CANCER BRIEFING PACKAGE

PC CODE: 108209 (Orthosulfamuron)

DATE OF PACKAGE: 9/7/2006

SUBMITTED BY: Jessica Kidwell
SIGNATURE AND DATE

For Archives

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

OFFICE OF PREVENTION,
PESTICIDES, AND TOXIC SUBSTANCES

MEMORANDUM

DATE: September 7, 2006

SUBJECT: *Cancer Assessment Review Committee Meeting on* ORTHOSULFAMURON

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on ORTHOSULFAMURON prepared by Karlyn Bailey.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday, September 20, 2006 at 10 am in Room S-10100, PY1.

Addressees:

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CANCER ASSESSMENT DOCUMENT

FOR COMMITTEE DELIBERATION

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
Orthosulfamuron

PC CODE 108209

September 20, 2006
Submitted by: Karlyn J. Bailey

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

TABLE OF CONTENTS

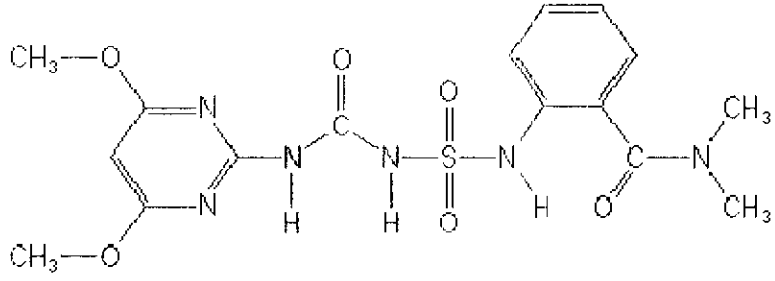
EXECUTIVE SUMMARY

I. INTRODUCTION

On September 20, 2006 the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs will meet to evaluate the carcinogenic potential of Orthosulfamuron.

II. BACKGROUND

Chemical Name:	Orthosulfamuron
IUPAC Name:	1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea
Other Name:	2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-*N,N*-dimethylbenzamide
CAS Registry No.:	213464-77-8
PC Code:	108209
Structure:	



Orthosulfamuron (1-(4,6-dimethoxypyrimidin-3[2-(dimethylcarbamoyl) phenylsulfamoyl] urea) is a systemic herbicide belonging to the sulfonylurea class of chemicals. It is being proposed for control of broadleaf weeds and sedges in rice. The mode of action for orthosulfamuron is through inhibition of the plant enzyme acetolactate synthase, which is also known as acetohydroxy acid synthase. Inhibition of this enzyme blocks branch-chain amino acid biosynthesis of valine, leucine, and isoleucine involved in plant growth processes leading to death of the plant.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. *Combined Chronic Toxicity/Carcinogenicity Study in Sprague-Dawley Rats*
Combined Carcinogenicity and Toxicity Study by Dietary Administration to Hans Wistar Rats for 104 Weeks (2004). Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project No. A2/05/05/02, AGR/131/033063, AGR131. July 20, 2004. MRID 46578913.

A. Experimental Design

Orthosulfamuron (IR5878; 98.6-98.8% a.i.; Batch Nos.: FCF/T/191-01 and G009/02) was administered in the diet to 70 Han Wistar (HsdBrl Han:Wist) rats /sex/dose at nominal concentrations of 0, 1, 5, 500, or 1000 mg/kg/day for up to 2 years. Twenty rats/sex/dose were sacrificed at Week 52, and the remaining survivors were sacrificed at Week 104.

B. Discussion of Survival and Tumor Data

Survival Analysis

TOXICITY PHASE

One 500 mg/kg/day female rat was killed in *extremis* during the first 52 weeks (week 20) of treatment. Histopathological examination indicated hepatocyte torsion, necrosis, and severe congestion; this death was considered unrelated to treatment.

CARCINOGENICITY PHASE

There were no statistically significant incremental changes in mortality with increasing doses of Orthosulfamuron in male rats **(Memo, L. Brunsman, 8/30/06, TXR# 0054345).** A total of 54 male and 66 females assigned to the carcinogenicity phase died or were sacrificed. The overall group distribution of these deaths was not affected by treatment. Survival in both sexes met the guideline requirements of 50% at week 78 and 25% at week 104.

Tumor Analyses

Males
Male rats had statistically significant trends in thyroid follicular cell adenomas, and adenomas and carcinomas combined, both at $p < 0.01$. There were statistically significant pair-wise comparisons of the 500 mg/kg/day dose group with the controls for thyroid follicular cell adenomas, and adenomas and carcinomas combined, both at $p < 0.05$. There were also statistically significant pair-wise comparisons of the 1000 mg/kg/day dose group with the controls for thyroid follicular cell adenomas, and adenomas and carcinomas combined, both at $p < 0.01$. The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test for pair-wise comparisons and the *ad hoc* Exact Test for trend since there were no statistically significant trends for mortality. **(Table 1, Memo, L. Brunsman, 8/30/06, TXR# 0054345).**

Historical control data for thyroid follicular cell adenoma in males were not provided by the Registrant (historical control data has been requested). Therefore, historical control data were obtained from Charles River Laboratories (Giknis and Clifford, 2001). In male Wistar Hans control rats, tested approximately 104 weeks, data were taken from 10 studies initiated prior to 1999. Of these 10 studies, all had adenomas. The percent affected ranged from 1.67-12.73%. Carcinomas were present in 7 out of 10 studies. The percent affected ranged from 1.67-3.64%.

Females

There were no treatment-related tumors observed in female rats.

Table 1. Orthosulfamuron – Han Wistar Rat Study (MRID 46578913)

Male Thyroid Follicular Cell Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (mg/kg/day)				
	0	1	5	500	1000
Adenomas (%)	1/50 (2)	2/50 (4)	1/50 (2)	7[a]/50 (14)	10/49 (20)
p =	0.00006**	0.50000	0.75253	0.02972*	0.00349**
Carcinomas (%)	0/50 (0)	1/50 (2)	1[b]/50 (2)	0/50 (0)	0/49 (0)
p =	0.3223	0.5000	0.5000	1.0000	1.0000
Combined (%)	1/50 (2)	3/50 (6)	2/50 (4)	7/50 (14)	10/49 (20)
p =	0.00030**	0.30865	0.50000	0.02972*	0.00349**

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 70, dose 500 mg/kg/day.
[b]First carcinoma observed at week 91, dose 5 mg/kg/day.

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 $p < 0.01$.

Historical controls: thyroid follicular cell adenoma, range = 1.67-12.73%
thyroid follicular cell carcinomas, range= 1.67-3.64%

C. Non-Neoplastic Lesions in Han Wistar Rats

The non-neoplastic lesions in male and female rats treated with orthosulfamuron are presented in Tables 2 and 3. In a combined chronic toxicity/carcinogenicity study, after 52 weeks (toxicity phase), increased ($p \leq 0.01$) incidences of minimal to moderate centrilobular hepatocyte vacuolation were observed in the ≥500 mg/kg/day males (50-95% treated vs 0% controls) and minimal to moderate centrilobular hepatocyte hypertrophy in the ≥500 mg/kg/day males (60-75% vs 10%) and 1000 mg/kg/day females (60% vs 0%). Additionally at Week 52, increased incidence ($p \leq 0.05$) in pancreatic acinar cell vacuolation was observed in the 1000 mg/kg/day males (95% treated vs 45% controls); however, other indications of toxicity were not evident, and an adverse effect at 104 weeks was also not substantiated.

In the carcinogenicity phase, increased ($p \leq 0.05$) incidences of the following findings (% treated vs % controls) were observed in the liver: (i) minimal to marked centrilobular hepatocyte vacuolation in the ≥500 mg/kg/day males (62-80% vs 30%); (ii) slight centrilobular hepatocyte hypertrophy in the 1000 mg/kg/day males (42% vs 6%); (iii) minimal to slight cystic degeneration in the 1000 mg/kg/day males (16% vs 0%); and (iv) slight to moderate focal sinusoidal dilatation in the 1000 mg/kg/day females (12% vs 0%). In the thyroid, increased incidences of minimal to marked cystic follicular cell hyperplasia (20% treated vs 4% controls; $p \leq 0.05$) were noted in the 1000 mg/kg/day males, and increased incidences of minimal follicular cell hypertrophy were observed in the ≥500 mg/kg/day males (42% each treated vs 24% control). An increased (NS) incidence in slight to moderate chronic progressive nephropathy of the kidney was observed in the ≥500 mg/kg/day males (46-52% treated vs 34% controls). Increased ($p \leq 0.05$) incidences in the following kidney lesions were observed (% treated vs % controls) in females: (i) slight to marked chronic progressive nephropathy at 1000 mg/kg/day (52% vs 24%); (ii) minimal to marked pelvic/papillary epithelium hyperplasia at ≥500 mg/kg/day (88-92% vs 70%); and (iii) minimal to marked papillary/pelvic epithelium mineralization at ≥5 mg/kg/day (88-94% vs 74%; NS at 5 and 500 mg/kg/day). An increased ($p \leq 0.05$) incidence of minimal to moderate hemosiderosis was observed in the spleen of the ≥500 mg/kg/day females (86-92% treated vs 66% controls).

Additionally, increased incidence of peri-islet pigment in the pancreas (22% treated vs 6% controls; $p \leq 0.05$) was noted in the 1000 mg/kg/day males; however, this isolated finding was considered incidental. Increased incidences of other findings in the treated groups relative to controls were minor and/or not corroborated by other clinical or pathological findings.

Table 2. **Non-Neoplastic Histopathological Findings in Male and Female Rats Treated With Orthosulfamuron in the Diet for up to 52 Weeks (toxicity phase).**

Microscopic lesion		Dose (mg/kg/day)				
		0	**1**	**5**	**500**	**1000**
Males						
Liver	Hepatocyte vacuolation, centrilobular (total)	0 (0)	0 (0)	0 (0)	10** (50)	19** (95)
	Minimal	0	0	0	10	5
	Slight	0	0	0	0	9
	Moderate	0	0	0	0	5
	Hepatocyte hypertrophy, centrilobular (total)	2 (10)	5 (25)	2 (10)	12** (60)	15** (75)
	Minimal	2	5	2	11	0
	Slight	0	0	0	1	14
	Moderate	0	0	0	0	1
Females						
Liver	Hepatocyte hypertrophy, centrilobular (total)	0 (0)	0 (0)	0 (0)	0 (0)	12** (60)
	Minimal	0	0	0	0	11
	Slight	0	0	0	0	1

a Data were obtained from Table 29F on pages 257-273 and pages 1549-2131 of MRID 46578913.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

Table 3. **Non-Neoplastic Histopathological Findings in Male Rats Treated With Orthosulfamuron in the Diet for up to 104 Weeks (carcinogenicity phase).**

Microscopic lesion		Dose (mg/kg/day)				
		0	**1**	**5**	**500**	**1000**
Liver	Hepatocyte vacuolation, centrilobular (total)	15/50 (30)	5/50* (10)	4/50** (8)	31/50** (62)	40/50** (80)
	Minimal	11	4	3	16	6
	Slight	4	1	1	12	28
	Moderate	0	0	0	2	6
	Marked	0	0	0	1	0
	Hepatocyte hypertrophy, centrilobular, slight (total)	3/50 (6)	4/50 (8)	3/50 (6)	8/50 (16)	21/50** (42)
	Cystic degeneration (total)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	8/50** (16)
	Minimal	0	0	0	0	3
	Slight	0	0	0	0	5

Microscopic lesion	Dose (mg/kg/day)				
	0	1	5	500	1000
Thyroid Cystic follicular cell hyperplasia	2/50 (4)	0/50 (0)	4/50 (8)	5/50 (10)	10/49* (20)
Minimal	1	0	1	1	1
Slight	0	0	1	2	5
Moderate	1	0	2	2	2
marked	0	0	0	0	2
Follicular cell hypertrophy, minimal (total)	12/50 (24)	11/50 (22)	16/50 (32)	21/50 (42)	21/49 (42)
Kidney Chronic progressive nephropathy (total)	17/50 (34)	12/50 (24)	7/50* (14)	26/50 (52)	23/50 (46)
slight	16	11	5	24	21
moderate	1	0	1	2	2
marked	0	1	1	0	0

a Data were obtained from Table 29F on pages 257-273 and pages 1549-2131 of MRID 46578913.
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

Adequacy of the Dosing for Assessment of Carcinogenicity

There were no significant treatment-related effects on mortality. One 500 mg/kg/day female died during the toxicity phase of the study. In the carcinogenicity phase animals, survival was 64-84% for all groups, and response was unrelated to dose. Thus, survival exceeded guideline requirements of 50% at Week 78 and 25% at Week 104 in both sexes.

At week 52, relative liver weights were increased (p≤0.01) in both sexes at 500 (↑9-11%) and 1000 (↑14-22%) mg/kg/day. Additionally in the 1000 mg/kg/day males, increases (p≤0.01) were observed in absolute liver weights (↑20%) and relative kidney weights (↑7%). There were also increased (p≤0.01) incidences of minimal to moderate centrilobular hepatocyte vacuolation observed in the ≥500 mg/kg/day males (50-95% treated vs 0% controls) and minimal to moderate centrilobular hepatocyte hypertrophy in the ≥500 mg/kg/day males (60-75% vs 10%) and 1000 mg/kg/day females (60% vs 0%).

During the second year (Weeks 52-104) of treatment at ≥500 mg/kg/day, body weight gains were significantly (p≤0.05) decreased (↓24-38%; not significant [NS] in the 500 mg/kg/day males), resulting in decreased (p≤0.05) overall (Weeks 0-104) body weight gains (↓11-20%). Relative kidney weights were increased (p<=0.01) by 11-14%, and an increased (NS) incidence in slight to moderate chronic progressive nephropathy of the kidney (46-52% treated vs 34% controls) was observed in males. In females, increased (p<=0.05) incidences (% treated vs % controls) of minimal to marked pelvic/papillary epithelium hyperplasia (88-92% vs 70%) and minimal to marked papillary/pelvic epithelium mineralization (90-94% vs 74%; NS at 500 mg/kg/day) were noted.

At 2 years, the following findings were noted in males: i) increased incidence of dark area(s) on the liver; ii) increased absolute liver weights; iii) increased incidence of slight centrilobular hepatocyte hypertrophy; and iv) increased incidence of minimal to slight cystic degeneration. In the females, the following findings were observed: i) increased relative liver weights; ii) increased incidence of slight to moderate focal sinusoidal dilatation; iii) increased blood urea; iv) increased relative kidney weights; and v) increased incidence of slight to marked chronic progressive nephropathy.

2. Carcinogenicity Study in CD-1 Mice

Carcinogenicity Study by Dietary Administration to CD-1® Mice for 78 Weeks (2002). Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project No. AGR/130. December 22, 2003. MRID 46578912

A. Experimental Design

Orthosulfamuron (IR5878; 98.0% a.i.; Batch #: FCF/T/172-00 (ex 20525/03/8)] was administered in the diet to 50 Crl:CD-1^{TM} (ICR)BR mice/sex/dose at nominal concentrations of 0, 100, 500, or 1000 mg/kg/day for up to 78 weeks.

B. Discussion of Survival and Tumor Data

Survival Analyses

There were no treatment-related effects observed on mortality. Survival in all groups was 70-88% at Week 65, and 58-76% at Week 78, exceeding guideline requirements of 50% at Week 65 and 25% at Week 78.

Tumor Analyses

There were no treatment-related tumors observed in male or female mice.

C. Non-Neoplastic Lesions in CD-1 Mice

The non-neoplastic lesions observed in male and female mice treated with orthosulfamuron are presented in Table 4. There were Increased ($p \leq 0.001$) incidences of slight to moderate centrilobular hepatocytes hypertrophy at ≥500 mg/kg/day (48-68%) compared to slight hypertrophy in controls (8%). Increased ($p \leq 0.05$) incidences of slight to marked centrilobular hepatocyte vacuolation was observed at 1000 mg/kg/day (50%), and slight to moderate vacuolation at 500 mg/kg/day (38%); both compared to slight to moderate vacuolation in controls (18%). Similar changes were not observed in females. Increased ($p \leq 0.01$) splenic white pulp cellularity was noted in the 1000 mg/kg/day females (28%) compared to controls (3%); however, this effect was no longer significant when animals that died prior to scheduled termination were included. No other treatment-related adverse effects were observed during non-neoplastic microscopic pathology.

TABLE 4: Non-Neoplastic Lesions in Mice after up to 78 weeks of treatment with orthosulfamuron in the diet

Dose (mg/kg/day)		0	100	500	1000
Centrilobular hepatocyte hypertrophy -	Total	8%	20%	48% ***	68% ***
	Slight	8%	18%	40%	54%
	Moderate	0	2%	8%	14%
Centrilobular hepatocyte vacuolation -	Total	18%	24%	38% *	50% **
	Slight	12%	18%	28%	24%
	Moderate	6%	6%	10%	24%
	Marked	0	0	0	2%

a Data were obtained from Text table 3 on page 28 and Table 10 F on page 147 of the study report. Percent difference from controls was calculated by the reviewers, n=50.

* Significantly different from controls, p≤0.05.

** Significantly different from controls, p≤0.01.

*** Significantly different from controls, p≤0.001.

C. Adequacy of Dosing for Assessment of Carcinogenicity

There were no significant treatment-related effects on mortality. At the doses tested, the number of surviving males and females in all groups met the minimal survival requirements of 50% at Week 65 and 25% at Week 78.

Absolute (↑12%, p≤0.05) and relative to body (↑14%, p≤0.05) liver weights were increased in the 1000 mg/kg/day males after 78 weeks of treatment when compared with controls. Decreased (↓8.0%, p≤0.05) absolute kidney weights were observed in the 1000 mg/kg/day females. Also, increases in absolute and relative to body uterus/cervix weights (↑60 and ↑76%, p≤0.05) were observed in the 1000 mg/kg/day females. However, the effects noted in kidney weights and uterus/cervix weights in the 1000 mg/kg/day females had no treatment-related histopathological correlate.

The following lesions were observed in the centrilobular hepatocytes of the males: increased (p≤0.001) incidence of slight to moderate hypertrophy at ≥500 mg/kg/day (48-68%) compared to slight in controls (8%); and increased (p≤0.05) incidence of slight to marked vacuolation at 1000 mg/kg/day (50%), and slight to moderate vacuolation at 500 mg/kg/day (38%), both compared to slight to moderate in controls (18%). Similar changes were not observed in females. Increased (p≤0.01) splenic white pulp cellularity was noted in the 1000 mg/kg/day females (28%) compared to controls (3%); however, this effect was no longer significant when animals that died prior to scheduled termination were included.

IV. TOXICOLOGY

1. Metabolism

EXECUTIVE SUMMARY: In a rat metabolism study (MRIDs 46578905 through 46578910), [^{14}C-U-phenyl] IR5878 (Lot No. 182) or [^{14}C-5-pyrimidinyl] IR5878 (Lot No. 180) in 0.5% aqueous carboxymethycellulose (radiochemical purity >97%) was administered by gavage to Sprague Dawley rats. In the preliminary study, 2 rats/sex received a single 250 mg/kg dose. In the main study, 4 rats/sex/dose received a single dose of 5 or 1000 mg/kg or 14 daily doses at 5 mg/kg (non-radioactive) followed by a single radioactive dose (5 mg/kg) on day 15. Additionally, a biliary excretion study was performed where 4 males and 7 females received a single dose at 5 mg/kg. Pharmacokinetic analyses of the absorption and distribution were performed, including blood kinetics, along with identification of the metabolites in the excreta.

