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## DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity Feeding - Mouse [OPPTS 870.4200 (§83-2)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: None
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Cyhalofop butyl, technical (97.1%)

SYNONYMS: XRD-537 BE; R-(+)-n-butyl-2-(4-(2-fluoro-4-cyanophenoxy) phenoxy)propanoate

CITATION: Harada, T., Ebino, K., Odanaka, Y., Maita, K. (1994) XRD-537 BE: 18-month oral chronic toxicity and oncogenicity study in mice. The Institute of Environmental Toxicology, Mitsukaido Laboratories, 4321, Uchimoriya-cho, Mitsukaido-shi, Ibaraki 303, Japan. Laboratory Study GHF-P-1384, IET 90-0166, June 2, 1994. MRID 45000418. Unpublished.

SPONSORS: Dow Chemical Japan Ltd., DowElanco Division, Seavans North, 2-1 Shibaura 1-chome, Minato-ku, Tokyo 105, Japan; Nichimen Corporation, 11-1, Nihonbashi 3-chome, Chuo-ku, Tokyo 103, Japan.

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 45000418), XRD-537 BE (97.1% purity, lot no. AGR 295713) was administered to groups of 76 male and 76 female ICR (Crj:CD-1) mice in the diet at concentrations of 0, 3, 10, or 100 ppm. Fifty-two mice/sex/group comprised the main study and were administered the test compound for 78 weeks. The satellite group, composed of 24 mice/sex/group were sacrificed at interim times of 26 weeks (10 mice/sex/group) and 52 weeks (10 mice/sex/group). The dietary concentrations of 3, 10, and 100 ppm resulted in daily compound intake of 0.31, 0.99, or 10.06 mg/kg/day, respectively, for males, and 0.29, 0.99, or 10.28 mg/kg/day, respectively, for females.

Treatment with XRD-537 BE did not result in increased mortalities or increases in incidences of clinical signs in treated groups compared with the control groups. At the end of 78 weeks, there were no effects of treatment on body weight, food consumption, food efficiency, hematology, clinical chemistry parameters, or organ weights for either sex. Treated mice that died during the study were emaciated compared with respective control groups; this effect was not present in animals that survived to terminal sacrifice.

Increases in liver weight for both sexes, observed at the 26-week interim sacrifice, were not significant at the 78-week terminal sacrifice. Grossly enlarged, dark-colored livers with histological correlates of hepatocellular swelling with minute eosinophilic granules, observed in main study males and females in the 100 ppm group and males in the 10 ppm group after 78 weeks, were considered liver hypertrophy, an adaptive response to chemical administration. A 6% decrease in brain weight in all treated female mice at the 52-week sacrifice was transient and considered incidental to treatment. Treatment-related kidney lesions in female mice in the 100 ppm main study group consisted of tubular dilatation ($p<0.01$), chronic glomerulonephritis ($p<0.05$), and hyaline casts ($p<0.01$). In male mice these kidney lesions were either not observed or incidences were not dose-related. Incidences of mucosal epithelial hyperplasia of the glandular portion of the stomach were increased in male mice in the 100 ppm group ($p<0.01$).

**The LOAEL for male and female mice is 100 ppm in the diet (males, 10.1 mg/kg/day; females, 10.3 mg/kg/day) based on effects on the kidney including tubular dilatation, chronic glomerulonephritis, and hyaline casts in females, and hyper-plasia of the stomach mucosal epithelium in males. The NOAEL for male and female mice is 10 ppm in the diet (approximately 1 mg/kg/day).**

There was no evidence of carcinogenic potential under the conditions of this study. The doses used in this study were inadequate for assessing carcinogenicity.

This carcinogenicity study in the mouse is **Unceptable/Guideline** and does not satisfy the guideline requirement for an carcinogenicity study [OPPTS 870.4200 (§ 83-2)] in mice.

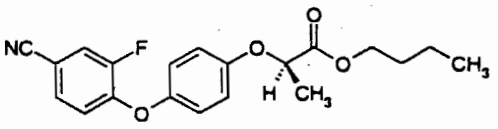
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. Test material: XRD-537 BE

   Description: Off-white powder
   Lot No.: AGR 295713
   Purity: 97.1%
   Stability of compound: stable for two years.
   CAS No.: 122008-85-9
   Structure:




2. Vehicle and/or positive control: The test material was dissolved in acetone and mixed with feed; the control diet was treated with acetone and prepared in the same manner.

3. Test animals:

Species: Mouse
Strain: ICR (Crj:CD-1)
Age and weight at study initiation: age: 5 weeks; average weight: males. 28.1±1.4 g; females, 22.9±1.2 g
Source: Charles River Japan, Inc, Atsugi Breeding Center. Shimofurusawa, Atsugi-shi, Kanagawa; Hino Breeding Center, Hino-cho, Gamoh-gun, Shiga
Housing: animals were housed four/cage in aluminum cages with wire mesh floors: the cages were placed in stainless steel racks with four tiers each. Cages were replaced and rotated every four weeks.
Diet: Certified diet MF Mash (Oriental Yeast Company), *ad libitum*
Water: Filtered, sterilized well water, *ad libitum*
Environmental conditions:
Temperature: 24±2°C
Relative humidity: 55±15%
Ventilation: air changes 15 times/hour
Light cycle: 12 hours light: 12 hours dark
Acclimation period: 9 days (males); 7 days (females)

B. STUDY DESIGN

1. In life dates: Start: October 17 and 25, 1991; end: April 15 and 23, 1993

2. Animal assignment

Animals whose body weights were ±20% of the mean weight and were free of clinical abnormalities were assigned to the test groups in Table 1 by means of a computerized randomization procedure.

