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DATA EVALUATION REPORT

STUDY TYPE: Dose-ranging (mice) for oncogenicity; Guideline 82-1

TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 407009-33

TEST MATERIAL: HWG 1608 TECHNICAL; mixed batch, FL No. 132; solid; purity of 96.9%

SYNONYMS: FOLICUR@;(terbuconazole); ethyltrianol(proposed)

STUDY NUMBER(S): report no. T 0018885(8 wk study); report no. T 6018539(5 day study); Lab. proj. ID no. 94211

SPONSOR: Mobay Corporation, Corporate Toxicology

TESTING LABORATORY: BAYER AG, Institut fuer Toxikologie Landwirtschaft, Fachbereich Toxikologie, D 5600 Wuppertal 1, FRG

TITLE OF REPORT: Range-finding toxicological study study with NMRI mice to establish dosage for a chronic study (feeding for eight weeks) and for determinations of enzyme induction in the liver (feeding for five days)

AUTHOR(S): Dr. W. Ramm

REPORT ISSUED: July 7, 1986

CONCLUSIONS: In an eight week dietary dose range-finding study, terbuconazole produced a slight but consistent depression in female but not male body weights at the HDT. The liver is a major target site for toxicity at both 500 and 2000 ppm as evidenced by increased absolute/relative organs weights, elevated total and indirect bilirubin, gross changes in liver appearance (paleness, lobulation) and increased histopathology findings (necrosis, vacuolization, degeneration, lipidosis). Other potential targets are the spleen (increase pigment deposition, increase serum Fe2+, heart (elevated relative weight), kidneys (increased presence of round cell infiltrates) and the adrenals (increased lipid concentrations, sinus dilation). Terbuconazole was an effective inducer of microsomal enzyme after 5 days of treatment at all dose levels evaluated (125, 500, 2000 ppm). Based upon these findings, dose levels of 20, 60 and 180 ppm were selected for the chronic study.

This study is designated as Core supplementary data.

MATERIALS AND METHODS: (photocopy of methods appended)

1. Eight week study

SPF-bred mice (4-5 weeks old) in groups of 5 males and 5 females per dose level were acclimatized for 7 days and then fed terbuconazole daily for 8 weeks at 0, 500 and 2000 ppm in their diet. Animals were inspected twice daily (once on weekends, public holidays) and alterations noted. Animal weights were recorded at start of study and then weekly with weekly food consumption and water intake being determined. Clinical chemistries were performed at study termination and included bilirubin and iron.

At study termination, gross and histopathology were conducted with the heart, testicles, ovaries, liver, lung, spleen, kidneys and adrenals weighed (see appended methods for organs/tissues examined). All organs/tissues in the control and 2000 ppm dose groups were examined plus the liver, lungs, spleen, kidneys and adrenals of the mid dose.

Arithmetic group means, standard deviations with 95 and 99% upper and lower confidence limits were determined. Collective values were evaluated for statistical significance with Mann, Whitney and Wilcoxon test.

2. Five day enzyme induction test

NMRI mice of the same strain (five of each sex/dose) were treated with 0, 125, 500 and 2000 ppm for five consecutive days via their diet. After five days the animals were sacrificed, the livers removed and frozen for determination of cytochrome P-450, triglycerides, N- and O-demethylase.

GLP CONCERNS: Signed and dated statements regarding data confidentiality and GLPs were included. Individual phases of the study and the final version of the report were not inspected by the QAU unit.

RESULTS/CONCLUSIONS:

Eight week study

MORTALITY/CLINICAL SIGNS

One female in the control (#6) and one male (# 15) in the 500 ppm group died during terminal blood sampling, apparently due to ether overdose or hypovolemia.

No clinical signs data were provided. The authors stated that mice at 500 and 2000 ppm did not differ in appearance and behavior from controls.

BODY WEIGHTS/FOOD CONSUMPTION/WATER INTAKE

There were no differences among the dose groups for either sex with regard to mean food consumption (g/animal/day) or water intake (ml/animal/day).

Mean body weights for males during the eight week period were similar but female body weights in the HDT as compared to the controls were consistently 1-2 grams lower (e.g., wk 2: control = 28.5 g vs 2000 ppm = 26.8g; wk 6: control = 28.8g vs 2000 ppm = 27.0g).

CLINICAL CHEMISTRIES

There was a statistically significant decrease in total bilirubin and indirect bilirubin concentrations in males at 500 and/or 2000 ppm as well as a statistically significant increase in serum iron (see below; form Table 3, p. 17 of report). No statistically significant changes were observed in treated females although mean iron values were elevated in the HDT as compare to the controls (54.3 vs 47.4, resp.).

