effect of the test material. Presented in the study is a "statistical analysis of time to tumor data" (pp. 5345-5346 of the study report) reproduced here as Exhibit 15, in which it is concluded: "The material is associated with increased interstitial cell
testicular tumors in male rats at all (emphasis added) doses measured based on Haseman's and Fisher's test; Haseman's at the 12000 ppm dose group; Fisher's at the 50, 500 and 6000 ppm groups." (p. 5346 of study report).

4) **Thyroid c-cells:** In the expanded incidence summary for thyroid c-cell carcinoma for males the recorded incidences are 1/69, 2/54, 6/53, 2/54 and 0/69 for the control, 100/50, 500, 6000 and 12000 ppm groups, respectively. According to the statistician's report (p. 5347 of the study report), the increase at 500 ppm is statistically significant by Fisher's exact test, and the dose trend is positive. Excessive mortality at 6000 and 12000 ppm among males may have compromised expression of the tumor at those doses.

5) **Pituitary Gland Pars Distalis Carcinoma:** In the expanded incidence summary for pars distalis carcinoma for females the recorded incidences are 0/66 (0%), 1/31 (3.2%), 3/34 (8.8%), 4/34 (11.8%) and 1/69 (1.8%) for the 0, 100/50, 500, 6000 and 12000 ppm groups, respectively. High mortality in females at 12000 ppm could have compromised expression of this tumor in that group. It appears there are increases at all other dose levels, manifesting a dose-response. According to the 1996 NTP historical control data base, the incidence of this tumor (described as "Pituitary Gland: Pars Distalis or Unspecified Site") in the oral feeding studies in female F344 rats is 14/1340 (1.04%). In 27 historical studies presented in this data base, the highest incidence in any study was 2/49 (4%), which occurred three times (the other two actually being 2/50). The study MRID submission did not report statistical analysis for this carcinoma, but did report a positive trend for adenoma. (Exhibit 16) Given the high incidence of this tumor in the 6000 ppm dose group, it could be argued that all animals in all dose groups should have been examined.

One must also conclude by inference from the statistical analysis of time to tumor data (Exhibit 16) that, in the case of males, two of the eight tumor types mentioned as
first analyzed were causes of death in this study, namely, lymphoreticular system mononuclear cell leukemia and pituitary pars distalis adenomas, and therefore were not analyzed secondarily by an incidental context method. Hence, the two tumorigenic responses were significantly increased when corrected for survivorship. We should also note from the statement in Exhibit 15 that among females mononuclear cell leukemia was statistically significantly increased in the 100/50 and 500 ppm dose groups, but with no dose response, according to the statistician.

Based upon an examination of tumor incidence summary data as tabulated in the study report, the following tumorigenic responses are being analyzed independently by the HED Statistics Team: mononuclear cell leukemia (male and female); liver adenomas, carcinomas, combined (male and female), interstitial cell tumors, one-year only data for "early onset"; thyroid follicular cell adenomas, carcinomas, combined (male only); pituitary, pars distalis adenomas, carcinomas, combined (male and female).

III. DISCUSSION

A. The critical findings in this study might be commented on as follows. At the time of initiation of the study, dosage levels employed were 0, 100, 500, 6000 and 12000 ppm. However, due to the finding of significant erythrocyte cholinesterase inhibition at 100 ppm among female rats at the 3-month time point, the decision was made at that time to reduce the low dose level from 100 ppm to 50 ppm for rats of both sexes. Subsequent to this reduction the LOEL for erythrocyte cholinesterase inhibition proved to be 500 ppm and the NOEL 50 ppm for females, while the respective values for males were 6000 ppm and 500 ppm. It could be argued that the study did not identify a true NOEL for females, as one cannot be certain that 50 ppm would have been a NOEL for the first three months of the study given the tendency for some degree of adaptation to occur after longer time periods of testing. In the case of plasma cholinesterase inhibition, for males the LOEL was 500 ppm and the NOEL 50 ppm (100 ppm during the first 3 months), while the respective values for females was 6000 ppm and 500 ppm. For brain cholinesterase inhibition, the LOEL was 6000 ppm and the NOEL 500 ppm for rats of both sexes. Cholinesterase inhibition in general exhibited a very shallow dose
response. Despite the finding of cholinesterase inhibition in both sexes as indicated, there were no obvious cholinergic clinical signs among males at any dose level. Among females, the only reported clinical sign was anogenital staining, evident throughout the study period at the 12000 ppm dose level only. To the extent that this was a cholinergic sign, the fact that it occurred in females only may be a reflection of the greater degree of brain cholinesterase inhibition at 12000 ppm in females, 28-49% over the first three time points, than in males, 15-19% over the corresponding period of the study.

