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

009307

FEB 24 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: HWG 1608 (Tebuconazole) technical. Oncogenicity data review.
Tox Chem No. 463
HED Project No. 2-1198
MRID No. 421750-01

From: Alberto Protzel, Ph.D. 2/20/92
Review Section III
Toxicology Branch II
Health Effects Division (H7509C)

To: Mr. Benjamin Chambliss/Ms. Susan Lewis (PM-21)
Fungicide-Herbicide Branch
Registration Division (H7505C)

Thru: James N. Rowe, Ph.D., Head 2/20/92
Review Section III
Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Chief
Toxicology Branch II
Health Effects Division (H7509C)

ACTION: Review of the following study on the chemical HWG 1608 (Tebuconazole) Technical submitted by Miles Inc. as a 6(a)(2) document:

HWG 1608. Toxic Dose Range Carcinogenicity Study in NMRI Mice (Supplement to Study T 6018953 - Cancerogenicity in NMRI Mice with Administration in Diet Over a 21-Month Period).

CONCLUSIONS:

The Registrant has previously tested HWG 1608 for oncogenicity in NMRI mice at dietary levels of 0, 20, 60, or 180 ppm for 21 months (MRID 407009-41. 1/25/88). In the review of this 1988 initial study (J.N Rowe, DER dated 12/24/88), the reviewer concluded that based on the findings reported in the study it appeared that the high-dose treatment (180 ppm) was not high enough to approximate the maximum tolerated dose (MTD) and the study was classified as CORE Supplementary.

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This conclusion was ratified by the HED RfD/Peer Review Committee following a meeting held on 3/5/91 (Memorandum from G.Z. Ghali, SACB, to S. Lewis, FHB, dated 7/11/91), and it was concluded that that the chemical should have been tested at a higher dose. The present study, designed to satisfy the MTD requirement, was conducted with NMRI mice administered HWG 1608 in the diet at higher doses.

HWG 1608 was administered to Bor:NMRI(SPF-Han) mice of both sexes for a period of up to 91 weeks in the diet at levels of 0, 500, and 1500 ppm resulting in mean respective compound intakes of 0, 84.9 and 279 mg/kg body weight/day (males) and 0, 103.1, and 356.5 mg/kg body weight/day (females). Statistically significantly decreased body weights and increased food consumption were reported that were consistent with decreased food efficiency at 500 and 1500 ppm in males and at 1500 ppm in females. Clinical chemistry values included dose-dependent increases in plasma GOT, GPT and AP for both sexes and were consistent with hepatotoxic effects at both 500 ppm and 1500 ppm. Relative liver weight increases reached statistical significance at both 500 and 1500 ppm in males and at 1500 ppm only in females. Histopathology included dose-dependent increases in hepatic panacinar fine fatty vacuolation, statistically significant at 500 and 1500 ppm in males and at 1500 ppm in females. Other histopathology included significant oval cell proliferation in both sexes at 1500 ppm and dose-dependent ovarian atrophy in females that was statistically significant at 500 and 1500 ppm.

Based on the above findings it is concluded that the MTD was achieved at or around 500 ppm.

Neoplastic histopathology consisted of statistically significant incidences vs controls of hepatocellular neoplasms: adenomas (35.4%) and carcinomas (20.8%) at 1500 ppm in males and carcinomas only (26.1%) at 1500 ppm in females. Comparison with historical controls indicates that the forementioned hepatocellular neoplasms are treatment related.

There was a dose-related increase in histiocytic sarcomas in both sexes. In males the incidences amounted to 1/48 (2.1%), 2/49 (4.2%) and 3/48 (6.3%) at 0, 500 and 1500 ppm, respectively. In females the incidences amounted to 1/47 (2.1%), 3/45 (6.7%), and 5/46 (10.9%) at 0, 500 and 1500 ppm, respectively. Although historical controls were not available it is noted that in the initial mouse study (MRID 407009-41), histiocytic sarcomas did not exceed 4% in males and 2.0% in females at any dose. Although the incidences of histiocytic sarcoma in the present study did not reach statistical significance vs. controls at any dose, it is not possible to fully assess the significance of these dose-related incidences in the absence of historical controls.

The study was classified as CORE Supplementary pending submission of the following:

- A signed and dated Quality Assurance Statement is required. Although a dated Quality Assurance statement was included, the corresponding signatures were not included; only the signature of the translator was available for review.

- Historical control data on the incidence of histiocytic sarcomas in male and female mice of the same strain under comparable conditions.

This study will be forwarded to the HED RfD/Peer Review Committee following receipt and analysis of the requested historical control data.

cc G. Ghali (HED/SACB)

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Reviewed by: Alberto Protzel, Ph.D.
Review Section III, Toxicology Branch II(H7509C)
Secondary Review by: James N. Rowe, Ph.D.
Review Section III, Toxicology Branch II(H7509C)

Alberto Protzel 2/20/92
James N. Rowe 2/20/92

DATA EVALUATION RECORD

009307

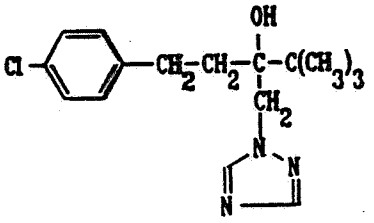
STUDY TYPE: Oncogenicity
Species: Mouse
EPA Guideline 83-2

TOX. CHEM. NO: 463P

EPA IDENTIFICATION NO.: EPA MRID No. 421750-01 (In 2 volumes)

TEST MATERIAL: HWG-1608

SYNONYMS/STRUCTURE: Tebuconazole; α-[2-(4-Chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H -1,2,4-triazole-1-ethanol



STUDY NUMBER: 16376-A (Supplement of Study 16376)

TESTING FACILITY: BAYER AG. Department of Toxicology. Friedrich-Ebert-Str. 217-233. D-56 Wuppertal 1. F.R. Germany [In-life phase and assessment] and Life Science Research in Eye, Suffolk, England [Histopathology].

TITLE OF REPORT: HWG 1608. Toxic Dose Range Carcinogenicity Study in NMRI Mice (Supplement to Study T 6018953 - Cancerogenicity in NMRI Mice with Administration in Diet Over a 21-Month Period).

AUTHOR: E. Bomhard and R. Burnett.

REPORT ISSUED: December 12, 1991 (Original study dated 1/25/88)

CONCLUSIONS:

HWG 1608 was administered to Bor:NMRI(SPF-Han) mice of both sexes for a period of up to 91 weeks in the diet at levels of 0, 500, and 1500 ppm resulting in mean respective compound intakes of 0, 84.9 and 279 mg/kg body weight/day (males) and 0, 103.1, and 356.5 mg/kg body weight/day (females).

Statistically significantly decreased body weights and increased food consumption were reported that were consistent with decreased food efficiency at 500 and 1500 ppm in males and at 1500 ppm in females.

Clinical chemistry values (statistically significant and dose-dependent increases

in plasma GOT, GPT and AP) for both sexes were consistent with hepatotoxic effects at both 500 ppm and 1500 ppm. A dose-dependent increase in absolute and relative liver weights was observed in both sexes at both interim and final sacifice. Relative liver weight increases reached statistical significance at both 500 and 1500 ppm in males and at 1500 ppm only in females.

Terminal sacrifice and interim non-neoplastic histopathology indicate the liver as a target organ at 500 and 1500 ppm in both sexes. Histopathological observations in males included a dose-dependant and statistically significant increases in hepatic panacinar fine fatty vacuolation at 500 and 1500 ppm and statistically significant increases in focal hyperplasia of hepatocytes and oval cell proliferation at 1500 ppm. In terminal sacrifice females, there was a dose-dependant increase in hepatic panacinar fine fatty vacuolation which was statistically significant at 1500 ppm. In addition, at 1500 ppm there were increases in periacinar hepatocyte hypertrophy, oval cell proliferation, and eosinophilic foci of hepatocyte alteration which were statistically significant. Additionally, terminal sacrifice non-neoplastic histopathology indicates the ovaries as posible targets in 500 and 1500 ppm females.

Based on the above information it is concluded that the MTD was reached at 500 ppm in this study.

The incidence of hepatocellular adenomas in males was 2/48 (4.2%) at 500 ppm and 17/48 (35.4%) at 1500, and was statistically significant ($p \leq 0.001$) vs. control incidences (3/47, 6.4%) only at the high dose. The incidence of male hepatocellular carcinomas was 0/48 at 500 ppm and 10/48 (20.8%) at 1500 ppm, and was statistically significant ($p \leq 0.01$) vs. control incidences (0/47, 0%) also only at the high dose. Thus, the combined incidence of benign/malignant tumors in high-dose males (56.2%), exceeds the reported 2-18% combined benign/malignant tumor incidence reported for historical controls and is considered to be compound related.

The incidence of hepatocellular adenomas in females was 0/45 at 500 ppm and 2/46 (4.2%) at 1500, and was not statistically significant vs. control incidences (0/47). On the other hand, the incidence of female hepatocellular carcinomas was 0/48 at 500 ppm and increased to 12/46 (26.1%) at 1500 ppm, to become statistically significant ($p \leq 0.001$) vs. control incidences (0/47). Although historical control data were not available for comparison, the statistically significant incidence of hepatocellular carcinomas in females (26.1%) observed in this study coupled to the small incidence at all doses in the initial study ($\leq$2%) indicate that the effect is treatment-related.