Absorption was rapid in all groups, regardless of sex, dose, or number of doses. T_{max} values were 12 min for the 5 mg/kg repeated dose group, 24 min-1 h for the single 5 mg/kg dose group, and 1-4 h for the single 1000 mg/kg dose group. Following a single 5 mg/kg dose of [^{14}C-U-phenyl] IR5878, 76-82% of the dose was absorbed and found in the urine/cage wash, bile, and carcass, indicating extensive absorption. The half-life (8-48 h) was similar regardless of sex, dose, or number of doses (8.9-13.3 h), with the exception of the females treated with a single 5 mg/kg dose of [^{14}C-5-pyrimidinyl] IR5878 (16.7 h).

Within 12 h of administration of the radiolabeled dose (5 or 1000 mg/kg single dose or multiple 5 mg/kg doses), approximately half of the dose was excreted, and excretion was almost complete at 72 h post-dose.

At 72 h post dose, overall recovery of the radioactive dose from both sexes was 92-100%. The majority of the dose was recovered in the feces (43-73%); 18-46% of the dose was found in the urine; and cage wash accounted for 1-5% dose. Minimal radioactivity was detected in the carcass and GI tract (<0.8% dose). In a preliminary study, <0.02% of the radioactive dose was isolated in the expired air; therefore, this route of excretion was not analyzed. A difference in the excretion profile was generally not noted based on sex, dose, or number of doses. Regardless of sex, dose, or number of doses, the distribution of radioactivity in tissues was similar. Comparison of the concentrations of radioactivity in tissues on the basis of radiolabel was not possible due to differing sampling times and/or differing T_{max} while a time course of tissue distribution was not performed. Excluding the GI tract, concentrations of radioactivity were highest in the liver, kidney, lung, and whole blood, with the lung having the lowest concentrations. At 5 mg/kg (single or multiple doses), these tissue concentrations were 1.74-23.83 µg equiv./g vs 2.41-5.10 µg equiv./g in whole blood. At 1000 mg/kg in all treated rats, these tissue concentrations were 161-435 µg equiv./g vs 305-495 µg equiv./g in whole blood. As relatively little radioactivity remained in the carcass at 72 h post-dose (≤0.70% dose), bioaccumulation is not suspected under the test conditions.

HPLC and HPLC-MS analyses were used to identify parent and a total of 10 metabolites in excreta from rats treated with [^{14}C] IR5878. Six to 7 metabolites were identified after treatment with each radiolabeled compound, and 3 of these metabolites were common to treatment with both radiolabeled compounds. Parent and identified metabolites in excreta accounted for 71-

86% dose in all animals, and overall recovery was 95-99% dose. Unidentified compounds accounted for 1-16% dose, but no single compound accounted for ≥5% dose.

The parent was found in 5 mg/kg animals (single and multiple doses) at 1-6% dose and in 1000 mg/kg animals at 33-56% dose. The predominant metabolite was 1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea (O-desm IR5878). It was found at 53-64% dose in all animals treated at 5 mg/kg and at 14-20% dose in all animals treated at 1000 mg/kg. This compound was found in similar quantities in the urine and feces. 2-Sulfoamino-N,N-dimethylbenzamide (DBS acid) was a primary metabolite in all animals treated with [^{14}C-U-phenyl] IR5878 and was found at 8-12% dose. This compound was found primarily in the feces. In all animals treated with [^{14}C-5-pyrimidinyl] IR5878, a fraction was isolated (primarily in feces) that contained (4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)urea (Pyr-O-Glucur DOP urea) and 1-(4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea (Pyr-O-Glucur IR5878). This fraction represented 9-18% dose. Additionally, 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(methylcarbamoyl)phenyl-sulfamoyl]urea (N-desm IR5878) was found at 5-8% dose in the urine of animals treated with 1000 mg/kg [^{14}C-5-pyrimidinyl] IR5878. All other identified metabolites each accounted for <5% dose. Identification of IR5878 metabolites indicates that metabolism is mainly occurring through O- and/or N-demethylations. Additionally, hydrolytic cleavage of the sulfamoylurea linkage yields DOP urea, and hydroxylation of the pyrimidinyl ring occurs followed by glucuronic acid or sulfate conjugation.

The metabolic profile of the liver, kidney, and bile was also evaluated. In the liver and kidneys of all animals treated with [^{14}C-U-phenyl] IR5878, the parent and O-desm IR5878 were found in the highest concentrations. In the liver and kidneys of all animals treated with [^{14}C-5-pyrimidinyl] IR5878, O-desm IR5878 and an unidentified fraction were generally found in the highest concentrations, as well as 2-amino-N,N-dimethylbenzamide (DB amine) in the male kidney. In the bile, O-desm IR5878 and an unidentified fraction were found in the highest concentration. Other identified compounds (same as found in the excreta) were not detected in the liver, kidney, or bile or were generally found at relatively low concentrations.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

2. Mutagenicity

Mutagenicity

Orthosulfamuron (IR5878; parent compound)

Orthosulfamuron (IR5878) was tested in four genetic toxicology studies. The results indicate that orthosulfamuron is not mutagenic in bacteria or cultured mammalian cells. It is also not clastogenic or aneugenic *in vivo* in a bone marrow mouse micronucleus assay, or *in vitro* in a chromosome aberration assay.

Orthosulfamuron Metabolites (IR7825, IR7863, and IR8181)

Several metabolites of orthosulfamuron were tested in genetic toxicology studies. The results indicate that the three metabolites tested are not mutagenic in bacteria or cultured mammalian cells. The metabolite IR 8181 is not clastogenic or aneugenic in vivo in a bone marrow mouse micronucleus assay (other metabolites were not tested). IR7825 and IR7863 were not clastogenic or aneugenic in an *in vitro* chromosome assay; however, IR8181 exposure resulted in evidence of chromosome aberrations induced over background in the presence and absence of S-9 activation.

GENE MUTATIONS

I. Orthosulfamuron (IR5878) was not mutagenic in independently conducted *Salmonella typhimurium* and *Escherichia coli* mammalian microsome reverse gene mutation assays (MRID 46219034) up to the limit dose (5000 µg/plate +/- S9). In addition, three metabolites (IR7825, IR7863, and IR8181) were not mutagenic in independently conducted *Salmonella typhimurium* mammalian reverse gene mutation assays (MRID 46578916, 46578919, and 46578923) tested up to the limit dose (5000 µg/plate +/- S9). Cytotoxicity, as indicated by a reduction in revertant colonies, was, however, seen at 5000 ug/plate +/- S9 in the majority of strains treated with IR 7825 (MRID 46578916). The studies are acceptable.

II. The test material (MRID 46219036) and its metabolites (MRID, 46578914, 46578920, and 46578925) were not mutagenic in mouse lymphoma L5178Y cell forward gene mutation assays up to the limit dose or the limit of solubility. The studies are acceptable.

CHROMOSOME ABERRATIONS

III. Orthosulfamuron (IR 5878) and two metabolites (IR7863 and IR 7825) did not produce a significant increase in either structural or numerical aberrations at doses up to the limit dose or the limit of solubility in human lymphocyte cytogenetic assays (MRID 46219035, 46578921, and 46578917, respectively). However, in another human lymphocyte cytogenetics assay with IR8181 (MRID 46578924) orthosulfamuron induced increases (exceeding historical control range of 0-4%) in the mean percent aberrant cells (excluding gaps) at 1250 and 2500 µg/mL (+S9) and at ≥1250 µg/mL (-S9). At these concentrations, relative mitotic indices were ≥ 50% of the control. The studies are acceptable.

IV. In *in vivo* mice micronucleus assays, the test material (MRID 46219037), and the metabolite IR8181(MRID 46578926), did not induce a clastogenic or aneugenic response in bone marrow cells harvested from male mice administered a single dose or doses up to the limit dose of 2,000 mg/kg. The studies are acceptable.

CONCLUSION:

The overall results indicate that neither the parent compound nor metabolites IR7863 or IR7825 are mutagenic. In contrast, metabolite IR8181 was positive for the induction of

structural chromosome aberrations at concentrations that were minimally cytotoxic. However, there is no concern for mutagenicity at this time because the evidence of *in vitro* mutagenicity is not expressed in the whole animal.

3. Structure-Activity Relationship

The closest structural analog to orthosulfamuron is cyclosulfamuron. Based on the structural similarities, it is likely that cyclosulfamuron shares the same properties as orthosulfamuron. Cyclosulfamuron is not currently registered by the EPA; therefore, toxicology data are not available at this time.

4. Subchronic, Chronic, and Reproductive Toxicity

a) Subchronic Toxicity

90-Day Oral Toxicity - Rat (870.3100)

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity study (MRID 46260103), IR5878 (98.0% a.i., Batch No. FCF/T/172-00 (ex 20525/03/8) was administered to ten Han Wistar rats/sex/dose in the diet at dose levels of 0, 250, 1500, and 9000 ppm (equivalent to 0, 19, 113, and 706 mg/kg bw/day, respectively, for males and to 0, 22, 131, and 773 mg/kg bw/day, respectively, for females). Concurrently, a recovery group of five Han Wistar rats/sex/dose were administered either 0 or 9000 ppm (equivalent to 0 or 706 mg/kg bw/day, respectively, for males and to 0 or 773 mg/kg bw/day, respectively, for females) during the 90-day study period and were then maintained for an additional four weeks on the control diet. There were no compound-related effects on mortality, body weights, body weight gains, food consumption, neurological observations and measurements, hematology, clinical chemistry, or gross pathology. Compared to controls, an increase in dorsal hair loss was seen in females, but this finding is common. Other treatment-related effects (transient increases in liver weight and hepatocellular hypertrophy in high-dose males compared to controls) indicate a minimal, adaptive response in the liver following exposure to IR5878 that is not considered adverse.
The LOAEL was not identified in this study (>706 mg/kg/day for males and >773 mg/kg/day for females). The NOAEL is the highest dose tested in this study (9000 ppm), which was equivalent to 706 mg/kg/day for males and 773 mg/kg/day for females.

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

90-Day Oral Toxicity - Mouse (870.3100)

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity study (MRID 46260102), IR5878 (98.0% a.i., Batch No. FCF/T/172-00 (ex 20525/03/8) was administered to ten CD-1 mice/sex/dose in the diet at dose levels of 0, 250, 1250, and

6000 ppm (equivalent to 0, 36, 187, and 865 mg/kg bw/day, respectively, for males and to 0, 47, 228, and 1096 mg/kg bw/day, respectively, for females).

There were no compound-related effects on clinical signs, mortality, body weights, clinical chemistry, gross pathology, or histopathology. Compared to controls, slight decreases in food consumption, MCH, and MCV in high-dose females and slight decreases in the lymphocyte count of high-dose males were noted, but these changes were not biologically significant.

The LOAEL was not identified in this study (>865 mg/kg/day for male mice and >1096 mg/kg/day for females). The NOAEL is the highest dose tested in this study (6000 ppm), which was equivalent to 865 mg/kg/day for male mice and 1096 mg/kg/day for females. Dosing in this study is considered adequate because the highest dose tested is sufficiently close to the limit dose of 1000 mg/kg/day.

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse.

b) Chronic Toxicity

Combined Chronic Toxicity/Carcinogenicity - Rat (870.4300)

EXECUTIVE SUMMARY - In this combined chronic toxicity/carcinogenicity study (MRID 46578913), IR5878 (Orthosulfamuron; 98.6-98.8% a.i.; Batch Nos.: FCF/T/191-01 and G009/02) was administered in the diet to 70 Han Wistar (HsdBrl Han:Wist) rats /sex/dose at nominal concentrations of 0, 1, 5, 500, or 1000 mg/kg/day for up to 2 years. Twenty rats/sex/dose were sacrificed at Week 52, and the remaining survivors were sacrificed at Week 104.

No treatment-related effects were observed on mortality, functional observational battery findings, food consumption, food efficiency, ophthalmoscopic examination, hematology, or urinalysis.

At 500 mg/kg/day and above, body weight gains were decreased ($p<=0.05$) in both sexes during the second year (Weeks 52-104) of treatment by 24-38% (not significant [NS] in the 500 mg/kg/day males), resulting in decreased ($p<=0.05$) overall (Weeks 0-104) body weight gains (decr. 11-20%).

Hepatotoxicity was observed at 500 mg/kg/day and above. At 1 year, relative to body liver weights were increased ($p<=0.01$) in both sexes by 9-22%. In the males, increased ($p<=0.01$) incidences were noted in minimal to moderate centrilobular hepatocyte vacuolation (50-95% treated vs 0% controls) and minimal to moderate centrilobular hepatocyte hypertrophy (60-75% vs 10%). At 2 years in the males, relative to body liver weights were increased ($p<=0.01$) by 9-23%, and increased ($p<=0.05$) incidence of minimal to marked centrilobular hepatocyte vacuolation was observed (62-80% treated vs 30% controls).

In the thyroid after 104 weeks, increased incidences of minimal to marked cystic follicular cell hyperplasia (20% treated vs 4% controls; p≤0.05) were noted in the 1000 mg/kg/day males, and increased incidences of minimal follicular cell hypertrophy were observed in the ≥500 mg/kg/day males (42% each treated vs 24% control).

Nephrotoxicity was observed at 500 mg/kg/day and above. In the carcinogenicity phase animals, incidences of perigenital yellow staining were generally increased in the males from Week 27 to termination (2-9%), and in the females from Week 32 to termination (2-24%), compared to 0 controls. At 2 years, relative kidney weights were increased (p<=0.01) by 11-14%, and an increased (NS) incidence in slight to moderate chronic progressive nephropathy of the kidney (46-52% treated vs 34% controls) was observed in the males. Additionally in the females, increased (p<=0.05) incidences (% treated vs % controls) of minimal to marked pelvic/papillary epithelium hyperplasia (88-92% vs 70%) and minimal to marked papillary/pelvic epithelium mineralization (90-94% vs 74%; NS at 500 mg/kg/day) were noted.

Additionally at 1000 mg/kg/day, increased perigenital yellow staining was noted from Week 66 to termination in the carcinogenicity phase males, from Week 3 to termination in the carcinogenicity phase females, and from Week 11 to termination in the toxicity phase females. At 1 year, increased absolute liver weights and relative kidney weights were observed in the males. At 2 years, the following findings were noted in the males: i) increased incidence of dark area(s) on the liver; ii) increased absolute liver weights; iii) increased incidence of slight centrilobular hepatocyte hypertrophy; and iv) increased incidence of minimal to slight cystic degeneration. In the females, the following findings were observed: i) increased relative liver weights; ii) increased incidence of slight to moderate focal sinusoidal dilatation; iii) increased blood urea; iv) increased relative kidney weights; and v) increased incidence of slight to marked chronic progressive nephropathy.

The LOAEL is 500 mg/kg/day, based on decreased body weight gains, slight hepatotoxicity and nephrotoxicity in both sexes and thyroid effects in males. The NOAEL is 5 mg/kg/day.

After 2 years, an increased (p≤0.05) incidence in thyroid follicular cell adenoma was observed in the >=500 mg/kg/day males (14-20% treated vs 2% concurrent controls), without an increased incidence of follicular cell carcinoma. In a special study (MRID 46578927), it was demonstrated that the compound results in the induction of UDP-GT, which is responsible for the degradation of T4. Pharmacokinetic data supported an increased elimination of T4. Thus, chronic stimulation of the thyroid would occur through the hypothalmic-pituitary-thyroid axis. This effect is not observed in humans because of differing hormone binding profiles and metabolic clearance rate of the thyroid hormones. Consequently, the effect on the thyroid is considered unimportant to humans.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4300; OECD 453) for a combined chronic toxicity/carcinogenicity study in rats.

Carcinogenicity Study - Mouse (870.4200b)

EXECUTIVE SUMMARY - In a carcinogenicity study (MRID 46578912), 50 Crl:CD-1™ (ICR)BR mice/sex/dose were exposed to IR5878 [Orthosulfamuron; 98.0% a.i.; Batch #: FCF/T/172-00 (ex 20525/03/8)] in the diet at nominal concentrations of 0, 100, 500, or 1000 mg/kg/day for up to 78 weeks.

No adverse treatment-related effects were observed on clinical signs, mortality, body weights, body weight gains, food consumption or food conversion efficiency, hematology, or gross pathology.

Absolute (incr. 12%, p =< 0.05) and relative to body (incr. 14%, p =< 0.05) liver weights were increased in the 1000 mg/kg/day males after 78 weeks of treatment when compared with controls. An increased (p =< 0.001) incidence of slight to moderate centrilobular hepatocyte hypertrophy was observed at >= 500 mg/kg/day (48-68%) compared to slight hypertrophy in controls (8%). Also, an increased (p =< 0.05) incidence of slight to marked liver vacuolation was observed at 1000 mg/kg/day (50%), and slight to moderate liver vacuolation at 500 mg/kg/day (38%), both compared to slight to moderate in controls (18%). Similar changes were not observed in females. It was stated that the observed increased incidence of centrilobular hepatocyte vacuolation, suggesting an effect on fat metabolism, in 500 and 1000 mg/kg/day compound-treated males was also observed in the corresponding rat carcinogenicity study MRID 46578913 (Huntingdon Life Sciences Report Number AGR 131/033063) with this test compound.

At 500 mg/kg/day, increased incidence of centrilobular hepatocyte vacuolation was mostly slight with moderate vacuolation observed in 10% of the animals. However, centrilobular hepatocyte vacuolation in the 1000 mg/kg/day males is more clearly defined with an increase in incidence as well as severity. This effect coupled with more pronounced increases in liver weights and centrilobular hypertrophy in the 1000 mg/kg/day males provide a weight of evidence sufficient to determine the LOAEL at 1000 mg/kg/day.

The LOAEL is 1000 mg/kg/day, based on increases in absolute and relative to body liver weights, centrilobular hepatocyte hypertrophy, and centrilobular hepatocyte vacuolation in males. The NOAEL is 500 mg/kg/day.

At the doses tested, there was not a treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on increased absolute and relative to body liver weights, and increased incidence of centrilobular hepatocyte hypertrophy and centrilobular hepatocyte vacuolation.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

5. *Mode of Action*

In a non-guideline study (MRID 46578927) in rats, the effect of orthosulfamuron (IR5878; Batch# G 009/02; 98.79% a.i.) on thyroid function was investigated. Potential direct pathways (using the perchlorate discharge test)and indirect mechanisms [determined by: (i) thyroxine pharmacokinetics using ^{125}I clearance; (ii) plasma thyroid hormone levels; and (iii) organ weights and histopathology of the liver and thyroid] were examined. In the study, the test substance was administered in the diet to 28 male Han Wistar rats/dose at doses of 0, 5, and 1000 mg/kg for up to 90 days. Out of the 28 rats/group placed on study, 6 rats/group were used for each of the three categories of parameters listed above. Propylthiouracil (200 mg/kg) was included as a positive control for the perchlorate discharge test and was administered daily via oral gavage to 15 rats for the final 16 days prior to termination on Day 90. Phenobarbital (75 mg/kg) was included as a positive control for the remaining parameters and was administered daily via oral gavage to 15 rats for 14 days prior to termination of Day 90.