	(UMOL/L)			
dose(ppm)	total Bili	direct Bili	indirect Bili	Fe++
0	3.8	1.2	2.6	41.1
500	2.5	1.8	1.0*	44.3
2000	2.5*	1.4	1.1*	51.7**

*,** statistically significantly different from controls ($p<0.05$;<0.01, resp.)

ORGAN WEIGHTS

Selected summary absolute (mg)/relative (mg/100 gm) organ weights are presented below:

DOSE(PPM)	LIVER		ADRENALS	
	Males	Females	Males	Females
0	1995/4802	1555/4971	7/18	14/46
500	2661*/6435*	2125*/6960*	8/19	13/43
2000	2846*/7204**	2090*/7394*	8/21	11*/38

Statistically significant increase in absolute and relative liver weights for both male and female mice were observed at 500 and 2000 ppm. In HDT females, absolute and relative adrenal weights were decreased (statistically significant for absolute weight). Not shown are compound treated male heart weights which were elevated with the relative weights significantly so ($p<0.05$; 488/control vs 601/500 and 593/2000 ppm, respectively).

GROSS PATHOLOGY/HISTOPATHOLOGY

Apparent compound-related gross changes were swollen or increased lobulation of the liver of males (500 ppm, males, females: 4/5, 0/5, resp.; 2000 ppm, males, females: 3/5, 0/5, resp.) and increase in pale livers, primarily in females (500 ppm, males, females: 1/5, 1/5, resp.; 2000 ppm, males, females: 1/5, 4/5, resp.). None of these changes were observed in controls.

A summary of histopathology findings (taken from Table 2, p. 61 of the report) is presented below:

Alterations	0 ppm	500 ppm	2000 ppm
LIVER			
-liver c. degeneration(grade)*	---	2.2	2.7
# affected	0/9	10/10	10/10
-individual necrosis	---	----	0.6
# affected	0/9	0/9	4/10
-focal necrosis	---	----	0.1
# affected	0/9	0/9	1/10
-large vacuoles	---	0.7	0.4
# affected	0/9	4/9	4/10
-fat content(ORO stain)	1.2	3.0	3.5
SPLEEN			
-pigment increased	---	---	1.0
#affected	0/9	9/9	10/10
KIDNEYS			
-round c. infiltrates	0.1	0.3	0.3
# affected	1/9	2/9	3/10
-round c. infiltrates in pelvis	0.3	0.2	0.6
# affected	3/9	2/9	6/10
ADRENALS			
-cortex c. lipid-rich (males)	---	1.5	3.0
# affected	0/5	4/4	5/5
-sinus dilation	0.4	0.4	1.1
# affected	2/9	2/10	5/10
cortical hyperplasia	0.5	0.6	0.3
# affected	3/9	6/9	3/10
brown degeneration of x-zone (females)	2.0	1.0	1.6
# affected	4/5	5/5	5/5

* Grades: 1 = slight; 2 = slight to moderate; 3 = moderate; 4 = moderate to severe; 5 = severe

The liver, spleen, possibly the kidneys and the adrenal glands appear to be sites of compound-induced toxicity at either the 500 and/or 2000 ppm dose levels in males and/or females. As indicated in the summary, there was an increase in the incidence and severity (grade) of liver cell degeneration, liver cell necrosis, focal necrosis and presence of vacuoles and lipid content at either or both doses. At the HDT, there was an increase in the incidence of pigmentation (iron-related pigment) and round cell infiltrates of the kidneys were apparently greater in the HDT as compared to the controls. The adrenals were also significantly affected as evidenced by an increase in cortical cell lipids (both dose groups) and sinus dilation (HDT) of males.

Five day enzyme induction study

Summary data for enzyme induction/triglycerides in liver are presented below (taken from Table 6, p. 22 of report):

Dose(ppm) Males	N-demethyl. (mU/g)	O-demethyl. (mU/g)	P-450 (nmol/g)	Trigly. (umol/g)
0	234.5	47.9	36.7	4.29
125	225.2	48.4	55.8**	12.71**
500	288.9	54.6	107.0**	18.84**
2000	222.1	86.1**	131.9**	23.78**
Females				
0	217.2	48.9	32.1	5.29
125	490.7**	64.4	45.6*	10.84
500	556.6**	77.6**	94.6**	23.45**
2000	364.2**	92.4**	110.5**	31.76**

Terbuconazole induced microsomal enzymes in both sexes at all dose levels as compared to controls. In males, there was a statistically significant increase in O-demethylase (HDT), P-450 and triglycerides (all dose levels). N-demethylase, O-demethylase, P-450 and triglycerides were all significantly elevated in female mice at all dose levels.