There was a dose-related increase in histiocytic sarcomas in both sexes. In males the incidences amounted to 1/48 (2.1%), 2/49 (4.2%) and 3/48 (6.3%) at 0, 500 and 1500 ppm, respectively. In females the incidences amounted to 1/47 (2.1%), 3/45 (6.7%), and 5/46 (10.9%) at 0, 500 and 1500 ppm, respectively. Although the incidences of histiocytic sarcoma in the present study did not reach statistical significance vs. controls at any dose, it is not possible to fully assess the significance of these dose-related incidences in the absence of historical control data.

CLASSIFICATION: CORE Supplementary. This study may be upgraded to minimum, allowing it to fulfill (in conjunction with the initial mouse study, MRID 407009-41) the requirements of Guideline 83-2a (Subdivision F) if the following information is submitted and is deemed acceptable:

- A signed and dated Quality Assurance Statement is required. Although a dated Quality Assurance statement was included, the corresponding signatures were not included; only the signature of the translator was included.

- Historical control data on the incidence of histiocytic sarcomas in male and female mice of the same strain under comparable experimental conditions. The data should encompass the 2-4 year period around the period in which the study was conducted.

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A. Materials: (A photocopy of the methods is included as Appendix I).

1. Test compound: HWG 1608 (Tebuconazole, technical grade). Description: Crystalline solid. Batch No.: 816896061. Purity: 96.2%. Contaminants: not listed.

2. Test animals: Species: mouse. Strain: SPF-bred NMRI mice of strain Bor:NMRI (SPF-Han). Age: approximately 6-7 weeks at the start of the study. Mean weight (at week 0): males, 34.0-34.8 g; females, 28.1-28.9 g. At week 0 the animals were within ±20% of the mean weight for each sex. Source: Winkelmann Breeders in Borchen.

B. Study Design:

1. Dose Selection:

THe Registrant has previously tested HWG 1608 for oncogenicity in NMRI mice administered the compound in the diet at levels 0, 20, 60, or 180 ppm for 21 months (MRID 407009-41, 1/25/88). In the review of this 1988 initial study (J.N Rowe, DER dated 12/24/88), the reviewer noted elevations in bilirubin and liver weights in addition to microscopic pathology findings at the mid and high doses. He concluded that based on the findings reported in the study it appeared that the high-dose treatment (180 ppm) was not high enough to approximate the maximum tolerated dose (MTD) and the study was classified as Supplementary. This conclusion was ratified by the HED RfD/Peer Review Committee following a meeting held on 3/5/91 (Memorandum from G.Z. Ghali, SACB, to S. Lewis, FHB, dated 7/11/91), and it was concluded that that the chemical should have been tested at a higher dose. The present study, designed to satisfy the MTD requirement, was conducted with NMRI mice administered HWG 1608 in the diet at 0, 500 or 1500 ppm based on:

- Failure to achieve the MTD at 180 ppm.
- Four- and 6-week exploratory studies. In these studies, hepatocellular degeneration/necroses and vacuolization in the liver cells were observed at 500 ppm and above.

2. Animal assignment:

The animals were assigned randomly to the test groups shown in Table 1. A 7-day (males) to 8-day (females) period of acclimation was allowed between receipt of shipment and start of treatment.

Table 1. Dosing groups for oncogenicity study of HWG 1608[a].

Group number	Dose (ppm)	Main Group	
		Males	Females
1 Control	0	60[b]	60
2 (LDT)	500	60	60
3 (HDT)	1500	60	60

[a] The original study [MRID 407009-41] was performed with HWG 1608 at dietary levels of 0, 20, 60, and 120 ppm.
[b] 10 Animals, in all groups, were used for interim sacrifice at 52 weeks and the remainder were sacrificed at 91 weeks. Dosing started on 8/22/88 (males) and 8/23/88 (females).

3. Diet preparation

Diets were prepared weekly. Conditions of storage of the treated diet prior to use were not specified. Samples of treated food were analyzed for homogeneity and stability prior to study initiation and for test article concentration at time of diet preparation for week 1 and at approximately every 3 months thereafter.

Analysis of concentration was performed on the sample right after preparation and after storing the sample for 7 days under conditions comparable to those of the actual feeding study. As shown in Table 2 actual concentrations of test material at nominal 500 ppm had a mean of 94% and a range of 87.2-105% of target; concentrations at nominal 1500 ppm had a mean of 96% and a range of 84.7-111% of target. In addition, the values were similar 7 days after preparation and storage under conditions similar to those of the feeding study. These values are within acceptable variability for test substance concentrations.

Table 2. Analytical concentrations of HWG 1608 during testing[a].

Dates[b]	Actual concentration (ppm) found at nomimal	
	500 ppm	1500 ppm
8/19/88	464/457[c]	1360/1330
10/7/88	481/436	1420/1230
12/9/88	443/461	1270/1430
6/2/89	458/518	1530/1480
6/30/89	455/458	1370/1320
11/24/89	479/466	1500/1460
1/5/90	444/489	1410/1420
5/4/90	478/479	1490/1530
5/18/90	525/496	1670/1590
Mean (ppm)	472	1430
Relative Standard Deviation	5.2	7.8
Mean (in % of target)	94	96

[a] Data obtained from p. 84 of the Study Report. The dates column indicates the date of diet preparation.
[b] Dosing started on 8/22/88 and the last day of necropsy was 5/25/90.
[c] The value preceding the slash (/) is the ppm shortly after diet preparation; the number following the slash is the ppm 7 days after diet preparation in samples kept under comparable conditions as those used in the experiment.

To analyze for homogeneity, 5 samples of 100-200g each were taken from a rectangular container from 5 locations. Three of the 5 samples were selected randomly and were analyzed. As shown in Table 3, blending appears to be homogeneous.

Table 3. Assessment of homogeneity in test diets[a]

Sample No.	Actual concentration (ppm) found at nominal	
	500 ppm	1500 ppm
1	423	1500
2	-	-
3	428	1430
4	432	-
5	-	1500
Mean	428	1480
Maximum deviation relative to mean (%)	1.1	3.2
Rel. standard deviation	1.1	2.7
Mean (in % of nominal)	95	98

[a] Data from pp. 84-85 of the Study Report.

To analyze for stability, prepared diets were stored for 14 days under conditions comparable to those used in the actual feeding study. As shown in Table 4, comparison of test article levels at 0 time and 10 days later indicate that 90, 88 and 94% of the low, middle, and high initial levels of target remain after 10 days of storage.

Table 4. Assessment of stability in test diets[a]

Storage period. (Days)	Actual concentration (ppm) found at nominal	
	500 ppm	1500 ppm
0	428	1470
14	428	1240
% of starting value	100	84

[a] Data from p. 86 of the Study Report.

Animals were allowed free access to tap water and to diet (Altromin 1321 flour, a "fixed formula" standard diet produced by Altromin GmbH in Lage).

5. Statistics -

i) Body weights, food consumption, hematological parameters, clinical chemistry parameters and organ weights were analyzed using the U test of Mann and Whitney and Wilcoxon's test.

ii) Mortality and clinical symptoms were analyzed using Fisher's exact test.

5. A statement of data confidentiality (none claimed) and a flagging statement according to the criteria of 40 CFR 158.34 [Nos. 1 and 2 (neoplasia)] were included. A signed and dated statement of compliance with GLP standards (40 CFR 160) was included [FIFRA was spelled FIRFA]. The Quality Assurance statement for the study was dated but not signed (it was signed and dated only by the translator).

C. Methods and Results:

1. Observations:

The animals were inspected at least twice daily (once daily on weekends and public holidays) for clinical signs. Individual animal examinations were performed once a week to include body surfaces, body openings, posture, general behavior, respiration and excreta.

Examination by the reviewer of the data summarized in Table 5 indicates that survival in all groups was 36% or higher through week 91 of the study (sacrifice was conducted on week 91). It appears that there might have been a tendency to higher mortality in females (36-40% survival) vs males (54-64% survival). No significant differences were found in mortality data in treated animals and their respective controls.

As shown in Table 6, there was an increase in the incidence of distended abdomen in the HDT vs controls in both sexes, (i.e. 23 vs 2 in males and 16 vs 8 in females. These instances of distended abdomen might be related to the occurrence of liver enlargement seen in the HDT mice. No other clinical signs of toxicological significance were observed.

Table 5. Mortality data of mice treated with HWG 1608. Data from pp. 33 and 140 to 146 of the Study Report.

Weeks	Dead/percent dead					
	Males (n=50/group)[a]			Females (n=50/group)		
	0	500 ppm	1500 ppm	0 ppm	500 ppm	1500 ppm
0-26	0/0	0/0	0/0	2/4	3/6	1/2
27-52	1/2	5/10	3/6	5/10	3/6	2/4
53-78	10/20	7/14	10/20	11/22	14/28	17/34
79-91	9/18	6/12	10/20	12/24	12/24	12/24
Total	20/40	18/36	23/46	30/60	32/64	32/64
% Survival[b]	[60]	[64]	[54]	[40]	[36]	[36]

[a] Initial number. Percent values were calculated by the reviewer.
[b] % survival at 91 weeks calculated by the reviewer as 100 - (% dead at 91 weeks). Numbers were placed in brackets to avoid confusion with the units of the column heading (i.e. dead/percent dead).