INDIRECT MECHANISM

Thyroid Hormone Concentrations

Mean thyroid hormone concentrations are presented in Table 5. On Day 90, TSH levels were increased at 1000 mg/kg (↑58%) compared to negative controls, with an even greater increase in the phenobarbital group (↑148%). In the text of the study report, it was stated that these increases were statistically significant; however, no symbols or p-values were indicated in the summary tables. There were no other differences in thyroid hormones that could be attributed to treatment.

On Day 30, concentrations of T3 were significantly decreased (↓20-23%; p-value not indicated) in the 5 and 1000 mg/kg groups compared to negative controls (note that a positive control phenobarbital groups was not included at this time point). However, the decrease in T3 was transient, in that concentrations at Day 90 were comparable to controls. Concentrations of T3 and TSH at all other time points and concentrations of rT3 and T4 at all time points were comparable to controls.

Table 5. Mean (± SD) thyroid hormone levels in male rats treated for up to 13 weeks with orthosulfamuron.[a]

Thyroid hormone	Orthosulfamuron			Phenobarbital
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg
Pre-treatment				
rev T3 (nmol/L)	0.13 ± 0.054	0.14 ± 0.036	0.13 ± 0.036	0.13 ± 0.018
T3 total (nmol/L)	1.80 ± 0.354	1.73 ± 0.185	1.77 ± 0.213	1.85 ± 0.251
T4 total (nmol/L)	52 ± 11.4	45 ± 8.5	49 ± 7.7	45 ± 8.3
TSH (ng/mL)	5.8 ± 0.88	6.1 ± 1.37	6.7 ± 1.03	6.1 ± 0.91

Thyroid hormone	Orthosulfamuron			Phenobarbital
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg
Day 30				
rev T3 (nmol/L)	0.24 ± 0.010	0.34 ± 0.105	0.24 ± 0.027	---
T3 total (nmol/L)	1.71 ± 0.416	**1.31 ± 0.097* (↓23)**	**1.37 ± 0.188* (↓20)**	---
T4 total (nmol/L)	66 ± 9.0	62 ± 17.2	57 ± 9.8	---
TSH (ng/mL)	14.7 ± 10.62	12.6 ± 2.70	16.2 ± 3.34	---
Day 76				
rev T3 (nmol/L)	---	---	---	0.25 ± 0.075
T3 total (nmol/L)	---	---	---	1.85 ± 0.316
T4 total (nmol/L)	---	---	---	50 ± 5.8
TSH (ng/mL)	---	---	---	7.8 ± 1.19
Day 90				
rev T3 (nmol/L)	0.18 ± 0.035	0.21 ± 0.057	0.18 ± 0.023	0.19 ± 0.062
T3 total (nmol/L)	1.71 ± 0.252	1.75 ± 0.128	1.78 ± 0.203	1.82 ± 0.179
T4 total (nmol/L)	48 ± 2.9	50 ± 2.6	47 ± 2.6	47 ± 4.1
TSH (ng/mL)	6.7 ± 0.80	7.6 ± 0.94	**10.6 ± 3.22* (↑58)**	16.6 ± 5.36* (↑148)

a Data were obtained from Table 5 on pages 48-51 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n = 6.

* Reported to be significantly different from controls on pages 31-32 of the study report; p-value not stated

Effects of Orthosulfamuron on the Liver and Thyroid (Pathology, Organ Weights, and Enzymes)

Macroscopic pathology - Enlarged thyroids were noted in the 5 mg/kg (1/6 rats) and 1000 mg/kg (2/6 rats) compared to 0/6 negative controls and 4/6 phenobarbital treated rats. No other macroscopic findings could be attributed to treatment.

Organ weights - Selected absolute organ weight data are presented in Table 6. Liver weights were increased at 1000 mg/kg (↑10%; p≤0.01) compared to negative controls, and an increase of an even greater magnitude was observed in the phenobarbital group (↑21%; p≤0.001). There were no treatment-related effects on thyroid/parathyroid weights or any other organs measured

Table 6. Mean (± SD) organ weights (g) in male rats treated for 13 weeks with orthosulfamuron [a]

Parameter	Orthosulfamuron			Phenobarbital
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg
Terminal body weight	372.4 ± 40.2	379.6 ± 38.4	336.3 ± 31.1	352.1 ± 14.0
Liver weight	13.44 ± 1.61	12.81 ± 1.80	**14.74 ± 1.32** (↑10)**	16.28 ± 1.30*** (↑21)
Thyroids + parathyroids	0.022 ± 0.003	0.023 ± 0.006	0.022 ± 0.004	0.025 ± 0.005

a Data were obtained from Table 10 on page 56 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n = 6.

** Significantly different from controls; p≤0.01

*** Significantly different from controls; p≤0.001

Microscopic pathology - Increased incidences of centrilobular hepatocyte hypertrophy were observed in the1000 mg/kg (4/6 rats, not significant; minimal severity) and phenobarbital (6/6 rats, p≤0.01; slight severity) groups compared to negative controls (0/6 rats; Table 7). Thus, both the severity and incidence of this finding were increased in the phenobarbital group compared to the 1000 mg/kg group. Similarly, increased incidences of minimal to slight follicular cell hypertrophy in the thyroid were observed in the1000 mg/kg (4/6 rats, not significant; minimal) and phenobarbital (6/6 rats, p≤0.05; minimal to slight) groups compared to negative controls (1/6 rats). Thus, both the severity and incidence of this finding were increased in the phenobarbital group compared to the 1000 mg/kg group. There were no other microscopic findings which could be attributed to treatment.

Table 7. Microscopic findings in male rats treated for 13 weeks with orthosulfamuron. [a]

Microscopic finding	Orthosulfamuron			Phenobarbital
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg
Liver, centrilobular hepatocyte hypertrophy				
Minimal	0	0	4	0
Slight	0	0	0	6
Total	0	0	4	6**
Thyroid, follicular cell hypertrophy				
Minimal	1	0	4	4
Slight	0	0	0	2
Total	1	0	4	6*

a Data were obtained from page 37 of the study report. n = 6.

* Significantly different from controls; p≤0.05

** Significantly different from controls; p≤0.01

Liver enzymes - The following liver enzymes were increased (p≤0.05) at 1000 mg/kg/day compared to negative controls, when expressed per mass of protein and/or per mass of liver (Table 8): (i) microsomal protein (↑32%); (ii) concentration of cytochrome P450 (↑28-69%); (iii) activity of PROD (↑2958-4020%); and (iv) activity of thyroxine UDP-GT (↑64-115%). With the exception of thyroxine UDP-GT, the concentration/activity of each of these liver

enzymes was increased ($p \leq 0.01$) in the phenobarbital group to a greater extent than the 1000 mg/kg/day group. There were no other treatment-related effects on liver enzymes.

Table 8. Mean (± SD) liver enzyme concentrations or activities in male rats treated for 13 weeks with orthosulfamuron. [a]

Parameter	Orthosulfamuron (mg/kg)			Phenobarbital
	0	5	1000	75 mg/kg
Microsomal protein (mg/g liver)	11.7 ± 1.3	12.5 ± 1.1	**15.4 ± 1.8** (↑32)**	18.4 ± 1.7*** (↑57)
Cytochrome P450				
nmoles/mg protein	0.537 ± 0.085	0.556 ± 0.047	**0.690 ± 0.049** (↑28)**	1.565 ± 0.136*** (↑191)
nmoles/g liver	6.31 ± 1.50	6.92 ± 0.73	**10.64 ± 1.81** (↑69)**	28.89 ± 4.81*** (↑358)
PROD [b]				
nmoles/min/mg protein	0.012 ± 0.001	0.010 ± 0.001	**0.367 ± 0.096* (↑2958)**	1.638 ± 0.245** (↑13550)
nmoles/min/g liver	0.138 ± 0.015	0.121 ± 0.014	**5.686 ± 1.831** (↑4020)**	30.208 ± 5.917*** (↑21790)
Thyroxine UDP-GT [c]				
pmoles/min/mg protein	0.325 ± 0.099	0.269 ± 0.097	**0.533 ± 0.096** (↑64)**	0.375 ± 0.083 (↑15)
pmoles/min/g liver	3.753 ± 1.090	3.311 ± 1.047	**8.070 ± 0.864** (↑115)**	6.968 ± 1.990*** (↑86)

a Data were obtained from Tables 13 through 16 on pages 59-62 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n = 6.
b 7-Pentoxyresorufin O-depentylase
c UDP-glucuronosyltransferase
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$
*** Significantly different from controls; $p \leq 0.001$

Thyroxine Clearance Test

Time course data for ^{125}I-Thyroxine concentrations in whole blood following a single intravenous dose are included in Table 9a. Pharmacokinetic parameters derived from these data are presented in Table 9b. In the 1000 mg/kg group, the following slight differences (not significant) compared to negative controls were noted: (i) decreased whole blood concentrations of radioactivity (↓4-19%); (ii) decreased AUC_{72} values (↓11%); (iii) increased systemic clearance (↑12%); (iv) increased volume of the central compartment (V_c; ↑12%); and (v) increased volume at steady state (V_{ss}; ↑9%). It was stated that the volume during the terminal phase (V_z) was also increased at this dose; however, these data were not presented in the summary table. Values for k^{-1} and $T_{1/2}$ at this dose were comparable to negative controls, and the 5 mg/kg group was comparable to negative controls for concentrations of radioactivity throughout the time course and for all derived pharmacokinetic parameters.

The phenobarbital (positive control) group responded as expected with decreased whole blood concentrations of radioactivity (↓29-46%) and AUC_{72} values (↓41%), increased V_c (↑75%) and V_{ss} (↑69%) values, and significantly ($p \leq 0.001$) increased systemic clearance (↑70%).

Table 9a. Mean (± SD) concentrations of radioactivity in whole-blood after administration of single intravenous doses of ^{125}I-Thyroxine in male rats.[a]

Time (Hours)	Control	Orthosulfamuron		Phenobarbital 75 mg/kg/day
		5 mg/kg/day	1000 mg/kg/day	
1	2.05 ± 0.29	2.09 ± 0.36	**1.96 ± 0.10 (↓4)**	1.21 ± 0.17 (↓41)
2	1.59 ± 0.22	1.62 ± 0.27	**1.53 ± 0.09 (↓4)**	0.99 ± 0.13 (↓38)
4	1.23 ± 0.17	1.24 ± 0.20	**1.17 ± 0.09 (↓5)**	0.79 ± 0.12 (↓36)
6	0.99 ± 0.21	1.11 ± 0.17	1.03 ± 0.08	0.67 ± 0.09 (↓32)
8	1.01 ± 0.14	0.96 ± 0.17	**0.82 ± 0.06 (↓19)**	0.56 ± 0.09 (↓45)
12	0.77 ± 0.10	0.78 ± 0.09	**0.64 ± 0.06 (↓17)**	0.46 ± 0.07 (↓40)
24	0.52 ± 0.06	0.52 ± 0.08	**0.45 ± 0.03 (↓13)**	0.28 ± 0.03 (↓46)
36	0.29 ± 0.04	0.31 ± 0.04	**0.25 ± 0.03 (↓7)**	0.16 ± 0.02 (↓41)
48	0.19 ± 0.02	0.19 ± 0.03	**0.17 ± 0.02 (↓11)**	0.11 ± 0.01 (↓42)
72	0.07 ± 0.01	0.07 ± 0.01	**0.06 ± 0.01 (↓14)**	0.05 ± 0.00 (↓29)

a Data were obtained from Table 6 on page 52 of the study report. n = 6. Percent differences from negative controls are included in parentheses.

Table 9b. Mean (± SD) pharmacokinectic parameters of whole-blood radioactivity after administration of single intravenous does of ^{125}I-Thyroxine in male rats.[a]

Parameter	Control	Orthosulfamuron		Phenobarbital 75 mg/kg/day
		5 mg/kg/day	1000 mg/kg/day	
AUC_{72} (%dose h/mL)	33.50 ± 3.98	34.10 ± 4.61	**29.66 ± 1.95 (↓11)**	19.63 ± 2.23 (↓41)
k (hours^{-1})	0.0396 ± 0.0023	0.0400 ± 0.0031	0.0395 ± 0.0034	0.0386 ± 0.0029
$T_{1/2}$ (hours)[b]	17.5	17.3	17.5	18.0
Cl (mL/hour)	2.86 ± 0.35	2.82 ± 0.38	**3.21 ± 0.23 (↑12)**	4.86 ± 0.51*** (↑70)
V_c (mL)	72.68 ± 11.43	70.98 ± 11.84	**81.43 ± 5.49 (↑12)**	126.96 ± 20.03 (↑75)
V_{ss} (mL)	66.29 ± 10.74	65.12 ± 11.54	**72.20 ± 4.98 (↑9)**	111.90 ± 16.91 (↑69)

a Data were obtained from Table 7 on page 53 of the study report. Percent differences from negative controls are included in parentheses.

b Calculated for ln2/(mean rate constant); n = 6.

*** Significantly different from the negative control group at $p \leq 0.001$. Significance level was not denoted in Table 7 on page 53, but was found in the text of the study report on page 32.

DIRECT MECHANISM

Perchlorate Discharge Assay

Potential direct effects of the test substance on the thyroid were examined using the perchlorate discharge assay. The positive control, propylthiouracil, acts as an inhibitor of the thyroid peroxidases responsible for the iodide organification necessary for T3/T4 synthesis. In contrast, perchlorate (ClO_4^-) exerts a direct affect on the thyroid by acting as a competitive inhibitor of iodide transport from the circulation into the follicular cells, thus limiting T3/T4 synthesis There was no evidence of a direct effect of the test substance on the thyroid. In the perchlorate discharge assay, propylthiouracil exhibited the expected results as a positive control for direct effects on the thyroid with the following differences ($p \leq 0.05$) from negative controls (Table 10a): (i) increased thyroid weight (↑154-315%); (ii) decreased radioactivity in the thyroid on a per weight basis (↓75-93%) and as a percent of the total dose (↓24-69%); (iii) increased radioactivity in whole blood on a per weight basis (↑12-46%) and as a percent of the total dose (↑30%; perchlorate group only); and thus (iv) decreased thyroid:whole blood ratio (↓75-95%).

Several significant ($p \leq 0.05$) differences from negative controls were noted in the groups treated with the test substance. However, none of these changes were considered to be toxicologically relevant because the direction of the change was opposite of that which would indicate prevention of iodide organification.

Comparison of the perchlorate and saline subgroups within each group revealed no effects of perchlorate on thyroid weight or radioactive iodide in the thyroid or whole blood in the groups treated with orthosulfamuron or in the negative controls, indicating no blockage of iodide uptake into the thyroid due to the test substance (Table 10b). The only differences between the perchlorate and saline subgroups were noted in the propylthiouracil group, with decreased radioactivity in the thyroid (↓63-68%) and increased radioactivity in whole blood (↑32-38%), resulting in a decreased thyroid:whole blood ratio (↓78%) in the perchlorate subgroups compared to the saline subgroups. These findings confirm that perchlorate, as a competitive inhibitor of iodide transport, displaced the free iodide present in the thyroid. The levels of free iodide were higher in this group because the process of organification of free iodide was inhibited by propylthiouracil.

Table 10a. Mean (± SD) thyroid weights and radioactivity in thyroid and whole blood in male rats treated for 13 weeks with orthosulfamuron. [a]

Parameter	Orthosulfamuron			Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	200 mg/kg
Thyroid weight (g)				
saline	0.0220 ± 0.0099	0.0154 ± 0.0042*(↓30)	0.0200 ± 0.0036	0.0558 ± 0.0124***(↑154)
perchlorate	0.0162 ± 0.0052	0.0128 ± 0.0020*(↓21)	0.0177 ± 0.0031	0.0672 ± 0.0079***(↑315)

Parameter	Orthosulfamuron			Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	200 mg/kg
Radioactivity				
Thyroid, % dose/g				
saline	430 ± 236	487 ± 128	**602 ± 85**(↑40)**	107 ± 23***(↓75)
perchlorate	461 ± 133	541 ± 150	**651 ± 70**(↑41)**	33.9 ± 14.9***(↓93)
Thyroid, total % dose				
saline	7.82 ± 1.73	7.43 ± 2.39	**11.9 ± 2.22***(↑52)**	5.98 ± 1.64 **(↓24)
perchlorate	7.28 ± 2.39	6.91 ± 2.08	**11.6 ± 2.97***(↑59)**	2.24 ± 0.92 **(↓69)
Whole blood, % dose/g				
saline	0.272 ± 0.035	0.289 ± 0.054	0.273 ± 0.053	0.305 ± 0.046***(↑12)
perchlorate	0.276 ± 0.018	0.285 ± 0.043	0.288 ± 0.058	0.404 ± 0.043***(↑46)
Whole blood, total % dose				
saline	7.34 ± 0.64	7.69 ± 1.24	6.83 ± 1.16	6.95 ± 0.81* (↓5)
perchlorate	7.40 ± 0.37	7.32 ± 0.78	7.16 ± 0.66	9.59 ± 0.99* (↑30)
Thyroid:whole blood ratio				
saline	1397	1653	**2217**(↑59)**	349***(↓75)
perchlorate	1616	1843	**2287**(↑42)**	78***(↓95)

a Data were obtained from Tables 8 and 9 on pages 54-55 of the study report. Percent differences from negative controls (calculated by reviewers) are included in parentheses. n = 6.

* Significantly different from controls; p≤0.05

** Significantly different from controls; p≤0.01

*** Significantly different from controls; p≤0.001

Table 10b. Mean (± SD) thyroid weights and radioactivity in thyroid and whole blood in male rats treated for 13 weeks with orthosulfamuron.[a]

Parameter	Orthosulfamuron			Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	200 mg/kg
Thyroid weight (g)				
saline	0.0220 ± 0.0099	0.0154 ± 0.0042	0.0200 ± 0.0036	0.0558 ± 0.0124
perchlorate	0.0162 ± 0.0052	0.0128 ± 0.0020	0.0177 ± 0.0031	0.0672 ± 0.0079

Parameter	Orthosulfamuron			Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	200 mg/kg
Radioactivity				
Thyroid, % dose/g				
saline	430 ± 236	487 ± 128	602 ± 85	107 ± 23
perchlorate	461 ± 133	541 ± 150	651 ± 70	33.9 ± 14.9*** (↓68)
Thyroid, total % dose				
saline	7.82 ± 1.73	7.43 ± 2.39	11.9 ± 2.22	5.98 ± 1.64
perchlorate	7.28 ± 2.39	6.91 ± 2.08	11.6 ± 2.97	2.24 ± 0.92*** (↓63)
Whole blood, % dose/g				
saline	0.272 ± 0.035	0.289 ± 0.054	0.273 ± 0.053	0.305 ± 0.046
perchlorate	0.276 ± 0.018	0.285 ± 0.043	0.288 ± 0.058	0.404 ± 0.043** (↑32)
Whole blood, total % dose				
saline	7.34 ± 0.64	7.69 ± 1.24	6.83 ± 1.16	6.95 ± 0.81
perchlorate	7.40 ± 0.37	7.32 ± 0.78	7.16 ± 0.66	9.59 ± 0.99*** (↑38)
Thyroid:whole blood ratio				
saline	1397	1653	2217	349
perchlorate	1616	1843	2287	78*** (↓78)

a Data were obtained from Tables 8 and 9 on pages 54-55 of the study report. Percent differences of perchlorate subgroup from saline subgroup (calculated by reviewers) are included in parentheses. n = 6.

