UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

October 5, 1999

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCE

SUBJECT: Cancer Assessment Review Committee Meeting on Tetraconazole

FROM: Sanjivani Diwan
Executive Secretary for
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Tetraconazole prepared by David Nixon.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday October 20,1999 at 10:00 am in room 813, CM2.

Addressees

K. Baetcke
L. Brennecke
L. Brunsman
W. Burnam
M. Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
M. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor
Y. Woo

(1)

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Tetraconazole: Evaluation of Carcinogenic Potential

TO: Sanjivani Diwan, PhD
Executive Secretary, Cancer Assessment Review Committee
Health Effects Division (7509C)

FROM: David Nixon, DVM
Toxicologist, RRB4
Health Effects Division (7509C)

THROUGH: Alberto Protzel, PhD
Senior Scientist, TOX1
Health Effects Division (7509C)

Attached is the Cancer Assessment Document for tetraconazole.

The issue of concern is the occurrence of benign and malignant liver cell tumors in mice.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF *TETRACONAZOLE*

DRAFT REPORT

October 20, 1999

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

4

DATA PRESENTATION: _____

DOCUMENT PREPARATION: _____

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

List the Committee members _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Consulting Pathologist

Statistician Analysis

OTHER ATTENDEES:

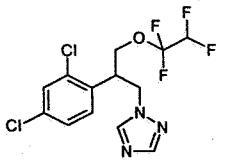
CONTENTS

I. INTRODUCTION

On October 20, 1999, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs is scheduled to meet to evaluate the carcinogenic potential of tetraconazole.

II. BACKGROUND INFORMATION

Tetraconazole is a triazole fungicide. The PC Code is 120603 and the CAS Number is 112281-77-3. It is recommended for agricultural use on sugar beets and turf and has an import tolerance set for bananas. Future uses may be included on grapes, peanuts, and wheat.



III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with tetraconazole in Crl CD (SD) rats

Reference: **Crome, S. J, *et al.* (1992) M 14360 Potential tumorigenic and toxic effects in prolonged dietary administration to rats. Laboratory name: Huntingdon Research Centre Ltd. Laboratory report number: AGR 74/911683. December 10, 1992. MRID 44305304. Unpublished. [Attachment 1]**

A. Experimental Design

In a carcinogenicity toxicity study (MRID 44305304), tetraconazole (94.6% a.i.) was administered to Crl:CD (SD) rats 50/sex/dose in the diet at dose levels of 0 (control) 10, 80 ,640 and 1280 ppm for males and 0 (control), 10, 80 and 640 ppm for females. This corresponds to 0, 0.6, 4.4, and 39.4 mg/kg/day for females and 0.0, 0.4, 3.4, 27.7 and 59 mg/kg/day for males. The study was 2 years in length and the animals were exposed to the test article throughout the duration of the study. An additional 20 rats/sex/dose were sacrificed and examined at 12 months.

B. Discussion of Tumor Data

The dietary administration of tetraconazole up to 1280 ppm in males and 640 ppm in females did not result in an overall treatment-related increased incidence of tumor formation in Crl:CD (SD) rats. Although an increase in the incidence of thyroid adenomas in males at doses ≥80 ppm was noted (4.2%, 9.1%, 13.8% and

11.6% at 0, 10, 80, 640 and 1260 ppm, respectively), these values did not achieve statistical significance and were well within the historical control range (mean of 9.7%; range of 0-19.6%) for male rats of this strain in studies conducted by the examining laboratory.

The statistical evaluation of mortality indicated significant decreasing trends with increasing doses of tetraconazole in male and female rats. [Attachment 2]

The statistical analyses of tumors in male and female rats were based upon Peto's Prevalence Test since there was statistically significant differential mortality with increasing doses of tetraconazole in both sexes. See Table 1 for male rat tumor analysis results. The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Table 1. Male Rats: Thyroid Follicular Tumor Rates[+] and Peto's Prevalence Test Results.

ppm	0	10	80	640	1280
mg/kg/day	0	0.4	3.4	27.7	59
TumorType					
Adenoma	1/24	1/29	3/33	4/29	5/43
%	(4)	(3)	(9)	(14)	(12)
p =	0.103	-	0.238	0.119	0.154
Carcinoma	0/24	1/29	0/33	2/29	1/43
%	(0)	(3)	(0)	(7)	(2)
p =	0.276	0.181	-	0.097	0.228
Combined	1/24	2/29	3/33	4[a]/29	6/43
%	(4)	(7)	(9)	(14)	(14)
p =	0.089	0.336	0.238	0.119	0.106

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
First thyroid follicular cell adenoma and carcinoma observed at Week 105, in final sacrifice animals, concurrently in all dose groups.
[a]Two animals in the 640-ppm dose group had both an adenoma and a carcinoma.
Note: Interim sacrifice animals are not included in this analysis. There were no thyroid follicular cell adenomas or carcinomas in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions

Non-neoplastic lesions are presented in Tables 2 and 3. The lesions observed appear to have no relevance in regards to tumor induction.

Table 2. Nonneoplastic histopathology findings in males at terminal sacrifice or dying an unscheduled death. Ter- terminal; Uns-unscheduled (50 animals examined in the control and high-dose groups)

Histopathology	control	10 ppm	80 ppm	640 ppm	1280 ppm
Liver- Centri. Hep. Enlarg.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=14 Uns=3	Ter=29 Uns=15	Ter=40 Uns=4
Liver- Midzon. Hep. Enlarg.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=16 Uns=0
Liver- Centri. Inflam. Cell	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=1	Ter=9 Uns=0
Liver- Fine Vac. Centri. Hep.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=1 Uns=1	Ter=4 Uns=8	Ter=25 Uns=1
Liver- Fine Vac. Midzon. Hep.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=16 Uns=2	Ter=32 Uns=1
Liver- Eosin. Vac. Hep.	Ter=3 Uns=3	Ter=3 Uns=1	Ter=5 Uns=3	Ter=12 Uns=0	Ter=10 Uns=1
Liver- Bile Duct Hyperplas.	Ter=6 Uns=6	Ter=9 Uns=3	Ter=11 Uns=6	Ter=9 Uns=8	Ter=26 Uns=3
Thyroid- Cystic Foll. Atrophy	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=6 Uns=0
Thyroid-Cystic Foll.	Ter=0 Uns=1	Ter=5 Uns=0	Ter=2 Uns=0	Ter=8 Uns=0	Ter=3 Uns=1
Piuitary- Enlarged Vac. Cells	Ter=7 Uns=4	Ter=1 Uns=8	Ter=5 Uns=2	Ter=12 Uns=7	Ter=17 Uns=2
Brain-Evidence of dorsal comp.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=1 Uns=0	Ter=24 Uns=0
Bone- Osseous Hyper. Of Cranium	Ter=0 Uns=0	Ter=0 Uns=0	Ter=2 Uns=1	Ter=3 Uns=5	Ter=42 Uns=3
Bone- Osseous Hyper. Of Parietal	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=4 Uns=4	Ter=41 Uns=2

Data from pages 174-207, MRID 44305304.

Table 3. Nonneoplastic histopathology findings in females at terminal sacrifice or dying an unscheduled death. Ter- terminal; Uns-unscheduled (50 animals examined in the control and high-dose groups)

Histopathology	Control	10 ppm	80 ppm	640 ppm
Liver- Centri. Hep. Enlarg.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=16 Uns=2	Ter=38 Uns=11
Liver- Fine Vac. Centri. Hep.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=2 Uns=1
Liver- Eosin. Vac. Hep.	Ter=3 Uns=0	Ter=3 Uns=2	Ter=15 Uns=2	Ter=15 Uns=1
Liver- Bile Duct Hyperplas.	Ter=4 Uns=3	Ter=4 Uns=3	Ter=10 Uns=1	Ter=12 Uns=4

Data from pages 208-231, MRID 44305304.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Under the conditions of this study, dosing is considered adequate to assess the carcinogenic potential of tetraconazole based on histopathology of the bone (osseous hypertrophy of the cranium/parietal bone), pale and thickened incisors, and decreased absolute and relative adrenal and pituitary weights in males at 640 ppm and decreased body weight (at terminal sacrifice) in females at 640 ppm and in males at 1280 ppm.

2. Carcinogenicity Study in Mice

Reference: Crome, S., M.E. Bellringer, *et al.* (1992) M 14360 Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice. Huntingdon Research Centre, Ltd., Cambridgeshire, England. Report # AGR73/920469, December 10, 1998. MRID # 44305305. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID 44305305) M14360 (95.05% a.i.) was administered to 50 Crl:CD-1 (ICR) mice/sex/dose in their diet at dose levels of 0, 10, 90, 800, 1250 ppm (for males: 0, 1.4, 12, 118, 217 mg/kg/day; for females: 0, 1.6, 14.8, 140, 224 mg/kg/day) for 80 weeks.

B. Discussion of Tumor Data

Neoplastic findings were noted in the liver where a statistically significant increased incidence of combined benign and malignant liver cell tumors was observed at 1250 ppm

(86% for males and 65% for females) and 800 ppm (49% for males and 22% for females) compared to the control (20% for males and 0% for females). The tumor incidence in animals receiving ≤90 ppm was found to be similar to that of controls. Statistical evaluation of liver tumors in both sexes revealed a significant increasing trend with differences in the pair-wise comparisons of the 1250-ppm dose group with the controls for benign, malignant and benign and/or malignant tumors combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 800-ppm dose group with the controls for liver benign tumors and for benign and/or malignant tumors combined, both at $p < 0.01$. Historical control data show that the incidence of hepatocellular adenomas ranged from 7.7% - 24% in male mice and 0% - 1.9% in females and the incidence of hepatocellular carcinomas ranged from 0% - 14% in males and was 0% in females.

Female mice had a significant increasing trend in ovarian benign luteomas at $p < 0.01$, but no significant differences in the pair-wise comparisons of any dose group. The incidence of ovarian tumors was 4.8 % and 12.5 % at 800 ppm and 1250 ppm, respectively. Historical control data ranged from 0 to 8 %. There was increased mortality in the 1250-ppm group, many of which died after 1 year post-dosing, in which none of the decedents had any incidences of luteomas. This may have resulted in an inflated percentage of luteoma incidences in the high dose group.

The statistical evaluation of mortality indicated significant increasing trends with increasing doses of tetraconazole in male and female mice.

The statistical analyses of tumors in male and female mice were based upon Peto's Prevalence Test since there was statistically significant differential mortality with increasing doses of tetraconazole in both sexes. See Tables 4 and 5 for male and female mice tumor analysis results, respectively. The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Table 4. Male Mice: Liver Tumor Rates[+] and Peto's Prevalence Test Results.

ppm	0	10	90	800	1250
mg/kg/day	0	1.4	12	118	217
Tumor Type					
Benign	9/49	8/50	6/49	22/49	34[a]/49
%	(18)	(16)	(12)	(45)	(69)
p =	0.000**	-	-	0.003**	0.000**
Malignant	1/48	2/47	2/47	4/48	20[b]/45
%	(2)	(4)	(4)	(8)	(44)
p =	0.000**	0.398	0.059	0.134	0.000**
Combined	10/49	9[c]/50	7[c]/33	24[d]/49	42[e]/49
%	(20)	(18)	(14)	(49)	(86)
p =	0.000**	-	-	0.002**	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First liver benign tumor observed at Week 34, 1250 ppm.
[b]First liver malignant tumor observed at Week 50, dose 1250 ppm.
[c]One animal in each of the 10- and 90-ppm dose groups had both an adenoma and a carcinoma.
[d]Two animals in the 800-ppm dose group had both an adenoma and a carcinoma.
[e]Twelve animals in the 1250-ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Female Mice: Liver Tumor Rates[+] and Peto's Prevalence Test Results.

ppm	0	10	90	800	1250
mg/kg/day	0	1.6	14.8	140	224
Tumor Type					
Benign % p =	0/49 (0) 0.000**	0/49 (0) -	0/50 (0) -	11/49 (22) 0.000**	26[a]/49 (53) 0.000**
Malignant % p =	0/48 (0) 0.000**	0/47 (0) -	0/50 (0) -	1/49 (2) 0.162	17[b]/44 (39) 0.000**
Combined % p =	0/49 (0) 0.000**	0/49 (0) -	0/50 (0) -	11[c]/49 (22) 0.000**	32[d]/49 (65) 0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First liver benign tumor observed at Week 57, 1250 ppm.
[b]First liver malignant tumor observed at Week 64, dose 1250 ppm.
[c]One animal in th 800-ppm dose group had both an adenoma and a carcinoma.
[d]Eleven animals in the 1250-ppm dose group had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Female Mice: Ovarian Tumor Rates[+] and Peto's Prevalence Test Results.

ppm	0	10	90	800	1250
mg/kg/day	0	1.6	14.8	140	224
Tumor Type					
Benign % p =	2/41 5) 0.006**	0/39 (0) -	0/41 (0) -	2/42 (5) -	4/32 (12) 0.121

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
First ovarian benign luteomas observed at Week 81, in final sacrifice animals, concurrently at all doses.

C. Non-neoplastic lesions

Gross pathology revealed slight to severe changes in the liver which correlated with the dose given. At 90 ppm, the liver appeared pale with accentuated lobular markings. At higher concentrations (800-1250 ppm), masses were found with raised, pale, or dark subcapsular areas. Masses were also found in the kidneys of male animals receiving 1250 ppm M14360. Dose-related increases in liver weights were noted in mice of both sexes given ≥90 ppm of the test material. Kidney weights were also found to increase slightly (9-11 % above controls) in males receiving ≥90 ppm.

Histopathological findings (Table 7) revealed liver toxicity including hepatocyte vacuolation, fat deposition, granulomatous inflammation, pigmented macrophages, generalized hepatocyte enlargement, and bile duct hyperplasia in mice receiving 1250 and 800 ppm of the test material. In addition, at the high dose of 1250 ppm, non-neoplastic changes were noted in the brain, lungs, kidneys, testes, epididymides, and ovaries. Thickening of compact bone of the cranium, in the ribs, collar bone (females only), and femur (females only) at 800 and 1250 ppm also appears to be treatment related.

Since tetraconazole does not appear to be genotoxic, other nongenotoxic mechanisms may play a role in tumorigenesis in the liver of the mouse when tetraconazole is administered orally.

None of the lesions observed appear to have any relevance in regards to induction of benign luteomas of the ovaries.

Table 7.

STATISTICAL ANALYSIS OF SELECTED HISTOPATHOLOGICAL LESIONS IN MICE

Findings Decedents	Control 0 ♂	Control 0 ♀	10 ppm ♂	10 ppm ♀	90 ppm ♂	90 ppm ♀	800 ppm ♂	800 ppm ♀	1250 ppm ♂	1250 ppm ♀
Liver										
Basophilic hepatocytes;	1	0	0	0	0	0	1	0	12	2
Eosinophilic hepatocytes; with/without further vacuolation	1	0	1	0	0	0	3	0	10	5
Generalised hcpatocyte vacuolation	0	0	0	2	1	0	6**	0	31**	12**
Granulomatous inflammation	1	0	2	1	2	0	4	2	21**	1
Fat deposition in hepatocytes	0	0	0	0	0	0	6**	1	24**	7*
Centrilobular hepatocyte enlargement	1	0	3	0	4	0	5	1	2	1
Gencralised hepatocyte enlargement	0	1	0	0	1	0	5*	1	33**	13**
Pigment macrophages	1	0	0	1	2	0	8**	3	34**	14**
Bile duct hyperplasia	0	0	0	0	0	0	5*	1	24**	9*
Pericholangitis	1	3	6	4	3	0	5	3	18*	7
Kidney										
Cortical scarring with atrophic tubules	4	3	5	3	3	2	2	5	31**	10
Subcapsular cortical scarring	0	-	0	-	0	-	0	-	2	-
Papillary necrosis	0	1	1	0	0	0	1	4	6	5
Brain										
Dorsal compression	0	0	0	0	0	0	0	0	2	1
Bone										
Animals examined	1	0	0	0	0	0	4	7	20	17
Thickening of compact bone in cranium	0	0	0	0	0	0	4	6	19	17
Myclofibrosis	0	0	0	0	0	0	2	3	6	5
Lung										
Prominent alveolar macrophages	4	0	6	1	2	0	2	5**	18	2
Pneumonitis	3	-	0	-	0	-	1	-	7	-
Thymus										
Animals examined	11	9	16	10	11	9	11	8	35	16
Involution	7	6	10	5	6	6	4	4	22	8
Testes										
Reduced spermatogenesis	I	-	1	-	2	-	2	-	23**	-
Tubular atrophy	0	-	0	-	1		0	-	19**	-
Interstitial cell hyperplasia	1	-	0	-	0		1	-	11	-
Epididymides										
Spermatogoa. absent	0	-	0	-	2		2	-	18**	-
Ovaries										
Corpora lutea. absent	-	5	-	2	-	4	-	7	-	13
Total number of animals examined[a]	12	9	17	11	11	9	11	8	36	18

* $p < 0.05$, ** $p < 0.01$

[a] For all tissues unless shown separately

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STATISTICAL ANALYSIS OF SELECTED HISTOPATHOLOGICAL LESIONS IN MICE

Findings	Control 0		10 ppm		90 ppm		800 ppm		1250 ppm	
Terminal	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver										
Basophilic hepatocytes	4	1	4	0	5	0	14**	9**	5*	11**
Eosinophilic hepatocytes with/without vacuolation	1	0	2	0	7*	0	26**	12	8*	24**
Generalized hepatocyte vacuolation	1	6	0	7	6	8	16**	32**	14**	32**
Granulomatous inflammation	6	2	1	2	4	4	24**	13**	12**	23**
Fat deposition in hepatocytes	2	25	5	18	9*	31	24**	33	8**	20
Centrilobular hepatocyte enlargement	3	0	2	0	14**	0	17**	7**	0	0
Generalized hepatocyte enlargement	3	0	1	0	6	0	21**	18**	14**	31**
Pigmented macrophages	1	2	0	1	3	4	4	1	13**	30**
Bile duct hyperplasia	0	0	0	0	2	0	0	1	11**	24**
Pericholangitis	14	14	12	16	13	16	20	23*	6	19*
Kidney										
Cortical scarring with atrophic tubules	8	8	2	5	2	6	8	4	5	6
Subcapsular cortical scarrmg	1	-	0	-	0	-	0	-	0	-
Papillary necrosis	0	1	1	0	0	1	0	0	0	1
Brain										
Dorsal compression	0	0	0	0	1*	0	0	0	4**	10**
Bone										
Ammals exammed	0	0	0	0	1	1	39	41	14	31
Thickening of compact bone in cranium	0	0	0	0	1	1	35	36	14	31
Thickening of compact bone in rib	0	0	0	0	0	0	4	1	1	8
Thickening of compact bone in collar bone	0	0	0	0	0	0	0	1	0	3
Myelofibrosis	0	0	0	0	0	1	2	0	3	4
Lung										
Prominent alveolar macrophages	5	1	4	3	7	6	6	2	4	6*
Pneumonitis	5	1	3	1	2	5	5	10**	3	8**
Thymus										
Animals examined	38	39	0	8	1	2	36	42	14	32
Involution	7	2	0	0	0	0	6	1	2	2
Testes										
Reduced spermatogenesis	5	-	5	-	6	-	14*	-	4	-
Interstitial cell hyperplasia	2	-	1	-	0	-	1	-	0	-
Epididymides										
Spermatogoa absent	0	-	1	-	4	-	12**	-	3*	-
Ovaries										
Corpora lutea absent	-	10	-	6	-	5	-	20*	-	13
Total number of animals examined[a]	38	41	33	39	39	41	39	42	14	32

*P <0.05, ** P <0.01

[a]For all tissues unless shown separately

STATISTICAL ANALYSIS OF SELECTED HISTOPATHOLOGICAL LESIONS IN MICE

Findings Combined	Control 0 ♂	♀	10 ppm ♂	♀	90 ppm ♂	♀	800 ppm ♂	♀	1250 ppm ♂	♀
Liver										
Basophilic hepatocytes	5	1	4	0	5	0	15*	9**	17**	13**
Eosinophilic hepatocytes with/without vacuolation	2	0	3	0	7	0	29**	12**	18**	29**
Generalised hepatocyte vacuolation	1	6	0	9	7*	8	22**	32**	45**	44**
Granulomatous inflammation	7	2	3	3	6	4	28**	15**	33**	24**
Fat deposition in hepatocytes	2	25	5	19	9*	31	30**	34	32**	27
Centrilobular hepatocyte enlargement	4	0	5	0	18**	0	22**	8**	2	1
Generalised hepatocyte enlargement	3	1	1	0	7	0	26**	19**	47**	44**
Pigmented macrophages	2	2	0	2	5	4	12**	4	47**	44**
Bile duct hyperplasia	0	0	0	0	2	0	5*	2	35**	33**
Pericholangitis	15	17	18	20	16	16	25*	26	24	26
Kidney										
Cortical scarring with atrophic tubules	12	11	7	9	5	8	10	9	36**	16
Subcapsular cortical scarring	1	-	0	-	0	-	0	-	2	-
Papillary necrosis	0	2	2	0	0	1	1	4	6*	6
Brain										
Dorsal compression	0	0	0	0	1	0	0	0	6*	11**
Bone										
Animals examined	1	0	0	0	1	1	43	48	44	48
Thickening of compact bone in cranium	0	0	0	0	1	1	39	42	33	48
Thickening of compact bone in rib	0	0	0	0	0	0	4	1	1	9
Thickening of compact bone in collarbone	0	0	0	0	0	0	0	1	0	3
Myelofibrosis	0	0	0	0	0	1	4	3	9	9
Lung										
Prominent alveolar macrophages	9	1	10	4	9	6	8	7*	22**	8*
Pneumonitis	8	-	3	-	2	-	6	-	10	
Thymus										
Animals examined	49	48	16	18	12	11	47	50	49	48
Involution	14	8	10*	5	6	6*	10	5	24*	10
Testes										
Reduced spermatogenesis	6	-	6	-	8	-	16*	-	27**	-
Tubular atrophy	0	-	0	-	1	-	0	-	19**	-
Interstitial cell hyperplasia	3	-	1	-	0	-	2	-	11*	-
Epididymides										
Spermatogoa absent	0	-	1	-	6*	-	14**	-	21**	-
Ovaries										
Corpora lutea absent	-	15	-	8	-	9	-	27*	-	26*
Total number of animals examined[A]	50	50	50	50	50	50	50	50	50	50

*P <0.05, ** P <0.01

[A]For all tissues unless shown separately

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D. Adequacy of Dosing for Assessment of Carcinogenicity

The doses were found to be adequate to test its carcinogenic potential based on increased mortality in both sexes at 1250 ppm (HDT) and decreased body weight gain and liver effects in both sexes at 800 and 1250 ppm.

IV. TOXICOLOGY

1. Metabolism

The following summary is from the radiolabeled triazole studies. Findings from the radiolabeled phenyl studies will follow later. Single oral doses of [^{14}C] triazole ring labeled M-14360 (MRID 44268117) were administered in a 0.75% (w/v) methylcellulose/HPLC water suspension to ten Sprague-Dawley rats of each sex at dose levels of 5 or 60 mg/kg/10 mL. The treated animals were placed individually in Nalgene^{R} metabolism cages. Urine and feces were collected from five rats/sex/dose for 168 hours at which time these animals were killed and their tissues and organs were harvested. The remaining five animals/sex/group were killed at peak blood levels of radioactivity occurring at 8-28 hours of post dosing and their tissues and organs were harvested. Radioactivity was measured in urine, feces, blood, tissues, organs, carcasses and cage washes from all animals.

Average total recovered radioactivity for either high or low single oral dose in males or females ranged from 95% to 102% of the administered dose (AD). Most of the radioactivity (75%) was recovered in the urine after 7 days. Recovered radioactivity in the feces ranged from 15% (high dose) to 18% (low dose) of the AD. In the urine and fecal samples, triazole was the major metabolite for both dose levels and sexes. M-14360 acid along with minor metabolites of M-14360 alcohol and its glucuoronide conjugate (M3) were also isolated from the urine. In the feces minor amounts of the parent material M-14360, the acid and alcohol were also isolated.

Radioactivity in the tissues was minimal after 7 days and accounted for less than 1.5% of the AD. However earlier sacrifices showed that the radioactive dose was absorbed and distributed throughout the various body organs and tissues. Heart, spleen, gonads, brain, kidneys, lungs or adrenals of either sex of both treatments had the lowest radioactive residues at any sacrifice time. The data indicate that M-14360 or its metabolites are not accumulated in rat tissues following a single oral dose.

The metabolic cleavage of M-14360 to yield triazole appears to be the major step in M-14360 metabolism. [Attachment 5] The study authors postulate that this step is

glutathione mediated. A metabolic pathway was proposed where the initial step is the formation of an aldehyde intermediate of M-14360 [See Attachment 5 for structure] following dealkylation of the fluoro-alkyl group of the molecule.

Following a single oral low or high dose, male rats produced in the urine more triazole than females (65-67% of the AD vs 48% in females), while urine from females had more of M-14360 acid (7-13% vs 3.5-4% for males), M-3 (2-4% vs 0.0-0.6% for males), and M-14360 alcohol (1.4-2.4% vs 0.0% for males). The same pattern, though not as pronounced, was also seen in the multiple dosing study.