Table 6. Selected clinical signs in mice treated with HWG 1608. Data from pp. 147-148 and 150-161 of the Study Report.

Parameter	Number with clinical sign					
	Males			Females		
	0	500	1500	0	500	1500
	Weeks 4-92					
Number in Group[a]:	60	60	60	60	60	60
Distended abdomen	2	2	23	8	9	16

[a] Includes 10 satellite animals.

2. Body weight:

The animals were weighed at the time of assignment of the animals to groups, 1 week before the start of treatment, weekly for weeks 1 through 16, and every four weeks thereafter.

Table 7 shows selected group mean body weights during treatment.

- o In males, there were statistically significant decreases in body weight at 500 ppm during the initial half of the testing period and at 1500 ppm throughout the treatment period up to week 84. As estimated by the reviewer, body weight weight depressions vs controls of up to 5.0-6.3% were observed at 500 ppm and of up to 9.0-11% at 1500 ppm.

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- **In females, there were sporadic statistically significant decreases in body weight at 500 ppm during the initial third of the study. At 1500 ppm there were statistically significant decreases in body weight in 39 of the first 59 weeks and in 7 of the last 8 weeks. As estimated by the reviewer, body weight depressions of up to 7.6-8.5% vs. controls were observed during the first 59 weeks in high-dose females.**

Table 7. Selected group mean body weights of mice treated with HWG 1608. Data from pp. 107-120 of the Study Report.

Week	Group mean body weights (g)					
	Males (n=60/group)[a]			Females (n=60/group)		
	0	500 ppm	1500 ppm	0 ppm	500 ppm	1500 ppm
0	34	34.3	34.8**	28.1	28.9**	28.9**
1	34.8	35.7**	35.1	28.7	29.1	28.6
12	40.6	39.4*	37.7**	32.3	31.4	30.7**
24	43.5	42.0*	40.0**	35.8	34.5	33.8**
36	44.3	41.9**	41.0**	36.5	36.1	35.2
48	45.2	43.4	41.6**	37.2	36.2	35.8
60	46.5	44.8	43.3**	38.1	39.1	37.2
72	46.1	44.7	43.9*	39.3	39.0	39.7
84	46.4	44.8	44.3	38.4	40.1	42.2**
91	46.5	44.4	46.6	41.3	39.3	44.1*

[a] The starting number was 60, including a satellite group of 10 animals. The satellite animals were sacrificed at 52 weeks.
* $p \leq 0.05$; ** $p \leq 0.01$.

3. Food consumption and compound intake:

Individual food consumption measurements were determined for 10 animals in each group on a weekly basis from week 1 through week 13 and every 4 weeks thereafter.

Examination of Table 8 indicates that food consumption per unit of body weight (in mg/kg/day) is significantly increased with respect to controls at 1500 ppm in both sexes and at 500 ppm in males.

Mean compound intake over the course of the study (mg/kg b.wt./day) was proportionally increased in both sexes as follows:

- Males: 84.9 and 279.0 for the 500, and 1500 ppm treatment groups, respectively. (From p. 37 of the Study Report).

- Females: 103.1 and 356.5 for the 500, and 1500 ppm treatment groups, respectively. (From p. 37 of the Study Report).

Table 8. Selected food consumption values of mice treated with HWG 1608. Data abstracted from pp. 98-103 and 162-170[a] of the Study Report.

Week	Mean food consumption values (g/kg body weight/day)					
	Males			Females		
	0	500 ppm	1500 ppm	0 ppm	500 ppm	1500 ppm
1	169	172	156	221	241	215
4	149	183*	214*	218	200	246
8	150	206**	242**	201	236	269**
17	152	197*	229**	229	286	360**
29	154	166	176*	179	200	226**
41	134	156*	164**	169	183	209*
53	116	143**	140**	146	172	163
65	140	157*	162*	161	177	218**
77	130	137	164**	165	170	201**
89	132	142	158**	156	187	195**

[a] It is noted that p. 163 (individual body weights weeks 18-76, males) was missing in the Study Report.
* $p \leq 0.05$ vs controls; ** $p \leq 0.01$ vs controls.

4. Opthalmological examinations:

No eye examinations were reported. None are required.

5. a. Hematology:

Hematology parameters were determined on 10/mice/sex/dose group during weeks 51/52 and 91/92 of the study. Blood samples were obtained from the retroorbital sinus plexus. The following parameters were determined: Differential blood count, erythrocyte morphology, erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular cell volume (MCV), thrombocyte count, and thromboplastin time. Group mean values are summarized in Tables 11 and 12.

Significant changes in blood parameters (Table 11) were observed at the high dose in both sexes, mainly in males. At sacrifice, in high-dose males, these changes included leucocytosis, and changes consistent with anemia (decreased erythrocytes, hemoglobin, MCHC, and hematocrit values). In addition, high-dose males had increased platelet counts and decreased thromboplastin times. In the case of high-dose females, a reduced hematocrit and leucocytosis were observed at 51 weeks, and only an increased platelet count at terminal sacrifice.

No consistent effects on the differential blood count (Table 12) were observed.

5b. Clinical Chemistry.

Clinical chemistry determinations were made in 10 animals/group at 91 weeks and in 8-10 animals/group at 52 weeks. The following parameters (in plasma, deproteinized blood, or serum) were determined: glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), alkaline phosphatase (AP), glucose, cholesterol, bilirubin total, creatinine, total protein, albumin, urea, inorganic phosphate, calcium, potassium, sodium and chloride. Group mean values are summarized in Table 13.

As shown in Table 13, there was a dose-dependent increase in the plasma activity of liver enzymes (GPT, GOT, AP) at the interim and final sacrifices. Except for the interim sacrifice in high-dose females, the increase in plasma activity of liver enzymes reached statistical significance vs controls for both interim and final sacrifice in high-dose animals of both sexes. In addition, statistical significance vs controls was observed at 500 ppm at both sacrifices for GPT in males and for GPT and GOT in females.

Cholesterol was statistically significantly decreased vs controls in both sexes at 500 and 1500 ppm for the interim sacrifice, and at 500 ppm for the final sacrifice. Albumin levels were transiently decreased at interim sacrifice at 500 ppm and 1500 ppm.

6. Urinalysis.

No urinalysis data were reported (none are required by EPA Guideline 83-2).

Table 11. Summary of hematological values in mice treated with HWG 1608. Data abstracted from pp. 41 and 213-218 of the Study Report.

Time	Hematological values[a]					
	Males			Females		
	0 ppm	500 ppm	1500 ppm	0 ppm	500 ppm	2000 ppm
Leucocytes ($x10^9/l$)						
51-Weeks	5.2	5.6	10.2**	3.9	3.7	10.7**
90/91-Weeks	6.6	5.0*	9.8*	7.6	4.3	9.5
Erythrocytes ($x10^{12}/l$)						
51-Weeks	8.90	9.11	8.51	8.40	8.99	7.49
90/91-Weeks	9.13	8.25	7.95*	8.36	8.63	7.51
Hemoglobin (g/l)						
51-Weeks	142	144	129**	139	148*	132
90/91-Weeks	143	150	117**	132	131	125
Hematocrit (l/l)						
51-Weeks	0.435	0.414	0.373**	0.422	0.432	0.381*
90/91-Weeks	0.427	0.375	0.373**	0.407	0.401	0.380
MCV (fl)						
51-Weeks	49	45*	44**	50	48	51
90/91-Weeks	47	46*	47	49	47	51
MCH (pg)						
51-Weeks	16.0	15.8	15.3	16.6	16.6	17.7
90/91-Weeks	15.7	18.3**	14.6*	15.8	15.3	16.7
MCHC (g/l)						
51-Weeks	327	348**	347**	331	344	347*
90/91-Weeks	334	402**	313**	324	328	328
Platelets ($10^9/l$)						
51-Weeks	1311	1323	1650**	1024	1274*	1284
90/91-Weeks	1678	1668	2223*	904	1374*	1771*
Thromboplastin (sec.)						
51-weeks	21.1	20.4	20.1*	20.7	19.7	19.2
90/91 weeks	19.0	18.9	16.8**	19.3	18.8	16.3

[a] The units used to report the data were left as reported by the authors, to avoid conversion errors.

* $p \leq 0.05$ vs controls; ** $p \leq 0.01$

Table 12. Differential count in mice treated with HWG 1608. Data extracted from pp. 41 and 219-226 of the Study Report.