* Perchlorate subgroup significantly different from saline subgroup at $p \leq 0.05$

** Perchlorate subgroup significantly different from saline subgroup at $p \leq 0.01$

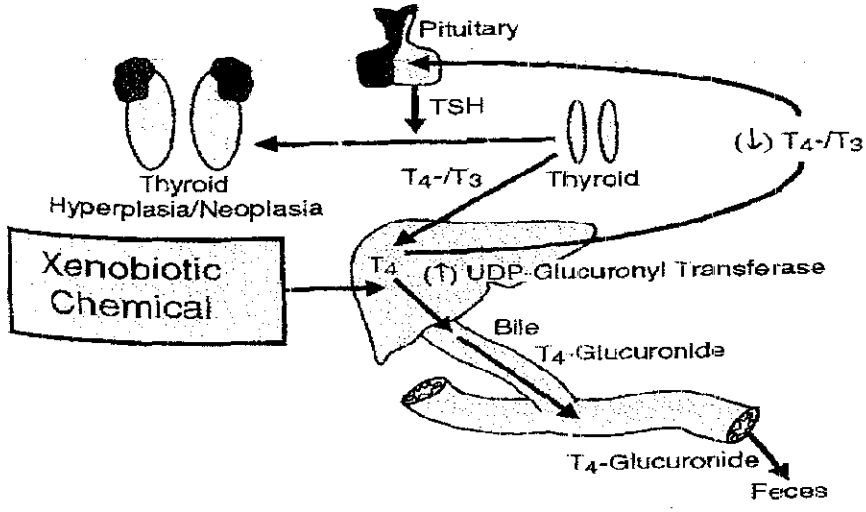
*** Perchlorate subgroup significantly different from saline subgroup at $p \leq 0.001$

Registrant's conclusion: Induction of UDP-GT results in increased elimination of T4 (Figure 1.), which is supported by the pharmacokinetic data. At 1000 mg/kg/day, ^{125}I-T4 exhibited slightly increased clearance compared to negative controls, indicated by decreased whole blood concentrations of radioactivity and AUC_{72} values and increased systemic clearance, V_c, V_{ss}, and V_z. The positive control group responded similarly, only with a greater magnitude difference from negative controls.

The increased elimination of T4 activates the negative feedback loop to stimulate the pituitary to release TSH. In the MOA study, TSH levels were increased at 1000 mg/kg on Day 90, with an even greater increase in the phenobarbital group. **TSH acts on the thyroid follicular cells to produce T3/T4; when this process is prolonged, it results in hypertrophy, hyperplasia, and eventually, tumor formation (Figure).** Although thyroid/parathyroid weights were comparable to controls in this study, enlarged thyroids were noted in the 5 mg/kg (1/6 rats) and 1000 mg/kg (2/6 rats) compared to 0/6 negative controls and 4/6 phenobarbital treated rats. Minimal follicular cell hypertrophy was noted at 1000 mg/kg (4/6 rats) compared to negative controls (1/6 rats). In the phenobarbital group, minimal to slight follicular cell hypertrophy was observed in all (6/6) rats.

In conclusion, there was no direct effect of the test substance on the thyroid, as indicated by the perchlorate assay. The increased organ weight, hypertrophy, and induction of UDP-GT in the liver and the increased TSH and enlarged thyroids and follicular cell hypertrophy support an indirect effect of the test substance on the thyroid.

Figure 1. Hepatic microsomal enzyme induction by the chronic administration of xenobiotic chemicals, leading to thyroid follicular cell hyperplasia and neoplasia.



Obtained from *Casarett & Doull's Toxicology: The Basic Science of Poisons*. Curtis D. Klassen. 6th edition, p. 729.

Determination of whether neoplasms are due to thyroid-pituitary imbalance

FACTOR 1. Consideration of whether the thyroid tumors associated with administration of orthosulfamuron can be attributed to disruption of the thyroid-pituitary hormonal balance.

a. Goitrogenic activity in vivo:

Thyroid: thyroid cystic follicular cell hyperplasia and hypertrophy were observed in mid (500 mg/kg/day) and high dose (1000 mg/kg/day) males in the chronic rat study; thyroid cysts were also seen at 500 mg/kg/day. Enlarged thyroids and thyroid follicular cell hypertrophy were observed in the special thyroid function study in rats (90 days). These effects were not observed in the 90-day feeding studies with rats, mice, or dogs.

Liver: hepatocellular hypetrophy was observed in both sexes in the chronic rat study at 500 and 1000 mg/kg/day. In addition, hepatocellular vacuolation (also seen at 500mg/kg/day), and cystic degeneration were seen in males at 1000 mg/kg/day. In females, focal sinusoidal dilatation was seen at 1000 mg/kg/day. Hepatocellular hypertrophy and vacuolation in males were also observed in a chronic mouse study. Subchronic studies in rats and dogs demonstrated similar effects on the liver (increased weights, hypertrophy) after exposure to orthosulfamuron. There were no incidences of hepatocellular hyperplasia noted in the study.

b. Clinical chemistry changes (eg., reduced thyroid hormone and increased TSH serum concentrations):

Thyroid: In the special thyroid function study in rats, orthosulfamuron induced significant increases in TSH levels at 1000 mg/kg (↑58%) compared to negative controls on Day 90 (In the text of the study report, it was stated that these increases were statistically significant; however, no symbols or p-values were indicated in the summary tables). In addition, concentrations of T3 (Day 30) were significantly decreased (↓20-23%; p-value not indicated) in the 5 and 1000 mg/kg groups compared to negative controls. However, the decrease in T3 was transient, in that concentrations at Day 90 were comparable to controls. Concentrations of T3 and TSH at all other time points and concentrations of rT3 and T4 at all time points were comparable to controls. There were no other differences in thyroid hormones that could be attributed to treatment. Thyroid hormone concentrations were not measured in the other subchronic/chronic studies with rats, mice, or dogs.

c. Specific evidence of reduced hormone synthesis (eg., inhibited iodine uptake) or increased thyroid hormone clearance (eg., enhanced biliary excretion):

The following effects were observed in the thyroid function studies:

- The following liver enzymes were increased ($p \leq 0.05$) at 1000 mg/kg/day compared to negative controls, when expressed per mass of protein and/or per mass of liver (Table 8): (i) microsomal protein (↑32%); (ii) concentration of cytochrome P450 (↑28-69%); (iii) activity of PROD (↑2958-4020%); and (iv) activity of thyroxine UDP-GT (↑64-115%). There were no other treatment-related effects on liver enzymes.

- At 1000 mg/kg/day, ^{125}I-T4 exhibited slightly increased clearance compared to negative controls, indicated by decreased whole blood concentrations of radioactivity (↓4-19%), decreased AUC_{72} values (↓11%), increased systemic clearance (↑12%), and increased volume of the central compartment (V_c; ↑12%) and volume at steady state (V_{ss}; ↑9%),

d. Evidence of progression (eg., hypertrophy/hyperplasia, nodular hyperplasia - neoplasia):

In the short term studies, there were no thyroid effects observed after 90-days of treatment in rats, mice, or dogs. In the special thyroid study, enlarged thyroids (no change in weight) and slight to minimal thyroid follicular cell hypertrophy were seen after 90 days (1000 mg/kg/day). In the two-year rat study, after 52 weeks, there were no incidences of thyroid hypertrophy/hyperplasia observed. At 104 weeks, thyroid cysts, follicular cell hypertrophy, and hyperplasia were seen. Thyroid follicular cell adenomas were first observed in males at week 70 at 500 mg/kg/day. The first carcinoma was observed at week 91 at 5 mg/kg/day. Significant increases in thyroid follicular cell tumors were evident in males at 500 and 1000 mg/kg/day by the end of the study.

e. Reversibility of effects after exposure is terminated:

Reversibility was not addressed in the special thyroid function study. In the 90-day feeding study in rats, animals were maintained an additional 4-weeks on control diet after exposure to orthosulfamuron. After the 4-week recovery period, liver effects observed during the study (eg., increased weights and hepatocellular hypertrophy) were no longer seen.

f. SAR to other thyroid tumorigens:

The closest structural analog to orthosulfamuron is cyclosulfamuron. Based on the structural similarities, it is likely that cyclosulfamuron shares the same properties as orthosulfamuron. However, cyclosulfamuron is not currently registered by the EPA; therefore, toxicology data are not available at this time.

<u>FACTOR II</u>. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

The genotoxicity data are negative. There is no indication that genotoxicity plays a role in the tumorigenic activity for this chemical.

<u>FACTOR III</u>. Evaluation of neoplasms other than thyroid follicular cell tumors (and relevant pituitary tumors).

There were no statistically significant increases in any other tumor types in either rats or mice for this chemical.

Conclusions: As indicated above, based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of orthosulfamuron may be due to a disruption in the thyroid-pituitary status.

Table 11. Comparison of Science Policy Council mandatory Criteria for Demonstration of Thyroid-pituitary Imbalance and Effects Observed After Orthosulfamuron Exposure.

SPC criterion/	Study	Observed effect	MRID
Cellular growth	13-week investigative	Enlarged thyroids at 5 and 1000 mg/kg/day, thyroid follicular cell hypertrophy at 1000 mg/kg/day, no increase in thyroid weight or evidence of cell proliferation.	46578927
	13-week toxicity	No increase in thyroid weight, or evidence of cell proliferation	46260103.
	104-week carcinogenicity	Thyroid cysts in males at 1000 mg/kg/day thyroid follicular cell hypertrophy in males at 500 and 1000 mg/kg/day, thyroid cystic follicular cell hyperplasia in males at 500, and 1000 mg/kg/day	46578913
Hormone changes	13-week investigative	Increase in TSH concentration (58%) on D90, transient decrease in T3 on D30 at 5 and 1000 mg/kg/day (not seen on D90).	46578927
	13-week toxicity	Hormones not measured	46260103
	104-week carcinogenicity	Hormones not measured	46578913
Site of action	13-week investigative	Increased liver weights and centrilobular hepatocyte hypertrophy at 1000 mg/kg/day. At 1000 mg/kg/day, there were increases in liver enzymes/activities • Microsomal protein (32%) • Cytochrome P450 (28-69%) • PROD activity (2958-4020%) • Thyroxine UDP-GT (64-115%)	46578927
	13-week toxicity	Increased liver weight and centrilobular hepatocyte hypertrophy at 706 mg/kg/day	46260103
	104-week carcinogenicity	Increased relative liver weights at 500 and 1000 mg/kg/day and absolute weights at 1000 mg/kg/day, centrilobular hepatocyte vacuolation and hypertrophy at 500 and 1000 mg/kg/day, cystic degeneration at 1000 mg/kg/day	46578913
Dose correlations	13-week investigative	Increased TSH at 1000 mg/kg/day, not seen at 5 mg/kg/day. Increased liver enzymes (P450, Microsomal) and activities (PROD, UDP-GT) at 1000 mg/kg/day, not seen at 5 mg/kg/day. Liver/thyroid effects at 1000 mg/kg/day, none seen at 5mg/kg/day.	46578927
	13-week toxicity	113 mg/kg/day-No liver effects 706 mg/kg/day-Increased liver weight and centrilobular hepatocyte hypertrophy in males.	46260103
	104-week carcinogenicity	Thyroid follicular cell adenoma at 500 and 1000 mg/kg/day, no thyroid adenoma at 5 mg/kg/day	46578913
Reversibility	13-week investigative	Reversibility not addressed in the study	465789271

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

1. Carcinogenicity

2. Mutagenicity

3. Structure-Activity Relationship

4. Mode of Action

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

VIII. BIBILIOGRAPHY

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DATA EVALUATION RECORD

IR5878 (ORTHOSULFAMURON)

Study Type: §83-5; Combined Chronic Toxicity/Carcinogenicity Study in Rats

Work Assignment No. 3-1-82 D (MRID 46578913)

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

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Registration Action Branch 2, Health Effects Division (7509C) **Date**_____
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509C) **Date**_____

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/carcinogenicity dietary study in rats; OPPTS 870.4300 [§83-5]; OECD 453.

PC CODE: 108209 **DP BARCODE:** D319264
TXR#: 0053612

TEST MATERIAL (PURITY): IR5878 (Orthosulfamuron; 98.6-98.8% a.i.)

SYNONYMS: 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N,N-dimethylbenzamide

CITATION: Webley, L. (2004) IR5878: combined carcinogenicity and toxicity study by dietary administration to Han Wistar rats for 104 weeks. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project ID.: AGR/131/033063, July 20, 2004. MRID 46578913. Unpublished.

SPONSOR: ISAGRO S.p.A., Centro Uffici San Siro, Fabbricato D-ala 3, Via Cladera 21, Milano, Italy

EXECUTIVE SUMMARY - In this combined chronic toxicity/carcinogenicity study (MRID 46578913), IR5878 (Orthosulfamuron; 98.6-98.8% a.i.; Batch Nos.: FCF/T/191-01 and G009/02) was administered in the diet to 70 Han Wistar (HsdBrl Han:Wist) rats /sex/dose at nominal concentrations of 0, 1, 5, 500, or 1000 mg/kg/day for up to 2 years. Twenty rats/sex/dose were sacrificed at Week 52, and the remaining survivors were sacrificed at Week 104.

No treatment-related effects were observed on mortality, functional observational battery findings, food consumption, food efficiency, ophthalmoscopic examination, hematology, or urinalysis.

At 500 mg/kg/day and above, body weight gains were decreased ($p<=0.05$) in both sexes during the second year (Weeks 52-104) of treatment by 24-38% (not significant [NS] in the 500 mg/kg/day males), resulting in decreased ($p<=0.05$) overall (Weeks 0-104) body weight gains (decr. 11-20%).

Hepatotoxicity was observed at 500 mg/kg/day and above. At 1 year, relative to body liver weights were increased (p<=0.01) in both sexes by 9-22%. In the males, increased (p<=0.01) incidences were noted in minimal to moderate centrilobular hepatocyte vacuolation (50-95% treated vs 0% controls) and minimal to moderate centrilobular hepatocyte hypertrophy (60-75% vs 10%). At 2 years in the males, relative to body liver weights were increased (p<=0.01) by 9-23%, and increased (p<=0.05) incidence of minimal to marked centrilobular hepatocyte vacuolation was observed (62-80% treated vs 30% controls).

Nephrotoxicity was observed at 500 mg/kg/day and above. In the carcinogenicity phase animals, incidences of perigenital yellow staining were generally increased in the males from Week 27 to termination (2-9%), and in the females from Week 32 to termination (2-24%), compared to 0 controls. At 2 years, relative kidney weights were increased (p<=0.01) by 11-14%, and an increased (NS) incidence in slight to moderate chronic progressive nephropathy of the kidney (46-52% treated vs 34% controls) was observed in the males. Additionally in the females, increased (p<=0.05) incidences (% treated vs % controls) of minimal to marked pelvic/papillary epithelium hyperplasia (88-92% vs 70%) and minimal to marked papillary/pelvic epithelium mineralization (90-94% vs 74%; NS at 500 mg/kg/day) were noted.

Additionally at 1000 mg/kg/day, increased perigenital yellow staining was noted from Week 66 to termination in the carcinogenicity phase males, from Week 3 to termination in the carcinogenicity phase females, and from Week 11 to termination in the toxicity phase females. At 1 year, increased absolute liver weights and relative kidney weights were observed in the males. At 2 years, the following findings were noted in the males: i) increased incidence of dark area(s) on the liver; ii) increased absolute liver weights; iii) increased incidence of slight centrilobular hepatocyte hypertrophy; and iv) increased incidence of minimal to slight cystic degeneration. In the females, the following findings were observed: i) increased relative liver weights; ii) increased incidence of slight to moderate focal sinusoidal dilatation; iii) increased blood urea; iv) increased relative kidney weights; and v) increased incidence of slight to marked chronic progressive nephropathy.

The LOAEL is 500 mg/kg/day, based on decreased body weight gains, slight hepatotoxicity, and slight nephrotoxicity in both sexes. The NOAEL is 5 mg/kg/day.

After 2 years, an increased (p≤0.05) incidence in thyroid follicular cell adenoma was observed in the >=500 mg/kg/day males (14-20% treated vs 2% concurrent controls), without an increased incidence of follicular cell carcinoma. In a special study (MRID 46578927), it was demonstrated that the compound results in the induction of UDP-GT, which is responsible for the degradation of T4. Pharmacokinetic data supported an increased elimination of T4. Thus, chronic stimulation of the thyroid would occur through the hypothalmic-pituitary-thyroid axis. This effect is not observed in humans because of differing hormone binding profiles and metabolic clearance rate of the thyroid hormones. Consequently, the effect on the thyroid is considered unimportant to humans.

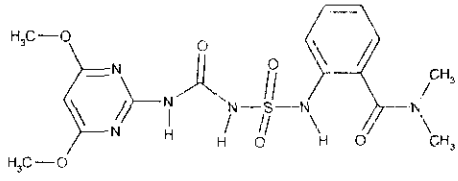
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4300; OECD 453) for a combined chronic toxicity/carcinogenicity study in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR5878

Description:	Off-white powder
Batch Nos.:	FCF/T/191-01 and G009/02
Purity:	98.6-98.8% a.i.
Stability of compound:	Stable in the diet for up to 15 days at room temperature
CAS #:	213464-77-8
Structure:	

2. Vehicle - Diet

3. Test animals

Species:	Rat
Strain:	Wistar (HsdBrl Han:Wist)
Age and body weight range at study initiation:	41-45 days, 124-188 g males; 104-156 g females
Source:	Harlan (UK) Ltd. (Bicester, Oxfordshire, England)
Housing:	5/sex/cage in suspended stainless steel cages with wire mesh floors and lid
Diet:	Powdered Rat and Mouse No. 1 Maintenance Diet (Special Diet Services Ltd., Witham, Essex, England), *ad libitum* except during urine collection and overnight before blood sampling
Water:	Tap water, *ad libitum* except during urine collection
Environmental conditions	
Temperature:	19-23°C
Humidity:	40-70%
Air changes:	Not reported
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	15 days

B. STUDY DESIGN

1. **In life dates** - Start: 06/29/01 End: 07/17/03

2. **Animal assignment** - The animals were randomly assigned to the test groups presented in Table 1, after animals at the extremes of the weight range were replaced.