There were also dose and sex differences in the quantitative and qualitative nature of the metabolites in the feces from single and repeated dose animals. In the multiple dosing, **triazole** (3.2-3.9% of the AD for males and females at the low dose vs 6.5-6.8% for the high dose), **M-14360 acid, M-14360 alcohol, M-14360, M6** and **others** were reported while in the single dosing only **triazole (5.6 - 10.4% of the administered low and high doses in both sexes), M-14360 acid** and **M-14360** were reported.

Ninety-five to ninety-eight percent of the urinary and fecal metabolites were identified.

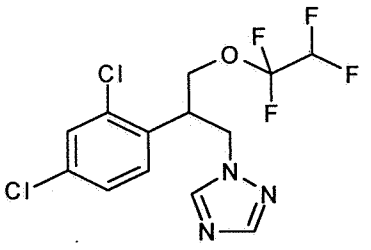
2. Mutagenicity:

Five acceptable genetic toxicology studies were available for review. The results from these studies indicate that tetraconazole was not mutagenic in *Salmonella typhimurium* (MRID 44335511), in cultured Chinese hamster ovary (CHO) cells (MRID 44335507), or in mouse lymphoma cells (MRID 44335508). There was also no evidence of clastogenicity *in vitro* (MRID 44335507) or *in vivo* (MRID 44335509) and tetraconazole did not induce unscheduled DNA synthesis (UDS) in human HeLa cells (MRID 44335510).

Overall, the data indicate that tetraconazole is negative for mutagenicity *in vitro* and *in vivo*. The acceptable studies satisfy the 1991 mutagenicity guideline requirements.

3. Structure-Activity Relationship

The structure of tetraconazole is provided below:



Caswell Number: 120603
CAS No.: 112281-77-3

Suitable analogs and their carcinogenic effects are summarized in Attachment 6.

4. Acute, Subchronic and Chronic Toxicity

A) Acute Toxicity

In an acute inhalation toxicity study (MRID #44305302), young adult albino rats (Sprague-Dawley) 5/sex were exposed by inhalation route to 83.5% (w/w) solution of M14360 in acetone for 4 hours to whole body at concentrations of 3.66 mg/L. A control group was exposed to acetone only. Animals were then observed for 14 days.

During the exposure period, all animals displayed signs of exposure to a mild irritant. These included wetness around the eyes, snout, mouth, irregular breathing, hunched body posture. Only one female was found dead on day 4 after exposure. **Necropsy findings included minimal hepatocyte enlargement or vacuolation in most test animals (9/10).**

B) Subchronic Toxicity

Rat

In a subchronic oral toxicity study (MRID 44335504), treatment-related increases in liver weights relative to body weight were seen in the 60 ppm (females only) and in 360 ppm treated males and females. Kidney weights relative to body weight were also significantly increased in the 360 ppm females rats. Macroscopic post mortem examination revealed two males at 360 ppm with enlarged livers and two males at 360 ppm and a single female at 10 ppm with swollen liver. Histological examinations

revealed minimal centrilobular hepatocyte enlargement in all rats receiving 360 ppm and 5/10 males and a single female receiving 60 ppm..

Mouse

In a subchronic mouse range-finding oral toxicity study (MRID 44778701), increased serum SGPT (+165% above controls) and SGOT (+56%), decreased serum BUN (-19%), slightly increased mean/absolute liver weights (15%/15%) and microscopic liver lesions (4/10 single cell necrosis, 1/10 necrosis, and 1/10 single cell degeneration vs. 0 in controls) were observed in females at 125 ppm (most of these findings showed a dose-related increase at 625 ppm). Two of ten males had single liver cell degeneration and only a slight (+39%; not significant) increase in SGPT. At 625 ppm, increased serum SGPT (+103%), decreased BUN (-15%) and increased absolute/relative liver weights (+77%/+75%), along with single cell necrosis and areas of necrosis (each 2/10), were also observed in males. In addition, midzonal hepatocyte hypertrophy and vacuolization in females (4/10 and 3/10, respectively) and liver cell degeneration in 1/10 females were also observed. Although the incidence of centrilobular hepatocyte hypertrophy was increased in both sexes at 25 ppm and higher, it was not considered toxicologically significant at that dose level, based on minimal severity and lack of other liver effects. Females appeared to be more sensitive at 125 ppm while significant changes in liver parameters and histopathology occurred in males at 625 ppm only.

C) Chronic Toxicity

Rat

Refer to Rat Combined Chronic Toxicity/Carcinogenicity Study on page 1 of this report.

The LOAEL is 640 ppm (27.7/39.4 mg/kg/day in male/female) based on histopathology of the bone (osseous hypertrophy of the cranium/parietal bone), pale and thickened incisors, and decreased absolute and relative adrenal and pituitary weights in males; decreased body weight (at terminal sacrifice) in females. The NOAEL is 80 ppm (3.4/4.4 mg/kg/day in male/female).

Mouse

Refer to Mouse Carcinogenicity Study on page 4 of this report.

The systemic toxicity LOAEL is 90 ppm (12 and 14.8 mg/kg/day for males and females, respectively), based on increased liver weight and hepatocyte vacuolation in both sexes

and increased kidney weights in males. The NOAEL is 10 ppm (1.4 and 1.6 mg/kg/day for males and females, respectively).

Dog

In a chronic toxicity study (MRID No. 44305303), Tetraconazole as M 14360 (94.6%) was administered to groups of four male and four female Beagle dogs/dose in the diet, at dose levels of 0, 22.5, 90, or 360 ppm (equivalent to achieved intakes of 0, 0.73, 2.95 or 12.97 for males or 0, 0.82, 3.33 or 14.50 mg/kg/day for females) for 52 weeks. [Attachment 7]

Exposure to M 14360 had no effect on feed consumption, hematological parameters or urinalysis. Treatment-related effects at the high dose included slight but nonsignificant body weight reductions in both sexes from study week 3 to termination; significantly increased alkaline phosphatase, γ-glutamyltransferase, alanine aminotransferase and ornithine carbamoyl transferase in both sexes from study week 13 to 52, increased absolute and relative liver and kidney weights for both sexes, and histopathological changes in both organs. In the mid-dose group, effects were manifested as increased absolute and relative kidney weights for males correlated with histopathological findings in the males (apparent hypertrophy in cortical tubules of the kidneys-1 male). No adverse effects were seen at the low dose.

Based on these considerations, the NOAEL is 22.5 ppm (equivalent to achieved intakes of 0.73 mg/kg/day for males or 0.82 mg/kg/day for females) and the LOAEL is 90 ppm (equivalent to achieved intakes of 2.95 mg/kg/day for males or 3.33 mg/kg/day for females), based on increased absolute and relative kidney weights and histopathological changes in the male kidney.

5. Mode of Action Studies

Liver Enzyme Induction

In a mouse subchronic toxicity study (MRID 44751309), tetraconazole 96.3% ai, Batch number FCF/T/113-94) was administered to 18 male and female Crl:CD-1 (ICR)BR mice per dose level at levels of 0, 20, 800, or 1250 ppm (0, 3.9, 150, or 225 mg/kg/day in males, 0, 4.6, 175, or 293 mg/kg/day in females) in the diet. [Attachment 8] The positive control was Phenobarbital (Na) salt, 75 mg/kg/day.

Data generated in this study indicate that tetraconazole administration for 4 weeks results in liver enzyme induction. Statistically significant increases were apparent in

females at the 20 ppm dose level based on increases in microsomal protein, cytochrome P450, and ethylmorphine N-demethylase. At all dose levels in males and females, 7-pentoxyresorufin O-dependentylase values were statistically elevated. At 800 and 1250 ppm, statistically significant findings were typically noted. However, dose response increases were not apparent in these findings at the 1250 ppm level as compared to the lower 800 ppm level.

In a rat subchronic toxicity study (MRID 44751310), tetraconazole 95.2% ai, Batch number FCF/T/122-95) was administered to 6 male and female Crl:CD BR rats per dose level at levels of 0, 10, 80, or 640 ppm (0, 0.8, 6.6, or 54.6 mg/kg/day in males, 0, 0.9, 7.6, or 57.6 mg/kg/day in females) in the diet. [Attachment 9] The positive control was Phenobarbital (Na) salt, 75 mg/kg/day.

Data generated in this study indicate that tetraconazole administration for 4 weeks results in liver enzyme induction at dose levels of 80 and 640 ppm. Induction at the 640 ppm dose level was similar to that induced by phenobarbital at 75 mg/kg/day.

Neither of these studies demonstrate a threshold level for initiation of hepatic carcinogenesis in the mouse as a result of oral administration of tetraconazole. Even though they show induction of Phase I and Phase II enzymes in the rat liver as a result of tetraconazole oral administration, no mechanism was demonstrated to explain the progression from enzyme induction to tumor initiation in the mouse.

V. WEIGHT-OF-THE-EVIDENCE CONSIDERATIONS

The Health Effects Division Carcinogenicity Peer Review Committee is asked to consider the following toxicology data in determining the carcinogenic potential of tetraconazole:

1) In a mouse carcinogenicity study (MRID 44305305) M 14360 (95.05% a.i.) was administered to 50 Crl:CD-1 (ICR) mice/sex/dose in their diet at dose levels of 0, 10, 90, 800, 1250 ppm (for males: 0, 1.4, 12, 118, 217 mg/kg/day; for females: 0, 1.6, 14.8, 140, 224 mg/kg/day) for 80 weeks. Male and female mice had significant increasing trends ($p = 0.000$ for both sexes) and significant differences in the pair-wise comparisons of the 1250-ppm dose group with the controls for liver benign (34/49,males; 26/49, females), malignant (20/45, males; 17/44, females) and benign and/or malignant tumors combined (42/49, males; 32/49, females), all at $p = 0.000$ for both sexes. There were also significant differences in the pair-wise comparisons of the 800-ppm dose groups with the controls for liver benign tumors (22/49, $p = 0.003$ for males, 11/49, $p = 0.000$ for females) and for benign and/or malignant tumors combined (24/49, $p = 0.002$ for males; 11/49, $p = 0.000$ for females).

Female mice had a significant increasing trend in ovarian benign luteomas at $p < 0.01$, but no significant differences in the pair-wise comparisons of any dose group.

Gross pathology revealed slight to severe changes in the liver which correlated with the dose given. At 90 ppm, the liver appeared pale with accentuated lobular markings. At higher concentrations (800-1250 ppm), masses were found with raised, pale, or dark subcapsular areas. Histopathological findings revealed liver toxicity including hepatocyte vacuolation, fat deposition, granulomatous inflammation, pigmented macrophages, generalized hepatocyte enlargement, and bile duct hyperplasia in mice receiving 1250 and 800 ppm of the test material.

The doses were found to be adequate to test its carcinogenic potential based on increased mortality in both sexes at 1250 ppm (HDT) and decreased body weight gain and liver effects in both sexes at 800 and 1250 ppm.

2) In a rat carcinogenicity study, administration of tetraconazole in the diet was not associated with a significant increase in liver tumors or other neoplastic findings. Under the conditions of this study, dosing is considered adequate to assess the carcinogenic potential of tetraconazole based on histopathology of the bone (osseous hypertrophy of the cranium/parietal bone), pale and thickened incisors, and decreased absolute and relative adrenal and pituitary weights in males at 640 ppm and decreased body weight (at terminal sacrifice) in females at 640 ppm and in males at 1280 ppm.

3) In the chronic dog study, treatment-related effects at the high dose included significantly increased alkaline phosphatase, γ-glutamyltransferase, alanine aminotransferase and ornithine carbamoyl transferase in both sexes from study week 13 to 52, increased absolute and relative liver and kidney weights for both sexes, and histopathological changes in both organs. There were no neoplastic findings.

4) Tetraconazole was not mutagenic, clastogenic, or aneugenic in any mutagenicity study.

5) Tetraconazole oral administration results in hepatic enzyme induction, but no clear evidence of a threshold effect on hepatic carcinogenesis in the mouse was demonstrated.

VI. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

VII. BIBLIOGRAPHY

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ATTACHMENTS

Attachment 1	Combined Chronic/Carcinogenicity Feeding Study - Rat
Attachment 2	Qualitative Risk Assessment on Combined Chronic/Carcinogenicity Rat Feeding Study and Carcinogenicity Mouse Feeding Study
Attachment 3	Carcinogenicity Feeding Study - Mouse
Attachment 4	Historical Control Data - Incidence of ovarian luteoma in female mice
Attachment 5	Figure 1 from Metabolism Study (MRID 44268117) - Rat
Attachment 6	SAR Table - Carcinogenic effects of suitable analogs of tetraconazole
Attachment 7	Chronic Oral Toxicity Feeding Study - Dog
Attachment 8	Liver Enzyme Induction Following Dietary Administration - Mouse (MRID 44751309)
Attachment 9	Liver Enzyme Induction Following Dietary Administration - Rat (MRID 44751310)

EPA Primary Reviewer: Roger Hawks
Reregistration Branch III (7509C)
Roger Hawks, Date 5/29/99
EPA Secondary Reviewer: Michelle Centra
Reregistration Branch III (7509C)
Michelle M. Centra, Date 6/15/99

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity feeding-rat; OPPTS 870.3200 [§83-2b]

DP BARCODE: D238518
P.C. CODE: 120603
SUBMISSION CODE:S528887

MRID No.: 44305304
CASE NO.: 288762

TEST MATERIAL (PURITY): Tetraconazole (94.6%)

SYNONYMS: M 14360

CITATION: Crome, S. J, *et al.* (1992) Potential tumorigenic and toxic effects in prolonged dietary administration to rats. Laboratory name Huntingdon Research Centre Ltd. Laboratory report number: AGR 74/911683. December 10, 1992. MRID 44305304. Unpublished.

SPONSOR: Sostram Corporation

EXECUTIVE SUMMARY: In a carcinogenicity toxicity study (MRID44305304), tetraconazole, (94.6% a.i.) was administered to Crl:CD (SD) rats 50/sex/dose in the diet at dose levels of 0 (control) 10, 80 ,640 and 1280 ppm for males and 0 (control), 10, 80 and 640 ppm for females. This corresponds to 0, 0.6, 4.4, and 39.4 mg/kg/day for females and 0.0, 0.4, 3.4, 27.7 and 59 mg/kg/day for males. The study was 2 years in length and the animals were exposed to the test article throughout the duration of the study. An additional 20 rats/sex/dose were sacrificed and examined at 12 months.

There were no compound-related effects on mortality. Group mean body weights at terminal sacrifice were decreased 7 % and 24% in 640 and 1280 ppm males respectively and were decreased 24% in 640 ppm females. Group mean food consumption for the entire study was decreased 5.1% and 15.2% in 640 and 1280 ppm males and 13.9% in 640 ppm females. At their respective high dose, both male and female serum glucose levels were reduced from 13.6% to 26% in males and 5.5 to 18.4% in females, depending on the time point. Packed cell volume, red blood cell counts and hemoglobin levels were reduced in both sexes at their respective high doses and also in males at 640 ppm. These were, at some time points statistically significant (SS) though the percentage wise decreases were never extreme (about 6% for PCV, 10% for Hb and 3% for RBC). Both the serum glucose and hematology alterations are likely to be attributable to the decreased food consumption and body weights seen in both sexes at these doses and are thus,

secondary to compound exposure. Significant increases in serum phosphorus were seen in 640 and 1280 ppm males, but no signficant alterations in phosphorus levels were seen in females of any dose. The only clinical signs likely related to compound exposure were: long upper incisors seen in 17 and 44 male rats at 640 and 1280 ppm, respectively (compared to incidences of 1, 0, and 1 at doses of 0, 10 and 80 ppm, respectively); pale lower incisors seen in 38 and 47 male rats at 640 and 1280 ppm, respectively (compared to 1, 3, and 3 males at doses of 0, 10 and 80 ppm, respectively); and pale lower incisors in the 640 ppm females (1, 0, 0 and 7 at doses of 0, 10, 80 and 640 ppm, respectively). Absolute adrenal weights were decreased, compared to controls, 18 and 33.6% in the 640 and 1280 ppm males, respectively, but only 3.4% in the 640 ppm females. Adrenal weights, relative to body weights, were decreased 12.8 and 25.65 in 640 and 1280 ppm males and 175 in 640 ppm females. Absolute pituitary weights were decreased, compared to controls, 34.2 and 46.8% in 640 and 1280 ppm males but only 9.4% in 640 ppm females. Pituitary weights, relative to body weights, were decreased 13.4% and 23% at 640 and 1280 ppm in males but were not greatly altered in females. The decreases in relative adrenal and pituitary weights in males were SS, but none of the decreases seen in females was. Both sexes did show an increase in relative liver weights at 640 and 1280 ppm from the males and 640 ppm for the females. These increases were SS. Necropsy findings reiterated the incisor findings seen as clinical observations in both sexes. Males, but not females, showed at the 1280 ppm dose (and to a much lesser extent the 640 ppm dose) necropsy findings of white cranium, thickened cranium and thickened parietal bones. No control animals displayed these signs but from 3 to 6 and from 25 to 34 640 and 1280 ppm males did. Liver findings at necropsy consisted of pale subcapsular areas in both sexes (6, 7, 8, 24 and 29 in control through 1280 ppm male groups and 5, 9, 11, and 17 in control through 640 ppm female groups) as well as accentuated lobular markings in the males only (2, 4, 2, 14, 25 in control through 1280 ppm groups). Histopathology findings supported the liver findings at necropsy for both sexes. Hepatic findings at histopathology were indicative of cellular proliferation and hypertrophy. Some findings, such as centrilobular hypertrophy, (incidences were 0, 0, 17, 34, and 44 in control through 1280 ppm males and 0, 0 17 and 39 in control through 640 ppm females) were increased in the 80 ppm groups. Most hepatic findings, however, were confined mostly to the 640 and 1280 ppm males and 640 ppm females. Fine vacuolation of hepatocytes and inflammatory cell foci in males were not seen at all in controls but were seen in anywhere from 18 to 62% of the 640 and 1280 ppm males. The skull bone findings at necropsy were supported by the histopathology findings which found an increased incidence of osseous hypertrophy of the parietal bones (8 and 43 incidences at 640 and 1280 compared to zero in controls) and osseous hypertrophy of the cranium (8 and 45 at 640 and 1280 compared to zero in controls). Additionally, in males only, an increased incidence of cystic follicular atrophy in the thyroid was seen (6 in 1280 ppm and zero in all other groups).

The findings in the liver at doses less than1280 ppm (cellular proliferation/hypertrophy) are indicative of an adaptive response rather than a toxicologic response. Only at the 1280 ppm dose, where increases in inflammatory foci are seen, can the liver responses be called toxicologically significant. The alterations in the skeletal system at 640 ppm in males can be considered toxicologically relevant.

The LOAEL is 640 ppm (27.7/39.4 mg/kg/day in male/female) based on histopathology of the bone (osseous hypertrophy of the cranium/parietal bone), pale and thickened incisors, and decreased absolute and relative adrenal and pituitary weights in males; decreased body weight (at terminal sacrifice) in females. The NOAEL is 80 ppm (3.4/4.4 mg/kg/day in male/female).

Under the conditions of this study, there was no evidence of a treatment-related increase in tumor incidence when compared to controls. **Therefore, tetraconazole is not a carcinogen in this study.** Dosing is considered adequate to assess the carcinogenic potential of tetraconazole, based on histopathology of the bone (osseous hypertrophy of the cranium/parietal bone), pale and thickened incisors, and decreased absolute and relative adrenal and pituitary weights in males at 640 ppm and decreased body weight (at terminal sacrifice) in females at 640 ppm and in males at 1280 ppm.

This carcinogenicity study in the rat is **Acceptable-Guideline**, and does satisfy the guideline requirement for a carcinogenicity study (83-2b) in the rat. Although this study was submitted to fulfill the Subdivision F guideline requirement for a carcinogenicity study (83-2b), it also satisfies the requirement for a combined chronic toxicity/carcinogenicity study (83-5) in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Tetraconazole
   Description: Brown viscous liquid
   Lot/Batch #: FCF/T/72
   Purity: 94.6% a.i.
   Stability of compound: Stable at room temp. in the dark
   CAS #: 112281-77-3

2. Vehicle and/or positive control: the test substance was dissolved in acetone which was subsequently allowed to evaporate away.

3. Test animals: Species: Rat
   Strain: Crl:CD (SD) BR
   Age and weight at study initiation: 28 days; Males $\bar{x}$= 139 gm, Females $\bar{x}$= 111 gm
   Source: Charles River Breeding Laboratories, Portage, MI, USA
   Housing: 5 rats to a cage in suspended wire mesh cages
   Diet: Powdered SDS Rat and Mouse Chow No. 1 modified maintenance diet ad libitum
   Water: Tap Water ad libitum
   Environmental conditions:
   Temperature: 21 ± 2°C
   Humidity: 50 ± 10%
   Air changes: Not Recorded
   Photoperiod: 12 hr light/12 hr dark
   Acclimation period: 15 days (8 days initially, followed by 7 days post veterinary examination)

B. STUDY DESIGN:

1. In life dates - start: July 28, 1989 end: August 6, 1991

2. Animal assignment

Animals were assigned randomly assigned to the test groups in table 1. Though assignments were random, animals were stratified by body weight such that the initial group mean body weights of the various groups were about equal.

TABLE 1: Study Design

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg/day)		Main Study 24 months		Interim Sacrifice 12 months	
		Males	Females	Males	Females	Males	Females
Control	0*	0	0	50	50	20	20
Low (LDT for males and females)	10	0.4	0.6	50	50	20	20
Mid (MDT1 for males, MDT for females)	80	3.4	4.4	50	50	20	20
Mid (MDT2, for males, HDT for females)	640	27.7	39.4	50	50	20	20
High (HDT for males)	1280	59.0	-	50	-	20	-
Health Check@	0	0	0	5	5	-	-

* Control was acetone added directly to the diet and then allowed to evaporate away.
@ These 10 animals were sacrificed prior to the initiation of treatment and were examined macroscopically for the purpose of looking for signs of infectious disease.

3. Dose Selection: Dose selection was based on the results of a 3-month rat subchronic study which has been submitted to the agency and found acceptable (MRID 44335504) and a 4-week preliminary toxicity study (AGR 21/871271) which has not been submitted to the agency. The doses tested in the 3-month study were: 0 (control), 10, 60 and 360 ppm. The LOAEL in the 3-month study was 60 ppm (4.1 and 5.5 mg/kg/day for males and females, respectively) based on findings of increased absolute and relative liver to body weight ratios in females and enlarged centrilobular hepatocytes in both sexes. Body weights in the 60 ppm group were not affected by compound exposure. The next highest dose of 360 ppm (23.9 and 28.7 mg/kg/day in males and females, respectively) resulted in significantly reduced (17% less than controls) female body weight gains and significantly increased (16.7% more than controls) male body weight gains.

4. Diet preparation and analysis

Diet was prepared weekly by dissolving test substance in acetone and then mixing that solution with Powdered SDS Rat and Mouse Chow No. 1. The acetone solvent was removed by evaporation on a rotary mixer. The resulting mixture, referred to in the study as a pre-mix, was blended in with the required amount of additional chow to

reach the desired concentration. The control diets had acetone alone added to them and then dissolved away.

Homogeneity and stability were determined from the first test compound mixture prepared, prior to the start of treatment. During the study, samples of treated food were analyzed every 3 months for concentration. Results are shown in Tables 2a, 2b, and 2c.

TABLE 2a. Homogeneity Analysis

Nominal Concentration	Top	Middle	Bottom
2 ppm	1.8; 2.06	1.79; 2.06	1.84; 2.23
160 ppm	168; 169	168; 171	169; 169
1280 ppm	1290; 1280	1290; 1300	1270; 1290

[a]Results presented in this table are obtained from duplicate samples.

TABLE 2b. Stability Analysis

Days in storage ▸ Nominal conc.▾	0	4	10	14
0 ppm	<0.2 ppm	<0.2 ppm	<0.2 ppm	<0.2 ppm
2 ppm	1.84	2.00	1.74	1.81
160 ppm	165	163	158	163
1280 ppm	1250	1250	1250	1250

[a]The values presented is this table are the means of duplicate samples.

TABLE 2c. Concentration Analysis

Nominal Conc.	Range of Means
10 ppm	9.62 - 11.1
80 ppm	74.6 - 84.1
640 ppm	608 - 673
1280 ppm	1160 - 1280

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. Homogeneity, stability, and concentration data were taken from pages 2166, 2167, 2170 and 2171.

5. Animals received fresh diet each week.

6. Statistics - Food and water consumption were analyzed using cumulative cage totals. Food and water consumption, clinical pathology and organ weight data were analyzed as such: if the relative frequency of the mode was 75% or more then the number of animals in each group with values different from the mode was analyzed using Fisher's exact test and Mantel's test. Bartlett's test was used to examine for heterogeneity of variance between treatments. If significance at the 1% was level was seen with this test then a logarithmic transformation was used to see if a more stable variance structure could be found. If log transformation could not remove the variance then the Kruskal-Wallis analysis of ranks was performed. This test was followed by the non-parametric equivalent of the Williams test - Shirley's test - to determine dose response relationship. If no significant heterogeneity was detected in the Bartletts test then a one way analysis of variance was carried out followed by students 't' test and Williams' test for a dose-response pattern.

   Organ weights were, additionally, analyzed by analysis of covariance and analysis of variance.