**TABLE 1: Study design**

| Test group | Dose Level (ppm) | Mean dose to animal (mg/kg/day) | | Number of Animals | | | |
|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | | Female | |
| | | | | Main | Satellite[a] | Main | Satellite[a] |
| 1 (control) | 0 | 0 | 0 | 52 | 24 | 52 | 24 |
| 2 (low dose) | 3 | 0.3112 | 0.2936 | 52 | 24 | 52 | 24 |
| 3 (mid dose) | 10 | 0.986 | 0.989 | 52 | 24 | 52 | 24 |
| 4 (high dose) | 100 | 10.06 | 10.28 | 52 | 24 | 52 | 24 |

Data taken from pp. 11 and 30, MRID 45000418.
[a]Ten animals/sex from the satellite group were selected for interim evaluations at 26 and 52 weeks.

3. Dose selection

Dietary concentrations were based on a previous subchronic study (IET 90-0164) in which groups of male and female mice were administered dietary levels of 0, 3, 30, 100, or 300 ppm. At necropsy, dark-colored livers were observed in both sexes at 100 and 300 ppm. Liver weights were increased in males receiving ≥30 ppm and in females administered 100 and 300 ppm. Kidney weights were increased in females receiving ≥30 ppm; hepatocellular swelling was observed in both males and females receiving ≥30 ppm. Urinalysis revealed significant decreases in pH and ketones in males at ≥30 ppm. Based on these results, 100 ppm was chosen as the high dose and was expected to result in systemic effects without substantially altering the life span.

4. Diet preparation and analysis

Diets were prepared prior to treatment and every 4 weeks thereafter. A premix was prepared by adding an appropriate amount of the test material, dissolved in acetone, to the basal diet. The dietary concentrations used in the study were prepared by diluting the premix with untreated diet in a mixer. The control diet was also treated with acetone and prepared in the same manner. Following a 30-minute period to allow the acetone to evaporate, the prepared test diets were sealed into plastic bags and stored in aluminum containers in the dark at 4°C. Animals were provided with fresh diet twice a week.

Prior to treatment, samples were taken from the top, middle, and bottom of the mixer for each dose level and analyzed for homogeneity. Samples taken from the middle of the mixer were analyzed for concentration at each preparation time (17 times). The stability and enantiomer ratio (R/S) of the test material in the diets were determined in a previous study (IET 90-0165). In that study, samples from a 600 ppm concentration were analyzed at the following time points: day 0, 42 days (following storage at 4°C in the dark), 57 days (following storage at 4°C in the dark for 42 days and then room temperature in the animal room for 15 days), and 64 days (following storage at 4°C in the dark for 42 days, room temperature in the animal room for 15 days, and exposed to air in a feeding jar for 7 days).

**Results**

**Homogeneity** – The ranges of concentrations of the test material in samples taken from different locations of the 3, 10, and 100 ppm dietary concentrations were 3.0-3.3, 9.3-9.8, and 86-94 ppm, respectively. Coefficients of variation were 5.4, 1.2, and 4.0%, respectively.

**Stability** – The enantiomer ratio following storage under the conditions outlined above was 98.9%/1.1% or 99.0%/1.0 at each time point. The R/S ratio claimed was 99.6%/0.4%.

**Concentration analysis** – Mean concentrations of the test substance from 17 sampling times for the 3, 10, and 100 ppm nominal concentrations were 3.0±0.19,

9.5±0.22, and 94±1.7 ppm. These concentrations were 100, 95, and 94% of nominal concentrations, respectively.

The analytical data indicated that the mixing procedure was adequate, that the variance between nominal and actual dosage to the animals was acceptable, and the test material was stable in the diet under storage conditions of the study.

5. Statistics

Body weight, food consumption, urine specific gravity, hematology , blood biochemistry, and organ weights were analyzed utilizing Dunnett's or Scheffe's method for multiple comparisons. Urine parameters (except specific gravity) were analyzed with the Mann-Whitney U test. Incidences of clinical signs, ophthalmology signs, and pathologic lesions were analyzed with Fisher's exact probability test. Statistical significance was flagged at $p<0.05$ or $p<0.01$. Statistical analyses were provided for total animals (main study and satellite animals) and animals sacrificed at 26, 52, and 78 weeks, but not for total main study animals (animals sacrificed at 78 weeks plus animals in the main study sacrificed *in extremis* or found dead).

C. METHODS

1. Observations

Animals in both the main and satellite groups were observed for mortality, morbidity, and clinical signs at least once a day. The date of onset, nature, severity, and duration of clinical signs were recorded. Detailed examinations including palpation for masses were performed once a week.

2. Body weight

All animals in both the main and satellite groups were weighed weekly for the first 13 weeks, once every 4 weeks from week 16 to study termination, and at necropsy.

3. Food consumption, compound intake, and food efficiency

Food consumption was calculated for animals in the main study. Food consumption was measured at the same time intervals as body weight measurements were taken: once a week during the first 13 weeks, once every 4 weeks from week 16 to study termination, and at necropsy. At each measurement interval, food consumption was measured over a 3-day period for each cage; this measurement was adjusted to a daily food consumption value per animal. Group mean values at each time period were reported as g/mouse/day. Chemical intake was calculated from the data on food consumption, nominal dietary level, and body weights. Food efficiency was calculated as: (g body weight gain/g food consumption) x 100.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were performed using a halogen ophthalmoscope on both main and satellite animals prior to treatment and on all surviving animals in the main control and high-dose groups at 78 weeks of treatment.