Table 1. Study design. [a]

Nominal concentration in diet (mg/kg/day)	Mean achieved dose (mg/kg/day; M/F) [b]	Toxicity phase (# rats/sex [c])	Carcinogenicity phase (# rats/sex [d])
0	0/0	20	50
1	1.0/1.0	20	50
5	5.1/5.2	20	50
500	510.8/520.3	20	50
1000	1026.0/1046.5	20	50

a Data were obtained from pages 17 and 149 of MRID 46578913.
b The mean actual intake was reported for Weeks 1-104. Similar results were reported at the end of 52 weeks.
c 20 rats/dose/sex were assigned to be sacrificed at Week 52 (referred to as the "toxicity phase" by the Sponsor).
d 50 rats/dose/sex were assigned to be sacrificed at Week 104 (referred to as the "carcinogenicity phase" by the Sponsor).

3. **Dose-selection rationale** - The Sponsor stated that the maximum tolerated dosage in a 4 week study (Huntingdon Life Sciences Report # AGR 125/000095) was 12,500 ppm, which resulted in changes in body weight gain, food consumption, hematology, clinical chemistry, and spleen weight. Furthermore, in a 13-week toxicity study (Huntingdon Life Sciences Report # AGR 128/012174) where the highest dose tested was 9000 ppm (equivalent to 706/773 mg/kg/day in males/females), no treatment-related adverse findings were observed. Based on the results of these two studies, the doses in Table 1 were selected.

4. **Treatment preparation, analysis, and administration** - Dietary formulations were prepared weekly by diluting one of two concentrated test material-feed mixtures (premix) with more feed to achieve the desired concentrations. Dietary formulations were stored at room temperature; however, during the first 13 weeks, part of the 1 mg/kg/day formulation was stored frozen until fed to the animals at mid-week. The Sponsor stated that homogeneity and stability in 50 and 12,500 ppm dietary formulations was established in a previous study (Huntingdon Life Sciences Report # AGR 127/003821). Homogeneity (top, middle, bottom) and stability (up to 15 days at 21°C) was determined in 5 and 30,000 ppm formulations in this study during the first 13 weeks. Concentration analyses were performed on each dietary formulations prepared for administration in Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 103.

Results: Homogeneity (% CV): 0.85-3.22%

Stability (% initial): 96-101%

Concentration (% of nominal): 95-109%

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

5. Statistics - The following statistical methods were applied to the data. Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$.

Parameter	Statistical procedure
Categorical data (including pathological findings)	Fisher's Exact test
Body weight gains and organ weights	Bartlett's test, Dunnett's test when variance was homogeneous or Behrens-Fisher test otherwise
Grip strength, motor activity, and clinical pathology	Frequency analysis (Mantel test for trend in proportions and pairwise Fisher's Exact test for each dose group against controls) was performed when 75% of the data across all groups were the same value. Otherwise, Bartlett's test was conducted and transformations were tried when necessary to achieve homogeneous variance. If the variance was homogeneous, the William's test (monotonic data) or Dunnett's test (data not monotonic) was performed. If the variance was heterogeneous, Shirley's test (data monotonic) or Steel's test (data not monotonic) were conducted.
Survival	Life tables and Kaplan-Meier survival curves, χ^2 tests
Tumors	Peto method. Tumors were categorized and selected tumors and groups were compared using life-table analysis. Time-to-tumor was analyzed using log-rank methods.

Assuming the data were tested for normal distribution, the reviewers considered these analyses appropriate.

C. METHODS

1. Observations

1a. Cageside observations - Animals were observed at least twice daily during the study for signs of toxicity.

1b. Clinical examinations - Detailed clinical observations, including palpation, were performed weekly.

1c. Neurological evaluations - A functional observational battery (FOB) was performed each week by technicians who were unaware of each animal's dose group assignment. Animals were removed from the home cage and assessed for physical condition and behavior both during handling and after being placed in a standard arena. Particular attention was paid to possible signs of neurotoxicity, such as convulsions, tremor, and abnormalities of gait or behavior. During Week 50, sensory reactivity (approach, touch, startle, and pain responses) and hind- and forelimb grip strength were evaluated in 10 rats/dose/sex. The scoring criteria were included in the Study Report on pages 23-24. No further details were provided.

Also during Week 50, the motor activity of 10 rats/sex/dose was measured using a Rodent activity Monitoring System (Pearson Technical Services, Framlingham, Suffolk, England). The cage floor locomotor activity and rearing activity of each animal was determined individually over ten 6-minute intervals.

2. Body weight - All animals were weighed prior to treatment, at treatment initiation, weekly until Week 16, once every 4 weeks thereafter, and at termination.

3. Food consumption, food efficiency, and compound intake - Mean weekly food consumption (g/animal/week) was reported for each cage weekly for the first 16 weeks and one week in every four thereafter. Food conversion efficiency (%) was reported weekly for the first 16 weeks, as well as a Weeks 1-16 average. Compound intake values (mg/kg/day) were calculated from the nominal dietary test material concentrations and food consumption and body weight data.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all animals prior to treatment and on 20 animals/sex from the control and 1000 mg/kg/day groups at Week 52 (toxicity phase animals).

5. Hematology and clinical chemistry - Animals were fasted overnight then anesthetized with isoflurane, and blood samples were collected from the retro-orbital sinus. Blood samples for hematology were collected from all surviving animals assigned to the toxicity phase at Weeks 13, 25, and 52, and from 10 animals/dose/sex at Weeks 78 and 104. Clinical chemistry analysis was performed on blood samples obtained at Weeks 25, 52 (toxicity phase animals), 78, and 104 from 10 animals/sex/dose. Blood smears were prepared from samples obtained from the tail vein of all surviving carcinogenicity phase animals not used for routine blood sampling during Weeks 52, 78, and 104. These samples were observed for abnormal morphology, unusual cell types, and leukocyte differential count. The CHECKED (X) parameters were examined for hematology and clinical chemistry analyses.

a. Hematology

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

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total cholesterol*
X	Potassium*		Globulins*
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma-glutamyl transferase (GGT)*		
	Sorbitol dehydrogenase		
	Glutamate dehydrogenase*		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

6. Urinalysis - For urine collection, animals were placed in individual metabolism cages overnight (approximately 16 hours) without food or water. Urine samples were collected from 10 mice/sex/dose assigned to the toxicity phase on Weeks 12, 24, and 51 and from 10 mice/sex/dose on Weeks 77 and 103. The following CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity / osmolality*	X	Bilirubin*
X	pH*	X	Blood/ red blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*		Urobilinogen

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

7. Sacrifice and pathology - Animals were killed on schedule or *in extremis* by carbon dioxide asphyxiation. All animals were subjected to a detailed necropsy, and the following CHECKED (X) tissues were collected. The (XX) organs were weighed in all animals sacrificed on schedule.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve* (sciatic)[c]
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes*
X	Jejunum*	XX	Thymus		GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal glands*+
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	XX	Thyroid*[a]
X	Rectum*	XX	Testes*+		Harderian gland
XX	Liver*+	XX	Epididymides*+	XX	Parathyroids*[a]
	Gall bladder* (not rat)	X	Prostate*		OTHER
X	Bile duct* (rat)	X	Seminal vesicle*	X	Bone (femur and sternum)
X	Pancreas*	XX	Ovaries*+	X	Skeletal muscle
	RESPIRATORY	XX	Uterus*+[a]	X	Skin*
X	Trachea*	X	Mammary gland*	X	All gross lesions and masses*
X	Lung*++	X	Vagina		
X	Nasal cavity*[b]	XX	Cervix[a]		
X	Pharynx*[b]				
X	Larynx*[b]				

* Required for carcinogenicity studies based on Guideline 870.4200
a The thyroids were weighed with the parathyroids after partial fixation; the uteri were weighed with the cervixes.
b Stored but not examined.
\+ Organ weight required in carcinogenicity studies
++ Organ weight required if inhalation route

Testes and epididymides were fixed in Bouin's solution prior to transfer to 70% industrial methylated spirit. Eyes were fixed in Davidson's fluid. The urinary bladder was initially inflated with Bouin's fluid. Other samples were stored in 10% neutral buffered formalin.

From the 1000 mg/kg/day groups and the controls, bone marrow samples were obtained from the femur of all toxicity phase animals and from 10 animals/sex/dose of the carcinogenicity phase animals. The samples were processed and the myeloid:erythroid ratio, cellularity, and composition of the marrow were determined.

All samples from all animals were prepared routinely and examined microscopically in the 1000 mg/kg/day group and the controls. In addition, the following samples were prepared routinely and examined microscopically: (i) all gross lesions; (ii) the kidney, liver, lungs, and pancreas of all toxicity phase animals that survived to scheduled sacrifice; and (iii) the kidney, liver, lungs, spleen (females only), pancreas (males only), and thyroid (males only) of all carcinogenicity phase animals that survived to scheduled sacrifice. Findings were reported as present or graded as minimal, slight, moderate, marked, or severe.

Microscopic findings were peer reviewed internally. A second peer review was performed by a consultant pathologist selected by the Sponsor. This comprised a cross-check of the pathology report and histological sections including at least 10% of the 1000 mg/kg/day and control groups and all target tissues and tumors. The conclusions of the pathology report were by consensus.

II. RESULTS

A. OBSERVATIONS

1. Mortality - No treatment-related effect was observed on mortality. One 500 mg/kg/day female died during the toxicity phase. In the carcinogenicity phase animals, survival was 64-84% for all groups, and response was unrelated to dose. Thus, survival exceeded guideline requirements of 50% at Week 78 and 25% at Week 104 in both sexes.

2a. Clinical signs of toxicity - At 500 mg/kg/day in the carcinogenicity phase animals, incidences of perigenital yellow staining were generally increased in the males from Week 27 to termination (2-9%), and in the females from Week 32 to termination (2-24%), compared to 0 controls. Additionally at 1000 mg/kg/day, the following increases in incidences perigenital yellow staining were observed (compared to 0 controls): i) 2-24% from Week 66 to termination in the carcinogenicity phase males; ii) 2-73% from Week 3 to termination in the carcinogenicity phase females; and iii) 5-35% from Week 11 to termination in the toxicity phase females.

In the toxicity phase females, incidences of tail skin exfoliation were increased at 500 (16-42%) and 1000 (35-75%) mg/kg/day during Weeks 32-50/51 compared to 0 controls. However, a similar finding was not noted in the males or in any of the carcinogenicity phase animals; therefore, this finding was considered incidental. No other clinical signs of toxicity were observed.

2b. Neurological evaluations - No treatment-related effects were observed during the functional observational battery. Rearing was increased ($p \leq 0.05$) in the 1000 mg/kg/day males at 12 minutes (↑103%) and over the total testing period (↑56%); however, a similar effect was not observed on locomotor activity. Additionally, a transient increase ($p \leq 0.05$) in locomotor activity was observed in the 1000 mg/kg/day females at 12 minutes (↑63%). Both of these findings were considered incidental. Habituation was demonstrated in all groups.

B. BODY WEIGHT AND BODY WEIGHT GAINS - Selected body weights and body weight gains from the carcinogenicity phase animals are presented in Table 2. At ≥500 mg/kg/day, body weight gains were decreased ($p \leq 0.05$) during the second year (Weeks 52-104) of treatment (↓24-38%; not significant [NS] in the 500 mg/kg/day males), resulting in decreased ($p \leq 0.05$) overall (Weeks 0-104) body weight gains (↓11-20%). Body weight gains were similar to controls during Weeks 0-52 in both the toxicity and carcinogenicity phase animals. No effects of treatment were observed on body weight gains at 1 or 5 mg/kg/day.

Body weights were not statistically analyzed.

Table 2. Selected mean (±SD) body weights and body weight gains (g) in rats treated with IR5878 for up to 2 years.[a]

Week(s)	Dose (mg/kg/day)				
	0	1	5	500	1000
Males					
0	160±11.7	160±12.5	158±10.2	159±10.7	160±12.2
13	369±38.0	355±33.8	362±36.8	354±35.5	357±36.7
52	504±58.8	485±48.8	492±51.0	482±57.6	482±52.2
76	539±73.3	534±56.8	538±57.7	521±69.8	516±56.5
104	577±85.2	565±69.1	562±67.9	529±75.3	526±68.4
BWG: 0-52	344±53.4	325±42.4	333±45.9	323±54.0	322±46.1
BWG: 52-104	74±39.0	80±30.1	69±31.3	56±43.4 (↓24)	50±32.7* (↓32)
BWG: 0-104	417±80.7	406±62.0	402±63.9	371±71.6* (↓11)	367±63.1** (↓12)
Females					
0	123±7.9	123±9.8	125±8.4	124±7.9	124±10.5
13	220±18.2	219±19.0	223±20.5	222±17.3	221±19.6
52	285±38.6	288±37.2	294±41.2	282±34.4	278±36.8
76	339±55.0	337±54.3	344±58.1	321±35.9	310±49.0
104	374±58.5	363±55.8	373±49.8	340±36.2	324±41.4
BWG: 0-52	162±33.5	164±31.0	169±35.1	158±31.3	154±29.7
BWG: 52-104	86±34.2	81±43.8	90±31.3	61±23.4** (↓29)	53±23.2** (↓38)
BWG: 0-104	251±54.1	239±50.9	250±45.7	216±34.0** (↓14)	201±35.8** (↓20)

a Data (n=32-50) were obtained on pages 138-142 from Table 19 of MRID 46578913. Percent difference from controls is included in parentheses.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - No treatment-related effect was observed on food consumption.

2. **Compound consumption**- The mean achieved dosages are shown in Table 1.

3. **Food efficiency** - No treatment-related effect was observed on food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION - No treatment-related effects were observed during ophthalmoscopic examination of the toxicity phase animals at Week 52.

E. BLOOD ANALYSES

1. **Hematology** - No adverse treatment-related effects were observed on hematology parameters. The following differences ($p \leq 0.05$) indicative of a mild anemia were noted (primarily at 1000 mg/kg/day), but were minor and/or transient: (i) decreases in hematocrit, hemoglobin concentrations, red blood cell counts, mean cell hemoglobin concentration, and mean cell volume; (ii) increased incidence of hyperchromasia; (iii) increased platelet counts; and (iv) decreased prothrombin times. All other differences ($p \leq 0.05$) were also minor, transient, and/or unrelated to dose.

2. **Clinical chemistry** - Blood urea was increased ($p \leq 0.01$) by 29% in the 1000 mg/kg/day females at Week 104. This finding was corroborated by microscopic findings in the kidney and was considered treatment-related. All other differences ($p \leq 0.05$) were minor, transient, and/or not dose-dependent.

F. URINALYSIS - No treatment-related effect was observed on urinalysis parameters. Minor decreases were observed in the urinary pH of the 1000 mg/kg/day males throughout the study. A transient increase ($p \leq 0.05$) of 1% was noted in the urine specific gravity of the 1000 mg/kg/day males at Week 77. All other values in the treated groups were similar to controls.

G. SACRIFICE AND PATHOLOGY

1. **Organ weights** - At 1 year, relative to body liver weights were increased ($p \leq 0.01$) in both sexes at 500 (↑9-11%) and 1000 (↑14-22%) mg/kg/day (Table 3a). Additionally in the 1000 mg/kg/day males, increases ($p \leq 0.01$) were observed in absolute liver weights (↑20%) and relative kidney weights (↑7%). Other differences ($p \leq 0.05$) in treated groups compared to controls were not considered treatment-related because toxicity was not corroborated by gross or microscopic pathology.

At 2 years, terminal body weights were decreased ($p \leq 0.05$) in the 500 and 1000 mg/kg/day groups by 9-13% (Table 3b). Relative liver weights were increased ($p \leq 0.01$) in the 500 mg/kg/day males (↑9%) and both sexes at 1000 mg/kg/day (↑17-23%). Additionally, absolute liver weights were increased ($p \leq 0.01$) in the 1000 mg/kg/day males (↑12%). Relative kidney weights were increased ($p \leq 0.01$) in the 500 mg/kg/day males (↑11%) and in both sexes at 1000 mg/kg/day (↑14% each). Other differences ($p \leq 0.05$) in treated groups compared to controls were not considered treatment-related because: (i) ovarian toxicity was not corroborated by gross or

microscopic pathology; (ii) relative weights of the spleen were comparable to controls; and because spleen weight scales with body weight, the decreases in absolute organ weights are likely due to the decreased terminal body weights in these animals; and (iii) absolute weights of brain and thyroids were comparable to controls, and because these organs do not scale with body weight, the increases in relative organ weights are likely due to the decreased terminal body weights in these animals.

Table 3a. Mean (±SD) liver and kidney weights in rats treated with IR5878 for up to 1 year.[a]

Organ		Dose (mg/kg/day)				
		0	1	5	500	1000
Males						
Terminal body weight (g)		472.5±57.9	484.9±46.1	470.2±72.2	464.2±50.3	463.8±45.6
Liver	absolute (g)	14.74±1.84	14.45±1.44	14.28±2.13	15.91±2.30	17.75±2.45** (↑20)
	relative (%)	3.13±0.28	2.99±0.26	3.04±0.21	3.42±0.27** (↑9)	3.82±0.33** (↑22)
Kidney	absolute (g)	2.36±0.27	2.45±0.22	2.30±0.31	2.45±0.27	2.49±0.33
	relative (%)	0.50±0.04	0.51±0.04	0.49±0.04	0.53±0.04	0.54±0.04** (↑7)
Females						
Terminal body weight (g)		288.5±28.5	268.3±26.1	298.3±38.2	269.0±25.7	266.6±24.7
Liver	absolute (g)	9.21±1.20	9.02±1.13	9.27±1.17	9.52±1.29	9.71±0.99
	relative (%)	3.20±0.27	3.36±0.24	3.12±0.26	3.55±0.41** (↑11)	3.65±0.29** (↑14)

a Data (n=19-20) were obtained from Tables 13A and 13B on pages 109-114 of MRID 46578913. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).

** Significantly different from controls; $p \leq 0.01$

Table 3b. Mean (±SD) liver and kidney organ weights in rats treated with IR5878 for up to 2 years. [a]

Organ		Dose (mg/kg/day)				
		0	1	5	500	1000
Males						
Terminal body weight (g)		576.4±86.0	557.5±70.3	561.0±66.9	526.6±77.7* (↓9)	524.5±67.9** (↓9)
Liver	absolute (g)	16.77±2.71	16.08±2.68	16.39±1.94	16.66±2.44	18.77±2.62** (↑12)
	relative (%)	2.92±0.32	2.88±0.31	2.94±0.35	3.19±0.44** (↑9)	3.59±0.38** (↑23)
Kidney	absolute (g)	2.96±0.45	2.79±0.33	2.91±0.37	2.97±0.36	3.06±0.38
	relative (%)	0.52±0.04	0.50±0.05	0.52±0.07	0.57±0.10** (↑11)	0.59±0.07** (↑14)
Females						
Terminal body weight (g)		370.9±59.5	360.8±52.9	367.2±48.6	335.0±35.3** (↓10)	323.6±42.7** (↓13)
Liver	absolute (g)	11.52±2.05	11.30±1.93	11.17±1.91	10.88±1.42	11.86±1.92
	relative (%)	3.12±0.35	3.14±0.33	3.06±0.52	3.26±0.36	3.66±0.31** (↑17)
Kidney	absolute (g)	2.20±0.51	2.21±0.32	2.20±0.30	2.14±0.26	2.21±0.42
	relative (%)	0.60±0.12	0.62±0.08	0.60±0.09	0.64±0.08	0.68±0.09** (↑14)

a Data (n=32-42) were obtained from Tables 27A and 27B on pages 174-179 of MRID 46578913. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

2. <u>Gross pathology</u> - At 1 year, the incidence of gross lesions in the treated groups was similar to controls.