Time	Hematological values					
	Males			Females		
	0 ppm	500 ppm	1500 ppm	0 ppm	500 ppm	2000 ppm
Lymphocytes (%)						
51-Weeks	78.9	77.7	79.2	83.1	73.7*	85.3
90-Weeks	71.3	61.5	73.8	69.9	68.6	69.1
Segmented forms (%)						
51-Weeks	19.4	20.4	19.5	13.8	22.3*	12.0
90-weeks	24.0	29.8	20.3	24.5	22.9	26.0
Eosinophiles (%)						
51-weeks	0.3	0.2	0.0	0.1	0.7	0.0
90-weeks	0.3	1.9*	0.0	0.1	0.4	0.1
Monocytes (%)						
51-Weeks	1.4	1.6	1.4	3.0	3.3	2.7
90-Weeks	4.5	6.8	5.9	5.3	8.2	4.7
Banded forms (%)						
51-Weeks	0.0	0.1	0.0	0.0	0.1	0.0
90-Weeks	0.0	0.2	0.0	0.1	0.0	0.1

* $p \leq 0.05$

Table 13. Summary of clinical chemistry values in mice treated with HWG 1608. Data abstracted from pp. 43-44, 126-131 and 227-238 of the Study Report.

Time	Clinical chemistry values[a]					
	Males			Females		
	0 ppm	500 ppm	1500 ppm	0 ppm	500 ppm	2000 ppm
GOT (U/l)						
51/52-Weeks	31.9	37.5	121.3**	38.3	47.2*	144.0**
90/91-Weeks	46.1	60.7	251.8**	36.9	59.0**	303.8**
GPT (U/l)						
51/52-Weeks	38.0	53.2*	236.3**	31.7	51.7*	272.5**
90/91-Weeks	74.9	123.1**	480.8**	39.2	64.9*	419.4**
Alkaline Phosphatase (U/l)						
51/52-Weeks	74	117.0*	181.0*	174.0	212.0	292.0[b]
90/91-Weeks	126.0	156.0	531.0**	182	328	517.0*
Glucose (mmole/l)						
51/52-Weeks	7.08	7.25	7.56	6.73	7.12	7.34*
90/91-Weeks	6.65	6.36	6.22	5.54	6.09	5.98
Cholesterol(mmole/l)						
51/52-Week	3.71	1.99**	1.66**	2.84	1.48**	1.92*
90/91-Weeks	3.88	1.57**	4.55	3.76	2.25**	3.59
Creatinine(µmole/l)						
51/52-Weeks	27.0	28.0	30.0	26.0	29.0	31.0*
90/91-Weeks	28.0	28.0	30.0	26.0	28.0	26.0
Bilirubin total (mcmole/l)						
51/52-Weeks	1.8	1.3**	1.3**	2.2	2.1	1.9
90/91-Weeks	2.1	1.6**	5.0	2.7	2.1*	4.9
Protein (g/l)						
51/52-Weeks	57.5	53.9*	59.6	58.5	56.8	59.3
90/91-Weeks	63.6	57.5**	63.2	60.9	60.0	65.3
Albumin(g/l)						
51/52-weeks	25.8	22.5**	23.4*	28.2	25.7*	24.6**
90/91 weeks	26.7	22.3**	25.5	30.2	25.8	27.9

[a] Data for urea were never significantly different from controls, thus they are not included in this table. The units used to report the data were left as reported by the authors, to avoid conversion errors.
[b] AP data for females were scattered (e.g. at 1500 ppm s.d. was 132).
* $p \leq 0.05$ vs controls; ** $p \leq 0.01$
(Continued)

Table 13. Summary of clinical chemistry values in mice treated with HWG 1608. Data abstracted from pp. 43-44, 126-131 and 227-238 of the Study Report (Continued).

Time	Clinical chemistry values[a]					
	Males			Females		
	0 ppm	500 ppm	1500 ppm	0 ppm	500 ppm	2000 ppm
Na^+ (mmole/l)						
52-Weeks	154	153	154	152	151	152
91-Weeks	153	154	156**	153	154	153
Ca^+ (mmole/l)						
52-Weeks	2.37	2.29	2.40	2.38	2.27*	2.41
91-Weeks	2.45	2.39	2.61**	2.49	2.40	2.64
Cl^- (mmole/l)						
52-Weeks	106	109	109	111	110	109
91-Weeks	109	109	108	109	112**	107
P(mmole/l)						
52-weeks	1.90	1.73*	2.14*	1.63	1.69	1.94**
90/91 weeks	1.60	1.75**	2.06**	1.62	1.64	1.93**

[a] Data for K^+ were never significantly different from controls, thus they are not included in this table.

7. Sacrifice and Pathology:

All animals that died or that were sacrificed moribund and those that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues listed below were collected for histological examination. The DOUBLE-CHECKED (XX) organs, in addition, were weighed in the case of animals sacrificed at the scheduled necropsies at 52 and 91 weeks.

Digestive System	Cardiovasc./Hemato.	Neurologic
X Tongue	X Aorta	XX Brain*+
X Salivary glands*	XX Heart*+	X Periph. nerve (sciatic)*
X Esophagus*	X Bone marrow (sternum)	X Spinal cord (3 levels)*
X Stomach*	X Lymph nodes*	X Eyes
X Duodenum*	X Spleen*+	X Optic nerve
X Jejunum*	X Thymus*	Glandular
X Ileum*	Urogenital	XX Adrenal gland*+
X Cecum*	XX Kidneys*+	- Lacrimal gland
X Colon*	X Urinary bladder*	X Mammary gland*
X Rectum*	XX Testes*+	X Parathyroid*
	X Seminal vesicles	X Pituitary*
XX Liver*+	X Epididymides	X Thyroid*
X Gall bladder*	X Prostate	Other
X Pancreas*	X Uterus*	X Skeletal muscle (thigh)*
Respiratory	X Ovaries*	X Skin*
X Trachea*	X Oviduct	X All gross lesions and masses*
X Lungs*+	X Vagina*	X Harderian gland
- Nose		X Head with nasal cavities.
- Pharynx	X Ureter	X Bone(femur)*
X Larynx	X Urethra	X Eyelids
- Nasal turbinates		X Extraorbital glands
		X Perianal glands
		X Tooth
		X Zymbal glands

* Required for oncogenicity studies (EPA Guideline 83-2)
+ Organ weights required in oncogenicity studies (EPA Guideline 83-2).

a. Organ weights

A summary of mean absolute and relative organ weights at interim and final sacrifice for males and females is presented below in Tables 14 and 15, respectively.

A dose-dependent increase in absolute and relative liver weights was observed in both sexes at both the interim and final sacrifice. In males (Table 14), the dose-related increases in absolute and relative liver weights reached statistical

significance at 500 and 1500 ppm. In females (Table 15), the dose-related increases in absolute and relative liver weights reached statistical significance only at 1500 ppm for both sacrifice periods. The increase in relative liver weights in females at 500 ppm was statistically significant only at interim sacrifice.

A dose-dependent decrease in absolute and relative kidney weights was observed in males at final sacrifice (Table 14); the decrease in absolute weights was statistically significant at 500 and 1500 ppm, whereas the decrease in relative weights reached statistical significance only at 1500 ppm. In females (Table 15), the decrease in absolute and relative kidney weights was statistically significant only for the interim sacrifice at 500 and 1500 ppm, but was not dose dependent.

There was statistically significant decrease in absolute and relative heart weights for females (Table 15) at interim sacrifice but the effect was not dose-dependent and was not observed in males.

Table 14. Mean absolute and relative organ weights in males at interim and final sacrifice. Data from pp. 47-48 and 239-248 of the Study Report.

Organ	Mean Absolute (mg)/Relative organ weight (mg/100 g b.w.)		
	0 ppm	500 ppm	1500 ppm
Body weight (g)			
52-Weeks[a]	43	48	43
91-Weeks[b]	46	44	47
Brain (mg)			
52-Weeks	533/1233	514/1074	508/1181
91-Weeks	512/1113	500/1133	487**/1055
Heart (mg)			
52-Weeks	280/653	267/556	252/583
91-Weeks	285/617	281/635	307/665
Testes[c] (mg)			
52-Weeks	271/618	287/597	262/611
91-Weeks	247/534	227/514	228/492
Liver (mg)			
52-Weeks	1963/4529	2737**/5666**	4159**/9606**
91-Weeks	2409/5214	2822**/6345**	8522**/18313**
Kidneys[c] (mg)			
52-Weeks	810/1880	763/1592	763/1770
91-Weeks	906/1947	797*/1800	771**/1668**
Adrenals[c] (mg)			
52-Weeks	9/21	12/26	10/24
91-Weeks	11/24	10/21	11/23

[a] Values for interim sacrifice used 5-6 animals.
[b] Values for final sacrifice used 27-31 animals.
[c] Organs occurring in pairs were weighed together.

Table 15. Mean absolute and relative organ weights in females at interim and final sacrifice. Data from pp. 47-48 and 239-248 of the Study Report.