5. Blood was collected from the posterior vena cava of mice under ether anesthesia. Samples were taken from 10 mice/sex/dose of the satellite group at 26 and 52 weeks (9 male mice in the 100 ppm group at 52 weeks) and from 10 animals/sex/group of main group mice at 78 weeks. The CHECKED (X) parameters were examined.

a. Hematology

| | | | |
|---|---|---|---|
| X | | X | |
| X | Hematocrit (HCT) | X | Leukocyte differential count* |
| X | Hemoglobin (HGB) | X | Mean corpuscular HGB (MCH) |
| X | Leukocyte count (WBC) | X | Mean corpuscular HGB concentration (MCHC) |
| X | Erythrocyte count (RBC) | X | Mean corpuscular volume (MCV) |
| X | Platelet count | | Reticulocyte count |
| | Blood clotting measurements (Thromboplastin time) (Clotting time) (Prothrombin time) | | |

* Minimum required for oncogenicity studies unless effects are observed, based on OPPTS 870.4200 Guidelines.

b. Clinical chemistry

The CHECKED (X) serum chemistry parameters were examined.

| | | | |
|---|---|---|---|
| X | | X | |
| X | Calcium | X | Total protein |
| X | Glucose | X | Total cholesterol |
| X | Urea nitrogen | X | Triglycerides |
| X | Albumin | X | Alkaline phosphatase |
| X | Globulin | X | Glutamic oxaloacetic transaminase |
| X | Albumin/globulin ratio | X | Glutamic pyruvic transaminase |

6. Urinalysis*

Urinalysis tests were conducted on 10 satellite mice/sex/group at 26 and 52 weeks and on 10 randomly selected mice/sex/group from the main group at 78 weeks. Fresh urine was obtained by pressing on the lower abdominal region of each mouse. The CHECKED (X) parameters were examined.

| | | | |
|---|---|---|---|
| X | | X | |
| | Appearance | X | Glucose |
| | Volume | X | Ketones |
| X | Specific gravity | | Bilirubin |
| X | pH | X | Blood |
| | Sediment (microscopic) | | Nitrites |
| X | Protein | X | Urobilinogen |

*Not required for carcinogenicity studies by OPPTS 870.4200 Guidelines.

7. Sacrifice and pathology

Necropsies were performed on all animals sacrificed in the satellite groups, on all animals in the main group at terminal sacrifice, and on all animals in the main group that died or were killed at unscheduled times during the treatment period. At the study terminations (26, 52, and 78 weeks), all animals were anesthetized with ether and sacrificed by exsanguination from the posterior vena cava. The CHECKED (X) tissues from all groups were collected for histopathological examination and were fixed in 10% neutral-buffered formalin. Preparations were stained with hematoxylin and eosin. The (XX) organs from all animals were weighed.

| X | DIGESTIVE SYSTEM | X | CARDIOVASC./HEMAT. | X | NEUROLOGIC |
|---|---|---|---|---|---|
| | Tongue | X | Aorta* | XX | Brain*+ |
| | Oral tissue | XX | Heart* | X | Peripheral. nerve* |
| X | Salivary glands* | X | Bone marrow* | X | Spinal cord (3 levels)* |
| X | Esophagus* | X | Lymph nodes* | X | Pituitary* |
| X | Stomach* | XX | Spleen* | X | Eyes* |
| X | Duodenum* | X | Thymus* | | |
| X | Jejunum* | | | | GLANDULAR |
| X | Ileum* | | UROGENITAL | XX | Adrenal gland* |
| X | Cecum* | XX | Kidneys*+ | | Lacrimal/Harderian glands |
| X | Colon* | X | Urinary bladder* | X | Mammary gland* |
| X | Rectum* | XX | Testes*+ | X | Parathyroids* |
| XX | Liver*+ | X | Epididymis | X | Thyroids* |
| X | Gall bladder* | X | Prostate | | Zymbal's gland |
| X | Pancreas* | X | Seminal vesicle | | |
| | | X | Coagulating gland | | OTHER |
| | RESPIRATORY | | Preputial gland | X | Bone* |
| X | Trachea* | X | Ovaries*+ | X | Skeletal muscle* |
| X | Lung* | X | Uterus* | X | Skin* |
| | Nose | X | Cervix | | Mediastinal tissue |
| | Pharynx | | Oviduct | | Mesenteric tissue |
| | Larynx | | Vagina | X | All gross lesions and masses* |

* Required for oncogenicity studies based on OPPTS 870.4200 Guidelines.
+ Organ weight required in oncogenicity studies.

# II. RESULTS

## A. OBSERVATIONS

### 1. Toxicity

The following clinical signs were recorded during daily observations. In the main group, the incidences of emaciation in male mice in the 3 and 10 ppm groups (both 11/52) were significantly higher ($p<0.01$) than in the control group (1/51) and the incidences of tactile hair loss (6/52) and dermal wounds (20/52) in male mice in the 3 ppm group were significantly higher than in the control group (0/51 and 11/51, respectively; both $p<0.05$). The incidence of emaciation in male mice in the 100 ppm group was 6/52 (not statistically significant). These clinical signs were not observed in female mice.

### 2. Mortality

The survival rates of mice in the main group were not affected by treatment (Table 2). One male mouse in the control group died in an accident during week 36.

**TABLE 2. Survival (percent) of male and female mice fed XRD-537 BE for 78 weeks (main group)**

| Dietary concentration (ppm) | | | |
|---|---|---|---|
| 0 | 3 | 10 | 100 |
| **Males** | | | |
| 38/51 (75)[a] | 35/52 (67) | 34/52 (65) | 42/52 (81) |
| **Females** | | | |
| 39/52 (75) | 35/52 (67) | 36/52 (69) | 40/52 (77) |

Data taken from p. 29, MRID 45000418.
[a]Number in parentheses represents % of initial number.