At 2 years, increased incidences of dark area(s) on the liver (15 treated vs 8 controls) and thyroid cysts (7 treated vs 0 controls; $p \leq 0.05$) were noted in the 1000 mg/kg/day males (Table 4). In the 1000 mg/kg/day females, an increased incidence of distended bile ducts (11 treated vs 3 controls; $p \leq 0.05$) was observed that was considered treatment-related, but not adverse. The incidences of pale areas on the lachrymal glands were increased in the ≥5 mg/kg/day males (14-50% treated vs 24% controls). As toxicity was not corroborated by histological evidence or by other gross lesions, these increases were considered incidental. Other findings were also considered unrelated to treatment because the differences were minor, unrelated to dose, or were not corroborated by histological evidence.

Table 4. Selected gross lesions (# affected/50) in rats treated with IR5878 for up to 2 years. [a]

Parameter	Dose (mg/kg/day)				
	0	1	5	500	1000
Males					
Liver, dark area(s)	8	3	4	8	15
Thyroid, cysts	0	2	2	4	7*
Females					
Bile ducts, distended	3	5	9	8	11*

a Data were obtained from Table 28C on pages 203-217 of MRID 46578913.
* Statistically different ($p \leq 0.05$) from the controls

3. Microscopic pathology

a. Non-neoplastic - At Week 52 (Table 5a), increased ($p \leq 0.01$) incidences of minimal to moderate centrilobular hepatocyte vacuolation were observed in the ≥500 mg/kg/day males (50-95% treated vs 0% controls) and minimal to moderate centrilobular hepatocyte hypertrophy in the ≥500 mg/kg/day males (60-75% vs 10%) and 1000 mg/kg/day females (60% vs 0%). Additionally at Week 52, increased incidence ($p \leq 0.05$) in pancreatic acinar cell vacuolation was observed in the 1000 mg/kg/day males (95% treated vs 45% controls); however, other indications of toxicity were not evident, and an adverse effect at 104 weeks was also not substantiated.

At Week 104, increased ($p \leq 0.05$) incidences of the following findings (% treated vs % controls) were observed in the liver: (i) minimal to marked centrilobular hepatocyte vacuolation in the ≥500 mg/kg/day males (62-80% vs 30%); (ii) slight centrilobular hepatocyte hypertrophy in the 1000 mg/kg/day males (42% vs 6%); (iii) minimal to slight cystic degeneration in the 1000 mg/kg/day males (16% vs 0%); and (iv) slight to moderate focal sinusoidal dilatation in the 1000 mg/kg/day females (12% vs 0%). In the thyroid, increased incidences of minimal to marked cystic follicular cell hyperplasia (20% treated vs 4% controls; $p \leq 0.05$) were noted in the 1000 mg/kg/day males, and increased incidences of minimal follicular cell hypertrophy were observed in the ≥500 mg/kg/day males (42% each treated vs 24% control). An increased (NS) incidence in slight to moderate chronic progressive nephropathy of the kidney was observed in the ≥500 mg/kg/day males (46-52% treated vs 34% controls). Increased ($p \leq 0.05$) incidences in the following kidney lesions were observed (% treated vs % controls) in females: (i) slight to marked chronic progressive nephropathy at 1000 mg/kg/day (52% vs 24%); (ii) minimal to marked pelvic/papillary epithelium hyperplasia at ≥500 mg/kg/day (88-92% vs 70%); and (iii) minimal to marked papillary/pelvic epithelium mineralization at ≥5 mg/kg/day (88-94% vs 74%; NS at 5 and 500 mg/kg/day). An increased ($p \leq 0.05$) incidence of minimal to moderate hemosiderosis was observed in the spleen of the ≥500 mg/kg/day females (86-92% treated vs 66% controls).

Additionally at Week 104, increased incidence of peri-islet pigment in the pancreas (22% treated vs 6% controls; $p \leq 0.05$) was noted in the 1000 mg/kg/day males; however, this isolated finding

was considered incidental. Increased incidences of other findings in the treated groups relative to controls were minor and/or not corroborated by other clinical or pathological findings.

Table 5a. Incidence (# affected [%]) of selected non-neoplastic microscopic lesions in rats treated with IR5878 in the diet for up to 1 year. [a]

Microscopic lesion		Dose (mg/kg/day) 0	1	5	500	1000
Males						
Liver	Hepatocyte vacuolation, centrilobular (total)	0 (0)	0 (0)	0 (0)	10** (50)	19** (95)
	minimal	0	0	0	10	5
	slight	0	0	0	0	9
	moderate	0	0	0	0	5
	Hepatocyte hypertrophy, centrilobular (total)	2 (10)	5 (25)	2 (10)	12** (60)	15** (75)
	minimal	2	5	2	11	0
	slight	0	0	0	1	14
	moderate	0	0	0	0	1
Females						
Liver	Hepatocyte hypertrophy, centrilobular (total)	0 (0)	0 (0)	0 (0)	0 (0)	12** (60)
	minimal	0	0	0	0	11
	slight	0	0	0	0	1

a Data (n=19-20) were obtained from Table 15 on pages 121-128 and pages 587-599 of MRID 46578913.
** Significantly different from controls; $p \leq 0.01$

Table 5b. Incidence (# affected/# examined [%]) of selected non-neoplastic microscopic lesions in male rats treated with IR5878 in the diet for up to 2 years. [a]

Microscopic lesion		Dose (mg/kg/day)				
		0	**1**	**5**	**500**	**1000**
Liver	Hepatocyte vacuolation, centrilobular (total)	15/50 (30)	5/50* (10)	4/50** (8)	31/50** (62)	40/50** (80)
	minimal	11	4	3	16	6
	slight	4	1	1	12	28
	moderate	0	0	0	2	6
	marked	0	0	0	1	0
	Hepatocyte hypertrophy, centrilobular, slight (total)	3/50 (6)	4/50 (8)	3/50 (6)	8/50 (16)	21/50** (42)
	Cystic degeneration (total)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	8/50** (16)
	minimal	0	0	0	0	3
	slight	0	0	0	0	5
Thyroid	Cystic follicular cell hyperplasia	2/50 (4)	0/50 (0)	4/50 (8)	5/50 (10)	10/49* (20)
	minimal	1	0	1	1	1
	slight	0	0	1	2	5
	moderate	1	0	2	2	2
	marked	0	0	0	0	2
	Follicular cell hypertrophy, minimal (total)	12/50 (24)	11/50 (22)	16/50 (32)	21/50 (42)	21/49 (42)
Kidney	Chronic progressive nephropathy (total)	17/50 (34)	12/50 (24)	7/50* (14)	26/50 (52)	23/50 (46)
	slight	16	11	5	24	21
	moderate	1	0	1	2	2
	marked	0	1	1	0	0

a Data were obtained from Table 29F on pages 257-273 and pages 1549-2131 of MRID 46578913.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

Table 5c. Incidence (# affected/# examined [%]) of selected non-neoplastic microscopic lesions in female rats treated with IR5878 in the diet for up to 2 years.[a]

Microscopic lesion		Dose (mg/kg/day)				
		0	**1**	**5**	***500***	***1000***
Liver	Sinusoidal dilatation, focal (total)	0/50 (0)	2/50 (4)	5/50 (10)	4/50 (8)	6/50* (12)
	slight	0	2	2	1	3
	moderate	0	0	3	3	3
Kidney	Chronic progressive nephropathy (total)	12/50 (24)	13/50 (26)	4/50 (8)	10/50 (20)	26/50** (52)
	slight	10	13	4	10	20
	moderate	0	0	0	0	4
	marked	2	0	0	0	2
	Hyperplasia, pelvic/papillary epithelium (total)	35/50 (70)	32/50 (64)	29/50 (58)	46/50** (92)	44/50* (88)
	minimal	23	25	18	18	16
	slight	12	6	10	24	15
	moderate	0	1	1	2	11
	marked	0	0	0	2	2
	Mineralization, papillary/pelvic epithelium (total)	37/50 (74)	34/50 (68)	44/50 (88)	45/50 (90)	47/50* (94)
	minimal	28	25	31	25	21
	slight	8	9	13	15	20
	moderate	1	0	0	3	6
	marked	0	0	0	2	0
Spleen	Increased hemosiderosis (total)	33/50 (66)	39/50 (78)	39/50 (78)	43/50* (86)	46/50** (92)
	minimal	17	17	18	12	14
	slight	16	18	18	21	25
	moderate	0	4	3	10	7

a Data were obtained from Table 29F on pages 257-273 and pages 1549-2131 of MRID 46578913.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

b. <u>Neoplastic</u> - Summary data for incidences of neoplastic lesions were reported in the Study Report in Table 15 on pages 121-128 and Tables 29A-29C on pages 218-228 and are included as an Appendix to this DER. Summary tables of selected neoplastic lesions are provided below (Table 6). There were no treatment-related increases in tumors after 1 year.

After 2 years, an increased ($p \leq 0.05$) incidence in thyroid follicular cell adenoma was observed in the ≥500 mg/kg/day males (14-20% treated vs 2% concurrent controls), without an increased incidence of follicular cell carcinoma. The incidences of other tumors were similar in the treated groups to the concurrent controls.

Table 6. Incidence (# affected/# examined [%]) of selected neoplastic microscopic lesions in rats treated with IR5878 in the diet for up to 2 years.[a]

Microscopic lesion		Dose (mg/kg/day)				
		0	1	5	500	1000
Males						
Thyroid	Follicular cell adenoma	1/50 (2)	2/50 (4)	1/50 (2)	7/50* (14)	10/49** (20)
	Follicular cell carcinoma	0/50 (0)	1/50 (2)	1/50 (2)	0/50 (0)	0/49 (0)

a Data were obtained from Table 29C on pages 225-228 of MRID 46578913.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The LOAEL was 500 mg/kg/day, based on liver, spleen, and kidney toxicity. Yellow staining in the perigenital area and decreased body weight gain were also observed. The NOAEL was 5 mg/kg/day. Increased incidences of thyroid follicular cell adenomas were noted in the ≥500 mg/kg/day males, but were considered secondary to enhanced hepatic metabolism and resultant disruption of hormonal feedback control of the thyroid.

B. REVIEWER COMMENTS - No treatment-related effects were observed on mortality, functional observational battery findings, food consumption, food efficiency, ophthalmoscopic examination, hematology, or urinalysis.

At ≥500 mg/kg/day, body weight gains were decreased ($p \leq 0.05$) during the second year (Weeks 52-104) of treatment (↓24-38%; not significant [NS] in the 500 mg/kg/day males), resulting in decreased ($p \leq 0.05$) overall (Weeks 0-104) body weight gains (↓11-20%). Body weight gains were similar to controls in both the toxicity phase animals and during Weeks 0-52 in the carcinogenicity phase animals. No effects of treatment were observed on body weight gains at 1 or 5 mg/kg/day.

Hepatotoxicity was observed. At 1 year, relative to body liver weights were increased ($p \leq 0.01$) in both sexes at 500 (↑9-11%) and 1000 (↑14-22%) mg/kg/day. Additionally, increased ($p \leq 0.01$) absolute liver weights were observed in the 1000 mg/kg/day males (↑20%). Increased ($p \leq 0.01$) incidences in minimal to moderate centrilobular hepatocyte vacuolation was noted in the ≥500 mg/kg/day males (50-95% treated vs 0% controls), and incidences of minimal to

moderate centrilobular hepatocyte hypertrophy were increased in the ≥500 mg/kg/day males (60-75% vs 10%) and 1000 mg/kg/day females (60% vs 0%).

At 2 years, increased incidences of dark area(s) on the liver (15 treated vs 8 controls) were noted in the 1000 mg/kg/day males. Relative to body liver weights were increased ($p \leq 0.01$) in the 500 mg/kg/day males (↑9%) and in both sexes at 1000 mg/kg/day (↑17-23%). Additionally, absolute liver weights were increased ($p \leq 0.01$) in the 1000 mg/kg/day males (↑12%). Increased ($p \leq 0.05$) incidences of the following microscopic findings (% treated vs % controls) were observed: (i) minimal to marked centrilobular hepatocyte vacuolation in the ≥500 mg/kg/day males (62-80% vs 30%); (ii) slight centrilobular hepatocyte hypertrophy in the 1000 mg/kg/day males (42% vs 6%); (iii) minimal to slight cystic degeneration in the 1000 mg/kg/day males (16% vs 0%); and (iv) slight to moderate focal sinusoidal dilatation in the 1000 mg/kg/day females (12% vs 0%).

Nephrotoxicity was observed. At 500 mg/kg/day in the carcinogenicity phase animals, incidences of perigenital yellow staining were generally increased in the males from Week 27 to termination (2-9%) and in the females from Week 32 to termination (2-24%) compared to 0 controls. Additionally at 1000 mg/kg/day, the following increases in perigenital yellow staining were observed (compared to 0 controls): i) 2-24% from Week 66 to termination in the carcinogenicity phase males; ii) 2-73% from Week 3 to termination in the carcinogenicity phase females; and iii) 5-35% from Week 11 to termination in the toxicity phase females.

At 1 year, increases ($p \leq 0.01$) were observed in relative kidney weights in the 1000 mg/kg/day males (↑7%).

At 2 years, blood urea was increased ($p \leq 0.01$) by 29% in the 1000 mg/kg/day females. Relative to body kidney weights were increased ($p \leq 0.01$) in the 500 mg/kg/day males (↑11%) and in both sexes at 1000 mg/kg/day (↑14% each). An increased (NS) incidence in slight to moderate chronic progressive nephropathy of the kidney was observed in the ≥500 mg/kg/day males (46-52% treated vs 34% controls). Increased ($p \leq 0.05$) incidences in the following kidney lesions were observed (% treated vs % controls) in females: (i) slight to marked chronic progressive nephropathy at 1000 mg/kg/day (52% vs 24%); (ii) minimal to marked pelvic/papillary epithelium hyperplasia at ≥500 mg/kg/day (88-92% vs 70%); and (iii) minimal to marked papillary/pelvic epithelium mineralization at ≥5 mg/kg/day (88-94% vs 74%; NS at 5 and 500 mg/kg/day). The mineralization observed in the 5 mg/kg/day females was not corroborated by other clinical or pathological evidence of toxicity and was generally minimal or slight in severity; therefore, this effect was not considered adverse at this dose.

At 2 years, increased incidences of thyroid cysts (14% treated vs 0% controls; $p \leq 0.05$) were noted in the 1000 mg/kg/day males. Increased incidences of minimal to marked cystic follicular cell hyperplasia (20% treated vs 4% controls; $p \leq 0.05$) were noted in the 1000 mg/kg/day males, and minimal follicular cell hypertrophy was observed in the ≥500 mg/kg/day males (42% each treated vs 24% control). In a concurrently submitted special study (MRID 46578927), it was demonstrated that administration of the test compound results in an induction of UDP-GT, which

is responsible for the degradation of thyroxine (T4). Pharmacokinetic data indicated an increased elimination of T4. Thus, chronic stimulation of the thyroid would occur through the hypothalmic-pituitary-thyroid axis, which could result in follicular cell hypertrophy and hyperplasia. This effect is not observed in humans because of differing thyroid hormone binding profiles and metabolic clearance rates of the thyroid hormones. Consequently, these effects on the thyroid were considered unimportant to humans.

The LOAEL is 500 mg/kg/day, based on decreased body weight gains, slight hepatotoxicity, and slight nephrotoxicity in both sexes. The NOAEL is 5 mg/kg/day.

After 2 years, increased ($p \leq 0.05$) incidences in thyroid follicular cell adenoma were observed in the ≥ 500 mg/kg/day males (14-20% treated vs 2% concurrent controls), without an increased incidence of follicular cell carcinoma. Again, the thyroid effects were secondary to metabolic induction of the liver and were not considered relevant to humans because of reasons stated above.

C. <u>STUDY DEFICIENCIES</u> - The following minor deficiencies were noted, but do not alter the conclusions of this review:

- Pituitary samples from all animals should have been examined microscopically.
- More than one lot of the test compound was used over the course of the study.

EPA Primary Reviewer: Kelly Schumacher, M.S. **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date**_____
EPA Secondary Reviewer: Alan Levy, Ph.D. **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date**_____

TXR#: 0052629

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity Feeding Study - Rats; OPPTS 870.3100 (§82-1a); OECD 408.

PC CODE: 108209 **DP BARCODE:** D306738
SUBMISSION NO.: S755326

TEST MATERIAL (PURITY): IR5878 (Orthosulfamuron, 98.0%)

SYNONYMS: 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)-phenylsulfamoyl] urea; benzamide, 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N-N-dimethyl-

CITATION: Webley, L. (2001). IR5878 Toxicity study by dietary administration to Han Wistar rats for 13 weeks followed by a 4 week recovery period. Huntingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, England PE28 4HS. Study No. AGR 128/012174, October 16, 2001. MRID 46260103. Unpublished.

SPONSOR: ISAGRO SpA, Centro Uffici San Siro - Fabbricato D, ala 3, Via Caldera, 21, 20153 Milano - Italy.

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity study (MRID 46260103), IR5878 (98.0% a.i., Batch No. FCF/T/172-00 (ex 20525/03/8) was administered to ten Han Wistar rats/sex/dose in the diet at dose levels of 0, 250, 1500, and 9000 ppm (equivalent to 0, 19, 113, and 706 mg/kg bw/day, respectively, for males and to 0, 22, 131, and 773 mg/kg bw/day, respectively, for females). Concurrently, a recovery group of five Han Wistar rats/sex/dose were administered either 0 or 9000 ppm (equivalent to 0 or 706 mg/kg bw/day, respectively, for males and to 0 or 773 mg/kg bw/day, respectively, for females) during the 90-day study period and were then maintained for an additional four weeks on the control diet.

There were no compound-related effects on mortality, body weights, body weight gains, food consumption, neurological observations and measurements, hematology, clinical chemistry, or gross pathology. Compared to controls, an increase in dorsal hair loss was seen in females, but this finding is common. Other treatment-related effects (transient increases in liver weight and

hepatocellular hypertrophy in high-dose males compared to controls) indicate a minimal, adaptive response in the liver following exposure to IR5878 that is not considered adverse.

The LOAEL was not identified in this study (>706 mg/kg/day for males and >773 mg/kg/day for females). The NOAEL is the highest dose tested in this study (9000 ppm), which was equivalent to 706 mg/kg/day for males and 773 mg/kg/day for females.