Organ	Mean Absolute (mg)/Relative organ weight (mg/100 g b.w.)		
	0 ppm	500 ppm	1500 ppm
Body weight (g)			
52-Weeks[a]	38	38	38
91-Weeks[b]	41	39	44
Brain (mg)			
52-Weeks	534/401	541/452	532/416
91-Weeks	526/1282	519/1327	506/1155**
Heart (mg)			
52-Weeks	258/670	209**/558**	213**/568**
91-Weeks	249/609	247/634	261/592
Liver (mg)			
52-Weeks	2131/5529	2426/6463*	4328**/11392**
91-Weeks	2524/6060	2623/6642	9405**/21141**
Kidneys[c] (mg)			
52-Weeks	629/1643	499**/1340**	512**/1368*
91-Weeks	541/1317	524/1342	594/1348
Adrenals[c] (mg)			
52-Weeks	16/42	15/39	20/53
91-Weeks	12/28	13/33	15**/34*

[a] Values for interim sacrifice used 8-9 animals.
[b] Values for final sacrifice used 17-20 animals.
[c] Organs occurring in pairs were weighed together.

b. Gross pathology

Gross pathology examination of satellite animals that were interim sacrificed (week 51) or which died or were sacrificed moribund during the dosing phase revealed in both sexes an increased incidence of apparently enlarged livers at 1500 ppm and a dose-dependent increase of pale livers (Table 16).

As summarized in Table 16, the incidence of apparently enlarged livers was statistically significantly increased in high-dose mice, amounting to 10/10 in 1500 ppm males vs. 0/10 in controls, and 5/10 in 1500 ppm females vs 0/10 in controls. The dose-dependent increase in pale livers was statistically significant in males at 500 ppm (5/10) and at 1500 ppm (9/10) vs controls (0/10) and in females at both 500 ppm (6/10) and 1500 ppm (8/10) vs controls (0/10). In addition, females at 1500 ppm showed an statistically significant increased incidence of areas of change in the liver amounting to 6/10 vs 0/10 in controls.

Gross pathology examination of main-group animals dead or sacrificed moribund

during the dosing phase and terminal sacrifice (after 91 weeks of dosing) revealed an statistically significant increase ($p \leq 0.001$) in the incidence of apparently enlarged livers and livers with irregular surfaces in both sexes. In addition, high-dose females had a significantly increased number of liver masses ($p \leq 0.05$) vs controls (Table 17).

As summarized in Table 17, the incidence of apparently enlarged livers appeared to be dose-dependant and reached statistical significance in high-dose mice, amounting to 35/50 in 1500 ppm males vs. 1/50 in controls, and 32/50 in 1500 ppm females vs 0/50 in controls. The incidence of irregular sufaces in livers did not appear to be dose-dependant but was statistically significant for high-dose mice amounting to 30/50 in males vs 1/50 in controls, and 28/50 in females vs 3/50 in controls. The increase in liver masses was significant only for females, amounting to 9/50 vs 1/50 for controls. Although the incidence of masses in males that died or were sacrificed moribund was significantly higher at 1500 ppm than in controls (Table 17), the overall incidence (D+T) was not significantly higher than in controls.

Table 16. Selected macroscopic observations in mice dosed with HWG 1608 (up to interim sacrifice). [Data from pp 270-276 (Interim sacrifice) and 278-283 (Died or terminated moribund)].

Finding	Incidence of macroscopic observations					
	0 ppm		500 ppm		1500 ppm	
	D[a]	I	D	I	D	I
Males						
No. examined	4	6	5	5	4	6
Kidneys						
Pale	0	0	0	0	0	1
Liver						
Accentuated lobular pattern	0	0	2	0	0	1
Appears large	0	0	0	0	4*	6**
Areas of change	0	0	1	0	0	3
Swollen	-[b]	0	-	0	-	0
Pale	0	0	2	3[c]	4*	5*
Perianal glands						
Cystic	0	0	0	0	0	2
Females						
No. examined	2	8	1	9	1	9
Kidney						
Pale	1	0	1	0	1	1
Areas of change	0	-	0	-	1	-
Liver						
Accentuated lobular pattern	0	0	0	3	1	3
Appears large	0	0	0	0	0	5*
Areas of change	0	0	0	0	1	5*
Irregular surface	0	-	0	-	1	0
Swollen	-	0	-	0	-	2
Pale	0	0	0	6**	0	8***

[a] D = Dead or sacrificed moribund. I = Sacrificed at interim sacrifice (51 weeks).
[b] No data, presumably the effect in question was not observed.
[c] When D + I (i.e. 2 + 3) are taken together (i.e. 5) $p \leq 0.05$.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table 17. Selected macroscopic observations in mice dosed with HWG 1608 (up to terminal sacrifice). [Data from pp 293-300 (Terminal sacrifice) and 302-313 (Died or terminated moribund)].

Finding	Incidence of macroscopic observations					
	0 ppm		500 ppm		1500 ppm	
	D[a]	T	D	T	D	T
Males						
No. examined	20	30	19	31	23	27
Heart (Auricle)						
Abnormal coloration	0	0	0	0	1	2
Liver						
Appears large	1	0	2	0	11**	24***
Areas of change	0	0	0	1	1	1
Irregular surface	1	0	0	0	5	25***
Masses	1	5	1	2	10**	2
Spleen						
Pale	0	0	0	0	3	2
Appears Small	0	0	0	0	2	2
Preputial gland						
Appears large	0	2	0	3	1	5
Females						
No. examined	30	20	33	17	32	18
Liver						
Appears large	0	0	5	0	15**	17***
Areas of change	0	0	0	0	3	0
Irregular surface	3	0	3	0	10	18***
Masses	1	0	0	1	9*	0
Ovary						
Masses	0	0	2	2	1	1

[a] D = Dead or sacrificed moribund. T = Sacrificed at terminal sacrifice.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

c. Microscopic pathology

Histopathology was done on all animals dying during treatment or sacrificed moribund and on animals sacrificed at interim (51 weeks) and at terminal (91 weeks) sacrifice.

Microscopic pathology examination of non-neoplastic/neoplastic lesions indicated the liver as the target site for test article-related toxicity in both sexes. Additionally, a dose-dependent increase in the incidence of ovarian atrophy was observed in treated females.

1. Non neoplastic Lesions

i. Interim Sacrifice

Microscopic examination of non-neoplastic lesions in interim sacrificed mice (and in those satellite mice that died or were sacrificed moribund) revealed dose-dependent increases of liver lesions in both males and females.

In males, as shown in Tables 18 and 20, there were dose-dependent increases in hepatocyte necrosis, panacinar fatty vacuolation, pigment laden Kupfer cells and ORO stained panacinar fat which were statistically significant ($p \leq 0.05$-0.001) at 500 and 1500 ppm, in addition to increases in bile duct hyperplasia and periportal fibrosis which were statistically significant at 1500 ppm. Incidences of these effects at 1500 ppm ranged from 5/10 (periportal fibrosis) to 10/10 (panacinar fine fatty vacuolation) vs 0/10 in controls. Additional dose-dependent effects (Table 18) included hyperkeratosis and acanthosis of the forestomach mucosa; this effect, however, did not reach statistical significance.

In females, as shown in Tables 19 and 20, panacinar fine fatty vacuolation had an incidence of 100% at both 500 and 1500 ppm and was statistically significantly elevated ($p \leq 0.001$) vs. controls which had no incidence of this effect. There were also dose-dependant increases in hepatocyte necrosis, bile duct hyperplasia, chronic inflammatory cells in the portal area, extramedullary hemopoiesis, all of which reached statistical significance at the high-dose ($p \leq 0.05$-0.001). Incidences of these effects at 1500 ppm ranged from 5/10 (extramedulary hemopoiesis) to 9/10 (hepatocyte necrosis) vs 0/10 in controls. In addition, there were increases in pigment-laden Kupfer cells and ORO-stained periacinar fat which were statistically significant ($p \leq 0.05$-0.001) and limited to the high-dose. In addition to effects on liver, there was a dose-dependent increase in hyperkeratosis and acanthosis of the forestomach mucosa; with an incidence of 8/10 at the high dose ($p \leq 0.01$) vs. 2/10 in controls.

Table 18. Selected microscopic non-neoplastic findings in male mice dosed with HWG 1608 (up to interim sacrifice). [Data from pp. 330-334 (Interim sacrifice) and 337-340 (Died or terminated moribund)].

Finding	Incidence of non-neoplastic findings					
	0 ppm		500 ppm		1500 ppm	
	D[a]	I	D	I	D	I
No. animals	4	6	5	5	4	6
Epididymides (Examined)	4	6	4	5	4	6
Reduced sperm content	0	0	0	0	0	2
Kidneys (Examined)	4	6	4	5	4	6
Dystrophic mineralization	0	0	0	0	0	1
Nephropathy	0	0	0	0	1	1
Liver (Examined)	4	6	4	5	4	6
Necrosis of individual hepatocytes	0	0	2	3[c]	4*	4[d]
Focal hyperplasia of hepatocytes	0	0	0	0	0	2
Panacinar fine fatty vacuolat. (Slight-marked)	0	0	4*	4*	4*	6**
Bile duct hyperplasia	0	0	0	1	4*	4
Periportal fibrosis	0	0	0	1	2	3
Extramedullary hemopoiesis	0	0	1	0	0	1
Pigment laden Kupfer cells	0	0	3	1	4*	4
ORO stain panacinar fat	0	1	2	4	4*	4
Stomach (Examined)	4	6	4	5	4	6
Keratinized region: hyperkeratosis and acanthosis	0	1	0	2	1	5
Spleen (Examined)	4	6	4	5	4	6
Hypocellularity of red pulp	0	0	0	0	3	1
Testes (Examined)	4	6	4	5	4	6
Mineralization in tubules	0	0	0	1	0	1
Degeneration of germinal epithelium	0	0	0	1	0	2
Perianal glands (Examined)	-[b]	0	-	0	-	2
Cystic distension	-	0	-	0	-	2

[a] D = Dead or sacrificed moribund. I = Sacrificed at interim sacr. (51 weeks)
[b] No data, presumably the effect in question was not observed.
[c] Statistically significant ($p \leq 0.05$) for D + T vs controls.
* $p \leq 0.05$, ** $p \leq 0.01$.