## B. BODY WEIGHT

Group mean body weights of male and female mice in the main group for selected weeks are summarized in Table 3. Body weights were comparable among groups for each sex throughout the study.

| TABLE 3. Group mean body weights and weight gains of main group male and female mice fed XRD-537 BE in the diet for 78 weeks (g) | | | | |
|---|---|---|---|---|
| Week of treatment | Dietary concentration (ppm) | | | |
| | 0 | 3 | 10 | 100 |
| **Males** | | | | |
| Body weight, week 0 | 28.1 ± 1.4[a] | 28.1 ± 1.4 | 28.1 ± 1.4 | 28.1 ± 1.4 |
| Body weight, week 52 | 51.7 ± 5.6 | 48.8 ± 6.3 | 50.7 ± 6.1 | 50.7 ± 7.0 |
| Body weight, week 78 | 50.6 ± 5.2 | 50.9 ± 7.0 (101)[b] | 48.6 ± 7.0 (96) | 49.4 ± 7.0 (98) |
| Body weight gain, 0-78[c] | 22.5 | 22.8 (101) | 20.5 (91) | 21.3 (95) |
| **Females** | | | | |
| Body weight, week 0 | 22.9 ± 1.2 | 22.9 ± 1.2 | 22.9 ± 1.3 | 22.9 ± 1.2 |
| Body weight, week 52 | 51.2 ± 6.2 | 50.5 ± 5.7 | 51.0 ± 8.0 | 49.5 ± 8.4 |
| Body weight, week 78 | 50.1 ± 6.3 | 48.8 ± 7.3 (97) | 51.7 ± 8.6 (103) | 49.3 ± 8.6 (98) |
| Body weight gain, 0-78[c] | 27.2 | 25.9 (95) | 28.8 (106) | 26.4 (97) |

Data taken from Tables 5 and 6, pp. 87-92, MRID 45000418.
[a]Mean ± Standard deviation
[b]Numbers in parenthesis represent % difference from the control; calculated by the reviewer.
[c]Calculated by reviewer.

## C. FOOD CONSUMPTION AND COMPOUND INTAKE

### 1. Food consumption

Mean daily food consumption did not differ among groups in the main study for either sex. Food intake was slightly, but significantly, increased for male mice in the 100 ppm group during week 1 and for female mice in the 100 ppm group during weeks 5, 11, and 60. Average food consumption values for males in the 0, 3, 10, and 100 ppm groups were 4.7, 4.9, 4.7, and 4.8 g/mouse/day, respectively. Average food consumption values for female mice in the 0, 3, 10, and 100 ppm groups were 4.2, 4.2, 4.3, and 4.4 g/mouse/day, respectively.

### 2. Compound consumption

The compound consumption was calculated based on feed consumption and body weight data. Doses are presented in Table 1.

### 3. Food efficiency

Food efficiency did not differ among groups for either sex in the main group. Overall food efficiency percentages for males in the 0, 3, 10, and 100 ppm groups were 3.7, 3.9, 4.0, and 3.8%, respectively. Overall food efficiency percentages for female mice in the 0, 3, 10, and 100 ppm groups were 3.4, 3.3, 3.4, and 3.4%, respectively.

D. BLOOD WORK

1. Hematology

Mean corpuscular hemoglobin concentration was significantly lower in the males in the 100 ppm group ($p<0.05$) after 26 weeks of treatment. Values in the 0, 3, 10, and 100 pm groups were 33.5, 33.0, 33.0, and 32.5 g/dl, respectively. A significantly lower mean corpuscular hemoglobin (MCH) value in female mice in the 10 ppm group after 26 weeks of treatment ($p<0.05$) was not dose-related.

2. Clinical chemistry

At the 52 week interim sacrifice there was a significant decrease in globulin (control, 2.71 g/dl; 100 ppm, 2.24 g/dl; $p<0.05$) and a significant increase in the albumin/ globulin ratio (control, 0.89; 100 ppm, 1.19; $p<0.01$) in male mice in the 100 ppm satellite group. These changes were not present in male mice at the 26 or 78 week observation period or in female mice at any observation period. No other significant changes in clinical chemistry parameters were observed in either sex.

E. URINALYSIS

Slightly higher urine pH values were noted for male mice in the 10 and 100 ppm groups at 26 weeks. pH values ranged up to 8.0 (1/10 males in the 10 ppm group and 4/10 males in the 100 ppm group; both $p<0.01$), whereas the highest value in the control group was 7.0. This difference was not noted at 52 or 78 weeks and was not noted in female mice at any time.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

The body weights at interim and terminal sacrifices and the corresponding absolute and relative (to body weights) organ weights are summarized in Table 4. In males in the 100 ppm group, absolute and relative liver weights were increased at the 26-week interim sacrifice, by 24 and 19%, respectively (both, $p<0.01$), but increases were no longer statistically significant at the 52 week and terminal sacrifice. In females in the 100 ppm group, only the relative liver weight was increased, by 13% over the control weight ($p<0.05$) at the 26 week interim sacrifice. Neither absolute nor relative liver weights of females were increased at the 52 and 78 week sacrifices.

At the 52 week interim sacrifice, the absolute weight of the brain in all treated female groups was significantly lower than the control value (all, $p<0.05$), but the relative weight was comparable to controls. The magnitude of decrease was similar in all treated groups (6%) and did not appear to be dose related. This finding was not observed at other sacrifice times or in males at any observation time (data not presented in Table 4).

**TABLE 4. Group mean final body weights (g) organ weights (g), and organ weights relative to body weight (%) in male and female mice fed XRD-537 BE for 26, 52 and 78 weeks**