   Mortality was analyzed using log rank methods as described in *Mantel, N. (1966), Cancer chemotherapy Reports, 50: 163-170.* When tumors were analyzed they were analyzed using the methods described in: *WHO International Agency for Research into Cancer (1980), Long Term and short Term Screening Assays for Carcinogens: A critical Appraisal. and, IARC monographs on the Evaluation of Carcinogenic Risk to Humans, Annex: R. Peto et al. Guidelines for simple Sensitive Tests for Carcinogenic Effects in Long-Term animal Experiments. Supplement 2, pp 311-426.*

C. METHODS:

1. Observations:

   Animals were inspected daily for signs of toxicity and mortality.

2. Body weight

   Animals were weighed at the time of allocation to treatment groups, on the first day of treatment and thereafter, once weekly.

3. Food consumption and compound intake

   Food consumption for each animal was determined and mean weekly diet consumption was calculated as grams of food/rat/week. Food efficiency (referred to as food conversion and efficiency of food utilization in this study) was calculated by

dividing food consumption for a selected time period by body weight gain during that time period. The study authors only calculated food conversion ratios for the first 6 months of the study (weeks 1-26). Compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination

Eyes were examined in all animals prior to the initiation of treatment. In addition, opthalmoscopic examination was performed at 52 weeks and at termination in the controls and the highest dose tested treatment groups.

5. Blood was collected from animals that had been fasted overnight. The collected blood was used for hematology and clinical analysis. Blood was collected at 13, 26, 52, 78 weeks and at termination. The animals were lightly anaesthetized with ether and blood was drawn from the orbital sinus of 10 males and 10 females in each group. Where possible, the same 10 males or females were used at each time point, although deaths sometimes required that another animals be used. 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 corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements	X	Cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

*** Minimum required for combined chronic toxicity/carcinogenicity studies (only on Cont. and HDT unless effects are observed based on Subdivision F Guidelines).**

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Minimum required for combined chronic toxicity/carcinogenicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was collected from animals which had their food and water removed the night before analysis. Urine samples were collected at the same time points as blood samples. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

* Minimum required for combined chronic toxicity/carcinogenicity studies based on Subdivision F Guidelines.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination. All animals sacrificed on schedule in the control and high-dose group, and those that died on study, had the CHECKED (X) tissues collected for histological examination. All of these tissues with the exception of the larynx and pharynx were subsequently examined histologically. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*
X	Colon*	XX	Kidneys*+		Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*++	XX	Testes*+		Parathyroids*++
	Gall bladder*	X	Epididymides	XX	Thyroids*++
X	Pancreas*	X	Prostate	X	Harderian Gland
		X	Seminal vesicle		
	RESPIRATORY	XX	Ovaries*+		OTHER
X	Trachea*	X	Uterus*	X	Bone*
X	Lung*	X	Vagina		Skeletal muscle*
X	Nose			X	Skin*
X	Pharynx			X	Entire Head
X	Larynx			X	All gross lesions and
				X	masses*

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

II. RESULTS:

A. Observations

1. Toxicity - Long upper incisors (indicating fast growing teeth) were seen in an increased incidence in male rats in the main study. The first reported incidence of this finding occurred at 9 weeks into the study in a male rat in the 1280 ppm group. Only one female (in the 640 ppm group) was observed to have this finding. Pale lower incisors was noted in many of the 640 and 1280 ppm males but in very few of the control, 10- and 80 ppm males. This finding did show a somewhat increased incidence in females as seven 640 ppm females had this finding compared to only one control.

Table 2. Incidence of incisor findings in main study rats. M=males; F=females					
	Control	10 ppm	80 ppm	640 ppm	1280 ppm
Long Upper incisors	1	0	1	17	44
Pale lower incisors	M=1 F=1	M=3 F=0	M=3 F=1	M=8 F=7	M=47

Data from page 35, current study

2. Mortality - Exposure to the test article did not result in an increase in mortality. Survival actually increased in the males as the dose increased and in the females there was increased survival at 80 and 640 ppm.

B. Body weight - Males in the 640 and 1280 ppm groups and females in the 640 ppm group showed reduced body weights compared to controls and the two lower dose groups. These reductions in body weight were evident by the end of the first week of the study and persisted to study termination. The reductions in body weights are evident in the reductions in body weight gains in these dose groups seen during the first 18 months of the study. During the final few months of the study, both sexes in the 640 and 1280 ppm groups began to gain as much or more weight than the controls, but they were not able to gain enough weight to catch up to the other groups.

Table 3. Mean group body weights at approximate 6 month intervals. All weights are in grams. M=males; F=females					
	Control	10 ppm	80 ppm	640 ppm	1280 ppm
Initial	M=190 F=143	M=192 (101)[a] F=145 (101)	M=191 (101) F=146 (102)	M=189 (100) F=145 (101)	M=188 (99)
1 week	M=242 F=168	M=243 (100) F=170 (101)	M=242 (100) F=169 (101)	M=227 (94) F=156 (93)	M=204 (84)
26 weeks	M=625 F=311	M=634 (101) F=313 (101)	M=610 (98) F=313 (101)	M=559 (89) F=259 (83)	M=468 (75)
52 weeks	M=739 F=381	M=754 (102) F=389 (102)	M=717 (97) F=392 (103)	M=659 (89) F=295 (77)	M=550 (74)
78 weeks	M=791 F=439	M=806 (102) F=477 (109)	M=775 (98) F=464 (106)	M=694 (88) F=328 (75)	M=585 (74)
104 weeks	M=768 F=464	M=775 (101) F=524 (113)	M=743 (97) F=508 (110)	M=711 (93) F=353 (76)	M=583 (76)

Data from pages 70-73, current study

[a]The values within the parentheses are the percent of control rounded values calculated by the reviewer.

Table 4. Mean group body weight gains at approximate 6 month intervals. All values are in grams. M=males; F=females

	Control	10 ppm	80 ppm	640 ppm	1280 ppm
0-26 wks	M=435# F=168	M=442 (102)[a] F=168 (100)	M=419 (96) F=167 (99)	M=370 (85) F=114 (68)	M=280 (64)
26-52 wks	M=114 F=70	M=120 (105) F=76 (109)	M=107 (94) F=79 (113)	M=100 (88) F=36 (51)	M=82 (72)
52-78 wks	M=52 F=58	M=52 (100) F=88 (152)	M=58 (112) F=130 (224)	M=35 (67) F=33 (57)	M=35 (67)
78-104 wks	M=-23 F=25	M=-31 F=47 (188)	M=-32 F=44 (176)	M=17 F=25 (100)	M=-2

#Calculated by reviewer using data from pages 70-73, current study
[a]The values within the parentheses are the percent of control rounded values calculated by the reviewer.

C. Food consumption and compound intake

1. Food consumption - Males in the 640 and 1280 ppm groups and females in the 640 ppm group consumed significantly less food during the course of the study than did controls. The reductions in food consumption in these groups was evident by the end of the first week of the study. Males in the 1280 ppm group consumed less than controls throughout the study while males in the 640 ppm group consumed less than controls only up to 90 weeks, after which their food consumption was similar to controls. Females in the 640 ppm group consumed less than controls throughout the course of the study.

Table 5. Mean food consumption at approximate 6 month intervals. All values are in grams. M=males; F=females

Week	Control	10 ppm	80 ppm	640 ppm	1280 ppm
1 week	M=201 F=138	M=200 (100)[a] F=136 (99)	M=192 (96) F=133 (96)	M=176 (88) F=118 (86)	M=149 (74)
26 wks	M=180 F=132	M=182 (101) F=126 (96)	M=175 (97) F=124 (94)	M=167 (93) F=110 (83)	M=151 (84)
52 wks	M=176 F=131	M=176 (100) F=134 (102)	M=175 (99) F=137 (105)	M=165 (94) F=114 (87)	M=150 (85)
78 wks	M=169 F=134	M=176 (104) F=144 (108)	M=178 (105) F=140 (105)	M=165 (98) F=120 (90)	M=155 (92)
104 wks	M=176 F=145	M=187 (108) F=159 (110)	M=175 (99) F=149 (103)	M=176 (100) F=128 (88)	M=157 (89)

0-104 wks	M=19198 F=14611	M=19582 (102) F=14949 (102)	M=19254 (100) F=14671 (100)	M=18226* (95) F=12584** (86)	M=16272** (85)

Data from pages 36 and 74-77, current study.
* statistically significant at $p < 0.05$
** statistically significant at $p<0.01$.
[a]The values within the parentheses are the percent of control rounded values calculated by the reviewer.

2. Compound consumption (time-weighted average) - Compound consumption across treatment groups started out at its highest levels at study initiation and slowly decreased as the study progressed to termination in male and female rats. This pattern is commonly seen in 2-year studies and is expected.

Table 6. Mean group compound consumption at approximate 6 month intervals. All values are in mg/kg/day. M=Males; F=Females

Treatment Week (s)	10 ppm	80 ppm	640 ppm	1280 ppm
1 week	M=1.3 F=1.2	M=10.1 F=9.7	M=77.4 F=71.3	M=139
26 wks	M=0.4 F=0.6	M=3.3 F=4.5	M=27.4 F=38.8	M=59
52 wks	M=0.3 F=0.5	M=2.8 F=4.0	M=22.9 F=35.3	M=50
78 wks	M=0.3 F=0.4	M=2.6 F=3.5	M=21.7 F=33.3	M=48
104 wks	M=0.3 F=0.4	M=2.7 F=3.3	M=22.5 F=33.1	M=49
0-104 wks	M=0.4 F=0.6	M=3.4 F=4.4	M=27.7 F=39.4	M=59

Data from pages 79-82, current study

3. Food efficiency - The study authors only calculated food efficiency (food conversion) ratios for the first 6 months the study (weeks 1-26). Males food conversion ratios were similar to control in the 10 and 80 ppm groups. Males food conversion ratios in the 640 ppm group were slightly above controls and ratios in the 1280 ppm group were about 26% above controls. Female ratios in the 10 and 80 ppm groups were similar to controls. In the 640 ppm group the food conversion ratios were approximately 26% above controls.

Table 7. Mean group food conversion ratios. M=males; F=females					
Treatment Interval (weeks)	**Control**	**10 ppm**	**80 ppm**	**640 ppm**	**1280 ppm**
weeks 1-26	M=11.6 F=21.1	M=11.4 (98)[a] F=20.9 (99)	M=11.8 (102) F=21 (100)	M=12.5 (108) F=26.6 (126)	M=14.6 (126)

Data from page 78, current study

[a]The values within the parentheses are the percent of control rounded values calculated by the reviewer.

D. Ophthalmoscopic examination - No treatment-related lesions were identified.

E. Blood work:

1. Hematology - Table 8 shows the hematology data for male rats at weeks 26, 52, 78 and 102. Packed cell volume (PCV), hemoglobin (Hb), red blood cell counts (RBC), mean cell hemoglobin concentration (MCHC), and Mean cell volume (MCV) were statistically significantly reduced (≤ 15%) in males in the 640 and 1280 ppm groups and to a lesser extent in the 640 ppm females (< 10%, data not shown). In addition, these parameters (with the exception of RBC) were also significantly reduced at week 13 in male rats at 640 and 1280 ppm. As the study progressed, the reductions became less pronounced and by study termination (week 102), most of the values were comparable to controls. Mean cell volume values remained significantly reduced at 102 weeks compared to the 13 week values.

   Male white blood cell counts (WBC) were reduced in the 1280 ppm group compared to controls. This reduction was especially evident at the later time points, but at no time point was statistically significant. Macrophage counts were statistically significantly reduced, compared to controls, in the male 1280 ppm group at the 78 and 102 week time point and this reduction may have contributed to the WBC reductions. Counts of neutrophils, leucocytes and eosinophils were also reduced, compared to controls, at these two time points, but not significantly.

   Female WBCs, unlike the males, were not reduced at 102 weeks at their HDT (640 ppm). Female WBCs were significantly reduced at 78 weeks though. At this time point lymphocyte counts were significantly reduced and platelet counts were significantly increased. Neutrophil, eosinophil and macrophage counts were also reduced, but not significantly at this dose and time point. WBCs, differential counts and platelet counts did not appear to greatly affected, statistically significantly or otherwise, in any other dose group or at any other time point.

Table 8. Male group mean hematology values. Values shown are groups mean and standard deviations. PCV and MCHC in %; Hb in g/dl; RBC x10^6/mm_3; MCV in fl.

Treatment	PCV	Hb	RBC	MCHC	MCV
Control	26=55±1.8 52=53±2.0 78=50±1.9 102=45±4.8	26=15.1±0.36 52=14.3±0.49 78=14.7±0.64 102=14.9±0.36	26=7.8±0.32 52=7.5±0.35 78=6.9±0.38 102=6.5±0.65	26=27.6±0.56 52=27.2±0.54 78=29.6±0.78 102=32.5±1.44	26=70±0.8 52=70±1.7 78=72±2.0 102=69±1.9
10 ppm	26=55±2.7 52=54±1.6 78=51±4.5 102=47±3.6	26=15.1±0.71 52=14.4±0.47 78=15±1.35 102=15±0.32	26=7.8±0.46 52=7.6±0.31 78=7.2±0.7 102=7.1±0.71	26=27.5±0.42 52=26.9±0.36 78=29.5±0.86 102=31.9±1.99	26=71±1.2 52=70±1.4 **78=71±1.4*** 102=67±2.3
80 ppm	26=54±3.1 52=53±1.6 78=48±3.5 102=44±1.9	26=14.6±1.07 52=14.3±0.5 78=14±1.64 102=14.7±0.22	26=7.7±0.43 52=7.6±0.26 78=6.8±0.58 102=6.5±0.49	26=27.0±0.90 52=27.1±0.37 78=29.4±1.88 102=33.5±1.06	26=70±1.4 52=70±1.5 **78=70±1.9**** 102=68±3
640 ppm	26=53±2.4 **52=50±1.8**** **78=45±6.5*** 102=44 ±3.3	**26=14.2±0.58*** **52=13.2±0.52**** 78=13.3±1.99 102=14.6±0.42	26=7.7±0.37 52=7.4±0.44 78=6.5±0.96 102=6.6±0.56	**26=26.5±0.45**** **52=26.6±0.53*** 78=29.9±0.87 102=33.6±1.63	26=69±2.0 **52=67±2.3**** **78=69±1.0**** **102=66±2.5***
1280 ppm	**26=52±1.8**** **52=48±1.2**** **78=45±1.9**** 102=44 ±2.2	**26=13.6±0.3**** **52=12.8±0.42**** **78=13.4±0.64**** **102=14.6±0.29***	**26=7.4±0.29*** **52=7.1±0.24**** 78=6.8±0.34 102=6.7±0.39	**26=26.3±0.96**** **52=26.5±0.45**** 78=29.7±0.42 102=33.3±1.01	26=70±1.5 **52=68±1.1**** **78=66±1.9**** **102=66±2.7****

Data from pages 85 to 87 and 474 to 493, current study.
*Statistically significant at $p<0.05$.
** Statistically significant at $p<0.01$.

Table 9. White blood cell counts.

Group	Control	10 ppm	80 ppm	640 ppm	1280 ppm
Males	26=13.3±2.2 52=12.4±4.4 78=10.8±2 102=10.9±1.6	26=11.2±1.4 52=10±1.4 78=11.5±2.4 102=10.4±3.4	26=13.4±4.3 52=9.9±2.1 78= 13.3±3.7 102=11.7±4.6	26=14.7±3.0 52=12.6±2.8 78=11.9±3.1 102=11.1±5.3	26=10.8±3.1 52=10.4±1.8 78=9.8±2.5 102=8.7±3.8
Females	26=8.3±2.1 52=6.9±1.5 78=6.6±1.2 102=8.9±2.6	26=10.1±2.4 52=7.7±2.7 78=6.8±1.5 102=8.4±3.2	26=9.0±1.6 52=7.2±2.5 78=6.8±2.3 102=9.5±3.2	26=9.9±1.8 52=6.5±0.8 **78=4.9±1.2*** 102=8.9±3.5	

Data from pages 85 to 90 and 474 to 493, current study.
*Statistically significant at $p<0.05$.

Table 10. Female group mean hematology values. Values shown are groups mean and standard deviations. PCV and MCHC in %; Hb in g/dl; RBC x10^6/mm_3; MCV in fl.

Treatment	PCV	Hb	RBC	MCHC	MCV
control	26=52±2.9 52=50±2.8 78=47±2.5 102=46±2.8	26=14.8±0.51 52=13.7±0.76 78=14.8±0.71 102=15.1±0.31	26=7.3±0.34 52=6.8±0.4 78=6.5±0.43 102=6.5±0.51	26=28.6±1.56 52=27.7±0.35 78=31.4±0.70 102=32.8±1.50	26=71±2.3 52=73±1.1 78=73±1.8 102=71±1.9
10 ppm	26=49±5.5 52=50±2.0 78=45±2.9 102=45±2.1	26=15.2±0.84 52=14.1±0.54 78=14±1.05 102=15.1±0.24	26=7.1±0.8 52=6.9±0.32 78=6.2±0.4 102=6.4±0.35	26=31.3±3.31 52=28.0±0.63 78=31.2±0.49 102=33.4±1.14	26=69±0.7 52=73±1.3 78=72±1.5 102=71±1.5
80 ppm	**26=48±2.9*** 52=48±2.5 78=47±2.5 102=46±3.4	26=14.4±0.72 52=13.4±0.60 78=14.6±0.80 102=15.1±0.32	26=6.9±0.43 52=6.6±0.37 78=6.4±0.39 102=6.4±0.47	26=30.1±0.44 52=27.8±0.46 78=31.3±0.56 102=33.4±1.76	26=70±0.7 52=73±0.9 78=73±1.2 102=72±1.3
640 ppm	**26=49±2.7*** 52=48±2.9 **78=44±1.4*** **102=43±3.1****	26=14.4±0.67 **52=13±0.87*** **78=13.5±0.49**** **102=14.7±0.28****	26=7.3±0.37 52=6.8±0.38 78=6.2±0.31 **102=6.0±0.59***	26=29.4±0.45 **52=26.9±0.45**** **78=30.3±0.79**** **102=34.7±2.06***	**26=68±1.2**** **52=72±1.4*** 78=72±1.9 102=71±3.0

Data from pages 88 to 90 and 474 to 493, current study.
* Statistically significant at $p<0.05$.
** Statistically significant at $p<0.01$.

2. Clinical Chemistry - Serum glucose (Glu) levels in both males and females showed statistically significant reductions compared to controls. In the males the reductions occurred at all time points from 13 weeks to 102 weeks at the HDT. In the MDT2 in males, the significant reductions occurred at weeks 13 and 52 while the other time points, while reduced, were not significant. In the females the only statistically significant reductions were at their HDT (640 ppm) at 26, 78 and 102 weeks. Serum glutamic-pyruvic transaminase (aka, alanine aminotransferase - 6GPT) levels were significantly increased in MDT2 and HDT males at 13 weeks and were significantly decreased in MDT1, MDT2 and HDT males at 102 weeks, compared to controls. In MDT and HDT females serum GPT levels were significantly decreased at all time points. Additionally, at 13 and 26 weeks, significant reductions were seen in females LDT serum GPT levels. Male cholesterol levels (chol) were decreased in a dose related-manner in the MDT1, MDT2 and HDT groups at the 52, 78 and 102 week time points. None of these reductions were statistically significant though. Female cholesterol levels, on the other hand, were significantly increased at 13 week in the HDT, at 26 weeks in the MDT and HDT, and at 52 weeks in the HDT. Females globulin levels were significantly increased at the HDT at all time points. Total protein and albumin levels were not significantly altered. Male globulin, albumin and total

protein levels, were occasionally significantly altered , but this effect was sporadic. Phosphorous (P) levels in the male HDT at all time points were significantly increased over controls. At the 52 and 78 week time points male (P) levels were also significantly increased. Female P levels were not significantly altered at any time point. Other parameters were occasionally significantly altered, but these alterations appeared sporadically at seemingly random times and doses.

Table 11. Male group mean clinical chemistry values. Glu in mg/dl; GPT in mU/ml; P in meq/l; chol in mg/dl.

Treatment	Glu	GPT	P	Chol
Control	26=125±15.2 52=135±18.2 78=129±17.4 102=103±12.8	26=29±19.3 52=54±17.8 78=47±49.5 102=53±42.5	26=3.5±0.14 52=2.9±0.13 78=3.0±0.15 102=2.9±0.29	26=75±15.1 52=113±29.2 78=156±54.8 102=178±26
10 ppm	26=121±8.8 52=128±11.7 78=124±11.6 102=106±16.2	26=23±6.5 52=36±17.8 78=53±41.4 102=34±12.5	26=3.4±0.21 52=2.8±0.15 78=3.0±0.24 102=2.9±0.29	26=80±15.2 52=111±23.3 78=151±66.4 102=181±26
80 ppm	26=116±8.9 52=122±12.4 78=125±12.8 102=99±11.4	26=25±27.2 52=71±34.3 78=46±41.1 **102=27±13.7***	26=3.4±0.2 52=2.9±0.24 78=3.1±0.27 102=3.1±0.20	26=80±12 52=123±25.2 78=131±44 102=169±92.8
640 ppm	26=113±17 **52=117±12.7**** 78=126±23.3 102=95±11.8	26=49±27.2 52=44±34.3 78=37±41.1 **102=29±11.7***	26=3.7±0.18 **52=3.3±0.33**** **78=3.5±0.27**** 102=3.1±0.43	26=92±15.2 52=110±19.2 78=139±44 102=165±36.6
1280 ppm	**26=93±21.8**** **52=109±15**** **78=103±16.1**** **102=89±13.6***	26=30±7.6 52=47±21.5 78=34±11.5 **102=27±8.6***	**26=4.2±0.34**** **52=3.5±0.3**** **78=3.4±0.2**** **102=3.2±0.23***	26=80±19.4 52=97±23.9 78=133±54.3 102=170±69.2

Data from pages 499 to 518, current study.

* Statistically significant at $p<0.05$.

** Statistically significant at $p<0.01$.

Table 12. Female group mean clinical chemistry values. Glu in mg/dl; GPT in mU/ml; Globulin in g/dl; chol in mg/dl.

Treatment	Glu	GPT	Globulin	Chol
Control	26=104±11.6 52=111±14.9 78= 122±18.7 102= 114±18.5	26=26±10.4 52=33±9.2 78=37±20.6 102= 38±16.3	26=3.7±0.43 52=4.1±0.31 78=4.0±0.42 102=4.2±0.24	26=91±15.7 52= 119±20.5 78= 141±33.4 102= 166±40
10 ppm	26=111±11.4 52=116±12.2 78=128±6.2 102= 118±13.6	**26=24±9.8*** 52= 28±6.9 78= 32±14.5 102= 36±8.3	26=3.7±0.41 52=4.2±0.26 78=4.0±0.35 102=4.3±0.45	26=90±13.7 52=121±31.6 78= 132±73 102=133±22
80 ppm	26=111±13.1 52= 124±10.1 78= 131±14.2 102= 112±13.8	**26=28±17.2**** **52=24±7.9*** **78= 21±5.2*** **102= 26±14.2***	26=4.0±0.38 52=4.3±0.3 78=4.1±0.3 102=4.4±0.28	**26=110±22.2*** 52=130±26.8 78= 127±42.1 102=174±52.6
640 ppm	**26=88±11.3**** 52= 101±16.7 **78= 105±8.8**** **102= 93±16.6****	26= 19±3.3 **52= 25±9.1*** **78= 22±12.6*** **102= 24±5.3***	**26=4.3±0.33**** **52=4.7±0.19**** **78=4.4±0.21*** **102=4.6±0.42***	26=121±19.4 **52=152±22.5**** 78=152 ±30.3 102=201±66.1

Data from pages 94 to 96, 502, 503, 507, 508, 512, 513, 517 and 518, current study.

* Statistically significant at $p<0.05$.

** Statistically significant at $p<0.01$.

F. Urinalysis - Urine protein levels in MDT2 and HDT males were significantly decreased, compared to controls, at the 13 and 26 week time points only. Female protein levels were not significantly altered at any time point. Significantly decreased urinary pH, compared to controls, was noted in MDT2 and HDT males at various time points. Female urinary pH was significantly decreased in the MDT and HDT at 102 weeks only.

Table 13: Urine pH and protein values. Protein in mg/dl.