Table 19. Selected microscopic non-neoplastic findings in female mice dosed with HWG 1608 (up to interim sacrifice). [Data from pp. 330-334 and 386-404 (Interim sacrifice) and 338-340 and 405-420 (Died or terminated moribund)].

Finding	Incidence of non-neoplastic findings					
	0 ppm		500 ppm		1500 ppm	
	D[a]	I	D	I	D	I
No. animals	2	8	1	9	1	9
Heart (Examined)	2	8	1	9	1	9
Myocardial mineralization	0	0	0	0	1	0
Kidneys (Examined)	2	8	1	9	1	9
Nephropathy	1	0	1	0	1	1
Liver (Examined)	2	8	1	9	1	9
Necrosis of individual hepatocytes (Minim.-Moderate)	-[b]	0	-	1	-	9***
Focal hyperplasia of hepatocytes	0	0	0	0	1	2
Panacinar fine fatty vacuolat. Minimal-Moderate	0	0	1	9***	1	9***
Centriacinar fatty vacuolation	0	0	0	9***	0	6***
Periacinar hepatocytic hypertrophy	0	-	0	-	1	-
Bile duct hyperplasia	0	0	0	2	1	5*
Chronic inflammatory cells in the portal area	0	1	1	3	1	7*
Periportal fibrosis	0	0	0	0	0	2
Extramedullary hemopoiesis	0	0	0	3	0	5*
Pigment laden Kupfer cells	0	0	0	0	0	8**
Eosinophilic focus/foci of hepatocyte alteration	-	0	-	0	-	3
ORO stain pericinar fat	0	-	0	-	1	-
Stomach (Examined)	2	8	1	9	1	9
Keratinized region: hyperkeratosis and acanthosis	1	1	0	6*	0	8*
Spleen						
Hypocellularity of red pulp	0	0	0	0	0	4

[a] D = Dead or sacrificed moribund. I = Sacrificed at interim sacrifice (51 weeks).
[b] No data, presumably the effect in question was not observed.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table 20. **Summary of statistically significant microscopic non-neoplastic findings for all satellite mice dosed with HWG 1608 (up to interim sacrifice). [Data from pp. 344-348 of the Study Report].**

Finding	Incidence of non-neoplastic findings		
	0 ppm	500 ppm	1500 ppm
Males			
No. animals	10	10	10
Liver (Examined)	10	9	10
Necrosis of individual hepatocytes (Minim.-Moderate)	0	5*	8***
Panacinar fine fatty vacuolat. Minimal-Moderate	0	8***	10***
Bile duct hyperplasia	0	1	8***
Periportal fibrosis	0	1	5*
Pigment laden Kupfer cells	0	4*	8***
ORO stain panacinar fat	0	6*	8**
Females			
Liver (Examined)	10	10	10
Focal inflammation with hepatocytic degeneration	0	5*	2
Necrosis of individual hepatocytes (Minim.-Moderate)	0	2	9***
Panacinar fine fatty vacuolat. Minimal-Moderate	0	10***	10***
Centriacinar fatty vacuolation	0	9***	6*
Bile duct hyperplasia	0	2	6*
Chronic inflammatory cells in the portal area	0	4	8**
Extramedullary hemopoiesis	0	3	5*
Pigment laden Kupfer cells	0	0	8***
ORO stain periacinar fat	0	0	5*
Stomach (Examined)	10	10	10
Keratinized region: hyperkeratosis and acanthosis	2	6	8*

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

ii. Terminal Sacrifice

Microscopic examination of non-neoplastic lesions in terminally sacrificed mice (and in those that died or were sacrificed moribund) revealed dose-dependent increases of liver lesions in both males and females and dose-dependent increases in ovarian atrophy in females.

In males, as shown in Tables 21 and 23, there was a dose-dependent increase in panacinar fine fatty vacuolation amounting to 14/48 at 500 ppm and to 25/48 at 1500 with $p \leq 0.001$ vs controls (0/47). In addition, there were increases in focal hyperplasia of hepatocytes (23/48) and oval cell proliferation (23/48) which were statistically significant ($p \leq 0.001$) and were limited to the high-dose. Extramedullary hemopoiesis increased in dose-dependent fashion and reached statistical significance ($p \leq 0.05$) at 1500 ppm. There was a dose-dependent decrease in dysplasia of the glandular region of the stomach with an incidence of 2/48 ($p \leq 0.001$) at 1500 ppm vs. 16/47 in controls.

In females, as shown in Tables 22 and 23, there was a dose-dependent increase in panacinar fine fatty vacuolation amounting to 4/45 at 500 ppm and to 19/46 ($p \leq 0.001$) at 1500 vs controls (1/47). In addition, there were increases in periacinar hepatocyte hypertrophy (13/46, $p \leq 0.001$), oval cell proliferation (17/46, $p \leq 0.001$), and eosinophilic foci of hepatocyte alteration (7/46, $p \leq 0.01$) which were statistically significant vs. controls (0/47) and were limited to the high-dose. Pigment-laden Kupfer cells cells increased in dose-dependent fashion and reached statistical significance ($p \leq 0.05$) at 1500 ppm (7/45). There was a dose-dependent increase in ovarian atrophy with an incidence of 17/44 ($p \leq 0.05$) at 500 ppm and 28/45 ($p \leq 0.001$) at 1500 vs. 7/47 in controls. Bilateral luteal cell hyperplasia was significantly elevated to 5/45 ($p \leq 0.05$) at 1500 ppm vs. controls (0/47). Although a dose-dependent increase in hyperkerathosis and acanthosis of the forestomach mucosa was observed in the interim sacrifice, this effect was not noted at terminal sacrifice.

Table 21. Selected microscopic non-neoplastic findings in male mice dosed with HWG 1608 (up to terminal sacrifice). [Data from pp. 353-359 and 442-464 (Final sacrifice) and 364-371 and 466-488 (Died or terminated moribund)].

Finding	Incidence of non-neoplastic findings					
	0 ppm		500 ppm		1500 ppm	
	D[a]	T	D	T	D	T
Total animals	20	30	19	31	23	27
-Adrenals (Examined)	17	30	17	31	21	27
Spindle cell hyperplasia	3	8	3	2*	1	2
-Lymph n. Mandibular (Examined)	17	29	15	29	20	27
Parafollicular hyperplasia	0	2	2	1	1	7
-Lymph n. Mesenteric (Examined)	17	30	17	31	21	27
Parafollicular hyperplasia	0	4	3	2	2	7
-Liver (Examined)	17	30	17	31	21	27
Necrosis of indiv. hepatocytes	1	2	0	11*	1	1
Focal hyperplasia of hepatocytes						
Slight	0	2	0	2	3	6
Moderate	0	2	0	0	5	4
Marked	0	1	0	0	1	3
Severe	-[b]	1	-	0	-	0
Total	0	6	0	2	9*	14**
Panacinar fine fatty vacuolat.						
Minimal	0	0	0	0	0	1
Slight	0	0	1	9	7	5
Moderate	0	0	0	4	2	6
Marked	-	0	-	0	-	4
Total	0	0	1	13***	9*	16***
Bile duct hyperplasia						
Slight	0	0	0	2	3	1
Moderate	0	0	1	0	0	1
Total	0	0	1	2	3	2
Oval cell proliferation						
Slight	0	0	0	0	6	14
Moderate	0	0	0	0	1	2
Total	0	0	0	0	7*	16***
Extramedullary hemopoiesis	0	0	0	2	4	3
Pigment laden Kupfer cells	0	1	0	0	4	2
-Lungs (Examined)	17	30	17	31	21	27
Interstitial pneumonitis	2	0	3	2	8	0
-Spleen (Examined)	17	30	17	31	21	27
Hypocellularity of red pulp	0	1	2	1	4	1
-Stomach (Examined)	17	30	17	31	21	27
Keratinized region: acanthosis and hyperkeratosis	1	5	2	6	5	3
-Urinary bladder (examined)	17	30	17	31	21	27
Transitional cell hyperplasia	1	1	1	3	2	0

[a] D = Dead or sacrificed moribund. T = Terminally sacrifice (91 weeks).
[b] No data, presumably the effect in question was not observed.
* $p \leq 0.05$, ** $p \leq 0.01$.

Table 22. Selected microscopic non-neoplastic findings in female mice dosed with HWG 1608 (up to terminal sacrifice). [Data from pp. 353-359 and 442-464 (Final sacrifice) and 364-371 and 466-488 (Died or terminated moribund)].