| Treatment period, body weight, organ weight | Dietary concentration (ppm) | | | |
|---|---|---|---|---|
| | 0 | 3 | 10 | 100 |
| **Males** | | | | |
| 26 Week interim sacrifice | | | | |
| Body weight | 47.5 ± 6.0[a,b] | 50.7 ± 3.8 | 47.7 ± 7.1 | 49.5 ± 6.7 |
| Liver, absolute weight | 2.24 ± 0.32 | 2.36 ± 0.24 | 2.38 ± 0.32 | 2.77 ± 0.44** (24) |
| Liver, relative weight | 4.75 ± 0.61 | 4.66 ± 0.38 | 5.02 ± 0.44 | 5.64 ± 0.81** (19) |
| 52 Week interim sacrifice | | | | |
| Body weight | 51.7 ± 7.5 | 52.5 ± 4.3 | 53.8 ± 6.4 | 53.3 ± 2.8 |
| Liver, absolute weight | 2.64 ± 0.44 | 2.71 ± 0.70 | 2.70 ± 0.26 | 3.14 ± 0.51 |
| Liver, relative weight | 5.11 ± 0.50 | 5.16 ± 1.26 | 5.04 ± 0.46 | 5.88 ± 0.69 |
| Brain, absolute weight | 0.523 ± 0.018 | 0.537 ± 0.035 | 0.517 ± 0.023 | 0.521 ± 0.016 |
| 78 Week terminal sacrifice | | | | |
| Body weight | 51.2 ± 3.5 | 50.6 ± 6.5 | 47.0 ± 7.0 | 53.0 ± 8.7 |
| Liver, absolute weight | 2.95 ± 1.15 | 3.23 ± 1.36 | 3.02 ± 1.00 | 3.35 ± 0.72 |
| Liver, relative weight | 5.80 ± 2.36 | 6.62 ± 3.48 | 6.42 ± 1.56 | 6.34 ± 0.85 |
| **Females** | | | | |
| 26 Week interim sacrifice | | | | |
| Body weight | 44.3 ± 6.9 | 45.0 ± 7.0 | 46.6 ± 6.1 | 44.5 ± 5.2 |
| Liver, absolute weight | 1.81 ± 0.18 | 1.82 ± 0.24 | 1.87 ± 0.27 | 2.08 ± 0.32 |
| Liver, relative weight | 4.12 ± 0.39 | 4.05 ± 0.25 | 4.03 ± 0.39 | 4.67 ± 0.55* (13) |
| 52 Week interim sacrifice | | | | |
| Body weight | 51.5 ± 7.9 | 56.7 ± 10.1 | 46.3 ± 8.3 | 49.8 ± 5.5 |
| Liver, absolute weight | 2.19 ± 0.35 | 2.42 ± 0.57 | 2.03 ± 0.46 | 2.22 ± 0.40 |
| Liver, relative weight | 4.28 ± 0.51 | 4.34 ± 1.13 | 4.41 ± 0.72 | 4.46 ± 0.61 |
| Brain, absolute weight | 0.551 ± 0.014 | 0.516 ± 0.030* | 0.520 ± 0.033* | 0.518 ± 0.024* |
| 78 Week terminal sacrifice | | | | |
| Body weight | 49.1 ± 7.7 | 50.3 ± 7.2 | 51.3 ± 5.4 | 49.7 ± 11.1 |
| Liver, absolute weight | 2.32 ± 0.47 | 2.44 ± 0.33 | 2.24 ± 0.48 | 2.45 ± 0.37 |
| Liver, relative weight | 4.82 ± 1.15 | 4.94 ± 0.97 | 4.38 ± 0.75 | 5.08 ± 0.92 |

Data taken from Tables 23 and 24, pp. 159-170, MRID 45000418.
[a]Based on 10 animals/sex/group.
[b]Mean ± standard deviation.
*$p < 0.05$, significantly different from the control.
**$p<0.01$, significantly different from the control.

2. Gross pathology

Macroscopic findings are summarized in Table 5. Emaciation was observed in male and female mice at the terminal sacrifice and in mice killed *in extremis*/found dead. Emaciation was not observed in either sex at the 26 or 52 weeks interim sacrifices. For males, incidences of emaciation at the terminal sacrifice for the 0, 3, 10, and 100 ppm groups were 0/38, 3/35, 5/34 ($p<0.05$), and 3/42, respectively. Respective incidences in males found dead or killed *in extremis* were 1/13, 7/17 ($p<0.05$), 6/18,

and 4/10. Total incidences of emaciation in males, reported in Table 5, indicate that statistical significance was attained in all treatment groups. For females, incidences of emaciation at the terminal sacrifice for the 0, 3, 10, and 100 ppm groups were 0/39, 2/35, 0/36, and 3/40, respectively. Respective incidences for females found dead or killed *in extremis* were 0/13, 8/17 ($p<0.01$), 6/16 ($p<0.05$), and 4/12 ($p<0.05$). Total incidences in females, reported in Table 5, indicate that statistical significance was attained in all treatment groups.

A thickened wall of the glandular portion of the stomach was observed in male mice in the 100 ppm group only at the 78 week terminal sacrifice (controls, 1/38; 100 ppm, 7/42; $p<0.05$). This lesion was not observed in male mice at the interim sacrifices or in male mice sacrificed *in extremis*/found dead. Therefore, the total incidences for controls and the 100 pm group were 1/71 and 7/71 ($p<0.05$), respectively. Statistics were not provided for the main study animals (1/52 vs 7/52). In female mice, this lesion was observed only in the control group at the terminal sacrifice (3/39) and in one mouse in the 3 ppm group sacrificed killed *in extremis*.

Total incidences of dark-colored livers were significant in both males (25/71; $p<0.01$) and females (13/71; $p<0.01$) in the 100 ppm group. This finding was observed in 7, 9, and 9 male mice at the 26 and 52 week interim sacrifices and the 78-week terminal sacrifice, respectively. In females, incidences at the respective times were 7, 0, and 5; a dark-colored liver was also observed in one female in this group that was found dead or sacrificed *in extremis*. Livers in male mice in the 10 and 100 ppm groups were grossly enlarged at the 26-week interim sacrifice, 5/10 ($p<0.05$) and 7/10 ($p<0.01$) compared with 0/10 and 0/10 in the control and 3 ppm groups, respectively, but incidences of gross enlargement in all groups were 0 at the 52 week interim sacrifice and no longer statistically significant at the 78 week sacrifice.