Increased mortality was observed among males in a dosing-related manner across the 500, 6000 and 12000 ppm dose levels. Increased mortality was observed among females at the 12000 ppm dose level only. Given the lack of cholinergic clinical signs among males even at the highest dose level and given the small inhibition of brain cholinesterase at the high dose level of 15-19%, as determined over the first 18-months of study, the elevated mortality that embraced the top three dose levels cannot likely be assigned in an essential way to cholinesterase inhibition, but was due to other chronic toxic effects of malathion. Specifically, increased mortality appears to have been primarily due to the effects of leukemia, chronic nephrotoxicity and the concerted effects of various other collective toxicologic burdens of the test material, which may indeed have included cholinesterase inhibition. It should be noted that among males at 500 ppm, while leukemia was a contributor to the increased mortality, the incidence of leukemia was not increased in the study group relative to that of the control group, suggesting that leukemia bearing animals were more vulnerable to other toxicologic effects of the test material. However explained, malathion exposure at the high dose level resulted in complete demise of male rats before term and resulted in increased mortality among males at the 6000 and 500 ppm dose level as well. The NOEL for mortality among male rats in this study was 50 ppm, an extremely low value given past findings with malathion. It is likely, however, that a higher NOEL for increased mortality for male rats, considerably closer to the 500 ppm dose level, could have been identified had such a dose been tested. Nevertheless, malathion by chronic exposure proved to be more toxic to male F344 rats than one might have anticipated. The MTD for male rats was exceeded. The fact that increased mortality occurred among females at 12000 ppm also indicates the MTD was exceeded for females. Since the mortality in both sexes was evidently the result of chronic exposure, it is unlikely that shorter term dose range-finding studies would have anticipated this outcome. However, in the 1979 National Cancer Institute study in the F344 rat (MRI# 43269) where doses of 0, 2000 and 4000 ppm of malathion (American Cyanamid 95% t.a.i.) were used, mortality was extensive at week 103, particularly among male rats.
With regard to the influence of extent of mortality on the acceptability of a carcinogenicity study, Office of Science and Technology Policy (1985) says: "A negative test is ordinarily accepted by regulatory agencies if: .... survival of all groups (per sex per dose) is no less than 50% ... at 104 weeks for rats" (p. 10414). By this criteria of acceptability, the finding among male rats of 100% and 74% mortality at the high and penultimate doses, respectively, would preclude the validity of the study to the extent that it is negative for carcinogenicity, at least in male rats. Among females, survival in all groups well exceeded 50% except the highest dose group where survival was 36%, and should be considered acceptable for females, or certainly equivocally so. As related to this interpretation, the FIFRA Subdivision F Guidelines for chronic toxicity/oncogenicity studies says: "At the termination of the experiment at 18 months in mice and 24 months in rats the survival in any groups should not fall below 25%", and further along "The highest dose level in rodents should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors". "For rodents, the incidence of fatalities in low and intermediate dose groups and in the controls should be low to permit a meaningful evaluation of the results." (p. 138)

Generally, various clinical chemistry and hematology parameters were altered in both males and females at the 6000 ppm and 12000 ppm dose levels, which serve to confirm a burden of the test material on the system at these doses. In addition, certain of the parameters were altered at 500 ppm, including decreased aspartate aminotransferase activity, alanine aminotransferase activity, and blood urea nitrogen in females at 500 ppm. Decreased blood urea nitrogen may have extended to the 100/50 ppm level in females. So there is evidence of effects on biochemical parameters for certain at 500 ppm and above, and possibly at 50 ppm.