Finding	Incidence of non-neoplastic findings					
	0 ppm		500 ppm		1500 ppm	
	D[a]	T	D	T	D	T
No. examined	30	20	33	17	32	18
-Liver (Examined)	27	20	28	17	28	18
Focal hyperplasia of hepatocytes						
Minimal	-[b]	1	-	0	-	0
Slight	0	0	0	0	2	0
Moderate	0	0	0	0	3	5
Marked	0	0	0	0	2	0
Total	0	1	0	0	7*	5
Panacinar fine fatty vacuolat.						
Minimal	1	0	1	0	0	0
Slight	0	0	1	2	7	4
Moderate	0	0	0	0	3	4
Marked	-	0	-	0	-	1
Total	1	0	2	2	10*	9***
Centriacinar fatty vacuolation	2	1	6	7*	2	2
Periacinar hepatoc. hypertrophy	0	0	0	0	6*	7**
Bile duct hyperplasia	0	0	0	0	0	1
Oval cell proliferation						
Total (Slight only)	0	0	0	0	8*	9*
Extramedullary hemopoiesis						
Minimal	2	1	0	0	0	0
Slight	0	1	0	0	6	6
Moderate	1	-	1	-	0	-
Total	3	2	1	0	6	6
Clear cell focus/foci	0	0	0	0	0	4
Pigment laden Kupfer cells	1	0	1	2	5	2
Eosinophilic focus/foci	0	0	0	0	2	5*
-Lungs (Examined)	27	20	28	17	28	19
Interstitial pneumonitis	5	1	3	0	5	1
-Ovaries (Examined)	27	20	27	17	28	17
Atrophy	3	4	11*	6	17***	11**
Bilateral: luteal cell hyperpla.	0	0	1	3	2	3
-Spleen (Examined)	26	20	27	17	28	18
Hypocellularity of red pulp	0	1	1	0	4	0
Extramedullary hemopoiesis	1	5	4	8	4	7
-Stomach (Examined)	26	20	28	17	28	18
Glandular region: dysplasia	5	9	2	8	5	5
Keratinized region: acanthosis and hyperkeratosis	3	9	8	8	8	5

[a] D = Dead or sacrificed moribund. T = Sacrificed at terminal sac. (91 weeks).
[b] No data, presumably the effect in question was not observed.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 23. Summary of microscopic statistically significant non-neoplastic findings in male and female mice dosed with HWG 1608 (up to terminal sacrifice). [Data from pp. 376-384].

Finding	Incidence of non-neoplastic findings 0 ppm	500 ppm	1500 ppm
Males			
No. animals	50	50	50
Adrenals (Examined)	47	48	48
Spindle cell hyperplasia	11	5	3*
Liver (Examined)	47	48	48
Necrosis of individual hepatocytes	3	11*	2
Focal hyperplasia of hepatocytes	6	2	23***
Panacinar fine fatty vacuolat.	0	14***	25***
Oval cell proliferation	0	0	23***
Extramedullary hemopoiesis	0	2	7*
Stomach (Examined)	47	48	48
Glandular region: dysplasia	16	7*	2***
Females			
No. animals	50	50	50
Liver (Examined)	47	45	46
Focal hyperplasia of hepatocytes	1	0	12*
Panacinar fine fatty vacuolat.	1	4	19***
Centriacinar fatty vacuolation	3	13**	4
Periacinar hepatocyte hypertrophy	0	0	13***
Oval cell proliferation	0	0	17***
Eosinophilic focus/foci	0	0	7**
Pigment laden Kupfer cells	1	3	7*
Ovaries (Examined)	47	44	45
Bilateral: luteal cell hyperplasia	0	4	5*
Atrophy	7	17*	28***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

2. Neoplastic Lesions

i. Interim sacrifice

Selected findings for interim microscopic pathology examination of neoplastic lesions are presented in Table 24. Historical control data were not submitted. No significant neoplastic lesions were observed at interim sacrifice.

Table 24. Selected microscopic neoplastic findings in mice of both sexes dosed with HWG 1608 (up to interim sacrifice). [Data from pp. 329 (Interim sacrifice) and 336 (Died or terminated moribund)].

	Incidence of neoplastic findings					
	0 ppm		500 ppm		1500 ppm	
Finding	D[a]	I	D	I	D	I
Males						
No. animals	4	6	5	5	4	6
Caecum (Examined)	-[b]	4	-	5	-	6
Leiomyoma	-	0	-	1	-	0
Females						
No. animals	2	8	1	9	1	9
Hemopoietic (Examined)	2	9	1	9	1	9
Malignant lymphoma	0	3	0	0	1	0

[a] D = Dead or sacrificed moribund. I = Sacrificed at interim sacrifice (51 weeks).
[b] No data, presumably the effect in question was not observed.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

ii. Terminal sacrifice

Selected findings for terminal microscopic pathology examination of neoplastic lesions are presented in Tables 25 and 27 (males) and 26 and 27 (females). The target organ is liver. Historical control data were not submitted.

The incidence of hepatocellular adenomas in males (Tables 25 and 27) was 2/48 at 500 ppm and 17/48 at 1500, and was statistically significant ($p \leq 0.001$) vs. control incidences (3/47) only at the high dose. The incidence of male hepatocellular carcinomas was 0/48 at 500 ppm and 10/48 at 1500 ppm, and was statistically significant ($p \leq 0.01$) vs. control incidences (0/47) also only at the high dose. Although there was an apparent dose-related increase in histiocytic sarcomas (1/47, 2/48 and 3/48 at 0, 500, and 1500 ppm, respectively), the effect did not reach statistical significance vs. controls at any dose tested.

The incidence of hepatocellular adenomas in females (Tables 26 and 27) was 0/45 at

500 ppm and 2/46 at 1500, and did not reach statistically significance vs. control incidences (0/47). On the other hand, the incidence of female hepatocellular carcinomas was 0/48 at 500 ppm and increased to 12/46 at 1500 ppm, to become statistically significant ($p \leq 0.001$) vs. control incidences (0/47). Although there was an apparent dose-related increase in histiocytic sarcomas (1/47, 3/45 and 5/45 at 0, 500, and 1500 ppm, respectively), the effect did not reach statistical significance vs. controls at any dose tested.

Historical control values for liver tumors for NMRI male mice were reported with the original mouse oncogenicity study (Table 11, p.65 of MRID No. 407009-41, as cited in the corresponding DER dated 12/24/88) for 6 comparable mouse studies covering the period of 1980-1984 as follows:

Study No.:	1	2	3	4	5	6
# Animals:	50	50	50	45	46	48
#(Benign + malignant):	7	3	9	5	1	6
% (Benign + malignant):	14	6	18	11	2	12

As shown in Table 27 the combined percent incidences of benign + malignant tumors in 0 ppm and 500 ppm male mice were 6.4% and 4.2%, these values were well within the above-reported historical range of combined benign/malignant tumors in NMRI male mice (2-18%). On the other hand, the combined percent incidence of benign/malignant tumors in 1500 ppm mice (56.2%, Table 27) clearly exceeds the historical control range.

Table 25. Selected microscopic neoplastic findings in male mice dosed with HWG 1608 (up to final sacrifice). [Data from pp. 350-351 (Final sacrifice) and 361-362 (Died or terminated moribund)].

Finding	Incidence of neoplastic findings					
	0 ppm		500 ppm		1500 ppm	
	D[a]	T	D	T	D	T
No. examined	20	30	19	31	23	27
Adrenals (Examined)	17	30	17	31	21	27
Pheochromocytoma (B)	0	0	0	1	1	1
Liver (Examined)	17	30	17	31	21	27
Hepatocellular adenoma	1	2	1	1	6	11**
Hepatocellular carcinoma	0	0	0	0	6*	4*
Lungs (Examined)	17	30	17	31	21	27
Pulmonary adenoma	2	6	1	5	5	5
Pulmonary carcinoma	1	0	0	1	1	1
Esophagus (Examined)	19	-[b]	18	-	22	-
Squamous cell papilloma	0	-	0	-	1	-
Testes (Examined)	17	-	17	-	21	-
Interstitial cell tumor	0	-	1	-	0	-
Hemopoietic (Examined)	18	30	18	31	21	27
Malignant lymphoma	4	2	6	1	3	4
Histiocytic sarcoma	1	0	0	2	1	2

[a] D = Dead or sacrificed moribund. T = Sacrificed at terminal sacrifice (91 weeks).
[b] No data, presumably the effect in question was not observed.
* $p \leq 0.05$, ** $p \leq 0.01$.

Table 26. Selected microscopic neoplastic findings in female mice dosed with HWG 1608 (up to final sacrifice). [Data from pp. 350-351 (Final sacrifice) and 361-362 (Died or terminated moribund)].