TABLE 5. Macroscopic findings in male and female mice fed XRD-537 BE in the diet for 78 weeks

| Finding or organ/tissue lesion | Dietary concentration (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 3 | 10 | 100 |
| **Males** | | | | |
| Emaciation | | | | |
| main study[a] | 1/51[b,c] | 10/52 | 11/52 | 7/52 |
| totals[d] | 1/71 | 10/72** | 11/72** | 7/71* |
| Stomach, thickened glandular wall | | | | |
| main study | 1/51 | 2/52 | 3/52 | 7/52 |
| totals | 1/71 | 2/72 | 3/72 | 7/71* |
| Liver, dark in color | | | | |
| 26-week interim sacrifice | 0/10 | 0/10 | 1/10 | 7/9** |
| 52-week interim sacrifice | 0/10 | 0/10 | 2/10 | 9/9** |
| main study | 0/51 | 0/52 | 0/52 | 9/52 |
| totals | 0/71 | 0/72 | 3/72 | 25/71** |
| **Females** | | | | |
| Emaciation | | | | |
| main study | 0/52 | 10/52 | 6/52 | 7/52 |
| totals | 0/72 | 10/72** | 6/72* | 7/72** |
| Stomach, thickened glandular wall | | | | |
| main study | 3/52 | 1/52 | 0/52 | 0/52 |
| totals | 3/72 | 1/72 | 0/72 | 0/72 |
| Liver, dark in color | | | | |
| 26-week interim sacrifice | 0/10 | 0/10 | 0/10 | 7/10** |
| 52-week interim sacrifice | 0/10 | 0/10 | 0/10 | 0/10 |
| main study | 0/52 | 0/52 | 0/52 | 6/52 |
| totals | 0/72 | 0/72 | 0/72 | 13/72** |

Data taken from the Tables 21 and 22, pp. 137-158, MRID 45000418.
[a]Mice at terminal sacrifice plus mice killed *in extremis*/found dead; statistics were not provided for the main study data.
[b]Number of mice with lesions/total number of mice examined.
[c]One animal died accidentally during week 36.
[d]Data includes animals sacrificed at 26, 52, and 78 weeks and animals killed *in extremis* or found dead.
*$p<0.05$, significantly different from control.
**$p<0.01$, significantly different from control.

3. Microscopic pathology

a. Non-neoplastic

Selected microscopic findings totaled from mice in the satellite and main study groups are summarized in Table 6. In the main study, incidences of hepatocellular swelling with minute eosinophilic granules were significantly increased over control values in male mice in the 100 ppm group (26/52; $p<0.01$) and in female mice in the 100 ppm group (12/52; $p<0.01$). In males in the 100 ppm group, the presence of this lesion was high at the 26 and 52 week interim sacrifices (10/10 and

9/9, respectively), with the percentage decreasing with time. This change was also present in males in the 10 ppm group at earlier interim sacrifice times, although in lower incidences compared with the 100 ppm group. Compared with males in the 100 ppm group, percentages of females in the 100 ppm group with this lesion were lower at the 26, 52, and 78-week interim sacrifice (4/10, 2/10, and 9/40 ($p<0.0.1$), respectively) and, in contrast to males, this finding was not observed in the 10 ppm treatment groups. Slight microgranuloma was significantly increased in female mice in the 10 and 100 ppm groups sacrificed at 78 weeks [incidences in order of ascending dose: 5/39, 9/35, 12/36 ($p<0.05$), and 14/40 ($p<0.05$); no incidences in female mice killed *in extremis* or found dead], but not when total mice were considered (incidences in order of ascending dose: 9/72,10/72, 13/72, and 15/72). This lesion was observed at low incidences in the female control and treated groups (0/10-2/10) at the earlier sacrifices. Incidences of microgranuloma were not dose-related in male mice. Centrilobular hepatocellular fatty changes were significantly decreased in male mice in the 100 ppm group ($p<0.01$) at the 78-week sacrifice; this lesions was not observed in female mice.

Kidney lesions were observed in female mice, primarily in the 100 ppm group. In the main study, incidences of chronic glomerulonephritis (12/52; $p<0.05$), tubular dilatation (10/52; $p<0.01$), and hyaline casts (16/52; $p<0.01$) were significantly higher compared with incidences in the control group (4/52, 1/52, and 4/52, respectively). Hyaline casts were also noted in the 3 ppm group of females. Most of these changes were not observed until the 78 week terminal sacrifice (casts) or in animals killed *in extremis* or found dead (tubular dilatation and chronic glomerulonephritis). Therefore, data for the interim sacrifices are not presented in Table 6. It should be noted that early changes of chronic glomerulonephritis were slightly lower in treated females than in controls so that incidences of early changes of chronic glomerulonephritis combined with incidences of chronic glomerulonephritis did not show a dose-response relationship (data not shown). These kidney lesions were not observed (tubular dilatation) or did not attain statistical significance (chronic glomerulonephritis and hyaline casts) in male mice.

Increased extramedullary hematopoiesis was observed in spleens of a significantly greater number of male mice in the 100 ppm group ($p<0.01$) as compared with controls, primarily in main study animals (0/10-2/10 incidence data not presented for interim sacrifices in Table 6). Incidences were approximately doubled over the control value in the 3 and 10 ppm groups of males, and attained significance in the 3 ppm group ($p<0.05$), but not in the 10 ppm group. Severity was generally slight in animals sacrificed on schedule and severe in animals found dead. Incidences in treated females were comparable to the control group.