The ocular effects testing component of this study which involved ophthalmoscopy, electroretinography (ERG) and histopathology of the retina did not yield any unequivocal effects of the test material. However, variability in ERG data was so great that if a compound-related effect of significant proportions occurred, it could have been missed. The F344 rat is considered a poor model for such testing. Effective testing for ocular toxicity remains outstanding.

With regard to pathology, organ weight data indicates that in male rats kidney, liver, spleen, heart and thyroid/parathyroid and in females kidney and liver may be target organs as evidenced by increased weight at the top two dose levels. Increased liver and thyroid/parathyroid weights may have extended to the 500 ppm dose level in males, and increased testes/epididymides weight
may have occurred at 6000 ppm. Decreased thyroid/parathyroid weight occurred in females at 12000 ppm and likely so at 6000 ppm.

Macroscopic examinations revealed increases of irregular surfaces of the kidney for male rats at 500, 6000 and 12000 ppm and in females at 12000 ppm. These findings correlate with increased mortality.

Microscopically there were no non-neoplastic correlates for the increased liver weight observed in either sex. Microscopic examination of the kidney revealed bilateral subacute-chronic inflammation/chronic nephropathy of high incidence in all groups. In terms of incidence there was no dosing related effect, but in terms of severity there was a NOEL for males of 500 ppm and for females 100/50 ppm. This microscopic finding does appear to provide a correlate of the macroscopic kidney effects with chronic nephropathy as a factor in excessive mortality.

Another outstanding finding in the study was that of nasal tissue effects. (hyperplasia, degeneration, replacement of the olfactory epithelium by ciliated and non-ciliated columnar epithelial cells) and nasopharyngeal hyperplasia. These effects were of high incidence in males and females at 6000 ppm and 12000 ppm. It is noteworthy that the olfactory epithelium as opposed to the respiratory epithelium was particularly affected in this study. Certain literature references as identified and discussed in the review suggest that unique effects on the olfactory epithelium might not be so surprising. The olfactory epithelium has certain metabolic capabilities not unlike those of the liver, and might be expected to metabolize a diester such malathion in a way similar to that of the liver. So it is quite likely that the microscopic lesions noted of the nasal epithelium result in some degree as the result of chemical modifications of malathion.

Squamous cell hyperplasia and hyperkeratosis of the epithelium covering the forestomach were seen in numerous decedents from the 6000 ppm and 12000 ppm dose levels of both sexes.

Congestion was a histopathologic finding for many tissues as discussed in this review. While the bulk of the findings were in the 6000 ppm and 12000 ppm dose group, congestion was of such frequency in the 500 ppm dose group, particularly in males, as to support a conclusion that 500 ppm is an effect level among males. This may be a corollary to increased mortality at 500 ppm. Among male rats in particular, tissues exhibiting increased incidence of congestion among tissues of the 500 ppm dose group were brain, salivary glands, kidneys (females also possibly affected), femoral marrow?
With regard to neoplastic findings, the study report as written acknowledges that among female rats there were statistically significant increases of hepatocellular tumors at 6000 ppm and 12000 ppm. Specifically, at 12000 ppm the combined incidence of hepatocellular adenomas and carcinomas was 10.9% and at 6000 ppm 5.5% versus 0% in the control group. We in addition conclude that increased combined incidences of hepatocellular adenomas and carcinomas in the 500 ppm (3.6%) and 100/50 ppm (3.6%) groups were positive extensions to these dose levels of the tumorigenic effects of malathion. The conclusion that one hepatocellular adenoma and one carcinoma in each of the two lowest dose groups are positive findings rests with the rarity of spontaneous hepatocellular tumors in female F344 rats in concert with the positive findings at the higher doses. In the most recent publication of the National Toxicology Program’s historical data, NIEHS (1996), the historical incidences in oral feeding studies among female F344 rats are 8/1351 (0.59%) for hepatocellular adenoma and 1/1351 (0.07%) for hepatocellular carcinoma. It is noteworthy that in this particular publication of the NTP data base, the single incidence of carcinoma in 1351 feeding study control females was actually the only incidence of hepatocellular carcinoma recorded in a total of 3621 control female F344 rats when other types of chronic studies, i.e., gavage (corn oil and water vehicle), oral (water vehicle), inhalation (air), are used.