Finding	Incidence of neoplastic findings					
	0 ppm		500 ppm		1500 ppm	
	D[a]	T	D	T	D	T
No. examined	30	20	33	17	32	18
Kidneys (Examined)	27	-[b]	28	-	28	-
Carcinoma	0	-	1	-	0	-
Liver (Examined)	27	20	28	17	28	18
Hepatocellular adenoma	0	0	0	0	1	1
Hepatocellular carcinoma	0	1	0	0	6*	7*
Hemangiosarcoma	-	0	-	1	-	0
Lungs (Examined)	27	20	27	17	28	18
Pulmonary adenoma	3	0	1	3	2	1
Pulmonary carcinoma	0	1	0	0	0	0
Mammary area (Examined)	27	20	28	17	29	18
Adenocarcinoma	0	1	2	0	0	1
Ovaries (Examined)	27	20	27	17	28	17
Granulosa cell tumor	0	1	3	2	0	0
Luteal cell tumor	0	1	1	0	0	1
Pancreas (Examined)	25	-	27	-	28	-
Islet cell adenoma	0	-	1	-	0	-
Hemopoietic (Examined)	27	20	28	17	28	18
Histiocytic sarcoma	1	0	3	0	3	2
Malignant lymphoma	13	8	12	4	10	4

[a] D = Dead or sacrificed moribund. T = Sacrificed at terminal sacrifice (91 weeks).
[b] No data, presumably the effect in question was not observed.
* $p \leq 0.05$, ** $p \leq 0.01$.

Table 27. Summary of microscopic neoplastic findings for all mice dosed with HWG 1608 (up to final sacrifice). [Data from pp. 373-374 of the Study Report and Tables 25 and 26 of this DER].

Finding	Incidence of neoplastic findings		
	0 ppm	500 ppm	1500 ppm
Males			
No. Animals	50	50	50
Liver (Examined)	47	48	48
Hepatocellular adenoma (%)	3 (6.4)	2 (4.2)	17*** (35.4)
Hepatocellular carcinoma (%)	0	0	10** (20.8)
Hemangiosarcoma	0	0	0
Hemopoietic tumor (Examined)	48	49	48
Histiocytic sarcoma (%)	1 (2.1)	2 (4.2)	3 (6.3)
Females			
No. of Animals			
Liver (Examined)	47	45	46
Hepatocellular adenoma (%)	0	0	2 (4.2)
Hepatocellular carcinoma (%)	1 (2.1)	0	12*** (26.1)
Hemangiosarcoma (%)	0	1 (2.2)	0
Hemopoietic tumor (Examined)	47	45	46
Histiocytic sarcoma (%)	1 (2.1)	3 (6.7)	5 (10.9)

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

D. Discussion:

HWG 1608 was administered to Bor:NMRI(SPF-Han) mice of both sexes for a period of up to 91 weeks in the diet at levels of 0, 500, and 1500 ppm resulting in mean respective compound intakes of 0, 84.9 and 279 mg/kg body weight/day (males) and 0, 103.1, and 356.5 mg/kg body weight/day (females).

Statistically significantly decreased body weights were reported in males at 500 ppm during the initial half of the study and at 1500 ppm levels throughout the treatment period. As estimated by the reviewer, body weight ~~weight~~ depressions vs controls of up to 5.0-6.3% were observed at 500 ppm and of up to 9.0-11% at 1500 ppm. In females, there were sporadic statistically significant decreases in body weight during the initial third of the study (at 500 ppm) and in 39 of the first 59 weeks and in 7 of the last 8 weeks (at 1500 ppm). As estimated by the reviewer, body weights depressions of up to 7.6-8.5% vs controls were observed in high-dose females during the first 59 weeks of treatment. Food consumption (in mg/kg/day) was significantly increased with respect to controls at 1500 ppm in both sexes and at 500 ppm in males. The increased food consumption per unit of body weight coupled with the observed decreases in body weights appears to reflect decreased

food efficiency.-

Hematology parameters in high-dose males at terminal sacrifice were consistent with anemia (statistically significant decreased erythrocytes, hemoglobin, MCHC, and hematocrit values); in high-dose females there was only an increase in platelet count (terminal sacrifice) and reduced hematocrit (interim sacrifice). Clinical chemistry values (statistically significant and dose-dependent increases in plasma GOT, GPT and AP) for both sexes were consistent with hepatotoxic effects at both 500 ppm and 1500 ppm.

Gross pathology examination indicated the liver as target organ at both 500 ppm and 1500 ppm. The incidence of apparently enlarged livers appeared to be dose-dependent and reached statistical significance in high-dose mice, amounting to 35/50 in 1500 ppm males vs. 1/50 in controls, and 32/50 in 1500 ppm females vs 0/50 in controls. A dose-dependent increase in absolute and relative liver weights was observed in both sexes at both interim and final sacifice. Relative liver weight increases reached statistical significance at both 500 and 1500 ppm at interim and final sacrifice in males and at 1500 ppm only for interim and final sacrifice in females.

Terminal sacrifice and interim non-neoplastic histopathology indicate the liver as a target organ at 500 and 1500 ppm in both sexes. Additionally, terminal sacrifice non-neoplastic histopathology indicates the ovaries as posible targets in 500 and 1500 ppm females.

In terminal sacrifice males, there was a dose-dependent increase in hepatic panacinar fine fatty vacuolation amounting to 14/48 at 500 ppm and to 25/48 at 1500 with $p \leq 0.001$ vs controls (0/47) for both doses. Extramedullary hemopoiesis, which increased in dose-dependent fashion, reached statistical significance ($p \leq 0.05$) at 1500 ppm only. In addition, there were increases in focal hyperplasia of hepatocytes (23/48) and oval cell proliferation (23/48) which were statistically significant ($p \leq 0.001$) and limited to the high-dose.

In terminal sacrifice females, there was a dose-dependent increase in hepatic panacinar fine fatty vacuolation amounting to 4/45 at 500 ppm and to 19/46 ($p \leq 0.001$) at 1500 ppm vs controls (1/47). Pigment-laden Kupfer cells cells increased in dose-dependant fashion and reached statistical significance ($p \leq 0.05$) at 1500 ppm (7/46). In addition, there were increases in periacinar hepatocyte hypertrophy (13/46, $p \leq 0.001$), oval cell proliferation (17/46, $p \leq 0.001$), and eosinophilic foci of hepatocyte alteration (7/46, $p \leq 0.05$) which were statistically significant vs. controls (0/47) and were limited to the high-dose.

In females there was also a dose-dependent increase in ovarian atrophy with an incidence of 17/44 ($p \leq 0.05$) at 500 ppm and 28/45 ($p \leq 0.001$) at 1500 ppm vs. 7/47 in controls. In the absence of historical data it is not clear if these histological observations of ovarian atrophy, may signal an effect of HWG 1608 on ovaries of treated female mice. Although a dose-dependent increase in hyperkerathosis and acanthosis of the forestomach mucosa was observed in the interim sacrifice, this effect was not observed at terminal sacrifice.

The incidence of hepatocellular adenomas and hepatocellular carcinomas in males and of hepatocellular carcinomas in females (Table 19) was a compound-related effect.

The incidence of hepatocellular adenomas in males was 2/48 (4.2%) at 500 ppm and 17/48 (35.4%) at 1500 ppm, and was statistically significant ($p \leq 0.001$) vs. control incidences (3/47, 6.4%) only at the high dose. The incidence of male hepatocellular carcinomas was 0/48 at 500 ppm and 10/48 (20.8%) at 1500 ppm, and was statistically significant ($p \leq 0.01$) vs. control incidences (0/47) also only at the high dose. Thus, the combined incidence of benign/malignant tumors in high-dose males, which is 56.2%, exceeds the reported 2-18% incidence reported for historical controls over the years 1980-1984 in 6 comparable mouse studies. The excess incidence in males of combined benign/malignant tumors over both historical and concurrent control values indicate that the effect is treatment related.

The incidence of hepatocellular adenomas in females was 0/45 at 500 ppm and 2/46 (4.2%) at 1500, and was not statistically significant vs. control incidences (0/47). On the other hand, the incidence of female hepatocellular carcinomas was 0/48 at 500 ppm and increased to 12/46 (26.1%) at 1500 ppm, to become statistically significant ($p \leq 0.001$) vs. control incidences (0/47). Although no historical control data were available for comparison, the statistical significant incidence of hepatocellular carcinomas in females (26.1%) in this study plus the low incidence at all doses in the initial study (2% or less) indicate that the effect is treatment related.

There was a dose-related increase in histiocytic sarcomas in both sexes. In males the incidences amounted to 1/48 (2.1%), 2/49 (4.2%) and 3/48 (6.3%) at 0, 500 and 1500 ppm, respectively. In females the incidences amounted to 1/47 (2.1%), 3/45 (6.7%), and 5/46 (10.9%) at 0, 500 and 1500 ppm, respectively. Although historical controls were not available it is noted that in the initial mouse study (MRID 407009-41), histiocytic sarcomas did not exceed 4% in males and 2.0% in females at any dose. Although the incidences of histiocytic sarcoma in the present study did not reach statistical significance vs. controls at any dose, it is not possible to fully assess the significance of these dose-related incidences in the absence of historical controls.

It is noted that although a dated Quality Assurance statement was included, the corresponding signatures were not included; only the signature of the translator was included.

Attachments

Attachment 1. Experimental procedure.

Tebuconazole tox review

Page _____ is not included in this copy.

Pages 42 through 59 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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