Treatment	Male pH	Male Protein	Female pH	Female Protein
Control	13=7.0±0.29 26=7.1±0.38 52=7.1±0.51 78=6.9±0.29 102=7.1±0.28	13=152±115.2 26=141±18.5 52=261±167.8 78=396±534.2 102=267±232.2	13=6.2±0.32 26=6.5±0.21 52=6.2±0.25 78=6.3±0.20 102=6.2±0.19	13=58±19.6 26=85±18.4 52=152±125.3 78=108±56.2 102=135±104.3
10	13=6.9±0.29 26=7.2±0.37 52=7.1±0.39 78=7.1±0.71 102=7.0±0.40	13=192±167.5 26=145±32.9 52=300±271.0 78=512±615.5 102=399±348.5	13=6.2±0.24 26=6.6±0.20 52=6.4±0.24 78=6.2±0.28 102=6.3±0.32	13=71±14.0 26=67±14.9 52=156±167 78=288±565.6 102=112±42.0
80	13=7.2±0.29 26=6.9±0.32 52=7.3±0.61 78=7.1±0.40 102=6.8±0.53	13=214±174 26=150±28.9 52=245±292.3 78=320±407.1 102=499±575.7	13=6.4±0.24 26=6.5±0.36 52=6.3±0.28 78=6.3±0.27 **102=6.6±0.36****	13=61±10.7 26=82±23.4 52=142±113.4 78=100±56.8 102=213±234.6
640	13=6.7±0.29 26=6.9±0.26 **52=6.7±0.16*** **78=6.6±0.26*** 102=6.8±0.34	**13=123±81**** 26=125±15.6 52=241±206.3 78=510±635 102=364±277.7	13=6.2±0.19 26=6.3±0.20 52=6.2±0.24 78=6.1±0.15 **102=6.5±0.25****	13=63±13.6 26=77±16.6 52=85±20.2 78=109±51.4 102=346±577.5
1280	**13=6.5±0.21**** 26=7.0±0.21 **52=6.6±0.27*** **78=6.6±0.18*** 102=7.1±0.49	**13=96±11**** **26=109±10**** 52=194±216.9 78=378±439.6 102=233±244.4		

Data from pages 97 to 102 and 519 to 543, current study.
* Statistically significant at $p<0.05$.
** Statistically significant at $p<0.01$.

G. Sacrifice and Pathology:

1. Organ weight - Absolute organ weights were not statistically significantly affected in any dose group compared to controls for any organ in male and female rats. Though not significant, male HDT absolute pituitary and adrenal weights were >30% decreased

compared to controls. Female HDT thyroid and adrenal weights were reduced 24 and 40.7%, respectively, compared to controls.

Relative to body weights, male pituitary and adrenal weights were statistically significantly reduced compared to controls at the HDT, while liver and kidney weights were significantly increased compared to controls. Male relative liver weights were also significantly increased at the MDT2. Female relative liver and kidney weights were statistically significantly increased at the HDT compared to controls, while HDT relative heart weights were significantly decreased. Though not statistically significant, female relative adrenal weights were reduced 17% compared to controls.
Male and female relative liver and kidney weights were statistically significantly increased at their respective HDTs at the interim (12 month) sacrifice. Absolute organ weights and other relative body weight in either sex at any doses, were similar to control values.

Table 14. Group mean absolute organ weights at terminal sacrifice. Values are in grams, except for adrenal weights which are in milligrams. M=Males; F=Females

Treatment	Pituitary (mg)	Adrenals (mg)	Liver (g)	Kidneys (g)
Control	M=41.33±60.33 F=36.3±31.7	M=81.9±32.3 F= 133.6±195.4	M=25.4±4.54 F=17.6±4.21	M=5.12±1.35 F=3.22±0.479
10 ppm	M=40.5±40.68 F=46.6±40.74	M=81.7±30.7 F=98.7±32.5	M=25.8±6.96 F=19.8±4.13	M=5.34±1.01 F=3.53±0.687
80 ppm	M=42.0±74.2 F=52.4±55.0	M=76.4±25.02 F=108.1±64.82	M=25.5±4.51 F=18.6±3.89	M=5.11±1.12 F=3.3±0.438
640 ppm	M=27.2±28.3 F=32.9±50.94	M=67.1±16.04 F=79.1±31.69	M=29.7±5.51 F=17±3.15	M=5.39±0.778 F=3.28±0.698
1280 ppm	M=22.6±24.63	M=54.4±23.8	M=27.6±4.24	M=5.22±0.617

Data from pages 553 to 561, current study.

Table 15. Group mean organ weights relative to body weights at terminal sacrifice. M=Males; F=Females					
Treatment	**Pituitary**	**Adrenals**	**Heart**	**Liver**	**Kidneys**
Control	M=28.3 F=31.0#	M=74.2 F=97.9	M=1.94 F=1.40	M=23.9 F=17.3	M=4.85 F=3.18
10 ppm	M=31.4 F=31.5	M=74.3 F= 88.7	M=1.91 F=1.42	M=24.0 F=17.9	M=5.07 F=3.33
80 ppm	M=24.5 F=28.7	M=71.3 F=92.8	M=2.00 F=1.43	M=24.5 F=17.2	M=4.91 F=3.16
640 ppm	M=21.8 F=27.9	M=64.7 F=81.0	M=1.92 F=1.30*	M=29.5** F=19.9**	M=5.31 F=3.57**
1280 ppm	M=15.5**	M=55.2**	M=1.97	M=30.7**	M=5.52**

Data from page 106, current study
* Statistically significant at $p<0.05$
** Statistically significant at $p<0.01$
# female pituitary organ weights relative to body weight were calculated by the reviewer using data from page 106.

2. Gross pathology - MDT2 and HDT Male animals who were sacrificed on schedule at 102 weeks and males who were found dead or underwent an unscheduled sacrifice, were found to have increased incidences of alterations of the bones of the head (including the teeth) and liver alterations. HDT females also had an increased incidence of liver findings, but no increases in bone or teeth alteration were seen. Pale, overgrown, and thickened incisors were observed in MDT2 and HDT males as can be sen in Table 16. Alteration of the skull - such as thickening or bleaching of the cranium - were seen and were especially evident in the HDT group. The brains of the HDT group were observed to have an increased incidence of dorsal depression, which may be attributable to the cranial bone alterations. Liver findings seen in increased incidence in MDT2 and HDT males were pale subcapsular area and accentuated lobular markings.

   Females in the HDT also had an increased incidence of pale subcapsular areas in the liver. A slight increase in incidence of dark subcapsular area of the liver was also observed in HDT females. A slight increase in incidence of thickened cervix was seen in HDT females.

   The pale and thickened incisors were also seen in increased incidence in the interim sacrifice males of the MDT2 and HDT. No animals in the controls, LDT or MDT1 had thickened incisors, but 10 animals in each in the MDT2 and HDT groups had this finding. Nineteen HDT and 3 MDT2 interim sacrifice males had pale incisors compared to zero controls and one each in the LDT and MDT1. Nineteen HDT and 2

MDT2 interim sacrifice males had the observation of white cranium. This finding was not observed in any animal from any of the other dose groups at interim sacrifice. The liver finding of lobular markings accentuated was seen 2 MDT1, 7 MDT2, and 11 HDT interim sacrifice males but in no control of LDT males.

Females, in contrast to the males, showed few alterations in any finding at interim sacrifice in any dose group compared to controls. Even the liver findings, which were increased at terminal sacrifice in the females, were not increased at interim sacrifice. Females at interim sacrifice did show an increase in the number of females in the MDT and HDT with no corpea lutea in the ovaries. There was a very slight increase in pale and thickened incisors in the HDT compared to controls.

Table 16. Necropsy findings in males at terminal sacrifice or unscheduled sacrifice (interim sacrifice not included). Each group contained 50 animals. Ter- terminal; Uns-unscheduled

	Con-trol	10 ppm	80 ppm	640 ppm	1280 ppm
Brain-dorsal depression	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=2 Uns=0	Ter=26 Uns=1
Incisors-pale	Ter=0 Uns=0	Ter=0 Uns=1	Ter=2 Uns=1	Ter=3 Uns=8	Ter=35 Uns=6
Incisors-overgrown	Ter=3 Uns=1	Ter=0 Uns=2	Ter=4 Uns=1	Ter=6 Uns=8	Ter=17 Uns=2
Incisors-Thickened	Ter=0 Uns=0	Ter=0 Uns=1	Ter=0 Uns=1	Ter=15 Uns=10	Ter=43 Uns=4
Liver-pale subcapsular area	Ter=1 Uns=5	Ter=4 Uns=3	Ter=6 Uns=2	Ter=19 Uns=5	Ter=28 Uns=1
Liver-lobular markings accentuated	Ter=0 Uns=2	Ter=1 Uns=3	Ter=1 Uns=1	Ter=8 Uns=6	Ter=24 Uns=1
Bone-white cranium	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=1	Ter=30 Uns=3
Bone-thickened cranium	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=1	Ter=1 Uns=3	Ter=32 Uns=2
Bone-parietal thickened area	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=3 Uns=3	Ter=25 Uns=0

Data from pages 112 to 118, current study.

Table 17. Necropsy findings in females at terminal sacrifice or unscheduled sacrifice (interim sacrifice not included). Each group contained 50 animals. Ter- terminal; Uns-unscheduled

	Control	10 ppm	80 ppm	640 ppm
Liver-pale subcapsular area	Ter=1 Uns=4	Ter=8 Uns=1	Ter=10 Uns=1	Ter=16 Uns=1
Liver-dark subcapsular area	Ter=15 Uns=12	Ter=18 Uns=12	Ter=26 Uns=2	Ter=29 Uns=5
Incisors- Thickened	Ter=0 Uns=0	Ter=0 Uns=1	Ter=0 Uns=0	Ter=3 Uns=1
Incisors- Pale	Ter=0 Uns=1	Ter=1 Uns=1	Ter=2 Uns=1	Ter=4 Uns=3
Cervix- thickened	Ter=7 Uns=1	Ter=9 Uns=0	Ter=9 Uns=2	Ter=10 Uns=1

Data from pages 119 to 123, current study.

3. Microscopic pathology -

a) Non-neoplastic - The alterations seen at terminal histopathology, or histopathology of animals that died an unscheduled death, is focused on the liver, thyroid and cranial bones in males and the liver in the female. The liver effects, though seen in both sexes, appeared to be more severe in the males. Males of the MDT2 and HDT groups displayed increased incidences of enlargement of both centrilobular and midzonal hepatocytes, fine vacuolization of midzonal hepatocytes, eosinophilic vacuolization of hepatocytes, bile duct proliferation and an increase in hepatic adipose deposits (detected by staining with Oil Red O). Increased incidence of enlargement of centrilobular hepatocytes was also seen in MDT1 males. HDT males showed an increased incidence of thyroid follicle cystic atrophy, compared to controls. An increase in cystic follicles of the thyroid was seen in all dose groups compared to control, but the increase was not dose-related. There was a slight increase the appearance of enlarged, vacuolated cells in MDT2 and HDT males compared to controls. There was an increase in osseous hypertrophy of both the cranium and parietal bones in both the MDT2 and HDT groups, but this was especially evident in the HDT group. Evidence of dorsal compression of the brain in the HDT was seen which was likely due to hypertrophy of the cranium.

Terminal sacrifice and unscheduled sacrifice females of the MDT and HDT groups displayed increased incidences of enlargement of centrilobular hepatocytes and eosinophilic vacuolization of hepatocytes. A very slight increase in fine vacuolization

of midzonal hepatocytes was seen at the HDT only. An increase in bile duct proliferation was seen at the HDT only.

Male rats killed at interim sacrifice displayed many of the same liver findings at the same doses as were seen in rats killed at terminal sacrifice. The bone and thyroid findings seen at terminal sacrifice were not seen at interim sacrifice. There was an increased incidence of pyelitis in the dosed males. The incidences were 4/19 in the HDT, 3/19 in the MDT2, 1/20 in the MDT1 and 1/19 in the LDT. No animals in the control group displayed this finding. The thickened and pale incisors seen at gross necropsy and clinical observations were confirmed at histology, but histology did not reveal any explanations for these effects.

Females in the HDT group sacrificed at 52 weeks showed an increase in centrilobular hepatocyte enlargement. There was also an increase in the number of females in the HDT at interim sacrifice who did not have corpea lutea in the ovaries. There was an increased incidence of squamous metaplasia in the endometrial glands of HDT and MDT females compared to controls (8/19 HDT, 5/20 MDT, 0/20 LDT and 2/20 .

Table 18. Nonneoplastic histopathology findings in males at terminal sacrifice or dying an unscheduled death. Ter- terminal; Uns-unscheduled (50 animals examined in the control and high-dose groups)

Histopathology	control	10 ppm	80 ppm	640 ppm	1280 ppm
Liver- Centri. Hep. Enlarg.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=14 Uns=3	Ter=29 Uns=15	Ter=40 Uns=4
Liver- Midzon. Hep. Enlarg.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=16 Uns=0
Liver- Centri. Inflam. Cell	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=1	Ter=9 Uns=0
Liver- Fine Vac. Centri. Hep.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=1 Uns=1	Ter=4 Uns=8	Ter=25 Uns=1
Liver- Fine Vac. Midzon. Hep.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=16 Uns=2	Ter=32 Uns=1
Liver- Eosin. Vac. Hep.	Ter=3 Uns=3	Ter=3 Uns=1	Ter=5 Uns=3	Ter=12 Uns=0	Ter=10 Uns=1
Liver- Bile Duct Hyperplas.	Ter=6 Uns=6	Ter=9 Uns=3	Ter=11 Uns=6	Ter=9 Uns=8	Ter=26 Uns=3

Thyroid- Cystic Foll. Atrophy	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=6 Uns=0
Thyroid-Cystic Foll.	Ter=0 Uns=1	Ter=5 Uns=0	Ter=2 Uns=0	Ter=8 Uns=0	Ter=3 Uns=1
Piuitary- Enlarged Vac. Cells	Ter=7 Uns=4	Ter=1 Uns=8	Ter=5 Uns=2	Ter=12 Uns=7	Ter=17 Uns=2
Brain-Evidence of dorsal comp.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=1 Uns=0	Ter=24 Uns=0
Bone- Osseous Hyper. Of Cranium	Ter=0 Uns=0	Ter=0 Uns=0	Ter=2 Uns=1	Ter=3 Uns=5	Ter=42 Uns=3
Bone- Osseous Hyper. Of Parietal	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=4 Uns=4	Ter=41 Uns=2

Data from pages 174-207, current study.

Table 19. Nonneoplastic histopathology findings in females at terminal sacrifice or dying an unscheduled death. Ter- terminal; Uns-unscheduled (50 animals examined in the control and high-dose groups)

Histopathology	Control	10 ppm	80 ppm	640 ppm
Liver- Centri. Hep. Enlarg.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=16 Uns=2	Ter=38 Uns=11
Liver- Fine Vac. Centri. Hep.	Ter=0 Uns=0	Ter=0 Uns=0	Ter=0 Uns=0	Ter=2 Uns=1
Liver- Eosin. Vac. Hep.	Ter=3 Uns=0	Ter=3 Uns=2	Ter=15 Uns=2	Ter=15 Uns=1
Liver- Bile Duct Hyperplas.	Ter=4 Uns=3	Ter=4 Uns=3	Ter=10 Uns=1	Ter=12 Uns=4

Data from pages 208-231, current study.

b) Neoplastic - There was an increase follicular adenomas of the thyroid in males of the MDT1, MDT2 and HDT. This increase was not great and did not achieve statistical significance. There were no other tumors in either sex which appeared to found at increased levels in dosed animals *vs* controls.

Table 20: Incidence of thyroid follicular adenomas in males at terminal sacrifice or unscheduled sacrifice (interim sacrifice not included). Each group contained 50 animals. Ter- terminal; Uns-unscheduled

Histopathology	Control	10 ppm	80 ppm	640 ppm	1260 ppm	Historical control
Thyroid-follicular adenoma	Ter=1 (4.2%) Uns=0	Ter=1 (3.4%) Uns=0	Ter=3 (9.1%) Uns=0	Ter=4 (13.8%) Uns=0	Ter=5 (11.6%) Uns=0	Ter= $\bar{x}$ =9.7% range= 0-19.6% Uns= $\bar{x}$ 1.9% range= 0-9.1

Data from pages 119 to 123, current study; Historical control data represents the mean and range of data from 8 two-year separate studies conducted in CD-1/SD rats in the examining laboratory.

III. DISCUSSION

A. The study author concludes that the main target organ in this study was the liver. The reviewer agrees with this assessment. The reviewer also believes that the thyroid in males (but not females) was adversely affected by tetraconazole exposure. Overall, the males appear to be more sensitive to the adverse effects of tetraconazole exposure.

Liver - males
Both the 640 and 1280 ppm males had histopathology findings of: hepatocyte enlargement; inflammatory cell foci; bile duct hyperplasia; cystic degeneration; eosinophilic hepatocytes; increased fat deposition; and fine vacuolation. At necropsy males in these two groups had increased incidence of pale livers and livers with lobular markings accentuated. Group mean liver weights relative to body weight were significantly increased at the 640 and 1280 ppm doses in males. There were no alterations of clinical chemistry parameters which were likely to have been induced by compound exposure. Males at the 80 ppm dose had hepatic alterations, but there were fewer types of alterations, fewer animals displayed these alterations, and they were generally less severe. Hepatocyte enlargement, cystic degeneration, and eosinophilic hepatocytes were seen in increased incidence in 80 ppm males. At the 10 ppm dose the only liver alteration observed at histopathology was an increased incidence of basophilic hepatocytes. Organ weights were not altered in either of these two dose groups and there were no alterations in clinical chemistry which would indicate a toxic effect on the liver.

Skeletal system - males
The other area which seemed to be affected by compound exposure in the males were ceratin aspects of the skeletal system (bones of the skull and the incisors). The bones of the cranium in the male 640 and 1280 ppm groups were found to have increased incidence of thickening and bleaching (white color), and the parietal bones were also thickened. These findings were evident in males of both dose groups but occurred at much higher frequency in the 1280 ppm group (see table 16). Histopathology findings of osseous hypertrophy of cranium and parietal bones at the 640 and 1280 ppm groups confirmed the findings in these bones seen at necropsy. Pale, thickened, and overgrown incisors were also seen in males of the 640 and 1280 ppm groups. The pattern here was similar to the pattern seen with the cranial bones in which the incidence in the 1280 ppm group is much higher than in the 640 ppm group. The 10 and 80 ppm dose groups did not show any toxicologically significant increases in these findings. No male in either the 10 or 80 ppm group displayed a thickened cranium or parietal bones, and only one male in the 80 ppm group showed a white cranium while no 10 ppm males displayed this finding. Only one male each in the 10 and 80 ppm group displayed thickened incisors. The incidence of overgrown incisors was less at 10 ppm than it was in the controls and at 80 ppm only one more male had overgrown incisors compared to controls (5 compared to 4). The incidence of pale incisors was very slightly decreased at 10 and 80 ppm compared to controls (0 in

control, 1 at 10 and 3 at 80 ppm).

Other alterations - 640 and 1280 ppm males
Body weights were decreased at the end of the end of the study in both these groups (7.5% at 640 ppm and 24% at 1280). Food consumption for the entire study was statistically significantly decreased in both groups (5% at 640 ppm and 15% at 1280). Both absolute and relative adrenal and pituitary weights were decreased in males of these dose groups. Relative liver weights were significantly reduced at these doses, but absolute weights were similar to controls. The statistically significant decrease in serum glucose levels at 1240 ppm was likely a secondary effect of treatment related to the decrease in food consumption and body weight. The statistically significant decreases seen in both groups, at various time points, of packed cell volume, hemoglobin, and red blood cell counts was also likely due to decreased food consumption and body weight. The statistically significant increases in serum phosphorus levels seen at time points in the 1280 ppm group may be related to compound exposure. There was an increase in cystic follicle atrophy in the HDT. The alterations in urinalysis values are not likely to be compound related.

Other alterations - 10 and 80 ppm males
Body weights and food consumption were similar to control values. There were no alterations in organ weights or hematology parameters which could be attributed to compound exposure. There was a statistically significant decrease in serum alanine aminotransferase (GPT) level at 102 weeks in the 80 ppm males. This effect is not considered to be of toxicological significance.

Liver - females
There was a dose-related increase in both light and dark subcapsular areas at necropsy in the females. This increase was very slight for both parameters at 10 ppm but it did increase with dose. Histopathology was able to confirm these findings only partially. There was an increase in centrilobular hepatocyte enlargement, bile duct hyperplasia and eosinophilic vacuolation of hepatocytes at both the 80 and 640 ppm groups. The 10 ppm group did not show an increase in bile duct hyperplasia and showed only a very slight increase in eosinophilic vacuolation of hepatocytes. No females at all were found to have centrilobular hepatocyte enlargement in this group. Relative liver weights were significantly decreased at the HDT in females. There were no alterations in clinical chemistry or hematology values which would be indicative of hepatic toxicity.

Skeletal system - females
The alterations of the cranial bones seen in the two high dose male groups were not seen in the females at any dose group. There were alterations in the incisors of high dose females, but a much lower percentage of females were observed to have these findings than in the males. Histopathology did not reveal the presence of osseous hypertrophy of the cranial bones in the females as it did in the males. There was a slight increase in thickened incisors in the 640 ppm group and a slight increase in pale incisors in all dose groups

compared to controls. The increases in pale incisors at 10 and 80 ppm were very small though (2 females in the 10 ppm group and 3 in the 80 ppm group compared to zero in controls).

Other alterations - females
Female body weights, body weight gains and food consumption were decreased at the 640 ppm dose only (24% decrease). Females at the high dose only showed a decrease in absolute pituitary and adrenal weights. Relative pituitary weights were not altered among groups in a dose-related manner. Relative adrenal weights were decreased at the HDT, but not significantly so. The statistically significant decrease in serum glucose levels at 640 ppm was likely a secondary effect of treatment related to the decrease in food consumption and body weight. The statistically significant decreases seen in both groups, at various time points, of packed cell volume, hemoglobin, and red blood cell counts was also likely due to decreased food consumption and body weight. The statistically significant increases in serum phosphorus levels seen at several time points in the 640 ppm group may be related to compound exposure. The alterations in urinalysis values are not likely to be compound related.

Conclusions and LOAEL and NOAEL
The livers and skeletal systems of both sexes were affected by compound exposure and the thyroid of males was also affected. The study authors note their belief that the effects seen in the liver at 80 ppm were of an adaptive, rather than a toxicological, nature. The reviewer agrees with this assessment. The findings such as hepatocyte enlargement and basophilic or eosinophilic hepatocytes may be indicative of an adaptive response as well as a toxicologic one. Levels of liver enzymes in the serum, such as alkaline phosphatase (AP) and aspartate aminotransferase (GOT), are not increased at the 80 ppm dose. Mean AP levels are actually 15% lower in 80 ppm males compared to controls and GOT level are also slightly lower. At the 1280 ppm dose in males finding such as inflammatory cell foci and bile duct hyperplasia are seen - findings not observed in increased incidence in the 10 or 80 ppm males.

The skeletal alterations seen in males at the 10 and 80 ppm groups are limited to only one male in each group with thickened incisors and 1 to 3 in each group with pale incisors. The increase in overgrown incisors from control to 80 ppm was very slight - 4 to 5. The higher dose groups displayed much larger numbers of males with incisor alterations and also had many males with white thickened skull bones. Additionally, histopathology supported the skull bone findings with the finding of increased incidence of osseous hypertrophy in the 640 and 1280 ppm groups. This finding was not increased in the two lower dose groups. The alterations in serum phosphorous levels seen in 640 and 1280 ppm males, but not in 10 or 80 ppm males, again emphasizes that the effects seen in the skeletal system at the two higher doses are likely compound-related and of toxicological significance, while the findings seen at the two lower doses may be compound-related but are not likely of toxicologic significance.

findings seen at the two lower doses may be compound-related but are not likely of toxicologic significance.

The LOAEL in males is 640 ppm based on histopathology of the bone (osseous hypertrophy of the cranium/parietal bone) seen at both terminal and unscheduled sacrifice, pale and thickened incisors, and decreased absolute and relative adrenal and pituitary weights. The NOAEL is 80 ppm in males.

The liver and skeletal effects in females were not as dramatic or extensive as the effects seen in males. The increase in enlarged hepatocytes and eosinophilic vacuolation at 80 ppm was likely to be an adaptive response and the increase in bile duct hyperplasia at this dose was minor (7 in controls *vs* 11 in the 80 ppm group). There are no clinical chemistry findings to support the histopathology findings, and liver weights, neither relative nor absolute, are not significantly altered at this dose.

The LOAEL in females is 640 ppm based on a 24% decreased body weight at terminal sacrifice compared to controls. The NOAEL is 80 ppm in females.

Under the conditions of this study, dosing is considered adequate to assess the carcinogenic potential of tetraconazole, based on histopathology of the bone (osseous hypertrophy of the cranium/parietal bone), pale and thickened incisors, and decreased absolute and relative adrenal and pituitary weights in males at 640 ppm and decreased body weight (at terminal sacrifice) in females at 640 ppm and in males at 1280 ppm. The reviewer agrees with the study author's conclusion that tetraconazole is not carcinogenic at dose levels tested. Although there is an increase in the incidence of thyroid adenomas in males at doses ≥80 ppm (4.2%, 9.1%, 13.8% and 11.6% at 0, 10, 80, 640 and 1280 ppm, respectively), these values did not achieve statistical significance and were well within the historical control range (mean of 9.7%; range of 0-19.6%) for male rats of this strain in studies conducted by the examining laboratory. **Therefore, tetraconazole exposure up to 1280 ppm in males and 640 ppm in females, is not carcinogenic in this study.**

B. Study deficiencies - Statistical analyses were not included in the study report for body weights and food consumption. This deficiency did not alter the interpretation of the data presented in the rat combined chronic toxicity/carcinogenicity study.

013540

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

MEMORANDUM June 30, 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Tetraconazole™ Qualitative Risk Assessment Based On Crl:CD(SD)BR Rat and Crl:CD-1(ICR) Mouse Dietary Studies

P.C. Code 120603

TO: David Nixon, Toxicologist
Toxicology Branch 1
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

Lori L. Brunsman

THROUGH: William L. Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

/s/ for W Burnam
6/30/99

Background

An oncogenicity study in Crl:CD(SD)BR rats was conducted by Huntingdon Research Centre, Ltd., Cambridgeshire, England, for Sostram Corporation, Landis International, Inc., Valdosta, Georgia, and issued December 10, 1992 (Laboratory Report No. AGR 74/911683; MRID No. 443053-04).