Among nasoturbinal tissues, there was one carcinoma reported in a male rat in the 12000 ppm group and one adenoma reported for a male rat in the 6000 ppm group. The study report acknowledges these two tumors to be related to treatment with malathion. The rationale for acknowledging these to be treatment-related rests with the very low incidence of such tumors in the F344 rat in concert with the fact that extensive non-neoplastic changes were seen in the nasoturbinal tissues, as discussed previously. More specifically, both of the tumors were of the olfactory epithelium. As noted in the discussion on non-neoplastic findings, various publications indicate that the olfactory epithelium of the rat is endowed with metabolic capabilities very similar to those of the liver. It might be anticipated therefore that the olfactory epithelium and the liver may respond similarly to a carcinogen. As discussed in this review, the NTP historical control data base for the F344 rat does not disclose a single incidence of tumor of the olfactory epithelium from among nearly 4000 control male rats, and none among a similar number of control females. So in view of the rarity of the tumor type, the finding of two in this one study merits a positive interpretation.

Also identified in the nasal turbinate sections were two additional tissues (female in this case) with malignant tumor, one appearing in the 12000 ppm group and the other in the 100/50 ppm group. These are described as
"squamous cell carcinomas arising from the squamous epithelium lining the alveolus of a tooth".

These tumors are also very rare historically. According to Haseman et al (1990), the incidence of squamous cell carcinoma of the oral mucosa (any site) of the F344 rat is one in nearly 4000 untreated or corn oil gavage female F344 controls. The likelihood that this finding was of the alveolus of the root of a tooth is probably low. To the extent that the tumors may possibly have a nasal tissue etiology or involvement, the same NTP data shows no incidence of squamous cell carcinomas of the nasal passages among the nearly 4000 control female F344 rats in the data base. So whether these tumors be classified as oral or nasal, the incidence is exceedingly rare, and in view of the rarity, the two same such findings observed in this study are concluded to be compound-related. The registrant should submit further information characterizing the anatomic location of these two tumors. Of additional concern is the fact that all rats in the low dose group were not examined histopathologically for nasal tissue effects. As explained in this review, the FIFRA Guidelines call for histologic examinations of rats from all dose groups for those tissues ordinarily examined in the control and high dose groups only, when hyperplasia and/or tumors are identified in the high dose group. The fact that nasal tissue sections for all rats in the low dose groups were not examined is considered a study deficiency. Furthermore, as discussed in the review more than two sections of the nasal mucosa should be examined.

Testicular interstitial cell tumor incidence was extensive in all groups including the control. This is not a surprising finding in itself as this tumor has a high historical incidence. The study report itself claims that "Statistically, the dietary administration of malathion was considered to be associated with increased incidence of this tumor" (p. 95 of the study report), but qualifies this on the grounds that nearly all will develop this tumor if allowed to complete their natural lifespan. However, that particular condition was not met and many male rats did not live out their normal lifespan, but nonetheless developed the tumors. This suggests decreased latency, a critical parameter in defining carcinogenicity. Furthermore, in the study report a "Statistical Analysis of Time to Tumor Data" is provided where the study statistician concluded "The material is associated with increased interstitial cell testicular tumors in male rats at all doses measured . . . " (p. 5346 of the study report). One must conclude from this treatment of the data that a NOEL was not identified for increases of this tumor type.