**TABLE 6. Non-neoplastic histopathology findings in male and female mice fed XRD-537 BE for 78 weeks**

| Organ/lesion | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 3 | 10 | 100 |
| **Males** | | | | |
| Liver, hepatocellular swelling, minute eosinophilic granules | | | | |
| 26-week interim sacrifice | 0/10[a] | 0/10 | 3/10 | 10/10** |
| 52-week interim sacrifice | 0/10 | 0/10 | 6/10** | 9/9** |
| main study[b] | 0/51 | 0/52 | 1/52 | 26/52‡ |
| Kidney | | | | |
| Tubular dilatation (main study) | 0/51 | 1/52 | 0/52 | 0/52 |
| Chronic glomerulonephritis (main study) | 0/51 | 3/52 | 3/52 | 4/51 |
| Hyaline casts (main study) | 4/51 | 4/52 | 4/52 | 8/51 |
| Spleen | | | | |
| Increased extramedullary hematopoiesis (main study) | 9/51 | 17/52† | 14/52 | 22/52‡ |
| Adrenal, increased brown pigment, cortico-medullary junction | | | | |
| main study | 12/51 | 8/52 | 9/52 | 4/52† |
| Stomach, mucosal epithelial hyperplasia | | | | |
| 26-week interim sacrifice | 3/10 | 2/10 | 0/10 | 3/10 |
| 52-week interim sacrifice | 4/10 | 1/10 | 1/10 | 1/9 |
| main study | 9/51 | 14/52 | 13/52 | 21/52‡ |
| **Females** | | | | |
| Liver, hepatocellular swelling, minute eosinophilic granules | | | | |
| 26-week interim sacrifice | 0/10 | 0/10 | 0/10 | 4/10* |
| 52-week interim sacrifice | 0/10 | 0/10 | 0/10 | 2/10 |
| main study | 0/52 | 0/52 | 0/52 | 12/52‡ |
| Kidney | | | | |
| Tubular dilatation (main study) | 1/52 | 6/52 | 0/52 | 10/52‡ |
| Chronic glomerulonephritis (main study) | 4/52 | 6/52 | 5/52 | 12/52† |
| Hyaline casts (main study) | 4/52 | 13/52† | 9/52 | 16/52‡ |
| Spleen | | | | |
| Increased extramedullary hematopoiesis (main study) | 7/52 | 12/52 | 9/52 | 5/52 |
| Adrenal, increased brown pigment, cortico-medullary junction | | | | |
| main study | 4/52 | 8/52 | 12/52† | 11/52† |
| Stomach, mucosal epithelial hyperplasia (main study) | 4/52 | 1/52 | 3/52 | 3/52 |

Data taken from Table 27, pp. 186-192 and Table 28, pp. 207-213; MRID 45000418.
[a]Number of mice with lesions/total number of mice examined.
[b]Mice at 78-week terminal sacrifice plus mice killed *in extremis*/found dead.
*$p<0.05$, significantly different from control.
**$p<0.01$, significantly different from control.
†$p<0.05$, significantly different from control; calculated by reviewer.
‡$p<0.01$, significantly different from control; calculated by reviewer.

In the main study, the incidences of increased brown pigment in the cortico-medullary junction of adrenal glands were significantly higher in females in the 10 and 100 ppm groups (12/52 and 11/52, both $p<0.05$) as compared with controls, but the incidence was significantly lower in males in the 100 ppm group (4/52, $p<0.05$) as compared with male controls (12/51). Although present in low

numbers at the 26 and 52 week interim sacrifices, this lesion was most prevalent in females in the 10 and 100 ppm groups at the 78 week terminal sacrifice.

Incidences of hyperplasia of the epithelial mucosa of the glandular stomach were significantly increased in male mice in the 100 ppm main group (controls, 9/51; 100 ppm, 21/52; $p<0.01$), but the incidence of this lesion was not statistically significant compared with the control group when all animals and all sacrifice times were considered (incidences of 16/71, 17/72, 14/72, and 25/71 in the 0, 3, 10, and 100 ppm groups, respectively). This lesion was present in low , non-dose related numbers of males at the interim sacrifices. Females were not affected; total incidences in the main study ranged from 1/52-4/52.

The incidences of mineralization of the testis were decreased in all treated male mice, attaining statistical significance in the 100 ppm group. The lack of mineralization is not an adverse effect and data are not presented in Table 6.

Other changes that attained statistical significance were not dose-related.

b. Neoplastic

Various types of neoplasms were observed in all treated groups of male and female mice, but there were no significant differences between any treated group and the respective control group. The most common lesions in male mice were hepatocellular adenomas, present in 17/71 control males and 20/71 high-dose males, followed by lung adenomas and adenocarcinomas. Incidences of hepatocellular adenomas were low in female mice (control, 1/72; 100 ppm, 3/72); incidences of lung adenomas and adenocarcinomas were similar to those in males. Malignant lymphomas were also present in female mice at incidences of ≤14%. No specific types of tumors showed early onset.

## III. DISCUSSION

### A. INVESTIGATOR'S CONCLUSION

The investigators concluded that treatment with XRD-537 BE at concentrations of 0, 3, 10, or 100 ppm in the diet for 78 weeks had no effect on mortality rates of male and female mice. Emaciation was not considered of toxicological significance as it was observed principally in animals that were found dead or moribund and was not dose related.

The study authors stated that treatment with XRD-537 BE at 100 ppm in the diet compared with the controls resulted in grossly enlarged, dark-colored livers; increased relative liver weights in both sexes at 26 weeks and increased absolute liver weight in males at 26 weeks; hepatocellular swelling with minute eosinophilic granules in both sexes at 78 weeks; microgranuloma in the liver of females at the 78 week sacrifice;

and epithelial hyperplasia of the glandular stomach in males sacrificed by design after 78 weeks.

Treatment with 10 ppm resulted in enlarged livers in males at 26 weeks: hepatocellular swelling with minute eosinophilic granules in males but not in females; and an increased incidence of hepatic microgranuloma in females at the 78 week sacrifice (but not in all females combined).

There were no treatment-related changes in either sex administered 3 ppm in the diet. Therefore, the study authors considered 3 ppm a "maximum no-effect level," 10 ppm was considered a "minimum toxic level," and 100 ppm was considered a "sure toxic level."

The study investigators concluded that there was no evidence of carcinogenic activity for ICR (Crj:CD-1) mice of either sex administered diets containing XRD-537 BE at concentrations of 3, 10, or 100 ppm for 78 weeks.

B. REVIEWER'S DISCUSSION

There were no dose-related clinical signs in mice fed the test material at concentrations of 3, 10, or 100 ppm in the diet for up to 78 weeks. At the end of 78 weeks, there were no significant treatment-related effects on survival, body weight, food consumption, food efficiency, hematology, clinical chemistry parameters, or organ weights. Treatment-related effects in the 100 ppm group included lower mean corpuscular hemoglobin concentration in male mice at the 26 week interim sacrifice, a decrease in globulin and an increase in the albumin/globulin ratio in male mice at the 52 week interim sacrifice, increased liver weights at 26 weeks in both sexes, and decreased brain weight in females at 52 weeks. Incidences and organ weight changes did not attain statistical significance at 78 weeks and were, therefore, considered transient effects or toxicologically non-significant.