The study design allocated groups of 50 rats per sex to dose levels of 0, 10, 80, 640, or 1280 (males only) ppm of Tetraconazole for 105 weeks. An additional 20 rats per sex per dose were designated for interim sacrifice at week 53.

An oncogenicity study in Crl:CD-1(ICR) mice was conducted by Huntingdon Research Centre, Ltd., Cambridgeshire, England, for Sostram Corporation, Landis International, Inc., Valdosta, Georgia, and issued December 10, 1998 (Laboratory Report No. AGR 73/920469; MRID No. 443053-05).

The study design allocated groups of 50 mice per sex to dose levels of 0, 10, 90, 800, or 1250 ppm of Tetraconazole for 81 weeks.

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Survival Analyses

The statistical evaluation of mortality indicated significant decreasing trends with increasing doses of Tetraconazole in male and female rats. Male and female mice had significant increasing trends for mortality with increasing doses of Tetraconazole. See Tables 1 and 2 for rat mortality test results, and Tables 4 and 5 for mouse mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

There were no significant compound-related tumors observed in male or female rats.

Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 1250 ppm dose group with the controls, for liver benign, malignant and benign and/or malignant tumors combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 800 ppm dose group with the controls for liver benign tumors and for benign and/or malignant tumors combined, both at $p < 0.01$.

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 1250 ppm dose group with the controls, for liver benign, malignant and benign and/or malignant tumors combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 800 ppm dose group with the controls for liver benign tumors and for benign and/or malignant tumors combined, both at $p < 0.01$. Female mice had a significant increasing trend in ovarian benign luteomas at $p < 0.01$.

The statistical analyses of the male and female rats and mice were based upon Peto's Prevalence Test since there was statistically significant differential mortality with increasing doses of Tetraconazole in both sexes of both species. See Table 3 for rat tumor analysis results. See Tables 6-9 for mouse tumor analysis results.

Table 1. Tetraconazole™ - Crl:CD(SD)BR Rat Study

Male Mortality Rates[t] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[i]	53-78	79-105[f]	Total
0	1/70	4/69	18/65	11/47	12/36	28/52**[n] (54)
10	1/70	1/69	19/68	4/49	16/45	22/51 (43)
80	3/70	0/67	20/67	3/47	11/44	17/50 (34)
640	1/70	4/69	19/65	3/46	14/43	22/51 (43)
1280	0/70	1/70	19/69	3/50	4/47	8/51**[n] (16)

[t]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

[n]Negative trend or negative change from control.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 2. Tetraconazole™ - Crl:CD(SD)BR Rat Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks					Total
	1-26	27-52	53[i]	53-78	79-105[f]	
0	0/70	0/70	20/70	8/50	14/42	22/50*[n] (44)
10	0/70	0/70	20/70	7/50	17/43	24/50 (48)
80	0/70	1/70	20/69	5/49	7/44	13/50 (26)
640	0/70	2/70	19/68	2/49	9/47	13/51 (25)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

[n]Negative trend.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Tetraconazole™ - Crl:CD(SD)BR Rat Study

Male Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	10	80	640	1280
Adenomas (%)	1/24 (4)	1/29 (3)	3/33 (9)	4/29 (14)	5/43 (12)
p =	0.103	-	0.238	0.119	0.154
Carcinomas (%)	0/24 (0)	1/29 (3)	0/33 (0)	2/29 (7)	1/43 (2)
p=	0.276	0.181	-	0.097	0.228
Combined (%)	1/24 (4)	2/29 (7)	3/33 (9)	4[a]/29 (14)	6/43 (14)
p =	0.089	0.336	0.238	0.119	0.106

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

First thyroid follicular cell adenoma and carcinoma observed at week 105, in final sacrifice animals, concurrently in all dose groups.

[a]Two animals in the 640 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no thyroid follicular cell adenomas or carcinomas in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	1-26	27-34	35-52	53-81[f]	Total
0	1/50	0/49	1/49	10/48	12/50** (24)
10	0/50	0/50	3/50	14/47	17/50 (34)
90	1/50	0/49	2/49	8/47	11/50 (22)
800	1/50	0/49	1/49	9/48	11/50 (22)
1250	0/50	2/50	5/48	29/43	36/50** (72)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 81.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-34	35-52	53-83[f]	Total
0	1/50	0/49	0/49	8/49	9/50* (18)
10	0/50	0/50	0/50	11/50	11/50 (22)
90	0/50	0/50	0/50	9/50	9/50 (18)
800	1/50	0/49	0/49	7/49	8/50 (16)
1250	0/50	0/50	0/50	18/50	18/50* (36)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 81.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Male Liver Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	10	90	800	1250
Benign (%)	9/49 (18)	8/50 (16)	6/49 (12)	22/49 (45)	34[a]/49 (69)
p =	0.000**	-	-	0.003**	0.000**
Malignant (%)	1/48 (2)	2/47 (4)	2/47 (4)	4/48 (8)	20[b]/45 (44)
p=	0.000**	0.398	0.059	0.134	0.000**
Combined (%)	10/49 (20)	9[c]/50 (18)	7[c]/49 (14)	24[d]/49 (49)	42[e]/49 (86)
p =	0.000**	-	-	0.002**	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First liver benign tumor observed at week 34, dose 1250 ppm.

[b]First liver malignant tumor observed at week 50, dose 1250 ppm.

[c]One animal in each of the 10 and 90 ppm dose groups had both an adenoma and a carcinoma.

[d]Two animals in the 800 ppm dose group had both an adenoma and a carcinoma.

[e]Twelve animals in the 1250 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 7. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Female Liver Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	10	90	800	1250
Benign (%)	0/49 (0)	0/49 (0)	0/50 (0)	11/49 (22)	26[a]/49 (53)
p =	0.000**	-	-	0.000**	0.000**
Malignant (%)	0/48 (0)	0/47 (0)	0/50 (0)	1/49 (2)	17[b]/44 (39)
p=	0.000**	-	-	0.162	0.000**
Combined (%)	0/49 (0)	0/49 (0)	0/50 (0)	11[c]/49 (22)	32[d]/49 (65)
p =	0.000**	-	-	0.000**	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First liver benign tumor observed at week 57, dose 1250 ppm.

[b]First liver malignant tumor observed at week 64, dose 1250 ppm.

[c]One animal in the 800 ppm dose group had both an adenoma and a carcinoma.

[d]Eleven animals in the 1250 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 8. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Female Ovarian Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	10	90	800	1250
Benign Luteomas (%)	2/41 (5)	0/39 (0)	0/41 (0)	2/42 (5)	4/32 (12)
p =	0.006**	-	-	-	0.121

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

First ovarian benign luteomas observed at week 81, in final sacrifice animals, concurrently in all dose groups.

Table 9. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Female Lymphoid Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	10	90	800	1250
Lymphomas (%)	2[a]/49 (4)	3/12[#] (25)	0/10[#] (0)	1/49 (2)	4/47 (9)
p =	0.372	-	-	-	0.327

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First lymphoma observed at week 20, dose 0 ppm.

[#]Not all animals in the 10 and 90 ppm dose groups had complete microscopic examinations for lymphoid tissues. Therefore, no appropriate statistical comparisons of these dose groups with the controls could be made.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

References

Cox, D.R. (1972) Regression Models and Life Tables (with discussion). J. Royal Stat. Soc. Ser. B. 34, 187-220.

Gart, J.J., D. Krewski, P.N. Lee, R.E. Tarone, and J. Wahrendorf (1986) The Design and Analysis of Long-Term Animal Experiments. In: Statistical Methods in Cancer Research, Volume III. IARC Scientific Publications No. 79. Lyon, France: International Agency for Research on Cancer, p. 18.

Peto, R., M. Pike, N. Day, R. Gray, P. Lee, S. Parish, J. Peto, S. Richard, and J. Wahrendorf (1980) Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic Effects in Long-Term Animal Experiments. In: Monographs on the long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2. Lyon, France: International Agency for Research on Cancer, pp. 311-426.

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

013594

MEMORANDUM June 30, 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Tetraconazole™ Qualitative Risk Assessment Based On Crl:CD(SD)BR Rat and Crl:CD-1(ICR) Mouse Dietary Studies

P.C. Code 120603

TO: David Nixon, Toxicologist
Toxicology Branch 1
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

Lori L. Brunsman

THROUGH: William L. Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

/1 for W Burnam
6/30/99

Background

An oncogenicity study in Crl:CD(SD)BR rats was conducted by Huntingdon Research Centre, Ltd., Cambridgeshire, England, for Sostram Corporation, Landis International, Inc., Valdosta, Georgia, and issued December 10, 1992 (Laboratory Report No. AGR 74/911683; MRID No. 443053-04).

The study design allocated groups of 50 rats per sex to dose levels of 0, 10, 80, 640, or 1280 (males only) ppm of Tetraconazole for 105 weeks. An additional 20 rats per sex per dose were designated for interim sacrifice at week 53.

An oncogenicity study in Crl:CD-1(ICR) mice was conducted by Huntingdon Research Centre, Ltd., Cambridgeshire, England, for Sostram Corporation, Landis International, Inc., Valdosta, Georgia, and issued December 10, 1998 (Laboratory Report No. AGR 73/920469; MRID No. 443053-05).

The study design allocated groups of 50 mice per sex to dose levels of 0, 10, 90, 800, or 1250 ppm of Tetraconazole for 81 weeks.

Table 1. Tetraconazole™ - Crl:CD(SD)BR Rat Study

Male Mortality Rates[†] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[i]	53-78	79-105[f]	Total
0	1/70	4/69	18/65	11/47	12/36	28/52**[n] (54)
10	1/70	1/69	19/68	4/49	16/45	22/51 (43)
80	3/70	0/67	20/67	3/47	11/44	17/50 (34)
640	1/70	4/69	19/65	3/46	14/43	22/51 (43)
1280	0/70	1/70	19/69	3/50	4/47	8/51**[n] (16)

[†]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

[n]Negative trend or negative change from control.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Tetraconazole™ - Crl:CD(SD)BR Rat Study

Male Thyroid Follicular Cell Tumor Rates[†] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	10	80	640	1280
Adenomas (%)	1/24 (4)	1/29 (3)	3/33 (9)	4/29 (14)	5/43 (12)
p =	0.103	-	0.238	0.119	0.154
Carcinomas (%)	0/24 (0)	1/29 (3)	0/33 (0)	2/29 (7)	1/43 (2)
p=	0.276	0.181	-	0.097	0.228
Combined (%)	1/24 (4)	2/29 (7)	3/33 (9)	4[a]/29 (14)	6/43 (14)
p =	0.089	0.336	0.238	0.119	0.106

[†]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

First thyroid follicular cell adenoma and carcinoma observed at week 105, in final sacrifice animals, concurrently in all dose groups.

[a]Two animals in the 640 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no thyroid follicular cell adenomas or carcinomas in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-34	35-52	53-83[f]	Total
0	1/50	0/49	0/49	8/49	9/50* (18)
10	0/50	0/50	0/50	11/50	11/50 (22)
90	0/50	0/50	0/50	9/50	9/50 (18)
800	1/50	0/49	0/49	7/49	8/50 (16)
1250	0/50	0/50	0/50	18/50	18/50* (36)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 81.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 7. Tetraconazole™ - Crl:CD-1(ICR) Mouse Study

Female Liver Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	10	90	800	1250
Benign (%)	0/49 (0)	0/49 (0)	0/50 (0)	11/49 (22)	26[a]/49 (53)
p =	0.000**	-	-	0.000**	0.000**
Malignant (%)	0/48 (0)	0/47 (0)	0/50 (0)	1/49 (2)	17[b]/44 (39)
p=	0.000**	-	-	0.162	0.000**
Combined (%)	0/49 (0)	0/49 (0)	0/50 (0)	11[c]/49 (22)	32[d]/49 (65)
p =	0.000**	-	-	0.000**	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First liver benign tumor observed at week 57, dose 1250 ppm.

[b]First liver malignant tumor observed at week 64, dose 1250 ppm.

[c]One animal in the 800 ppm dose group had both an adenoma and a carcinoma.

[d]Eleven animals in the 1250 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

References

Cox, D.R. (1972) Regression Models and Life Tables (with discussion). J. Royal Stat. Soc. Ser. B. 34, 187-220.

Gart, J.J., D. Krewski, P.N. Lee, R.E. Tarone, and J. Wahrendorf (1986) The Design and Analysis of Long-Term Animal Experiments. In: Statistical Methods in Cancer Research, Volume III. IARC Scientific Publications No. 79. Lyon, France: International Agency for Research on Cancer, p. 18.

Peto, R., M. Pike, N. Day, R. Gray, P. Lee, S. Parish, J. Peto, S. Richard, and J. Wahrendorf (1980) Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic Effects in Long-Term Animal Experiments. In: Monographs on the long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2. Lyon, France: International Agency for Research on Cancer, pp. 311-426.

Thomas, D.G., N. Breslow, and J.J. Gart (1977) Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381.

EPA Reviewer: Suhair Shallal, Ph.D. Date 6/9/99
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Yung Yang, Ph.D. Date 6/9/99
Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity- Mouse; OPPTS 870.4100 [§83-2]

DP BARCODE: D238518 SUBMISSION CODE: S528887
P.C. CODE: 120603 ID NO.: 7E4830
CASE NO.: 288762

TEST MATERIAL (PURITY) : M 14360 (Tetraconazole), (95.05 %)

SYNONYMS: 1H-1,2,4-Triazole; 1[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetraflouroethoxy) propyl]-1,2,4-triazole

CITATION: Crome, S., M.E. Bellringer, et al, (1992) M 14360 Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice. Huntingdon Research Centre, Ltd., Cambridgeshire, England. Report # AGR73/920469, December 10, 1998. MRID # 44305305. Unpublished.

SPONSOR: Sostram Corp., Landis International, Inc., Valdosta, GA

EXECUTIVE SUMMARY:

In a carcinogenicity study (MRID 44305305) M14360 (95.05% a.i.) was administered to 50 Crl:CD-1 (ICR) mice/sex/dose in their diet at dose levels of 0, 10, 90, 800, 1250 ppm (for males: 0, 1.4, 12, 118, 217 mg/kg/day; for females: 0, 1.6, 14.8, 140, 224 mg/kg/day) for 80 weeks.

Significantly increased mortality was observed at the highest dose (72% for males and 34% for females) compared to the control (24% for males and 16% for females). Increased incidence of swollen, hard, or dark abdomens was noted in mice (both sexes) receiving 1250 ppm of the test material. Although absolute mean body weights were comparable among groups, decreased body weight gains were observed at 800 ppm (73% of the control in both sexes) and at 1250 ppm (73% and 80% of the control for males and females, respectively) at the end of the study.

Gross pathology revealed slight to severe changes in the liver which correlated with the dose given. At 90 ppm, the liver appeared pale with accentuated lobular markings. At higher concentrations (800-1250 ppm), masses were found with raised, pale, or dark subcapsular areas. Masses were also found in the

kidneys of male animals receiving 1250 ppm M14360. Dose-related increase of liver weights were noted in mice of both sexes given ≥90 ppm of the test material. Kidney weights were also found to increase slightly (9-11 % above controls) in males receiving ≥90 ppm.

Histopathological findings revealed liver toxicity including hepatocyte vacuolation, fat deposition, granulomatous inflammation, pigmented macrophages, generalised hepatocyte enlargement, and bile duct hyperplasia in mice receiving 1250 and 800 ppm of the test material. In addition, at the high dose of 1250 ppm, non-neoplastic changes were noted in the brain, lungs, kidneys, testes, epididymides, and ovaries. Of particular interest is the sizeable increase in the absence of corpora lutea in the ovaries and in the absence of sperm in the epididymides at 800 and 1250 ppm and the effects on the testes (reduced spermatogenesis, tubular atrophy, and interstitial cell hyperplasia) at 1250 ppm. Thickening of compact bone of the cranium, in the ribs, collar bone (females only), and femur (females only) at 800 and 1250 ppm also appears to be treatment related.

Neoplastic findings were noted in the liver where a statistically significant increased incidence of combined benign and malignant liver cell tumors was observed at 1250 ppm (84% for males and 64% for females) and 800 ppm (48% for males and 22% for females) compared to the control (20% for males and 0% for females). The tumor incidence in animals receiving ≤90 ppm was found to be similar to that of controls. Historical control data show that the incidence of hepatocellular adenomas ranged from 7.7% to 24% in males and 0% to 1.9% in females and the incidence of hepatocellular carcinomas ranged from 0% to 14% in males and was 0% in females.

The systemic toxicity LOAEL is 90 ppm (12 and 14.8 mg/kg/day for males and females, respectively), based on increased liver weight and hepatocyte vacuolation in both sexes and increased kidney weights in males. **The NOAEL is 10 ppm (1.4 and 1.6 mg/kg/day for males and females, respectively).**

There was evidence of increased incidence of combined benign and malignant liver tumors in mice of both sexes treated with M14360 at 800 ppm (48% for males and 22% for females) and 1250 ppm (84% for males and 64% for females) compared to the control (20% for males and 0% for females). The doses were found to be adequate to test its carcinogenic potential based on the reduction of body weight gain and increased mortality at the highest dose.

This study is classified Acceptable/ Guideline and satisfies the guideline requirement for a carcinogenicity study (83-2; OPPTS 870.4100) 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: M14360
   Description: technical, brown, viscous liquid
   Lot/Batch #: FCF/T/80-89
   Purity: 95.05 % a.i.
   Stability of compound: Not provided; the report stated that the compound was stable til May 1992

2. Vehicle: Acetone/ diet

3. Test animals: Species: Mouse
   Strain: Crl:CD-1 (ICR)
   Age and weight at study initiation: 28 days; (19-28) g females, (22-34) g males
   Source: Charles River Breeding Laboratories, Portage, Michigan
   Housing: solid bottom polypropylene cages, 2 animals/cage/sex
   Diet: SDS Rat and Mouse No.1 modified maintenance diet (ground) ad libitum
   Water: tap water ad libitum
   Environmental conditions: Temperature: 21 ± 2°C
   Relative Humidity: 50 ± 10 %
   Air changes: not provided
   Photoperiod: 12 hrs. light/ 12 hrs dark
   Acclimation period: 14 days

B. STUDY DESIGN:

1. In life dates - start: June 1, 1990 end: December 31, 1991

2. Animal assignment

   Animals were randomly assigned to cages, stratified by bodyweight, in such a way that initial cage means were

approximately equal (Table 1). Two animals were housed in each cage at the start of the study.

Table 1. Study design

Test Group	Dietary Concentration (ppm)	Mean Achieved dose (mg/kg/day) M/F[a]	Number of Animals	
			Male	Female
Control	0	0	50	50
Low	10	1.4/1/6	50	50
Mid	90	12.0/14.8	50	50
Mid	800	118/140	50	50
High	1250	217/224	50	50

a Data were extracted from study report, page 31

3. Dose Selection

The rationale for dose selection was not reported. However, since a significant increase of mortality was observed at the hi-dose (1250 ppm) of both sexes, the dose selection was acceptable.

4. Diet preparation and analysis

Diet was prepared on week 1, 13, 26, 39, 57, 65 and 78 by mixing appropriate amounts of test substance with SDS Rat and Mouse No.1 modified maintenance diet (ground) and was stored at ambient temperature. Homogeneity and stability were tested prior to the beginning of the study. Specimen batches of diet formulation containing 0, 2, 160, and 2180 ppm of M14360 were sampled during blending from the top, middle and bottom of the mixture. A portion of these samples was then mixed and used to determine the stability of the test material. Each dose sample was then divided into four portions (-20°C, and room condition for 4, 10 and 14 days) and tested for stability. During the study, samples of treated food were analyzed *after each preparation* for stability and concentration.

Results -

Homogeneity Analysis [a]:

nominal conc. (ppm)	Homogeneity
2	1.74-2.30
160	167-172
1280	1260-1310

[a] data derived from MRID 44305305, p. 1678

Stability Analysis [b]:

nominal conc. (ppm)	Storage time (days)			
	0	4	10	14
0	ND	ND	ND	ND
2	1.84	2.00	1.74	1.81
160	165	163	158	163
1280	1250	-------	1220	1220

[b] Data derived from MRID 44305305, p. 1679

Concentration Analysis:

The deviation of mean results from nominal concentrations was within ± 9% for doses >90 ppm and within ± 12% for the 10 ppm dose level.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

C. METHODS:

1. Observations

Animals were inspected daily for 4 weeks, then once weekly thereafter, for signs of toxicity and mortality.

2. Body weight

Animals were weighed once prior to treatment, once on the first day of treatment, then once weekly.

3. Food consumption and compound intake

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency(body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination

Data not provided

5. Hematology

Blood smears were obtained from the tail vein of 10 males and 10 females from the control group and the group receiving 1250 ppm during week 52 and all surviving mice from these groups during week 80 were examined for a differential WBC. The CHECKED (X) parameters were examined.

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

* Required for chronic studies based on Subdivision F Guidelines

6. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination [*note if not all collected tissues were examined*]. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*+		GLANDULAR
X	Rectum*	X	Urinary bladder*	X	Adrenal gland*
XX	Liver*+	XX	Testes*+		Lacrimal gland
X	Gall bladder*	X	Epididymides	X	Mammary gland*
X	Pancreas*	X	Prostate	X	Parathyroids*++
		X	Seminal vesicle	X	Thyroids*++
	RESPIRATORY	X	Ovaries*+		
X	Trachea*		Uterus*		
X	Lung*				OTHER
	Nose				Bone*
	Pharynx			X	Skeletal muscle*
	Larynx			X	Skin*
					All gross lesions and masses*

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

7. Statistics

The sexes were analyzed separately. Body weight, food consumption and organ weight data were analyzed with Fisher's exact test to detect general difference between treatments and with Mantel's test to detect dose-related trends in the numbers of such animals. Bartlett's test was applied to test for heterogeneity of variance between treatments. If no significant heterogeneity was detected, an one-way analysis of variance was carried out followed by Student's t test and William's test for a dose-related response. If significant heterogeneity of variance was present, the Kruskal-Wallis analysis of ranks was used, followed by the non-parametric equivalent of the William's test. Mortality was analyzed using log rank methods. For selected tumors, incidence rats were analyzed according to the IARC recommendation.

II. **RESULTS**

A. Observations

1. Clinical signs - An increased incidence of swollen or hard and dark abdomens was noted in mice of both sexes

receiving 1250 ppm of the test material. These findings are summarized in the Table 2.

2. Mortality - There was a three-fold increase in the number of deaths for males receiving 1250 ppm test material. There was a two-fold increase in the number of deaths for females receiving 1250 ppm test material (Table 2).

Table 2. Mortality and Clinical signs

	Males / Dose (ppm)					Females / Dose (ppm)				
	Cont	10	90	800	1250	Cont	10	90	800	1250
# of deaths (%Mortality)	12/50 (24%)	16/50 (32%)	11/50 (22%)	11/50 (22%)	36/50 (72%**)	8/50 (16%)	10/50 (20%)	8/50 (16%)	8/50 (16%)	17/50 (34%*)
Observation- swollen abdomen	2	5	3	8	31	1	2	5	3	10
hard abdomen	2	1	0	1	5	0	0	0	1	1
dark abdomen	1	0	0	2	11	1	1	1	1	2

Data extracted from MRID 44305305, p. 28
* p=0.029; ** p<0.001

B. Body weight/ body weight gain

Absolute mean body weights were comparable among groups in both sexes (Table 3). Decreased body weight gains were observed at 800 ppm (73% of the control in both sexes) and 1250 ppm (73% and 80% of the control for males and females, respectively) at the end of the study.

Table 3. Selected mean body weights and body weight gains (g) in mice fed M 14360 for 80 weeks[a]

Males					
Dosing Weeks	Dietary Level (ppm)				
	0	10	90	800	1250
0	29	29	28	28	28
1	30	30	30	30	28
2	31	32	31	31	30
16	40	40	39	37	36
52	45	46	45	40	40
80	44	46	43	39	39
wt gain					
0-1	1	1	2	2	0
0-13	9	10	11	9	7
0-80	15	17	15	11	11
(% control)		(113%)	(100%)	(73%)	(73%)
Females					
Dosing Weeks	Dietary level (ppm)				
	0	10	90	800	1250
0	22	23	23	22	22
1	23	23	23	24	23
2	24	25	25	25	24
16	30	31	30	30	29
52	35	36	35	33	32
80	37	38	36	33	34
wt gain					
0-1	1	0	0	2	1
0-13	8	7	7	8	8
0-80	15	15	13	11	12
(% control)		(100%)	(87%)	(73%)	(80%)

a Data were extracted from the study report, pages 51 and 52.

C. Food consumption and compound intake

1. Food consumption Food consumption was similar to all treated groups and controls during the first week of treatment. Subsequently, males receiveing 1250 ppm showed increased food consumption in comparison with controls. Calculated food efficiency suggested a slightly impaired food efficiency in males at high dose.