As mentioned under the discussion of mortality, leukemia was a principle cause of early mortality in this study, although an increased incidence of leukemia was evidently not a dosing-related effect. However, as explained in
this review, among male rats of the 0, 100/50, 500 and 6000 ppm groups, there was a dosing-related increased mortality from leukemia among those rats harboring the condition. Evidently, rats with leukemia are more likely to die of leukemia as the result of a competing dosing-related toxicologic burden of the test material. This effect appears to be evident at 500 ppm and 6000 ppm, and perhaps so at 100/50 ppm, and at least constitutes an element of supporting evidence of chronic toxicity of the test material at all doses.

B. Study Deficiencies

1. A NOEL for erythrocyte cholinesterase inhibition among female rats was not identified for the first three months of testing. Although following reduction of dosage level in the low dose group from 100 ppm to 50 ppm at the three month time point yielded a subsequent (six months) 50 ppm NOEL, it cannot be claimed that 50 ppm was a NOEL during the first three months. Additional testing is necessary in the female F344 rat in order to identify a three month NOEL for erythrocyte cholinesterase inhibition.

2. The MTD was exceeded in both sexes as evidenced by increased mortality at 500, 6000 and 12000 ppm in males and at 12000 ppm in females. Among females, the lower doses of 100/50, 500 and 6000 ppm may satisfy Guideline requirements (although 6000 ppm may not be an MTD) in terms of MTD, but among males there is the deficiency of adequate dosing at and below an MTD to satisfy Guideline requirements.

3. A NOEL was not identified for increased food consumption particularly among males during the first three months, i.e., prior to reduction in dose from 100 ppm to 50 ppm. There is no certainty that 50 ppm would not have elicited the same effect during the first three months of testing, since this may be a period of adaption. This could be a cholinergic appetitive effect of malathion.

4. A NOEL was not identified for the microscopic finding of hyperplasia of the parathyroid gland for rats of either sex.

5. A NOEL was not identified for increased incidences of hepatocellular adenomas/carcinomas in female rats.

6. While there were no significant increases in liver tumor neoplasia in the case of males, high mortality in the 6000 and 12000 ppm groups may have precluded expression of a tumorigenic response, particularly if
late occurring. Since the next lowest dose level tested (500 ppm) was substantially lower than the 6000 ppm dose level (more than 10 fold lower), malathion was not tested for potential carcinogenicity at adequate dose levels for males in this study. Hence this study does not satisfy as a negative study for liver carcinogenicity among male F344 rats, or for that matter for carcinogenicity at any other anatomic site among male rats. This constitutes a major study deficiency. In the case of females, increased mortality at 12000 ppm also constitutes a study deficiency in terms of assessing carcinogenicity.

7. The absence of histopathologic examination of nasoturbinal tissue sections for the low dose (100/50 ppm) group for rats of both sexes is a study deficiency. In view of extensive hyperplasia of the olfactory epithelium at the 6000 and 12000 ppm doses in both sexes, in concert with the finding of rare tumors of the olfactory epithelium in male rats at 6000 ppm (one adenoma) and 12000 ppm (one carcinoma), there is under FIFRA Guideline requirements incumbency to examine all dose groups. The need for this is further reinforced by the finding of rare squamous cell carcinomas that appeared one each in the 12000 ppm and 100/50 ppm (unscheduled death animal) in the nasoturbinal tissue slides of female rats. These latter tumors may strictly be viewed as oral cavity tumors, but even that assessment requires some clarification. Not only should nasoturbinal tissue slides be examined in the low dose groups for tumors in question, but the number of sections taken should be increased from two per rat in each study group to five or six sections per rat.

8. A NOEL for decreased latency of interstitial cell testicular tumors was not identified. Although similar and high incidences of interstitial cell testicular tumors were observed in all the control and treated male groups in this study, the latency or time to tumor appeared to be decreased in the treated groups.
BIBLIOGRAPHY