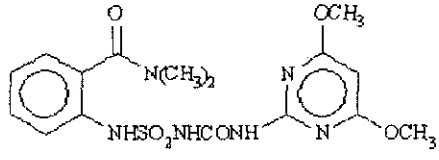
This study is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** IR5878

Description:	White crystalline powder
Lot/Batch #:	Batch No. FCF/T/172-00 (ex 20525/03/8)
Purity:	98.0 % a.i.
Compound Stability:	Stable in rodent diet for 15 days at freezing temperature and for 8 days at room temperature
CAS No. of TGAI:	213464-77-8
Structure	

2. **Vehicle and/or positive control:** The test material was incorporated into the diet. No positive control was used in this study.

3. **Test animals:**

Species:	Rat	
Strain:	*Han Wistar*	
Age/weight at study initiation:	Approximately 37-41 days of age / males: 135-138 g, females: 108-112 g	
Source:	Harlan UK Limited, Bicester, Oxfordshire, England	
Housing:	Housed five of the same sex per cage in stainless steel cages with stainless steel mesh lids and floors	
Diet:	Rat and Mouse No. 1 Maintenance Diet (Special Diets Services Ltd., Witham, Essex, England), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	40-70%
	Air changes:	>15 times/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimat on period:	11 days	

B. STUDY DESIGN:

1. **In life dates:** Start: November 20, 2000
   End: February 19-20, 2001 (*Main group*); March 19, 2001 (*Recovery group*)

2. **Animal assignment:** Animals were randomly assigned to the test groups noted in Table 1. Three males with body weights at the extreme ends of the weight range of the other rats and two males with ophthalmic lesions were replaced by five spare males from the same batch of animals. At the end of the regular study, the recovery groups were held for an additional four weeks, during which they were fed the control diet.

TABLE 1: Study design

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg/day)[a]	# Male	# Female
Control	0	0	10	10
Low	250	♂ (19) ♀ (22)	10	10
Mid	1500	♂ (113) ♀ (131)	10	10
High	9000	♂ (706) ♀ (773)	10	10
Recovery Group				
Control	0	0	5	5
High	9000	♂ (706) ♀ (773)	5	5

[a]Data from page 51 of MRID 46260103.

3. **Dose selection rationale:** The dose levels were selected based on the results from a four-week oral toxicity study in rats (Report No. AGR125/000095) where dietary administration of up to 12500 ppm resulted in changes in body weight gains, food consumption, hematology, clinical chemistry, and spleen weights. These changes were also seen at 2500 ppm. Because the high dose, 12500 ppm, was considered sufficiently close to the maximum tolerated dose, 9000 ppm was set as the high dose in the 90-day study.

4. **Diet preparation and analysis:** Premix was prepared weekly by mixing appropriate amounts of test substance with Rat and Mouse No. 1 Maintenance Diet. Test diets were then prepared weekly, three days in advance, by diluting the concentrated premix and were stored frozen. Homogeneity and stability of IR5878 in the feed, at concentrations of 50 and 12500 ppm, were tested in an earlier study (Report No. AGR 127/003821); however, the results of this study were not provided in MRID 46260103. During the 90-day study, samples of treated food from each test group were analyzed on weeks 1, 6, and 12 for active ingredient concentration using a solvent extraction method, followed by reverse phase HPLC analysis with external calibration.

Results:

Homogeneity analysis: Homogeneity was confirmed at nominal concentrations of 50 and 12500 ppm in an earlier study (Report No. AGR 127/003821); however, the results of this study were not reported in MRID 46260103.

Stability analysis: Stability of IR5878 in the diet was confirmed for 15 days with freezer storage and for 8 days with storage at room temperature in an earlier study (Report No. AGR 127/003821); however, results of the stability analysis were not reported in MRID 46260103.

Concentration analysis: Dietary concentrations were 97-99%, 94-98%, and 92-96% of target concentrations for the low-, mid-, and high-dose groups, respectively.

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

5. **Statistics:** Body weights and organ weights were tested for homogeneity of variance using Bartlett's test. If significant, a Behrens-Fisher test was used for pairwise comparisons; if not significant, Dunnett's test was used. Gross pathological and histopathological findings were analyzed using Fisher's exact test to compare each treated group with controls.

For clinical pathology data, if 75% of the findings (across all treatment groups) were the same number, then a frequency analysis was conducted. Treatment groups were compared using a Mantel test and pairwise Fisher's Exact tests.

If Bartlett's test was not significant at the 1% level, parametric analysis was conducted. Then, if the F1 test was not significant at the 1% level, Williams' test was applied, but if it was significant at that level, Dunnett's test was used.

If Bartlett's test was significant at the 1% level, the data were subjected to transformation to obtain equality of variance and then tested again using Bartlett's test. If still significant, non-parametric analysis was applied. Then, if the H1 test was not significant at the 1% level, Shirley's test was applied, but if it was significant at that level, Steel's test was used.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice a day for signs of toxicity and mortality.

1b. Clinical examinations: Clinical observations were conducted weekly.

1c. Neurological evaluations: The following observations were made on ten animals/group/sex prior to initiation of the study and weekly during the dosing period and on all of the recovery phase animals in the final week of the recovery period: exophthalamos, fur condition, lacrimation, piloerection, reactivity to handling, ease of removal from cage, salivation, vocalization, activity counts, arousal, convulsion, defecation counts, gait, grooming, palpebral closure, posture, rearing, tremor, twitching, and urination. The following measurements were recorded prior to study initiation and

during week 12 of the dosing period for 10 animals/group/sex and during week 4 of the recovery period for all of the recovery phase animals: approach response, auditory startle reflex, body temperature, body weight, grip strength, landing footsplay, tail pinch response, pupil reflex, righting reflex, touch response, and motor activity.

2. **Body weight:** Animals were weighed prior to initiation of the study, on the first day of dosing, weekly during the dosing and recovery periods, and before necropsy.

3. **Food consumption and compound intake:** Food consumption for each cage was determined, and mean weekly diet consumption was calculated as g food/animal/week. Group mean food efficiencies were calculated on a weekly basis as 100*(g body weight gain per week/g food consumption per week). Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. **Ophthalmoscopic examination:** Eyes of all animals were examined before dosing (week -1) using indirect ophthalmoscopy. Eyes of all surviving control and high-dose animals were also similarly examined during week 13. In both cases, a 0.5% tropicamide solution was instilled prior to examination.

5. **Hematology and clinical chemistry:** After an overnight fast, blood was collected from the retro-orbital sinus of all main study animals during week 13 and from all recovery study animals in week 4 of the recovery period. During sampling, animals were held under isoflurane anesthesia. The CHECKED (X) parameters were examined.

a. Hematology:

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
X	Blood clotting measurements*		
X	(Thromboplastin time)		
-	(Fibrinogen)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
- Not examined

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
-	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	-	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
-	Cholinesterase (ChE)	X	Triglycerides
-	Creatine phosphokinase	-	Serum protein electrophoresis
-	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
-	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
-	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
- Not examined

6. **Urinalysis:** Urine was collected from fasted main study animals during week 13 and from fasted recovery phase animals during week 4 of the recovery period. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)	-	Nitrate
X	Protein*	-	Urobilinogen

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
- Not examined

7. **Sacrifice and pathology:** All animals were sacrificed by carbon dioxide inhalation and were subjected to gross pathological examination; the CHECKED (X) tissues were collected for histological examination. The head, larynx, nose, pharynx, salivary gland (one only), sciatic nerve (one only), and skeletal thigh muscle (one only) were collected and preserved, but these organs were not examined histologically. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
-	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	-	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroid*
X	Rectum*	X	Urinary bladder*	XX	Thyroid*
XX	Liver*	XX	Testes*+		
-	Gall bladder* (not rat)	XX	Epididymides*+		
-	Bile duct	X	Prostate*		OTHER
X	Pancreas*	X	Seminal vesicles*	X	Bone (sternum and femur)
	RESPIRATORY	XX	Ovaries*+	X	Skeletal muscle
X	Trachea*	XX	Uterus*+	X	Skin*
X	Lung*	X	Mammary gland*	X	All gross lesions and masses*
X	Nose*			X	Head
X	Pharynx*				
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies
- Not examined

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** The only treatment-related clinical sign of toxicity observed was hair loss from the dorsal body surface, beginning on week 1 for both males and females. Hair loss was found in 3/10, 2/10, and 6/15 of the low-, mid-, and high-dose males, respectively, compared to 0/15 of control males. In females, 4/10, 4/10, and 12/15 animals were affected in the low-, mid-, and high-dose groups, respectively, compared to 2/15 of control females. Although no hair loss was seen in control and high-dose males during the recovery period, 2/5 of the control females and 5/5 of the high-dose females also exhibited hair loss during the four week recovery phase.

2. **Mortality:** No treatment-related mortalities occurred during the study. One control female had overgrown and maloccluded teeth and was sacrificed during week four for humane reasons.

3. **Neurological evaluations:** Mean forelimb grip strength values were lower in all treated males compared to controls during treatment week 12. However, no dose-response was observed, and none of the differences between treated and control males were statistically significant. Forelimb grip strength in females and hindlimb grip strength in both males and females were similar to controls at all treatment levels.

   As shown in Table 2, a statistically significant increase in mean landing footsplay was seen in high-dose females compared to control females in week 4 of the recovery phase. Prior to treatment, no difference in mean landing footsplay between ten control and ten high-dose females was observed. However, an examination of the individual data indicated that the landing footsplay values for just those females that were examined on recovery week 4, in addition to treatment weeks 0 and 12, differed before the initiation of the study. Specifically, on treatment week 0, footsplay ranged from 42-75 mm in the five control females and from 67-94 mm in the five high-dose females maintained throughout the recovery phase of the study. No differences in landing footsplay were seen between any of the treated females on treatment week 12 compared to the control females or between any of the treated males compared to control males on treatment week 12 and recovery week 4.

   As shown in Table 3, statistically significant decreases were seen in the number of high-beam breaks made on treatment week 12 by low- and mid-dose males compared to controls. However, prior to the initiation of the study, the number of high-beam breaks made by the these low- and mid-dose males were also lower compared to controls, although the differences were not statistically significant. No dose-response was seen; the number of high-beam breaks made by high-dose males did not differ from controls. Compared to controls, the number of high-beam breaks made by females and the number of low-beam breaks made by both males and females were unaffected by all levels of treatment.

TABLE 2. Mean landing footsplay (mm ± SD) in rats fed IR5878 for 13 weeks[a]						
Dose (ppm)	Males			Females		
	Treatment Week 0[b]	Treatment Week 12[b]	Recovery Week 4[c]	Treatment Week 0[b]	Treatment Week 12[b]	Recovery Week 4[c]
0	80 ± 13	128 ± 11	120 ± 20	70 ± 21	106 ± 17	75 ± 17
250	91 ± 21	125 ± 22	N/A	82 ± 11	121 ± 14	N/A
1500	81 ± 25	123 ± 19	N/A	84 ± 19	117 ± 10	N/A
9000	81 ± 17	133 ± 13	125 ± 11	81 ± 9	108 ± 17	101 ± 15 *

[a] Data obtained from pages 336-338 in the study report.
[b] n = 10, including those 5 animals observed at recovery week 4
[c] n = 5
* Significantly different from controls, p≤0.05

TABLE 3. Mean motor activity (beam breaks ± SD) over 60 minutes in rats fed IR5878 for 13 weeks[a]						
Dose (ppm)	Males			Females		
	Treatment Week 0[b]	Treatment Week 12[b]	Recovery Week 4[c]	Treatment Week 0[b]	Treatment Week 12[b]	Recovery Week 4[c]
High beam breaks						
0	90 ± 26	147 ± 41	161 ± 105	99 ± 45	131 ± 42	111 ± 44
250	58 ± 35	95 ± 38 **	N/A	83 ± 56	107 ± 38	N/A
1500	73 ± 59	107 ± 30 *	N/A	79 ± 30	99 ± 41	N/A
9000	101 ± 51	125 ± 44	103 ± 36	83 ± 39	114 ± 37	87 ± 70
Low beam breaks						
0	410 ± 124	697 ± 180	640 ± 204	358 ± 144	623 ± 130	482 ± 198
250	291 ± 159	516 ± 182	N/A	358 ± 100	555 ± 136	N/A
1500	368 ± 125	594 ± 121	N/A	417 ± 148	584 ± 161	N/A
9000	454 ± 254	624 ± 248	664 ± 83	376 ± 139	663 ± 173	563 ± 328

[a] Data obtained from pages 342-346 in the study report.
[b] n = 10, including those 5 animals observed at recovery week 4
[c] n = 5
* Significantly different from controls, p≤0.05
** Significantly different from controls, p≤0.01

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weights and total body weight gains are given in Table 4. There were no treatment-related effects on body weights or body weight gains in the main or recovery phases of this study.

TABLE 4. Mean body weights and body weight gains (BWG) during 90 days of treatment[a]								
Dose (ppm)	Body weights (g ± SD)				BWG (g) - Treatment Period	Body weights (g ± SD)		BWG (g) - Recovery Period
	Week 0	Week 1	Week 7	Week 13		Recovery Week 0	Recovery Week 4	
Male								
0	138 ± 8	175 ± 11	318 ± 26	367 ± 32	230	365 ± 26	384 ± 28	19
250	136 ± 6	176 ± 9	318 ± 29	369 ± 38	233	N/A	N/A	N/A
1500	135 ± 4	172 ± 6	306 ± 21	356 ± 28	221	N/A	N/A	N/A
9000	136 ± 6	173 ± 8	311 ± 28	367 ± 40	231	398 ± 53	422 ± 52	24
Female								
0	108 ± 8	127 ± 12	188 ± 22	211 ± 22	103	196 ± 11	204 ± 10	7
250	111 ± 6	132 ± 10	197 ± 27	221 ± 25	110	N/A	N/A	N/A
1500	112 ± 5	132 ± 7	194 ± 13	214 ± 16	102	N/A	N/A	N/A
9000	112 ± 6	133 ± 8	196 ± 16	221 ± 16	109	230 ± 17	239 ± 17	9

[a] Data obtained from pages 43-45 in the study report.

C. FEED CONSUMPTION AND COMPOUND INTAKE:

1. **Feed consumption:** There were no treatment-related effects on food consumption.

2. **Compound Intake:** Time-weighted average compound intakes are shown in Table 1.

3. **Feed Efficiency:** There were no treatment-related effects on food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related ophthalmological findings.

E. BLOOD ANALYSES:

1. **Hematology:** Selected hematology data are given in Table 5. Mean red blood cell (RBC) counts were marginally decreased in high-dose females in the main study compared to controls, but this decrease (-6%) is not biologically meaningful. Additionally, no significant decreases in mean RBC counts were seen in the low- or mid-dose main study females, the high-dose females following a four week recovery period, or in any of the treated male groups. White blood cell and lymphocyte counts were slightly decreased in all treated male and female groups following 13 weeks of treatment with IR5878; however, these parameters were comparable to controls for both males and females following a four week recovery period.

TABLE 5. Selected hematology findings in rats fed IR5878 for 13 weeks[a]			
Dose (ppm)	Red blood cells (10^{-12}/L ± SD)	White blood cells (10^{-9}/L ± SD)	Lymphocytes (10^{-9}/L ± SD)
Male Main Group - Treatment Week 13			
0	8.6 ± 0.3	8.5 ± 1.2	6.4 ± 0.9
250	8.7 ± 0.3	7.0 ± 1.4 *	5.4 ± 1.5 *
1500	8.5 ± 0.4	6.9 ± 1.1 **	5.3 ± 0.8 *
9000	8.6 ± 0.2	6.7 ± 1.4 **	4.8 ± 1.1 **
Male Recovery Group - Recovery Week 4			
0	N/A	7.6 ± 1.7	5.7 ± 1.7
9000	N/A	7.1 ± 1.2	5.3 ± 0.9
Female Main Group - Treatment Week 13			
0	7.9 ± 0.4	5.5 ± 1.0	4.5 ± 0.9
250	7.7 ± 0.5	4.7 ± 1.2 *	3.6 ± 0.9 *
1500	8.0 ± 0.3	4.5 ± 0.6 *	3.4 ± 0.5 **
9000	7.4 ± 0.3 *	4.0 ± 0.6 **	3.1 ± 0.6 **
Female Recovery Group - Recovery Week 4			
0	7.7 ± 0.3	4.1 ± 1.8	3.3 ± 1.2
9000	7.6 ± 0.3	5.1 ± 0.7	3.9 ± 0.7

[a] Data obtained from pages 55-60 in the study report.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p\leq0.01$

2. **Clinical chemistry:** In treated males compared to controls, statistically significant changes were seen in aspartate aminotransferase, creatine, phosphorous, total protein, albumin, and albumin/globulin values; however, none of these changes are considered biologically significant. Similarly, in treated females compared to controls, statistically significant changes were found in chlorine and phosphorous levels, but neither change was biologically meaningful.

F. URINALYSIS: There were no treatment-related changes reported.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Compared to controls, liver and kidney weights relative to body weights were marginally increased in high-dose males following 13 weeks of treatment, as shown in Table 6. However, male absolute liver and kidney weights in the main study and male absolute and relative liver and kidney weights following four weeks of recovery were all comparable to controls.

   Absolute kidney weights in high-dose females were marginally increased compared to controls after the four week recovery period. However, female absolute and relative liver and

kidney weights in the main study, female relative kidney and liver weights following recovery, and female absolute liver weights following recovery were all comparable to controls.

TABLE 6. Absolute and relative organ weights in rats fed IR5878 for 13 weeks[a]

Dose (ppm)	Body weight (g)	Liver (g)	Liver/body (g/100)	Kidney (g)	Kidney/body (g/100)
Male Main Group - Treatment Week 13					
0	369 ± 35	12.2 ± 1.6	3.3 ± 0.2	2.0 ± 0.2	0.5 ± 0.0
250	369 ± 39	12.2 ± 1.5	3.3 ± 0.1	2.0 ± 0.2	0.5 ± 0.0
1500	356 ± 29	11.6 ± 1.3	3.2 ± 0.2	1.9 ± 0.2	0.5 ± 0.0
9000	351 ± 20	13.5 ± 1.1	3.9 ± 0.2 **	2.0 ± 0.1	0.6 ± 0.0 *
Male Recovery Group - Recovery Week 4					
0	384 ± 29	13.5 ± 1.7	3.5 ± 0.2	2.1 ± 0.1	0.5 ± 0.0
9000	422 ± 53	13.9 ± 1.1	3.3 ± 0.2	2.2 ± 0.2	0.5 ± 0.1
Female Main Group - Treatment Week 13					
0	215 ± 26	7.9 ± 1.1	3.7 ± 0.3	1.3 ± 0.2	0.6 ± 0.0
250	222 ± 28	8.1 ± 1.2	3.6 ± 0.3	1.4 ± 0.2	0.6 ± 0.0
1500	216 ± 16	8.0 ± 0.5	3.7 ± 0.2	1.3 ± 0.1	0.6 ± 0.0
9000	216 ± 17	8.1 ± 0.7	3.7 ± 0.1	1.4 ± 0.2	0.6 ± 0.0
Female Recovery Group - Recovery Week 4					
0	204 ± 11	6.9 ± 0.7	3.4 ± 0.2	1.3 ± 0.1	0.6 ± 0.0
9000	234 ± 14 **	8.0 ± 1.2	3.4 ± 0.4	1.5 ± 0.1 **	0.6 ± 0.0

[a]Data obtained from pages 70-77 in the study report.
* Significantly different from controls, $p \leq 0.05$
** Significantly different from controls, $p \leq 0.01$

A marginal increase in the adrenal gland absolute weights following 13 weeks of dosing occurred only in low-dose (not in mid- or high-dose) females compared to controls.