2. Compound consumption (time-weighted average) - The amount of M14360 in mg/kg/day is shown in the Table 1. The value for control were not detected, except in week 53, where it was found to be 3.6 ppm. The problem was traced back to a contaminated batch of acetone used in preparing the control feed. As this is a negligible amount given only at this time during the 80 week study, it is thought to have little or no effect on the outcome of the data

3. Food efficiency - It was calculated as the weight of food consumed per unit gain in bodyweight. These ratios were calculated up to week 13 of the treatment period. Food efficiency was lowered due to a reduced bodyweight gain at the concentrations of 800 and 1250 ppm for males and at the 800 ppm level for females.

D. Ophthalmoscopic examination - No data submitted.

E. Blood work

Hematology - There were no treatment related changes in the differential blood counts for treated versus control animals.

F. Sacrifice and Pathology

1. Gross pathology - Liver pathology revealed slight to severe changes which correlated with the dose given. At 90 ppm, the liver appeared pale with accentuated lobular markings. At higher concentrations (800-1250 ppm), masses were found with raised, pale, or dark subcapsular areas. Masses were also found in the kidneys of male animals receiving 1250 ppm M14360. The brains of animals of both sexes (800-1250 ppm) were found to have a dorsal depression; this was also true for males in the 90 ppm dose group. The testes of males receiving $\geq$ 90 ppm were found to be flaccid, blue and small. No corpora lutea were visible in ovaries of females receiving $\geq$ 90 ppm. Bones of the cranium were found to be discolored and thicker than the control group.

2. Organ weight - Dose-related increases of mean absolute liver weights were noted for mice (both sexes) given 90 ppm and above (Table 4). Treatment with 10 ppm had no effect on liver weight. Increased mean kidney weights were noted in both sexes of mice given 800 or 1250 ppm and in males only given 90 ppm. There is a slight decrease in brain weights at 1250 ppm.

Table 4. Selected mean organ weights in mice fed M 14360.

Parameters	Males / Dose (ppm)					Females / Dose (ppm)				
	0	10	90	800	1250	0	10	90	800	1250
Body wt(g)	42.8	43.9	42.0	38.0	38.6	35.6	37.4	35.2	32.7	32
Brain (g) (% control)	0.476	0.485 (102%)	0.481 (101%)	0.462 (97%)	0.449** (94%)	0.498	0.503 (101%)	0.490 (98%)	0.485 (97%)	0.462** (93%)
Liver (g) (% control)	2.14	2.15 (100%)	2.39** (112%)	5.21** (243%)	10.00** (467%)	1.70	1.88 (110%)	1.97* (116%)	3.74** (220%)	6.24** (367%)
Liver/BW (Rel. Wt)	0.05	0.05	0.06	0.14	0.26	0.05	0.05	0.06	0.11	0.20
Kidney (g) (% control)	0.75	0.76 (101%)	0.83** (111%)	0.86** (115%)	0.88** (117%)	0.46	0.45 (98%)	0.47 (102%)	0.52** (113%)	0.54** (117%)
Kidney/BW (Rel. Wt.)	0.02	0.02	0.02	0.03	0.02	0.01	0.01	0.01	0.02	0.02

Data extracted from Table 6, pages 61-62 of the report.
* $p<0.05$; ** $p<0.01$

3. Microscopic pathology -

a) Non-neoplastic - Significant histopathological findings were summarized in Appendix 1. Most of the findings were associated with liver toxicity including hepatocyte vacuolation, fat deposition, granulomatous inflammation, pigmented macrophages, generalised hepatocyte enlargement, and bile duct hyperplasia in mice receiving 1250 and 800 ppm of the test material. In addition, at the high dose of 1250 ppm, non-neoplastic changes were noted in the brain, lungs, kidneys, testes, epididymides, and ovaries. Thickening of compact bone of the cranium, in the ribs, collar bone (females only), and femur (females only) at 800 and 1250 ppm also appears to be treatment related.

b) Neoplastic - Treatment-related neoplastic findings were confined to the liver and are presented in the Table 5. Significantly increased incidence of benign and/or malignant liver cell tumors was observed in males and females at 1250 ppm and 800 ppm compared to the control. The tumor incidence in animals receiving ≤90 ppm was found to be similar to that of controls. Historical control data (Appendix 2) show that the incidence of

hepatocellular adenomas ranged from 7.7% to 24% in males and 0% to 1.9% in females and the incidence of hepatocellular carcinomas ranged from 0% to 14% in males and was 0% in females.

Table 5. Incidence of Liver Tumors[a]

Finding	Tumor incidence in males				
	0 ppm	10 ppm	90 ppm	800 ppm	1250 ppm
No. animals examined	50	50	50	50	50
Benign	9 (18%)	8 (16%)	6 (12%)	22 (44%)	34 (68%)
Malignant	1 (2%)	2 (4%)	2 (4%)	4 (8%)	20** (40%)
Combined	10 (20%)	9 (18%)	7 (14%)	24* (48%)	42** (84%)

Finding	Tumor incidence in females				
	0 ppm	10 ppm	90 ppm	800 ppm	1250 ppm
No. animals examined	50	50	50	50	50
Benign	0	0	0	11 (22%)	26 (52%)
Malignant	0	0	0	1 (2%)	17** (34%)
Combined	0	0	0	11** (22%)	32** (64%)

[a]Data was extracted from pp. 74-77 and pp. 1696-1702 from MRID 44305305.

*p<0.01 **p<0.001

III. DISCUSSION

A. In a carcinogenicity study (MRID 44305305) M14360 (95.05% a.i.) was administered to 50 Crl:CD-1 (ICR) mice/sex/dose in their diet at dose levels of 0, 10, 90, 800, 1250 ppm for 80 weeks.

Significantly increased mortality was observed at the highest dose. The compound appeared to affect males more than females

at the 1250 ppm dose level; males had a 72% mortality rate whereas females had a 34% mortality rate. Increased incidence of swollen, hard, or dark abdomens was noted in mice (both sexes) receiving 1250 ppm of the test material. Although absolute mean body weights were comparable among groups, decreased body weight gain were observed at 800 ppm (73% of the control in both sexes) and at 1250 ppm (73% and 80% of the control for males and females, respectively) at the end of the study.

Gross pathology revealed slight to severe changes in the liver which correlated with the dose given. At 90 ppm, the liver appeared pale with accentuated lobular markings. At higher concentrations (800-1250 ppm), masses were found with raised, pale, or dark subcapsular areas. Masses were also found in the kidneys of male animals receiving 1250 ppm M14360. Dose-related increase of liver weights were noted for mice of both sexes given ≥90 ppm of the test material. Kidney weights were also found to increase slightly (9-11 % above controls) in males receiving ≥ 90 ppm.

Significant histopathological findings were associated with liver toxicity including hepatocyte vacuolation, fat deposition, granulomatous inflammation, pigmented macrophages, generalised hepatocyte enlargement, and bile duct hyperplasia in mice receiving 1250 and 800 ppm of the test material. In addition, at the high dose of 1250 ppm, non-neoplastic changes were noted in the brain, lungs, kidneys, testes, epididymides, and ovaries. Of particular interest is the sizeable increase in the absence of corpora lutea in the ovaries and in the absence of sperm in the epididymides at 800 and 1250 ppm and the effects on the testes (reduced spermatogenesis, tubular atrophy, and interstitial cell hyperplasia) at 1250 ppm. Thickening of compact bone of the cranium, in the ribs, collar bone (females only), and femur (females only) at 800 and 1250 ppm also appears to be treatment related.

Neoplastic findings were noted in the liver where a statistically significant increased incidence of combined benign and malignant liver cell tumors was observed in males and females at 1250 ppm and 800 ppm compared to the control. The tumor incidence in animals receiving ≤90 ppm was found to be similar to that of controls. Historical control data show that the incidence of hepatocellular adenomas ranged from 7.7% to 24% in males and 0% to 1.9% in females and the incidence of hepatocellular carcinomas ranged from 0% to 14% in males and was 0% in females.

The systemic toxicity LOAEL is 90 ppm (12.0 and 14.8 mg/kg/day for males and females, respectively), based on increased liver weight in both sexes, increased kidney

weights in males and hepatocyte vacuolation in both sexes. The NOAEL is 10 ppm (1.4 and 1.6 mg/kg/day for males and females, respectively).

There was evidence of increased incidence of combined benign and malignant liver tumors in mice of both sexes treated with M14360 at 800 ppm (48% for males and 22% for females) and 1250 ppm (84% for males and 64% for females) compared to the control (20% for males and 0% for females). The chemical was administered at doses sufficient to test its carcinogenic potential in mice.

B. Study Deficiencies - None

Appendix

STATISTICAL ANALYSIS OF SELECTED HISTOPATHOLOGICAL LESIONS

Findings Decedents	Control 0 ♂	 ♀	10 ppm ♂	 ♀	90 ppm ♂	 ♀	800 ppm ♂	 ♀	1250 ppm ♂	 ♀
Liver										
Basophilic hepatocytes;	1	0	0	0	0	0	1	0	12	2
Eosinophilic hepatocytes; with/without further vacuolation	1	0	1	0	0	0	3	0	10	5
Generalised hepatocyte vacuolation	0	0	0	2	1	0	6**	0	31**	12**
Granulomatous inflammation	1	0	2	1	2	0	4	2	21**	1
Fat deposition in hepatocytes	0	0	0	0	0	0	6**	1	24**	7*
Centrilobular hepatocyte enlargement	1	0	3	0	4	0	5	1	2	1
Generalised hepatocyte enlargement	0	1	0	0	1	0	5*	1	33**	13**
Pigment macrophages	1	0	0	1	2	0	8**	3	34**	14**
Bile duct hyperplasia	0	0	0	0	0	0	5*	1	24**	9*
Pericholangitis	1	3	6	4	3	0	5	3	18*	7
Kidney										
Cortical scarring with atrophic tubules	4	3	5	3	3	2	2	5	31**	10
Subcapsular cortical scarring	0	-	0	-	0	-	0	-	2	-
Papillary necrosis	0	1	1	0	0	0	1	4	6	5
Brain										
Dorsal compression	0	0	0	0	0	0	0	0	2	1
Bone										
Animals examined	1	0	0	0	0	0	4	7	20	17
Thickening of compact bone in cranium	0	0	0	0	0	0	4	6	19	17
Myclofibrosis	0	0	0	0	0	0	2	3	6	5
Lung										
Prominent alveolar macrophages	4	0	6	1	2	0	2	5**	18	2
Pneumonitis	3	-	0	-	0	-	1	-	7	-
Thymus										
Animals examined	11	9	16	10	11	9	11	8	35	16
Involution	7	6	10	5	6	6	4	4	22	8
Testes										
Reduced spermatogenesis	1	-	1	-	2	-	2	-	23**	-
Tubular atrophy	0	-	0	-	1		0	-	19**	-
Interstitial cell hyperplasia	1	-	0	-	0		1	-	11	-
Epididymides										
Spermatozoa. absent	0	-	0	-	2		2	-	18**	-
Ovaries										
Corpora lutea. absent	-	5	-	2	-	4	-	7	-	13
Total number of animals examined[a]	12	9	17	11	11	9	11	8	36	18

* $p < 0.05$, ** $p < 0.01$

[a] For all tissues unless shown separately

STATISTICAL ANALYSIS OF SELECTED HISTOPATHOLOGICAL LESIONS

Findings	Control 0		10 ppm		90 ppm		800 ppm		1250 ppm	
Terminal	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver										
Basophilic hepatocytes	4	1	4	0	5	0	14**	9**	5*	11**
Eosinophilic hepatocytes with/without vacuolation	1	0	2	0	7*	0	26**	12	8*	24**
Generalized hepatocyte vacuolation	1	6	0	7	6	8	16**	32**	14**	32**
Granulomatous inflammation	6	2	1	2	4	4	24**	13**	12**	23**
Fat deposition in hepatocytes	2	25	5	18	9*	31	24**	33	8**	20
Centrilobular hepatocyte enlargement	3	0	2	0	14**	0	17**	7**	0	0
Generalized hepatocyte enlargement	3	0	1	0	6	0	21**	18**	14**	31**
Pigmented macrophages	1	2	0	1	3	4	4	1	13**	30**
Bile duct hyperplasia	0	0	0	0	2	0	0	1	11**	24**
Pericholangitis	14	14	12	16	13	16	20	23*	6	19*
Kidney										
Cortical scarring with atrophic tubules	8	8	2	5	2	6	8	4	5	6
Subcapsular cortical scarrmg	1	-	0	-	0	-	0	-	0	-
Papillary necrosis	0	1	1	0	0	1	0	0	0	1
Brain										
Dorsal compression	0	0	0	0	1*	0	0	0	4**	10**
Bone										
Ammals exammed	0	0	0	0	1	1	39	41	14	31
Thickening of compact bone in cranium	0	0	0	0	1	1	35	36	14	31
Thickening of compact bone in rib	0	0	0	0	0	0	4	1	1	8
Thickening of compact bone in collar bone	0	0	0	0	0	0	0	1	0	3
Myelofibrosis	0	0	0	0	0	1	2	0	3	4
Lung										
Prominent alveolar macrophages	5	1	4	3	7	6	6	2	4	6*
Pneumonitis	5	1	3	1	2	5	5	10**	3	8**
Thymus										
Animals examined	38	39	0	8	1	2	36	42	14	32
Involution	7	2	0	0	0	0	6	1	2	2
Testes										
Reduced spermatogenesis	5	-	5	-	6	-	14*	-	4	-
Interstitial cell hyperplasia	2	-	1	-	0	-	1	-	0	-
Epididymides										
Spermatozoa absent	0	-	1	-	4	-	12**	-	3*	-
Ovaries										
Corpora lutea absent	-	10	-	6	-	5	-	20*	-	13
Total number of animals examined[a]	38	41	33	39	39	41	39	42	14	32

*P <0.05, ** P <0.01

[a]For all tissues unless shown separately

STATISTICAL ANALYSIS OF SELECTED HISTOPATHOLOGICAL LESIONS

Findings Combined	Control 0		10 ppm		90 ppm		800 ppm		1250 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver										
Basophilic hepatocytes	5	1	4	0	5	0	15*	9**	17**	13**
Eosinophilic hepatocytes with/without vacuolation	2	0	3	0	7	0	29**	12**	18**	29**
Generalised hepatocyte vacuolation	1	6	0	9	7*	8	22**	32**	45**	44**
Granulomatous inflammation	7	2	3	3	6	4	28**	15**	33**	24**
Fat deposition in hepatocytes	2	25	5	19	9*	31	30**	34	32**	27
Centrilobular hepatocyte enlargement	4	0	5	0	18**	0	22**	8**	2	1
Generalised hepatocyte enlargement	3	1	1	0	7	0	26**	19**	47**	44**
Pigmented macrophages	2	2	0	2	5	4	12**	4	47**	44**
Bile duct hyperplasia	0	0	0	0	2	0	5*	2	35**	33**
Pericholangitis	15	17	18	20	16	16	25*	26	24	26
Kidney										
Cortical scarring with atrophic tubules	12	11	7	9	5	8	10	9	36**	16
Subcapsular cortical scarring	1	-	0	-	0	-	0	-	2	-
Papillary necrosis	0	2	2	0	0	1	1	4	6*	6
Brain										
Dorsal compression	0	0	0	0	1	0	0	0	6*	11**
Bone										
Animals examined	1	0	0	0	1	1	43	48	44	48
Thickening of compact bone in cranium	0	0	0	0	1	1	39	42	33	48
Thickening of compact bone in rib	0	0	0	0	0	0	4	1	1	9
Thickening of compact bone in collarbone	0	0	0	0	0	0	0	1	0	3
Myelofibrosis	0	0	0	0	0	1	4	3	9	9
Lung										
Prominent alveolar macrophages	9	1	10	4	9	6	8	7*	22**	8*
Pneumonitis	8	-	3	-	2	-	6	-	10	
Thymus										
Animals examined	49	48	16	18	12	11	47	50	49	48
Involution	14	8	10*	5	6	6*	10	5	24*	10
Testes										
Reduced spermatogenesis	6	-	6	-	8	-	16*	-	27**	-
Tubular atrophy	0	-	0	-	1	-	0	-	19**	-
Interstitial cell hyperplasia	3	-	1	-	0	-	2	-	11*	-
Epididymides										
Spermatozoa absent	0	-	1	-	6*	-	14**	-	21**	-
Ovaries										
Corpora lutea absent	-	15	-	8	-	9	-	27*	-	26*
Total number of animals examined^	50	50	50	50	50	50	50	50	50	50

*P <0.05, ** P <0.01
^For all tissues unless shown separately

Dr. J. R. French
SIPCAM AGRO USA, INC.
70 Mansell Court,
Suite 230
Roswell,
Georgia,
USA, 30076
18th March 1999

Item #3

Dear Dr French

I enclose historical control data derived from 20 mouse tumorigenicity studies performed at Huntingdon Life Sciences with study start dates between 1988 and 1992.

Historical control data for Study number: AGR 73/920469

Incidence of liver cell tumours in control mice of strain Crl:CD-1

Study code*	88A	88B	88C	88D	8911	8912	8913	8914	8915	8916
Males										
Hepatocellular adenoma	5	4	9	8	5	7	5	8	9	12
Hepatocellular carcinoma	3	3	1	1	1	0	1	2	1	1
Number of livers examined	51	52	52	60	52	52	52	52	52	50
Females										
Hepatocellular adenoma	0	1	0	0	0	1	0	0	0	0
Hepatocellular carcinoma	0	0	0	0	0	0	0	0	0	0
Number of livers examined	52	52	52	60	52	52	52	52	52	50

*- First two digits are year of study start date

Study code*	8917	8918	9001	9002	9102	9103	9104	9111	9101	92A
Males										
Hepatocellular adenoma	6	10	5	10	6	11	4	8	4	7
Hepatocellular carcinoma	1	2	0	2	8	5	5	1	4	6
Number of livers examined	50	56	52	50	56	56	50	50	50	50
Females										
Hepatocellular adenoma	0	0	1	0	0	0	0	0	0	0
Hepatocellular carcinoma	0	0	0	0	0	0	0	0	0	0
Number of livers examined	50	56	52	50	56	56	50	50	50	50

* - First two digits are year of study start date

Yours sincerely

Dr. John M. Offer
Consultant Pathologist
Department of Pathology

SIPCAM AGRO USA, INC.

March 22, 1999

Suku Oonithan (7505C), PM-21
Registration Division, Fungicide Branch
U.S. Environmental Protection Agency
Crystal Mall#2, 6th Floor
1921 Jefferson Davis Highway
Arlington, VA 22202

Subject: Tetraconazole; Historical Controls Data
Huntingdon Study AGR 73/920469 (MRID# 44305305)

Dear Mr. Oonithan:

In accordance with our phone conversation on March 22, 1999, enclosed you will find the original letter from Dr. John Offer of Huntingdon Life Sciences which itemizes the historical controls data for the subject study. This is the same information which was contained on the telefax that I had transmitted to you last week, and which is being herewith supplied in an effort to provide the Agency with the most legible version of the same information. Please transmit this information to the appropriate reviewer(s) in HED who may be addressing this study so that these data may be considered within the context of the DER that will be generated.

Please let me know how the reviews for all of the tetraconazole studies are progressing, and about when we may receive confirmation of approvals for the pending actions concerning tetraconazole on bananas, turfgrass and sugarbeets.

Sincerely,

John R. French

John R. French, Ph.D.
Technical Director

SIPCAM AGRO USA, INC. • 70 MANSELL COURT • SUITE 230 • ROSWELL, GEORGIA 30076
PHONE 770-587-1032 • FAX 770-587-1115

Huntingdon Life Sciences

Historical control data for Study Number AGR 73/920469

Incidence of ovarian luteoma in control female mice of strain Crl:CD-1

animals w/luteo
animals w/ovaries

Study code*	88A	88B	88C	88D	8911	8912	8913	8914	8915	8916
Luteoma	2	1	0	0	2	0	0	1	1	2
Number of ovaries examined	51	50	52	60	51	52	52	51	52	50

*- First two digits are year of study start date

Study code*	8917	8918	9001	9002	9102	9103	9104	9111	9101	92A
Luteoma	4	0	1	0	3	1	1	0	1	1
Number of ovaries examined	50	56	52	50	56	56	50	50	50	50

* - First two digits are year of study start date

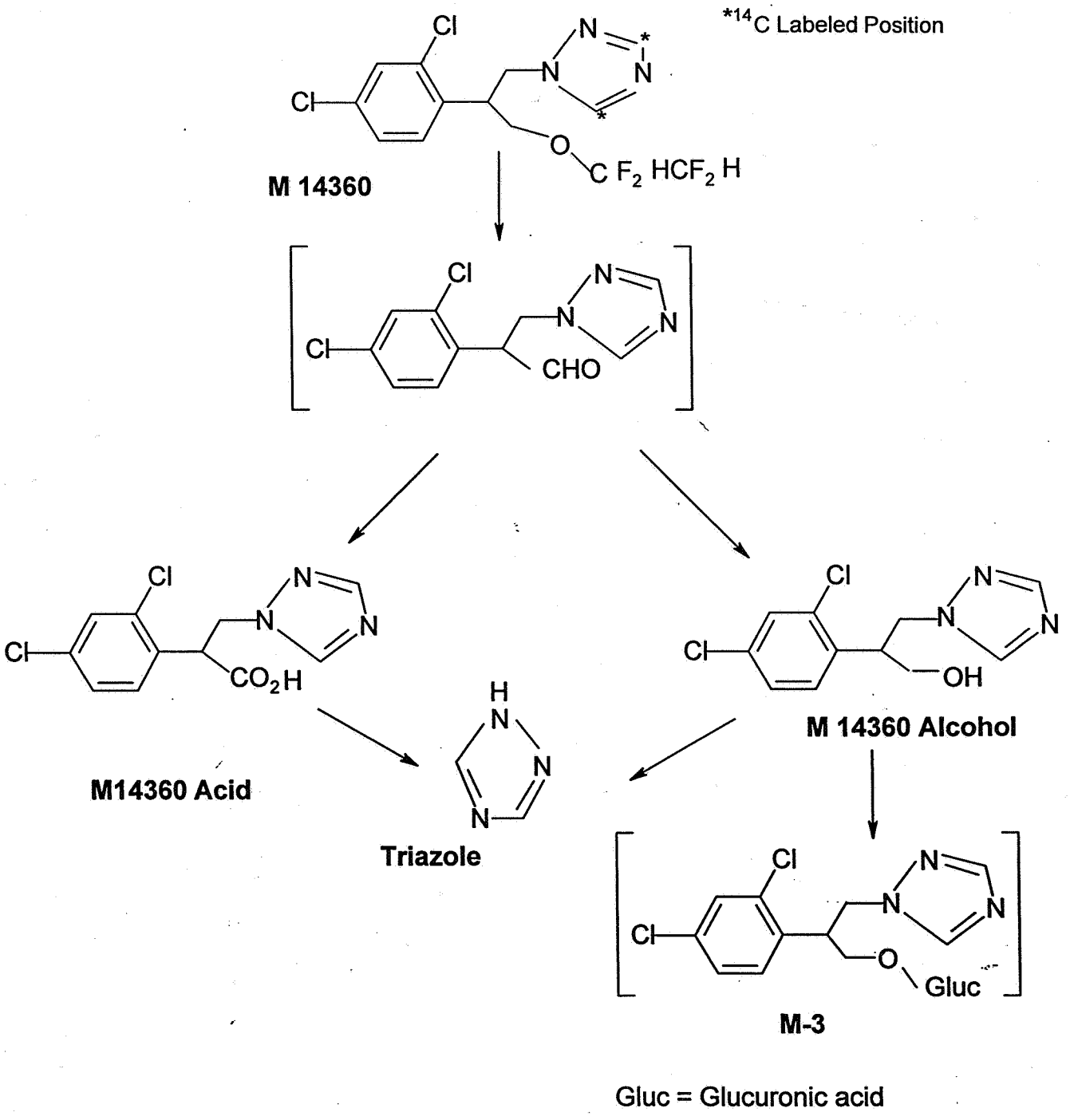
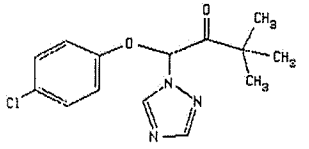
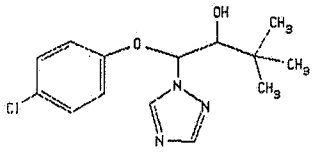
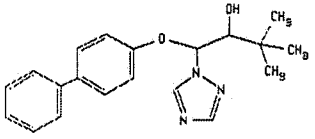
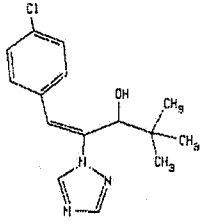
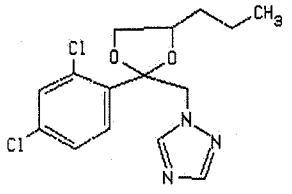
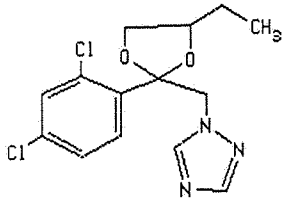
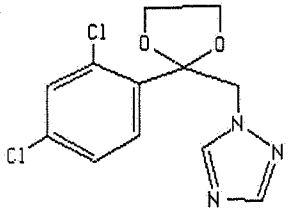
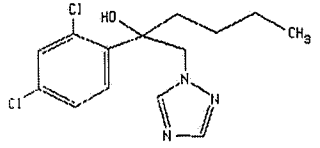
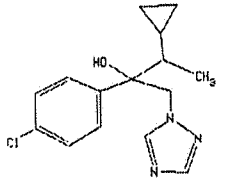
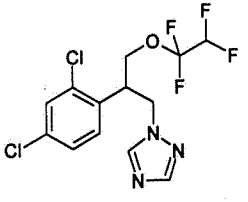


FIGURE 1. Proposed Metabolic Pathway of [^{14}C]-triazole M-14360 in Rats
(reproduced from MRID 44268117, Lab No.3786-91-0093-AM-001)

Table 1. Related Conazole Compounds.