At autopsy, gross examination revealed emaciation in all treated groups of both sexes, but primarily in mice that died or were found moribund. The reviewer agrees with the study authors that this observation is of no or questionable toxicological significance because incidences were not clearly dose related and food consumption, food efficiency, body weights, and survival were not affected over the course of the study.

Mucosal epithelial hyperplasia of the glandular portion of the stomach appeared to be accelerated and more severe in aging male mice, with statistical significance attained in the 100 ppm group in the main sacrifice group. This lesion correlated with the thickened wall of the glandular stomach which could be observed grossly. This lesion indicates a treatment-related irritant effect of the test material in male mice. Incidences were low and similar in female control and treated groups. The study authors note that this lesion occurs with a high frequency in aged animals and was not present at the shorter time periods. The lesion is treatment related, but incidences were low, onset was late in the study, it occurred in only one sex, and it is a common

lesion of aging mice. Thus, the reviewer considers this lesion a minimal adverse effect.

Although statistical significance for increases in absolute and relative liver weights were not attained in any treatment group of either sex by the end of the study (indicating minimal hypertrophy), the livers in the treated groups were described as grossly enlarged and dark colored; microscopically, hepatocellular swelling with minute eosinophilic granules was observed. These treatment-related findings are consistent with liver hypertrophy which is an adaptive response to chemical intake. Liver hypertrophy may be accompanied by cell inclusions, in this case eosinophilic granules, the composition of which is unknown. Focal granuloma, also observed in female mice, is a common incidental finding in the liver of aging mice. Severity of this lesion in the female 100 ppm group was slight. Focal hepatocellular necrosis was not increased in treated mice and there were no corresponding enzyme, hematology or clinical chemistry changes, indicating that the function of the liver was not impaired. Therefore, the reviewer considers the effects on the liver an adaptive response rather than a toxicologically significant response. The reason for dark colored livers is unknown, but this observation may be an artifact of sacrifice.

Kidney lesions were confined to female mice in the 100 ppm group and consisted of tubular dilatation, chronic glomerulonephritis, and increased incidences of hyaline casts. While these findings were significantly higher for all females combined compared with the control group, the tubular dilatation and glomerulonephritis were confined primarily to females killed *in extremis* or found dead. Incidences were not significantly elevated in females at the 78 week terminal sacrifice. Furthermore, incidences of early changes of chronic glomerulonephritis were slightly lower in the treated female groups than in the control group, indicating that progression to the chronic state was either slightly accelerated in the higher dose groups or was related to death from other causes. Although found primarily in dead or moribund females in the 100 ppm group, survival in this group was not compromised compared with controls, and therefore the effect may be incidental to treatment. There were no related effects on clinical chemistry or urinary parameters in females. The toxicological significance of tubular dilatation and hyaline (protein) casts is unknown. Protein casts are usually associated with nephropathy in male rats and mice. Although some of the kidney lesions in the 100 ppm group may have been incidental to treatment, taken together, the lesions indicate a treatment-related toxicological effect. The significantly increased incidence of hyaline casts in the 3 ppm group was not considered treatment related as incidences were slightly lower in the 10 ppm group.

Although the incidence of increased brown pigment in the cortico-medullary junction of the adrenal gland was significantly elevated in female mice in the 10 and 100 ppm groups, particularly at 78 weeks, this lesion was significantly lower in male mice in the 100 ppm group compared with male controls. Pigmentation deposition in the cortico-medullary junction of the adrenal gland may be related to aging processes. This lesion was not accompanied by fatty deposition in the cortico-medullary

junction. Therefore, the toxicological significance of deposition of brown pigment in the adrenal gland is unknown.

Likewise, extramedullary hematopoiesis of the spleen was increased in only one sex (males), primarily at the 78 week sacrifice and in males found dead or moribund during the study. Statistical significance was attained in the 3 ppm group ($p<0.05$) and 100 ppm group ($p<0.01$) but not in the 10 ppm group (indicating a poor dose-response relationship). The proportion of animals with mild, moderate, and severe splenic hematopoiesis was similar among the control and treated groups. Mild hematopoiesis of the bone marrow was observed in many of the same animals. Hematology parameters were not affected and urobilinogen in the urine was not increased in the treated groups. Extramedullary hematopoiesis of the spleen is observed in aging mice. The fact that incidences were increased in the treated groups may be incidental to treatment.

**The LOAEL for male and female mice is 100 ppm in the diet (males, 10.1 mg/kg/day; females, 10.3 mg/kg/day) based on effects on the kidney including tubular dilatation, chronic glomerulonephritis, and hyaline casts in female mice, and hyperplasia of the stomach mucosal epithelium in male mice. The NOAEL for male and female mice is 10 ppm in the diet (approximately 1 mg/kg/day).**

Treatment of male and female ICR (Crj:CD-1) mice for up to 78 weeks with XRD-537 BE in the diet did not result in a statistically significant increase in the number of animals with primary neoplasms at any anatomical site. Common age related neoplasms included hepatocellular adenomas and lung adenomas/adenocarcinomas in male mice and lung adenomas/adenocarcinomas and malignant lymphomas in female mice. The doses used in this study were inadequate for assessing carcinogenicity.

C. STUDY DEFICIENCIES

Statistics should have been provided for main study animals (animals sacrificed at 78 weeks as well as animals in the main study found dead or sacrificed *in extremis*). The reviewer felt that total incidences, for which the study authors provided statistics, were not as important as main study incidences because some lesions did not appear until late in the study; animals sacrificed early, before lesions appeared, were effectively censored. The reviewer calculated probabilities for the main study animals and based LOAELs and NOAELs on main study animals.