Compound	Structure	Carcinogenic Effect	Carcinogen Class
Bayleton PC 109901 Tx.# 862AA		**NMRI Mouse** Only hepatocellular adenoma, at 1800 in (22%)♂ & (18%)♀ $p<0.05$ for trend and paired comps. Hist. Conts.: 18.4% ♂, and 2.0% ♀. **Wistar rat** Do. rel. trend in TFC adenomas in ♂ & comb. w. cystic hyperplasia in ♂ & ♀; Pairwi. comparisons not significant.	**C NQ** (7/31/90).
Baytan PC 127201 T.# 074A		**CF1-W74 mouse**, 2000 ppm: Hepatocellular adenomas and hyperplastic nodules ($p<0.01$) in ♀. No increase in ♂. Adrenal adenomas noted in ♀ LDT and HDT but not in hist. conts. No elevation in carcinomas. **Rat**, 125-2000 ppm, increases in thyroid adenoma.	**Weak C** SAP 12/23/87.
Baycor PC 112403 T.# 087AA		**Mouse**: up to 500 ppm: (-) **Rat**: up to 500 ppm : (-)	
Uniconazole PC 128976 T.# 207H		**Crl:CD-1(ICR)BR mouse** Incr. incidence of hepatocell. adenomas and carcinomas in HDT males only. **Crl:CD-1(ICR)SD rat** No increase in neoplastic findings	**C NQ**

Propiconazole PC 122101 T# 323EE		**CD-1 mouse** Statistically significant trend and pairwise comparisons in liver adenomas and combined. For carcinomas 2 pathologists were significant, the third was not.	**C NQ**
Etaconazole PC			
Azaconazole PC 128882 T# 321A		**Mouse**, 25,100 &400 ppm. There is the question of whether the MTD was reached. No oncogenicity effect.	

Compound	Structure	Carcinogenic Effect	Carcinogen Class
Hexaconazole PC 128925 T# 480G		**CD-1/Alpk mouse**, 5, 40 & 200 ppm. No oncogenicity effect. Should be seen with caution because MTD was not reached. No oncogenicity effect. **ALpk:APfSD (Wistar derived) rats**, 10, 100, 1000 ppm.There was a significant ($p<0.01$) dose-related trend and a significant pair-wise comparison with controls at the HDT for benign Leydig cell tumors in the testes. The incidence at the HDT (16%) exceeded historical control values of up to 6.0%	**C Q**
Cyproconazole PC 128993 T# 272E		**CD-1 mouse**, 5,15, 100 & 200 ppm. Significant incidence of adenomas & carcinomas at the MDT and HDT in males and at the HDT in females.	**B2**
Tetraconazole PC		**CD-1 mouse**, 10, 90, 800 & 1250 ppm. Significant increasing trend in both sexes with differences in the pair-wise comparisons of the 1250-ppm dose group with the controls for benign, malignant and benign and/or malignant tumors combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 800-ppm dose group with the controls for liver benign tumors and for benign and/or malignant tumors combined in both sexes, both at $p < 0.01$.	Not Classified

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901

ATTACHMENT 1

TETRACONAZOLE GUIDELINE SERIES 83-1: CHRONIC FEEDING (DOG)

EPA Reviewer: Nancy McCarroll
Toxicology Branch 1 (7509C)
EPA Secondary Reviewer: David Nixon
Toxicology Branch 1 (7509C)

Nancy McCarroll, Date 5/27/99
David Nixon, Date 5/27/99

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity [feeding]-[Dogs]; OPPTS 870.4100 [§83-1b]

DP BARCODE: D238518 SUBMISSION CODE: S528887
P.C. CODE: 120603 TOX. CHEM. NO.:

TEST MATERIAL (PURITY): M 14360 (94.6%)

SYNONYMS: Tetraconazole;1-[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetra-fluoroethoxy)propyl]-1,2,4-triazole

CITATION: Makin, A., McLean, T.A., Buist, D.P., Crook, D., Morrow, J., Lewis, D.J. and Gopinath, C. (1990). M 14360 Dietary Toxicity Study in Beagle Dogs (Final Report - Repeated Daily Dosage for 52 weeks); Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England; Study No. AGR 72-G/901546 Study Completion Date: June 25, 1990. Unpublished; MRID No. 44305303.

SPONSOR: Sostram Corp., Valdosta, GA

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID No. 44305303), Tetraconazole as M 14360 (94.6%) was administered to groups of four male and four female Beagle dogs/dose in *the diet,* at dose levels of 0, 22.5, 90, or 360 ppm (equivalent to achieved intakes of 0, 0.73, 2.95 or 12.97 for males or 0, 0.82, 3.33 or 14.50 mg/kg/dayfor females) for 52 weeks.

Exposure to M 14360 had no effect on feed consumption, hematological parameters or urinalysis. Treatment-related effects at the high dose included slight but nonsignificant body weight reductions in both sexes from study week 3 to termination; significantly increased alkaline phosphatase, γ-glutamyltransferase, alanine aminotransferase and ornithine carbamoyl transferase in both sexes from study week 13 to 52, increased absolute and relative liver and kidney weights for both sexes, and histopathological changes in both organs. In the mid-dose group, effects were manifested as increased absolute and relative kidney weights for males correlated with histopathological findings in the males (apparent hypertrophy in cortical tubules of the kidneys-1 male). No adverse effects were seen at the low dose.

Based on these considerations, **the NOAEL is 22.5 ppm (equivalent to achieved intakes of 0.73 mg/kg/day for males or 0.82 mg/kg/day for females) and the LOAEL is 90 ppm (equivalent to achieved intakes of 2.95 mg/kg/day for males or 3.33 mg/kg/day for females), based on increased absolute and relative kidney weights and histopathological changes in the male kidney.**

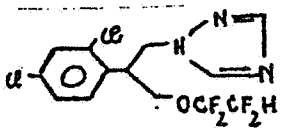
This chronic toxicity study in the dog is **acceptable,** and does satisfy the guideline requirement for a chronic oral study (83-1b) in the dog.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: M 14360
   Description: Thick, viscous brown liquid
   Lot/Batch #: FCF/T/72
   Purity: 94.6 % a.i.
   Stability of compound: Stored at room temperature; reported to be stable until at least July 1990
   CAS #: 112281-77-3
   Structure:



2. Vehicle and/or positive control: Basal diet (Standard Diet A:Special Diets Service)

3. Test animals: Species: Dogs
   Strain: Beagle
   Age at study initiation: 35-39 weeks
   Weight at study initiation: 10.7-13.9 ♂ 8.9-11.8 kg ♀
   Source: Consort Ltd., Harewood Park, Harewood End, Herefordshire, England
   Housing: Individually
   Diet: 400 gm of standard dry feed were provided daily to each dog.
   Water: Provided ad libitum
   Environmental conditions:
   Temperature: Not reported
   Humidity: Not reported
   Air changes: Not reported
   Photoperiod: Not reported
   Acclimation period: At least 20 weeks

B. STUDY DESIGN:

1. In life dates - start: 6/22/89 end: 6/25/90

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1.

TABLE 1: Assignment of Animals

Test Group	Conc. in Diet *(ppm)*	Dose to animal *(mg/kg/ day)*	Main Study 52 weeks	
			male	female
Control	0	0	4	4
Low (LDT)	22.5	0.73 ♂ 0.82 ♀	4	4
Mid (MDT)	90	2.95 ♂ 3.33 ♀	4	4
High (HDT)	360	12.97 ♂ 14.50 ♀	4	4

3. Dose selection rationale: Dosage levels used in the study were selected based on the findings of a preliminary dose-range finding test (Report No. AGR 51/89813); no details were provided.

4. Diet preparation and analysis

Diets were prepared weekly by mixing appropriate amounts of test substance with acetone and adding the appropriate amount of the prepared solution to basal diet (Standard Diet A:Special Diets Service). Acetone was removed by placing the mixtures on a rotary evaporator at 40°C. Prepared test feed was stored at room temperature, protected from light. Stability was determined prior to study start and on storage days 0, 1, 10, and 18. During the study, samples of treated food from all dosage groups were analyzed for concentration at study weeks 1, 13, 26, 39 and 52. Samples from the top, middle and bottom of diets containing target concentrations of 22.5 and 360 were analyzed for homogeneity.

Results:

Homogeneity Analysis: The test material was evenly distributed throughout the feed as indicated by the percentage recovery (92-102%).

Stability Analysis: The formulated diets were stable for up to 18 days at room temperature with marginal loss of the test substance. Recovery ranged from 94 to 98%.

98%.

Concentration Analysis: Actual concentrations of the test material were within 99.0 to 101.2 % of the nominal levels.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics: Data from male and female dogs were analyzed separately and combined using the following procedures: body weight, food consumption, organ weight and clinical pathology data were untransformed and/or transformed and evaluated by Bartlett's test, ANOVA, Kruskal-Wallis, Student's t and/or Williams'tests.

C. METHODS:

1. Observations:

Animals were inspected daily for signs of toxicity and mortality.

2. Body weight:

Animals were weighed prior to dosing, and once weekly throughout the pre-dosing and dosing period.

3. Food consumption and compound intake

Food consumption for each animal was determined daily and mean daily diet consumption was calculated as g food/day. Weekly compound intake (mg/kg/day) values were calculated from the consumption and group mean mid-week body weight data.

4. Ophthalmoscopic examination

Eyes were examined prior to initiation of the study and at weeks 13, 26 and 52.

5. Blood was collected from fasted animals (all animals in all treatment and control groups) once before dosing and at weeks 13, 26 and 52 for hematology and clinical chemistry from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
x	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*	x	Reticulocyte count
x	Blood clotting measurements*		
x	(Activated partial Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines

b. Bone marrow: In addition, bone marrow smears were prepared for each animal prior to sacrifice by sternal puncture.

c. Clinical Chemistry

X	ELECTROLYTES		OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*	X	Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)	x	Ornithine Carbamoyl Transferase (OCT)
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected from fasted animals (all animals in all treatment and control groups) prior to initiation of the study and at weeks 13, 26 and 52. The CHECKED (X) parameters were examined.

X		X	
	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen

* Required for chronic studies

7. Sacrifice and Pathology

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

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph.nerve*
x	Esophagus*		Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	xx	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+		Lacrimal gland
x	Rectum*	x	Urinary bladder*	x	Mammary gland*
xx	Liver*+	xx	Testes*+	xx	Parathyroids*++
x	Gall bladder*		Epididymides	xx	Thyroids*++
xx	Pancreas*	xx	Prostate		
			Seminal vesicle		OTHER
	RESPIRATORY	xx	Ovaries*+	x	Bone*
x	Trachea*	xx	Uterus*	x	Skeletal muscle*
xx	Lung*			x	Skin*
	Nose			x	All gross lesions and masses*
	Pharynx			x	Vagina
	Larynx				

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

II. RESULTS

A. Observations: There were no clinical signs considered by the investigators to be related to treatment with M 14360 and no unscheduled deaths occurred throughout the course of the study.

B. Body weight: There was no significant difference between control and treatment groups body weight during the 52-week exposure. However, as shown in Table 2, a consistent decline in the body weight of the high-dose males and females was apparent. In contrast to the study authors statement that weight loss was generally associated with the first few weeks of dosing, the decline was generally persistent from study week 3 to termination and was more pronounced in the females, with body weight decrements ≥10% of control at weeks 7 to 52. Body weight gain data summarized in Table 3 indicate that despite the weight loss in the control animals from weeks 13 to 52, overall body weight gain for the high-dosage group males and females generally lagged behind the control throughout the study.

TABLE 2: Summary of Mean Body Weights of Dogs Administered Dietary Concentrations of M 14360 For 52 Weeks

Week	Body weight (kg) by Dietary Level (ppm)[A]			
	MALES			
	0	22.5	90	360
0	11.8	12.1	12.0	12.0 (102)[B]
3	11.9	12.3	12.2	11.4 (96)
5	12.0	12.4	12.4	11.4 (95)
7	12.0	12.5	12.4	11.2 (93)
13	12.1	12.5	12.6	11.4 (94)
26	11.9	12.4	12.2	11.4 (96)
39	11.7	12.3	12.0	11.0 (94)
52	11.0	11.7	11.9	10.4 (95)

[A] Data were extracted from Study Report, MRID No. 44305303, Appendix 1b, pp 89 and 90.
[B] Values in parentheses represent percent of control.

TABLE 2: Summary of Mean Body Weights of Dogs Administered Dietary Concentrations of M 14360 For 52 Weeks

Week	Body weight (kg) by Dietary Level (ppm)[A]			
	FEMALES			
	0	22.5	90	360
0	10.8	10.2	10.4	10.7 (99)[B]
3	10.9	10.2	10.6	10.1 (93)
5	11.0	10.4	10.5	10.0 (91)
7	11.1	10.4	10.7	9.9 (89)
13	11.2	10.4	10.8	9.5 (85)
26	11.0	10.5	10.8	9.5 (86)
39	10.7	10.9	10.7	9.5 (89)
52	10.2	10.3	10.6	9.2 (90)

[A] Data were extracted from Study Report, MRID No. 44305303, Appendix 1b, pp 89 and 90.
[B] Values in parentheses represent percent of control.

TABLE 3: Summary of Body Weight Gain for Dogs Administered Dietary Concentrations of M 14360 For 52 Weeks

Week	Body Weight Gain (kg) by Dietary Level (ppm)[A]			
	MALES			
	0	22.5	90	360
0-3	0.1	0.2	0.2	-0.6
3-13	0.2	0.2	0.4	0.0
13-39	-0.4	-0.2	-0.6	-0.4
39-52	-0.7	-0.6	-01	-0.6
0-52	-0.8	-0.4	-0.1	-1.6

[A] Data were extracted from Study Report, MRID No. 44305303, Appendix 1b, pp 89 and 90, and calculated by our reviewers.

TABLE 3: Summary of Body Weight Gain for Dogs Administered Dietary Concentrations of M 14360 For 52 Weeks

Week	Body Weight Gain (kg) by Dietary Level (ppm)[A]			
	FEMALES			
	0	22.5	90	360
0-3	0.1	0.0	0.2	-0.6
3-13	0.3	0.2	0.2	-0.6
13-39	-0.5	0.5	-0.1	0.0
39-52	-0.5	-0.6	-0.1	-0.3
0-52	-0.6	0.1	0.2	-1.5

[A] Data were extracted from Study Report, MRID No. 44305303, Appendix 1b, pp 89 and 90, and calculated by our reviewers.

C. Food consumption and compound intake:

1. Food consumption: Feed consumption was unaffected by treatment.

2. Compound consumption: Achieved intakes, calculated from mid-weekly body weights and weekly feed consumption are presented in Table 1.

3. Food efficiency: Feed efficiency was not determined in this study.

D. Ophthalmoscopic examination: No treatment-related effects on the appearance of the eyes were reported.

E. Blood work

1. Hematology: With the exception of significantly prolonged activated partial thromboplastin times for dogs receiving 360 ppm at weeks 13 and 26 (males and females combined and for separate sexes)and 52 (males and females combined and only females), no adverse effects on hematological parameters were reported.

2. Clinical chemistry: Data from the analysis of clinical chemistry parameters are summarized in Table 4 and indicate that significant increases in male and/or female ALK, GPT and γ-GT were generally noted at weeks 13, 26 and 52. OCT was also significantly increased at week 26 (males and females) and at week 52 (females). As further shown in Table 4, albumin for both males and females in the high dosage group was significantly lower than control at weeks 13 and 52 and cholesterol levels were generally higher than control for both sexes, with significant increases recorded at week 26 and 52 (males only). Overall, these findings suggest an adverse effect on the liver. In addition, phosphorous (♂ from week 26 to termination and in ♀ from week 13 to termination) was significantly increased.

Table 4. Summary of Relevant Clinical Chemistry Parameters in Dogs Receiving Dietary Concentrations of M 14360 [A]

Males

Parameter	Dietary level (ppm)			
	0	22.5	90	360
AP [B]				
Week -3	160 mU/mL	168 mU/mL	146 mU/mL	187 mU/mL
Week 13	122	134	133	554**
Week 26	119	137	143	623**
Week 52	127	154	145	722**
γ-GT [B]				
Week -3	2 mU/mL	2 mU/mL	3 mU/mL	2 mU/mL
Week 13	2	2	2	3*
Week 26	3	3	3	4
Week 52	3	3	3	5**
SGPT [a]				
Week -3	20 mU/mL	26 mU/mL	19 mU/mL	25 mU/mL
Week 13	27	26	23	36
Week 26	22	31	20	42**
Week 52	22	26	20	53**
OCT[B]				
Week -3	3.2 mU/mL	5.4 mU/mL	2.4 mU/mL	3.8 mU/mL
Week 13	4.8	3.3	6.0	5.0
Week 26	2.9	3.3	4.9	6.8**
Week 52	3.6	3.4	3.7	7.9

[A] Data were extracted from Study Report, MRID No. 44305303, Table 7a and 7b, pp 59-62.

[B] Abbreviations:

AP=Alkaline phosphatase γ-GT= γ-Glutamyltransferase

SGPT= Alanine aminotransferase OCT= ornithine carbamoyl transferase

* Significant different ($p<0.05$) than control * *Significant different ($p<0.01$) than control

Table 4. Summary of Relevant Clinical Chemistry Parameters in Dogs Receiving Dietary Concentrations of M 14360 [A] (cont.)

Males

Parameter	Dietary level (ppm)			
	0	22.5	90	360
Albumin				
Week -3	3.0 g/dL	2.8 g/dL	2.9 g/dL	2.7 g/dL*
Week 13	3.0	2.8	2.9	2.5**
Week 26	2.8	2.7	3.0	2.7
Week 52	2.7	2.6	2.8	2.4*
Cholesterol				
Week -3	137 mg/dL	126 mg/dL	147 mg/dL	145 mg/dL
Week 13	133	126	154	172
Week 26	125	110	146	181*
Week 52	99	90	112	140*

[A] Data were extracted from Study Report, MRID No. 44305303, Table 7a and 7b, pp 59-62.

* Significant different ($p<0.05$) than control * *Significant different ($p<0.01$) than control

Table 4. Summary of Relevant Clinical Chemistry Parameters in Dogs Receiving Dietary Concentrations of M 14360 [A] (cont.)

Females

Parameter	Dietary level (ppm)			
	0	22.5	90	360
AP [B]				
Week -3	141 mU/mL	165 mU/mL	155 mU/mL	155 mU/mL
Week 13	103	120	161	580**
Week 26	122	116	198	671**
Week 52	121	138	169	553**
γ-GT [B]				
Week -3	2 mU/mL	2 mU/mL	2 mU/mL	2 mU/mL
Week 13	2	3	2	4*
Week 26	3	3	3	5*
Week 52	3	3	3	5
SGPT [a]				
Week -3	19 mU/mL	25 mU/mL	25 mU/mL	27 mU/mL*
Week 13	22	24	24	42**
Week 26	29	22	26	51
Week 52	23	22	29	36**
OCT[B]				
Week -3	2.4 mU/mL	4.6 mU/mL	3.7 mU/mL	4.0 mU/mL
Week 13	4.8	4.2	4.4	4.6
Week 26	2.7	3.6	5.7	7.9**
Week 52	2.5	3.3	4.4	6.9*

[A] Data were extracted from Study Report, MRID No. 44305303, Table 7a and 7b, pp 59-62.

[B] Abbreviations:

AP=Alkaline phosphatase γ-GT= γ-Glutamyltransferase

SGPT= Alanine aminotransferase OCT= ornithine carbamoyl transferase

* Significant different ($p<0.05$) than control * *Significant different ($p<0.01$) than control

Table 4. Summary of Relevant Clinical Chemistry Parameters in Dogs Receiving Dietary Concentrations of M 14360 [A] (cont.)

Females

Parameter	Dietary level (ppm)			
	0	22.5	90	360
Albumin				
Week -3	3.1 g/dL	3.0 g/dL	2.9 g/dL*	2.9 g/dL*
Week 13	3.2	3.1	2.9	2.9*
Week 26	3.1	3.1	3.0	3.0
Week 52	3.1	2.8	2.8	2.7*
Cholesterol				
Week -3	157 mg/dL	147 mg/dL	142 mg/dL	133 mg/dL
Week 13	151	145	154	161
Week 26	166	145	159	156
Week 52	123	114	141	168

[A] Data were extracted from Study Report, MRID No. 44305303, Table 7a and 7b, pp 59-62.

* Significant different ($p<0.05$) than control * *Significant different ($p<0.01$) than control

F. Urinalysis - As shown in Table 5, urinary protein values for the high-dosage group were generally higher than control for males at weeks 13 and 52 and for females at weeks 3, 13, 26 and 52 with values reaching statistical significance for the females at weeks 13 and 26. However, these values were not generally dose-related and the study investigators stated that the majority of individual values were within normal ranges.

Table 5. Summary of Relevant Urinalysis Parameters in Dogs Receiving Dietary Concentrations of M 14360 [A]

Males

Parameter	Dietary level (ppm)			
	0	22.5	90	360
Volume				
Week -3	243 mL	183 mL	175 mL	284 mL
Week 13	265	99	242	352
Week 26	322	337	293	317
Week 52	251	189	202	302
Ph [B]				
Week -3	6.6	6.4	6.5	6.4
Week 13	6.9	6.2	6.2	6.1
Week 26	6.8	5.8	5.9	6.2
Week 52	5.6	5.2	5.3	5.6
Specific gravity				
Week -3	1028	1033	1029	1028
Week 13	1029	1039	1031	1026
Week 26	1026	1027	1022	1021
Week 52	1031	1035	1030	1027
Protein				
Week -3	13 mg/dL	22 mg/dL	21 mg/dL	17 mg/dL
Week 13	30	27	29	35
Week 26	38	30	20	31
Week 52	27	39	27	44

[A] Data were extracted from Study Report, MRID No. 44305303, Table 8a and 8b, pp 65-68.

Table 5. Summary of Relevant Urinalysis Parameters in Dogs Receiving Dietary Concentrations of M 14360 [A] (cont.)

Females

Parameter	Dietary level (ppm)			
	0	22.5	90	360
Volume				
Week -3	194 mL	143 mL	99 mL	146 mL
Week 13	212	108	147	179
Week 26	301	269	210	226
Week 52	280	193	189	315
Ph [B]				
Week -3	6.5	6.1	6.3	6.4
Week 13	6.2	6.0	6.5	6.4
Week 26	6.1	6.1	6.5	6.2
Week 52	5.4	5.4	5.5	5.7
Specific gravity				
Week -3	1032	1035	1041	1040
Week 13	1035	1036	1039	1031
Week 26	1021	1028	1031	1033*
Week 52	1032	1035	1036	1021
Protein				
Week -3	14 mg/dL	28 mg/dL	20 mg/dL	20 mg/dL
Week 13	16	19	24	34**
Week 26	13	18	17	42**
Week 52	20	28	27	24

[A] Data were extracted from Study Report, MRID No. 44305303, Table 8a and 8b, pp 65-68.

* Significant different ($p<0.05$) than control * *Significant different ($p<0.01$) than control

G. Bone marrow smears: Bone marrow smears prepared for all animals prior to sacrifice were reported to be normal in cellularity, distribution and morphology.

H. Sacrifice and Pathology

1. Organ weight - Marked increases in absolute and relative (to body weight) liver and kidney weights were observed for males and females in the mid-and high-dosage groups. The effect on absolute organ weight at the high dose was significant for both sexes. Although no significant difference occurred at 90 ppm, male absolute and relative kidney weight was increased 17 and 9%, respectively. Similarly, in the mid-dose females, absolute and relative liver weight was increased 11 and 10%, respectively (Table 6). Absolute and relative prostate weights were also increased at 360 ppm (17 and 91%, respectively). Organ weights were unaffected by treatment with the low dose.

TABLE 6. Summary of Relative and Absolute Liver and Kidney Weights for Dogs Administered Dietary Concentrations of M 14360 For 52 Weeks

MALES					
Dietary Levels (ppm)	Body Weight (kg)	Liver (Absolute) (g)	Liver (Relative) (%)	Kidney (Absolute) (g)	Kidney (Relative) (%)
0	10.9	382.4	3.51	57.5	0.53
22.5	11.3	356.9	3.16	58.8	0.52
90	11.6	404.4 (6%↑)	3.49	67.0 (17%↑)	0.58 (9%↑)
360	10.3	537.7* (41%↑)	5.22 (49%↑)	98.4* (71%↑)	0.96 (81%↑)
FEMALES					
0	10.2	331.3	3.25	51.7	0.51
22.5	10.0	337.5	3.38	51.0	0.51
90	10.6	369.1 (11%↑)	3.48 (10%↑)	53.7	0.51
360	9.1	457.8*(38%↑)	5.03 (55%↑)	76.5* (48%↑)	0.84 (65%↑)

[A] Data extracted from Study Report, MRID No. 44305303, Table 9, p 71 and Appendix 7a and b, pp 129-136.
* Significantly different than the control ($p<0.01$).

2. Gross pathology : Gross necropsy revealed pale discoloration and accentuated lobular markings of the left lateral, left medial and caudate lobes of the liver in single females receiving 90 or 360 ppm. No other gross lesion related to treatment were observed.

3. Microscopic pathology : The incidence of microscopic lesions is presented in Table 7. As shown, effects on the liver were mild, generally confined to the high-dose group and consisted of minimal to moderate centrilobular fat (1 high-dose male and female and 1 mid-dose female), hepatocyte enlargement (1 male and 2 females in the high-dose group), few eosinophilic intracellular inclusion in hepatocytes (3 males and 2 females at 360 ppm) and centrilobular hepatocyte rarefaction (I male and 2 females at 360 ppm and 1 female at 90 ppm). Histopathology noted in the kidneys was also mild, generally confined to the high-dose group and consisted of cortical tubular hypertrophy (2 males and one female at 360 ppm), apparent cortical tubular hypertrophy (1 male and 2 female at 360 ppm and 1 male at 90 ppm) and apoptotic bodies in cortical tubules (2 males and 4 females at 360 ppm).

Based on the overall results, the study authors concluded that the NOEL was 22.5 ppm in dogs.

III. DISCUSSION

The data presented in this study show that the principal target organs for M 14330 toxicity in the dog are the liver and the kidney. Effects on the liver at 360 ppm included significantly increased ALK, GPT and γ-GT in males and females at weeks 13, 26 and 52 and significantly increased OCT at week 26 (both sexes) and at week 52 (females); significantly increased absolute liver weights and marked increases in relative liver weights in males and females; and histopathology findings. Effects on the kidney at the high dose were significant with a marked increase in relative kidney weight (≥65%↑ compared to the control) seen in both sexes. Overall, the data indicate that the effects on clinical chemistry parameters (liver only) and organ weights were correlated with histopathology findings at the high dose. At the mid-dose, nonsignificant increases in both absolute and relative kidney weights (17% and 9%↑, respectively) in males correlated with histopathological findings (apparent hypertrophy in cortical tubules of the kidneys-1 male). No adverse effects were seen at the low dose.

Based on these considerations, the NOAEL is 22.5 ppm (equivalent to achieved intakes of 0.73mg/kg/day for males or 0.82 mg/kg/day for females) and the LOAEL is 90 ppm (equivalent to achieved intakes of 2.95 mg/kg/day for males or 3.33 mg/kg/day for females), based on effects on absolute and relative kidney weights and histopathological changes in males.

IV. STUDY DEFICIENCIES: The appearance of the urine, as required by guideline, was not reported. However, it is doubtful if this deficiency altered the outcome of the study.

Table 7. Incidence of Histopathology in Dogs Receiving M 14360 in the Diet for 52 Weeks

Parameter	Histopathology Incidence by Dietary Level[A] (ppm) 0	22.5	90	360
		Males		
Liver				
Minimal/marked centrilobular fat	0/4	0/4	0/4	1/4
Hepatocyte enlargement	0/4	0/4	0/4	1/4
Eosinophilic intracytoplasmic inclusion in hepatocytes (few)	0/4	0/4	0/4	3/4
Centrilobular hepatocyte rarefaction	1/4	0/4	0/4	1/4
		Females		
Liver				
Minimal/marked centrilobular fat	0/4	0/4	1/4	1/4
Hepatocyte enlargement	0/4	0/4	0/4	2/4
Eosinophilic intracytoplasmic inclusion in hepatocytes (few)	0/4	0/4	0/4	2/4
Centrilobular hepatocyte rarefaction	0/4	0/4	1/4	2/4
		Males		
Kidney				
Cortical tubular hypertrophy	0/4	0/4	0/4	2/4
Apparent cortical tubular hypertrophy	0/4	0/4	1/4	1/4
Apoptotic bodies in cortical tubules (few)	0/4	0/4	0/4	2/4
		Females		
Kidney				
Cortical tubular hypertrophy	0/4	0/4	0/4	1/4
Apparent cortical tubular hypertrophy hypertrophy	0/4	0/4	0/4	2/4
Apoptotic bodies in cortical tubules (few)	0/4	0/4	0/4	4/4

[A] Data were extracted from the Study Report, Table 10, pp. 73-80.

EPA Reviewer: Laurence D. Chitlik, DABT L. Chitlik, Date 4/26/99
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Brian Dementi, Ph.D. Brian Dementi, Date 8/30/99
Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Liver Enzyme Induction following Dietary Administration to Mice for 4 Weeks

OPPTS Number: 870.4200 (FIFRA) OPP Guideline Number: §83-5

TEST MATERIAL (PURITY): Tetraconazole; 96.3% ai

CITATION: Waterson, Lynne A. (1996) Effect of Tetraconazole on Hepatic Enzyme Activities After 4 Weeks of Treatment in Mice. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, PE186ES, England. Report Number AGR 90/952463. September 2, 1996. MRID 44751309. Unpublished.

SPONSOR: Isagro S.p.A., Centro Direz. Milano Oltre, Pal. Raffaello, Via Cassanese, 224, 20090 Segrate, Milan, Italy

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44751309), Tetraconazole 96.3% ai, Batch number FCF/T/113-94) was administered to 18 male and female Crl:CD-1 (ICR)BR mice per dose level at levels of 0, 20, 800, 1250 ppm in the diet. The positive control was Phenobarbital (Na) salt, 75 mg/kg/day.

Data generated in this study indicate that Tetraconazole administration for 4 weeks results in liver enzyme induction. Statistically significant increases were apparent in females at the 20 ppm dose level based on increases in microsomal protein, cytochrome P450, and ethylmorphine N-demethylase. At all dose levels in males and females, 7-pentoxyresorufin O-depentylase values were statistically elevated. At 800 and 1250 ppm, statistically significant findings were typically noted. However, dose response increases were not apparent in these findings at the 1250 ppm level as compared to the lower 800 ppm level.

This subchronic toxicity study in rats is an **acceptable** non-guideline study in mice.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Tetraconazole Technical
   Description: Viscous yellow/brown liquid
   Lot/Batch #: Batch no. FCF/T/113-94
   Purity: 96.3% ai
   Storage conditions: Room temperature in the dark; reported to be stable until September 1996

2. Vehicle and/or positive control: The test material was dissolved in acetone and blended with diet. The positive control was Phenobarbital (sodium salt) dissolved in deionized distilled water.

3. Test animals: Species: mouse
   Strain: Crl:CD-1 (ICR) BR
   Age and weight at study initiation: Six weeks at start of dosing with a range of 24 to 35 grams for males and 19 to 27 grams for females.
   Source: Charles River Breeding Laboratories, Margate, Kent, UK.
   Housing: Two to a cage.
   Diet: ground SDS Rat and Mouse No 1 maintenance diet
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 19-25 degrees C
   Humidity: 48 to 67%
   Air changes: not noted
   Photoperiod: 12-hour light/dark cycle
   Acclimation period: 11 to 13 days

B. STUDY DESIGN:

1. In life dates - Start: July 24, 1995
   End: August 23, 1995

2. Animal assignment

   One hundred ninety mice were assigned to groups in this study. Animal numbers 181 to 190 were sacrificed for

a health check. Animals were assigned to groups as noted in the following table:

TABLE 1: STUDY DESIGN

Test Group	Dose to Animal (PPM)	Animals Assigned	
		Male	Female
1	0	18	18
2	Phenobarbital (Na) salt 75 mg/kg/day*	18	18
3	20	18	18
4	800	18	18
5	1250	18	18

* Positive control

Animals 181 to 190 were sacrificed prior to administration of the test material in order to assess the health status of the test animals

The positive control was administered as a solution using a graduated syringe and a metal dosing cannula inserted into the stomach. The dose volume administered was calculated according to the most recent body weight and adjusted to the nearest 0.01 ml. A constant volume of 5 ml/kg was maintained.

Statistics

The report stated on page 16 relative to food consumption and bodyweight data:

"If the data consisted predominantly of one particular value(relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analyzed, Fisher (1950) and Mantel (1963). Otherwise:

A test was applied to test for heterogeneity of variance between treatments, Bartlett (1937). Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, an analysis of ranks was used, Kruskal and Wallis (1952/3).

Except for pre-dose data, analyses of variance were followed by Student's t test and Williams' test (1971/2) for a dose related response, although only the one thought most appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalent of these tests, Shirley, (1977)."

C. METHODS

1. Observations

Animals were examined at least once daily. A detailed palpation was performed weekly. Morbidity checks were performed early in the day and in the afternoon.

2. Body weight

Animals were weighed at the time of allocation to groups, one week prior to the beginning of the treatment period, on the first day of treatment and weekly thereafter.

3. Food consumption

Food consumption for each cage of animals was recorded weekly from 1 week prior to dosing. Food intake per mouse was calculated on a g/mouse/week basis.

Intake of tetraconazole was determined at weekly intervals.

4. Analysis of Diet formulations

Prior to the study, the formulation procedure was checked by chemical analysis to confirm that the method was acceptable and that the homogeneity and stability of the formulation was satisfactory under study conditions. Analysis of diet formulations was performed by the HRC Dept of Analytical Chemistry.

Results: Analyses of diet formulations prepared for

Weeks 1 and 4 showed concentrations to be within 8% of the nominal value.

5. Sacrifice and Pathology

At the conclusion of the test period, all animals were sacrificed. Animals were killed by cervical dislocation and a macroscopic examination was performed. The livers were removed, weighed and 3 were pooled per sex and treatment group and a microsomal subcellular fraction was prepared from each pool. These were stored at -75 degrees C. The following parameters were measured: protein, cytochrome P450 concentrations and activities of 7-ethoxyresorufin O-deethylase (a marker for CYP1A), 7-pentoxyresorufin O-dependentylase (a marker of CYP2B), ethylmorphine N-demethylase (a marker for CYP3A), lauric acid 11-and 12-hydroxylase (a marker for CYP2E and 4A, respectively) and p-nitrophenol UDP-glucuronyltransferase.

The investigators noted that there was a suspected mix-up of one liver in one pool between the female phenobarbital and male 20 ppm Tetraconazole groups, and therefore analyses from only 5 pools were reported for both of these groups.

II. **RESULTS**

A. Observations

1. Mortality - No animals died during this study.

2. Clinical Signs - No animals in the Tetraconazole test groups exhibited any clinical signs associated with treatment.

B. Body weight and weight gain

Overall group mean body weight gain for males at the 20, 800, or 1250 ppm levels and females receiving 1250 ppm were lower as compared to untreated controls for weeks 0-4 of the study, ($p \leq 0.05$) except at the 20 ppm level which demonstrated statistical significance for only the first 2 weeks. The investigators reported that statistically significant reductions were attained by males receiving 800 or 1250 ppm and females receiving 1250 ppm.

Males and females receiving the positive control showed significantly lower gains during the treatment period.

C. Food consumption

For the 4 week study period, food intake for males was reduced at the 1250 ppm dose level. This effect resulted from differences mainly during the first 2 weeks of treatment. Food consumption in other groups, including the positive controls, was similar to the untreated controls, (Table 2, page 23 of the report).

Intake of the test material was calculated for the treatment groups over the 4 week period. Males received 3.9, 150 and 225 mg/kg/day and females received 4.6, 175, and 293 mg/kg/day in the 20, 800 and 1250 ppm dose groups, respectively, (Table 3, pg.23 of the study report).

D. Sacrifice /Pathology and Hepatic Enzyme Assays:

Significant increases in enzyme induction were observed in both male and female mice receiving tetraconazole in the diet (with the exception of lauric acid 11-hydroxylase which significantly decreased). The investigators concluded that the P450 induction was greater in females than in male mice. However they noted the exception of 7-pentoxyresorufin O-depentylase activity (male control activities were lower than female control activity). At 800 ppm, the effects were generally similar or greater than at 1250 ppm Tetraconazole.

No gross pathology findings were noted by the investigators.

Table 2, ENZYME INDUCTION Males (females)

Parameter	Phenobarbital 75 mg/kg/day	20 ppm	800 ppm	1250 ppm
Relative liver wt	1.3 (1.3)	1.0 (1.1)	2.1**(2.2**)	2.7**(2.6**)
Microsomal protein mg/g liver mg/total liver	 1.4 (1.4) 1.5 (1.6)	 1.1(1.1*) 1.1(1.2**)	 1.3**(1.4**) 2.6**(3.1**)	 1.2**(1.5**) 2.6**(3.7**)
Cytochrome P450 nmoles/mg protein nmoles/g liver	 1.7 (2.0) 2.3 (2.9)	 1.1(1.2**) 1.2*(1.4**)	 2.7**(2.6**) 3.5**(3.7**)	 2.7**(2.7**) 3.1**(4.1**)
7-Ethoxyresorufin O-deethylase nmoles/min/mg protein nmoles/min/g liver	 2.7(5.3) 3.7 (7.5)	 0.9(1.1) 1.0(1.2)	 0.9(1.3*) 1.2 (1.8**)	 1.1 (1.3*) 1.3*(2.0**)
7-Pentoxyresorufin O-depentylase nmoles/min/mg protein nmoles/min/g liver	 70.3(22.9) 88.8(32.4)	 5.0**(2.0*) 4.9**(2.2**)	 7.7**(2.3*) 9.1**(3.2**)	 6.0**(1.8*) 6.5**(2.7**)
Ethylmorphine N-demethylase umoles/hr/mg protein umoles/hr/g liver	 4.4(6.9) 6.0(9.9)	 1.2(1.5**) 1.3(1.7*	 2.3**(3.1**) 3.0**(4.4**)	 2.3**(2.6**) 2.7**(4.0**)
Lauric acid 11-hydroxylase nmoles/min/mg protein nmoles/min/g liver	 1.6 (1.8) 2.1 (2.6)	 0.9 (0.9) 1.0 (1.0)	 0.7**(0.5**) 0.9 (0.8**)	 0.6**(0.4**) 0.7**(0.7**)
Lauric acid 12-hydroxylase nmoles/min/mg protein nmoles/min/g liver	 1.1 (0.7) 1.4 (1.0)	 1.0(1.1) 1.1(1.2)	 1.1(1.8) 1.4(2.5**)	 1.2(1.3) 1.4(2.1**)
p-Nitrophenol UDP-glucuronyltransferase umoles/hr/mg protein umoles/hr/g liver	 1.0(1.2) 1.4(1.7)	 1.0(1.1) 1.1(1.2*)	 1.2**(1.4**) 1.6**(1.9**)	 1.2**(1.2**) 1.4**(1.8**)

Data extracted from Tables 1 and 2, pages 96 and 97 of the report.

III. DISCUSSION

Tetraconazole appears to act as a phenobarbitone-type inducer. A dose response was not apparent between the mid and high dose levels.

Statistically significant increases were seen in all enzymes assayed (except Lauric acid 11-hydroxylase) at 800 and 1250 ppm. Cytochrome P450, 7-pentoxyresorufin O-dependentylase, ethylmorphine N-demethylase (females) and p-Nitrophenol UDP-glucuronyltransferase (females) were also increased at 20 ppm.

IV. Study deficiencies

The positive control was not analyzed for concentration.

EPA Reviewer: Laurence D. Chitlik, DABT L Chitlik, Date 4/28/99
Toxicology Branch (7509C)
EPA Secondary Reviewer: Brian Dementi, Ph.D. Brian Dementi, Date 8/30/99
Toxicology Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Liver Enzyme Induction following Dietary Administration to CD Rats for 4 Weeks

OPPTS Number: 870.4200 (FIFRA) OPP Guideline Number: §83-5

TEST MATERIAL (PURITY): Tetraconazole; 95.2% ai)

CITATION: Waterson, Lynne A. (1998) Investigation of Liver Enzyme Induction Following Dietary administration to CD Rats for 4 Weeks. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, PE186ES, England. Report Number AGR 112/974061. April 2, 1998. MRID 44751310. Unpublished.

SPONSOR: Tetraco S.r.l., Centro Direz. Milano Oltre, Pal. Raffaello, Via Cassanese, 224, 20090 Segrate, Milan, Italy

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44751310, Tetraconazole 95.2% ai, Batch number FCF/T/122-95) was administered to 6 male and female Crl:CD BR rats per dose level at levels of 0, 10, 80, 640 ppm in the diet. The positive control was Phenobarbital (Na) salt, 75 mg/kg/day.

Data generated in this study indicate that Tetraconazole administration for 4 weeks results in liver enzyme induction at dose levels of 80 and 640 ppm. Induction at the 640 ppm dose level was similar to that induced by phenobarbital at 75 mg/kg/day.

This subchronic toxicity study in rats is an **acceptable** non-guideline study in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:Tetraconazole Technical
   Description: Viscous yellow/brown liquid
   Lot/Batch #: Batch no. FCF/T/122-95
   Purity: 95.2% ai
   Storage conditions: 4 degrees C in the dark

2. Vehicle and/or positive control: The test material was dissolved in acetone and blended with diet. The positive control was Phenobarbital (sodium salt) dissolved in deionized distilled water.

3. Test animals: Species: Crl:CD BR rats
   Strain: rat
   Age and weight at study initiation: Eight weeks at start of dosing with a range of 295 to 338 grams for males and 184 to 213 grams for females.
   Source: Charles River Breeding Laboratories, Margate, Kent, UK.
   Housing: Three to a cage.
   Diet: SDS rat maintenance diet
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 20-22 degrees C
   Humidity: 46 to 86%
   Air changes: not noted
   Photoperiod: 12-hour light/dark cycle
   Acclimation period: a six day period followed by a 7 day period

B. STUDY DESIGN:

1. In life dates - Start: October 22, 1997
   End: December 2-4, 1997

2. Animal assignment

   Seventy rats were used in this study. Initially, five males and females were sacrificed for a health check. Animals were assigned to groups as noted in the following table:

TABLE 1: STUDY DESIGN

Test Group	Dose to Animal (ppm)	Animals Assigned	
		Male	Female
1	0	1-6	31-36
2	10	7-12	37-42
3	80	13-18	45-48
4	640	19-24	49-54
5	Phenobarb (Na) salt	25-30	55-60
Health Check*		61-65	66-70

* Animals sacrificed prior to administration of the test material in order to assess the health status of the test animals

The positive control, phenobarbital (Na salt) was administered as a solution using a syringe and rubber catheter inserted via the mouth into the stomach. A constant dosage volume of 5 ml/kg was used.

3. Statistics

The report stated on pages 17 and 18 relative to food consumption and bodyweight data:

"If the data consisted predominantly of one particular value(relative frequency of the mode exceeded 5%), the proportion of animals with values different from the mode was analyzed, Fisher (1950) and Mantel (1963). Otherwise:

A test was applied to test for heterogeneity of variance between treatments, Bartlett (1937). Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, an analysis of ranks was

used, Kruskal and Wallis (1952/3).

Analyses of variance were followed by Student's t test and Williams' test (1971/2) for a dose related response, although only the one though most appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalent of these tests, Shirley, (1977)."

C. METHODS

1. Observations

Animals were examined at least once daily. A detailed palpation was performed weekly. Morbidity checks were performed early in the day and in the afternoon.

2. Body weight

Animals were weighed one week prior to the beginning of the treatment period, one the first day of treatment and weekly thereafter.

3. Food consumption

Food consumption for each cage of animals was recorded weekly from 1 week prior to dosing. Food intake per rat was calculated on a g/rat/week basis.

Intake of tetraconazole was determined at weekly intervals.

4. Analysis of Diet formulations

Analysis of diet formulations was performed by Huntingdon Life Sciences Department of Analytical Chemistry. Analyses of diet formulations prepared for Weeks 1 and 3 showed concentrations to be within 8% of the nominal value.

5. Sacrifice and Pathology

At the conclusion of the test period, all animals were sacrificed. The sacrifices took 3 days to complete. Groups 2 to 4 continued to received test material until the day of sacrifice. Animals were killed by carbon dioxide asphyxiation and then received a macroscopic examination. The liver was removed, weighed and placed

in isotonic ice-cold buffer, pH7.4. The liver was then processed. The thyroids were examined, removed and weighed and fixed in 10% neutral buffered formalin. The skull was examined an d preserved for possible future examination. Abnormal tissues were not preserved.

A microsomal subcellular fraction was prepared from each liver by differential centrifugation and stored as aliquots at -75 degrees C. The hepatic microsomal parameters measured included protein, cytochrome P{450 concentrations and activities of 7-pentoxyresorufin O-depentylase, ethylmorphine N-demethylase and p-nitrophenol UDP-glucuronyltransferase.

II. RESULTS

A. Observations

1. Mortality - No animals died during this study.

2. Clinical Signs - No animals in the Tetraconazole test groups exhibited any clinical signs associated with treatment. In the positive control group, animals showed lethargy and unsteady gait.

B. Body weight and weight gain

Overall group mean body weight gain for males at the 640 ppm dose level was significantly lower as compared to untreated controls for weeks 0-4 of the study ($p \leq 0.05$) The weight gain for this group was apparently depressed during the first week of treatment ($p \leq 0.01$, Williams' test) but thereafter, gain was comparable to controls. Food consumption was also reduced during the first week of treatment. A reduction in body weight gain was also noted in females at the high dose level, but statistical significance was not attained.

Males receiving the positive control showed significantly lower gains during the treatment period ($p \leq 0.01$, Student's t test)

C. Food consumption

Males in the 640 ppm dose group showed statistically significant lower mean food consumption during week 1. Statistical significance was not reached in females. For the remainder of the study, mean food consumption was comparable

in all groups. Positive control males demonstrated statistically significant lower food consumption throughout the test period.

Over the four week dosing period, mean tetraconazole intake was 0.8, 6.6, and 54.6 in males and 0.9, 7.6, and 57.6 mg/kg/day for females in the 10, 80, and 640 mg/kg/day dose groups, respectively (Table 3, pg. 27 of the submitted report).

D. Sacrifice and Pathology:

No gross pathology findings were noted which could be associated with treatment with the test material. Both males and females at the 640 ppm dose level showed higher liver-to-body weight ratios as compared to controls ($p<0.01$), but mean weights were not significantly elevated as compared to controls. Thyroid weights were comparable in all groups. Animals receiving the positive control showed higher liver-to-body weight ratios and elevated mean thyroid weights.

E. Hepatic Enzyme Assays

At the 10 ppm level, an increase in 7-pentoxyresorufin O-depentylase activity in males was noted. No effects in females at 10 ppm were noted. At 80 ppm, males showed increases in all parameters except microsomal protein concentration. Females at the 80 ppm level demonstrated a significant increase in 7-pentoxyresorufin O-depentylase activity. At 640 ppm, significant effects were apparent in both sexes. The positive control, phenobarbital elicited greater effects on Phase I, but a reduced effect on p-nitrophenol UDP-glucuronyltransferase activity as compared to the test material at 640 ppm.

After 4 weeks of administration, Tetraconazole induced cytochromes P450, including CYP2B and 3A as well as UDP-glucuronyltransferase.

Table 2, ENZYME INDUCTION Males (Females)

Parameter	Phenobarbital 75 mg/kg/day	10 ppm	80 ppm	640 ppm
Relative liver wt	1.1** (1.3**)	1.0 (1.0)	1.0 (1.1)	1.2** (1.2**)
Microsomal protein mg/g liver mg/total liver	 1.5** (1.2*) 1.6** (1.5**)	 1.0 (0.9) 1.0 (0.9)	 1.1(1.0) 1.1(1.0)	 1.4**(1.1) 1.6**(1.3*)
Cytochrome P450 nmoles/mg protein nmoles/g liver	 2.1** (1.6**) 3.2** (2.0**)	 1.2(1.1) 1.2(0.9)	 1.3*(1.1) 1.4*(1.0)	 1.7**(1.4**) 2.5**(1.5*)
7-Pentoxyresorufin O-dependentylase nmoles/min/mg protein nmoles/min/g liver	 23** (424**) 37** (481**)	 1.6*(1.0) 1.7*(0.8)	 2.1**(12**) 2.4**(11**)	 8.9**(105**) 14**(107**)
Ethylmorphine N-demethylase nmoles/min/mg protein Nmoles/min/g liver	 3.1**(5.5**) 4.8**(6.9**)	 1.2 (0.7) 1.3 (0.6)	 1.4*(1.1) 1.5*(1.1)	 2.0**(4.6**) 3.0**(5.0**)
p-Nitropphenol UDPGT nmoles/min/mg protein nmoles/min/g liver	 3.4**(2.0**) 5.2**(2.5**)	 1.4(1.1) 1.5(1.0)	 1.6*(1.3) 1.8(1.3)	 3.7**(2.2**) 5.4**(2.4**)

Data extracted from tables 1 and 2, pages 120 and 121 of the test report.

III. DISCUSSION

Tetraconazole appears to act as a phenobarbitone-type inducer. At the high dose level, enzyme induction is similar to that of rats receiving 75 mg/kg/day of phenobarbital.

Such effects were generally seen at 640 ppm in both sexes, while for the enzyme 7-pentoxyresorufin O-dependentylase significant increases extend to 80 ppm (females) and to the lowest dose, 10 ppm (males). Also, increases in cytochrone P450, ethylmorphine N-demethylase and p-Nitrophenol UDP-glucuronyltransferase were seen at 80 ppm in males.

IV. Study deficiencies

The positive control was not analyzed for concentration.