2. **Gross pathology**: There were no treatment-related gross pathology findings.

3. **Microscopic pathology**: On treatment week 13, slight centrilobular hepatocyte hypertrophy was seen in 7/10 of the high-dose males (Table 7), which correlates with the increase seen in relative liver weights. Hypertrophy was also seen in 1/10 of the mid-dose males on treatment week 13, but none was seen in low-dose or control males. No hepatocellular hypertrophy was observed in females after 13 weeks or treatment or in males or females after a 4 week recovery period.

TABLE 7. Incidence of centrilobular hepatocyte hypertrophy in rats fed IR5878 for 13 weeks[a]				
Dose (ppm)	Male Main Group - Treatment Week 13	Male Recovery Group - Recovery Week 4	Female Main Group - Treatment Week 13	Female Recovery Group - Recovery Week 4
0	0/10	0/5	0/10	0/5
250	0/10	N/A	0/10	N/A
1500	1/10	N/A	0/10	N/A
9000	7/10 **	0/5	0/10	0/5

[a] Data obtained from pages 85 and 89 in the study report.
** Significantly different from controls, $p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study authors concluded that, in Han Wistar rats, dietary exposure to IR5878 caused dorsal hair loss in females, slight decreases in female red blood cell counts, hematocrit, and hemoglobin values; decreases in male and female white blood cell and lymphocyte counts and in phosphorous levels; changes in male creatinine concentration, triglyceride levels, aspartate amino-transferase and alkaline-phosphatase activities, and urine composition; increased male liver and kidney weights; and an increase in the incidence of slight centrilobular hypertrophy of male hepatocytes. The investigators found no evidence of neurotoxicity.

The investigators report that the effects seen in male liver (i.e., increased liver weight and hepatocellular hypertrophy) were not seen following the four week recovery. The study authors determined that the NOAEL was 1500 ppm, which is equivalent to 113 mg/kg/day for male rats and to 131 mg/kg/day for female rats.

B. **REVIEWER COMMENTS:** Dietary exposure to IR5878 in rats did not cause any treatment-related effects on mortality, body weights, body weight gains, food consumption, neurological observations and measurements, hematology, clinical chemistry, or gross pathology. The statistically significant increases in the relative kidney weights of males and of absolute kidney weights in females (following recovery) are not biologically significant.

Dorsal hair loss in female rats is considered a treatment-related effect of IR5878 exposure; however, hair loss is a common clinical sign with unclear toxicological significance.

The treatment-related increase in relative liver weights seen in males administered 9000 ppm is considered to be an adaptive response, because liver weights were comparable to controls following a four week recovery period, there were no biologically significant changes in liver enzymes, there were no gross pathological effects in the liver, and there were no histopathological effects in the liver other than slight transient centrilobular hepatocyte hypertrophy.

The LOAEL was not identified in this study (>706 mg/kg/day for males and >773 mg/kg/day for females). The NOAEL is the highest dose tested in this study (9000 ppm), which was equivalent to 706 mg/kg/day for males and 773 mg/kg/day for females.

This study is **Acceptable/Guideline** and **satisfies** the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

C. **STUDY DEFICIENCIES:** Homogeneity of the test compound was confirmed at nominal concentrations of 50 and 12500 ppm in an earlier study (Report No. AGR 127/003821). While samples of the actual prepared diet used in MRID 46260103 were not tested for homogeneity, this does not invalidate the study results.

DATA EVALUATION RECORD

IR5878 (ORTHOSULFAMURON)

Study Type: §83-2b; Carcinogenicity Study in Mice

Work Assignment No. 3-1-82 C (MRID 46578912)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date**_____
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:**_____
Registration Action Branch 1, Health Effects Division (7509C) **Date**_____
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity study in mice [feeding]; OPPTS 870.4200b [§83-2b]; OECD 451.

PC CODE: 108209 **DP BARCODE:** D319264
TXR#: 0053612

TEST MATERIAL (PURITY): IR5878 (Orthosulfamuron; 98.0% a.i.)

SYNONYM: 1-[2-(dimethylcarbamoyl) phenylsulfamoyl]-3-(4,6-dimethoxypyrimidin-2-yl)urea

CITATION: Webley, L. (2003) IR5878: Carcinogenicity study by dietary administration to CD-1 mice for 78 weeks. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project ID.: AGR/130, December 22, 2003. MRID 46578912. Unpublished.

SPONSOR: ISAGRO S.p.A., Centro Uffici San Siro, Fabbricat D-ala 3, Via Caldera 21, Milano, Italy

EXECUTIVE SUMMARY - In a carcinogenicity study (MRID 46578912), 50 Crl:CD-1™ (ICR)BR mice/sex/dose were exposed to IR5878 [Orthosulfamuron; 98.0% a.i.; Batch #: FCF/T/172-00 (ex 20525/03/8)] in the diet at nominal concentrations of 0, 100, 500, or 1000 mg/kg/day for up to 78 weeks.

No adverse treatment-related effects were observed on clinical signs, mortality, body weights, body weight gains, food consumption or food conversion efficiency, hematology, or gross pathology.

Absolute (incr. 12%, p =< 0.05) and relative to body (incr. 14%, p =< 0.05) liver weights were increased in the 1000 mg/kg/day males after 78 weeks of treatment when compared with controls. An increased (p =< 0.001) incidence of slight to moderate centrilobular hepatocyte hypertrophy was observed at >= 500 mg/kg/day (48-68%) compared to slight hypertrophy in controls (8%). Also, an increased (p =< 0.05) incidence of slight to marked liver vacuolation was observed at 1000 mg/kg/day (50%), and slight to moderate liver vacuolation at 500 mg/kg/day (38%), both compared to slight to moderate in controls (18%). Similar changes were

not observed in females. It was stated that the observed increased incidence of centrilobular hepatocyte vacuolation, suggesting an effect on fat metabolism, in 500 and 1000 mg/kg/day compound-treated males was also observed in the corresponding rat carcinogenicity study MRID 46578913 (Huntingdon Life Sciences Report Number AGR 131/033063) with this test compound.

At 500 mg/kg/day, increased incidence of centrilobular hepatocyte vacuolation was mostly slight with moderate vacuolation observed in 10% of the animals. However, centrilobular hepatocyte vacuolation in the 1000 mg/kg/day males is more clearly defined with an increase in incidence as well as severity. This effect coupled with more pronounced increases in liver weights and centrilobular hypertrophy in the 1000 mg/kg/day males provide a weight of evidence sufficient to determine the LOAEL at 1000 mg/kg/day.

The LOAEL is 1000 mg/kg/day, based on increases in absolute and relative to body liver weights, centrilobular hepatocyte hypertrophy, and centrilobular hepatocyte vacuolation in males. The NOAEL is 500 mg/kg/day.

At the doses tested, there was not a treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on increased absolute and relative to body liver weights, and increased incidence of centrilobular hepatocyte hypertrophy and centrilobular hepatocyte vacuolation.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

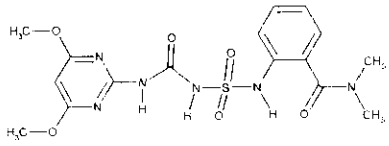
COMPLIANCE - Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR5878

Description: White powder
Lot/Batch #: FCF/T/172-00 (ex 20525/03/8)
Purity (w/w): 98.0% a.i.
Stability of compound: Stable in the diet for a maximum of 15 days at room temperature
CAS #: 213464-77-8
Structure:



2. Vehicle - Diet

3. Test animals

Species:	Mouse
Strain:	Crl:CD-1™ (ICR)BR
Age and group mean weights at Week 0:	Approximately 41 to 45 days; 29.2 - 40.8 g males; 21.4 -32.1 g females
Source:	Charles River (UK) Ltd. (Margate, Kent, England)
Housing:	Two/sex/cage, in polypropylene cages with stainless steel mesh lids
Diet:	Rat and Mouse No. 1 Maintenance Diet in powdered form (Special Diet Services Ltd., Witham, Essex, England), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	19-23°C
Humidity:	40-70%
Air changes:	At least 15/hour
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	15 days

B. STUDY DESIGN

1. In life dates - Start: 07/05/01 End: 01/10/03

2. Animal assignment/dose levels - The animals were randomly assigned to the test groups shown in Table 1.

Table 1. Study design. [a]

Nominal Dose (mg/kg/day)	Achieved Dose (mg/kg/day; M/F)	Terminal Sacrifice (78 Weeks) (#/sex)
0	0/0	50
100	102.2/104.2	50
500	515.5/514.2	50
1000	1023.7/1044.8	50

a Data were obtained from pages 13 and 51 of the study report.

3. **Dose-selection rationale** - The sponsor stated that the dose-selection rationale was based on a 4-week (Huntingdon Life Sciences Report Number AGR 126/000096) and 13-week (Huntingdon Life Sciences Report Number AGR 129/012173) dietary studies where 997/1332 mg/kg/day and 865/1096 mg/kg/day M/F of test compound, respectively, were administered. Minimal changes in body weight gain, liver weights, and food conversion efficiency were observed in treated males and no reportable effects were noted in females. No further information was provided. Based on the results of these two studies, the limit dose (1000 mg/kg/day) was considered appropriate as the high-dose for this study.

4. **Diet preparation and analysis** - Dietary formulations were prepared weekly by mixing the appropriate amount of test material with a small amount of diet to form a premix. The premix was further diluted with diet to achieve the desired concentration. Dietary formulations were stored at ambient temperature until presented to the animals. It was stated that homogeneity and stability of the test substance in the diet were verified prior to the study in diets ranging from 50 to 12,500 mg/kg/day (Huntingdon Life Sciences Report Numbers AGR 127/003821; not provided). In a carcinogenicity study in rats (MRID 46578913), reviewed concurrently, homogeneity and stability in diets ranging from 5 to 30,000 mg/kg/day was confirmed for up to 15 days at ambient temperature. Concentration analyses were performed on samples of each dose level at Weeks 1, 13, 26, 39, 52, 65, and 77 of the study.

Results:

Homogeneity (% CV): 0.85-3.22%

Stability (% initial): 96-101%

Concentration Analysis (% of nominal): 95.7 - 105.4%

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

5. Statistics - Data were subjected to the statistical procedures listed below.

Parameter	Statistical procedure
Continuous data	Bartlett's test for homogeneity of variance; then treated groups were compared with controls (incorporating adjustments for multiple comparisons) using tests dependent on the outcome of Bartlett's test.
Categorical data, including pathological findings	Fisher's Exact test
Body weight gains and organ weights	Bartlett's test, then Behrens-Fisher for pairwise comparisons if significant with Bartlett's. If not significant with Bartlett's, then Dunnett's test was used.
Clinical pathology	1) If 75% of the data across all groups were the same value, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for trend in proportions and pairwise Fisher's Exact test. 2) If Bartlett's test for homogeneity of variance was not significant at 1%, then parametric analysis was applied. If the F1 test for dose-response monotonicity was not significant at 1%, then Williams' test for trend was applied. If the F1 test was significant, then Dunnett's test was applied instead. 3) If Bartlett's test was significant at 1%, then logarithmic and square-root transformations were applied. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for dose-response monotonicity was not significant at 1%, Shirley's test for trend was applied. If the H1 test was significant, then Steel's test was performed instead.

Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

C. METHODS

1. Observations

1a. Cageside observations - Animals were observed twice daily for signs of toxicity and mortality.

1b. Clinical examinations - Detailed clinical examinations were performed weekly on all animals. These examinations included palpation with attention to any superficial swellings.

2. Body weight - All animals were weighed at the start of the study, weekly for 15 weeks, at Week 18, and generally every 4 weeks thereafter. Body weight gains were reported as a group mean value (Weeks 0-78) at the end of the study.

3. Food consumption, food conversion efficiency, and compound intake - Mean food consumption (g/animal/week) was determined weekly for 15 weeks, at Week 18, and generally every 4 weeks thereafter.

Group mean food conversion efficiency (%) values were calculated for weeks 0-14. Compound intake (mg/kg bw/day) was calculated from the food consumption, nominal dose, and body weight gain data.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were not performed and are not required by the Guidelines (OPPTS 870.4200b/OECD 451).

5. Hematology and clinical chemistry - Hematology parameters were evaluated in all surviving animals at Weeks 52 and 77. Smears from the control and 1000 mg/kg/day animals were examined for differential determinations. Blood was collected from the tail veins of non-fasted animals. The following CHECKED (X) parameters were examined.

a. Hematology

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

* Minimum required for carcinogenicity studies (Cont. and HDT unless effects are observed) based on Guideline 870.4200 & OECD 451.

b. Clinical chemistry - Clinical chemistry was not performed and is not required by the Guidelines (OPPTS 870.4200b/OECD 451).

6. Urinalysis - Urinalysis was not performed and is not required by the Guidelines (OPPTS 870.4200b/OECD 451).

7. Sacrifice and pathology - At the end of the treatment period, all surviving animals were killed by carbon dioxide asphyxiation. All animals that died or were sacrificed *in extremis* and those sacrificed on schedule were subjected to a detailed gross pathological examination, and the following CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*–
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow (sternum)*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*+
X	Cecum*	XX	Kidneys*+	X	Lacrimal/Harderian gland
X	Colon*	X	Urinary bladder*	X	Parathyroids*
X	Rectum*	XX	Testes*+	X	Thyroids*
XX	Liver*+	XX	Epididymides*+		
X	Gall bladder* (not rat)	X	Prostate*		OTHER
	Bile duct* (rat)	X	Seminal vesicle*	X	Bone (sternum and femur)
X	Pancreas*	XX	Ovaries*+	X	Skeletal muscle (thighs)
	RESPIRATORY	XX	Uterus (with cervix)*+	X	Skin*
X	Trachea*	X	Mammary gland*	X	All gross lesions and masses*
X	Lung*+–	X	Vagina	X	Joint (femur)
X	Nose*			X	Head
X	Pharynx*				
X	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200
– Organ weight required in carcinogenicity studies
+ Organ weight required if inhalation route

All the collected tissues were routinely processed and examined histologically for control and 1000 mg/kg/day animals and for all animals discovered dead or sacrificed *in extremis*. Additionally, the liver, lungs, and kidneys were examined for all animals killed at scheduled termination. All abnormal tissues were also examined.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity - No treatment-related clinical signs of toxicity were observed.

2. Mortality - No treatment-related effect was observed on mortality. Survival in all groups was 70-88% at Week 65, and 58-76% at Week 78, exceeding guideline requirements of 50% at Week 65 and 25% at Week 78.

B. BODY WEIGHT: No treatment-related effects were observed on body weights or body weight gains (Table 2). Differences in the treated groups relative to controls were sporadic and unrelated to dose.

Table 2. Selected mean (±SD) body weights and overall body weight gains (g) in mice treated with IR5878 in the diet for up to 78 weeks[a]

Weeks on Study	Dose (mg/kg/day)			
	0	100	500	1000
Males				
1	36.4±2.57	37.0±2.37	37.5±2.74	36.3±2.08
26	53.4±7.21	53.3±6.41	54.3±6.80	51.7±6.88
54	56.7±8.60	58.4±8.13	57.3±8.23	55.4±7.63
78	56.4±9.74	57.1±8.86	56.4±7.88	54.9±7.94
BWG: 0-78	22.3±8.94	22.2±8.27	21.5±7.04	20.7±7.39
Females				
1	26.8±1.98	26.0±1.97	26.6±2.17	26.7±1.84
26	37.4±6.48	36.5±5.66	38.4±6.03	37.2±5.99
54	43.6±8.14	41.3±7.11	43.3±8.73	42.0±8.91
78	44.8±8.38	44.9±7.25	45.2±7.68	44.4±9.38
BWG: 0-78	19.5±7.71	19.9±6.83	19.3±7.61	18.9±8.69

a Data were obtained from Table 3, pages 41-44 of the study report.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - No treatment-related effects were observed on food consumption or food conversion efficiency.

2. Compound consumption - The mean achieved dosages based on actual dose levels and mean food consumption are shown in Table 1.

D. BLOOD ANALYSES: No treatment-related effects were observed on hematology. At Week 52, a decrease (↓12%; $p \leq 0.05$) in percent neutrophils was observed in the 1000 mg/kg/day females when compared with controls. No other effects were observed during Week 52 in the remaining treatment groups. During Week 77, an increase in percent eosinophils (↑59%; $p \leq 0.05$) and a decrease in percent monocytes (↓26%; $p \leq 0.05$) were observed in the1000 mg/kg/day males when compared with controls. No other effects were observed at Week 77 in the remaining treatment groups. These minor changes were not considered to be treatment-related.

E. SACRIFICE AND PATHOLOGY

1. Organ weights - Selected organ weight data are shown in Table 3. Absolute (↑12%, $p \leq 0.05$) and relative to body (↑14%, $p \leq 0.05$) liver weights were increased in the 1000 mg/kg/day males after 78 weeks of treatment when compared with controls. Decreased (↓8.0%, $p \leq 0.05$) absolute kidney weights were observed in the 1000 mg/kg/day females. Also, increases in absolute and

relative to body uterus/cervix weights (↑60 and ↑76%, $p \leq 0.05$) were observed in the 1000 mg/kg/day females. However, the effects noted in kidney weights and uterus/cervix weights in the 1000 mg/kg/day females had no treatment-related histopathological correlate. No other dose-related changes in organ weights were observed.

Table 3. Selected mean absolute and relative (to body) organ weights (±SD) in mice treated with IR5878 for up to 78 weeks[a]

Organ	Dose (mg/kg/day)			
	0	100	500	1000
Males				
Terminal body weight (g)	56.1±9.3	57.1±8.6	56.4±8.2	54.9±7.5
Liver - absolute (g)	2.763±0.502	2.908±0.605	3.001±0.573	3.102±0.647 (↑12%)*
relative (to body) (%)	4.9466±0.6335	5.1419±1.0334	5.3437±0.8544	5.6561±0.8873 (↑14%)**
Females				
Terminal body weight (g)	44.1±8.1	44.3±7.6	44.6±8.5	44.4±9.2
Kidneys - absolute (g)	0.522±0.097	0.498±0.060	0.505±0.078	0.480±0.069 (↓8.0%)*
relative (to body) (%)	1.2128±0.2901	1.1515±0.2068	1.1646±0.2422	1.1047±0.1687
Uterus/Cervix - absolute (g)	0.498±0.261	0.751±1.121	0.749±0.726	0.797±0.791 (↑60%)*
relative (to body) (%)	1.1575±0.6621	1.7284±2.3168	1.8008±1.8408	2.0375±2.2808 (↑76%)*

a Data were obtained from Tables 8A and B on pages 54-59 of study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls, $p \leq 0.05$
** Significantly different from controls, $p \leq 0.01$

2. **Gross pathology** - No treatment-related macroscopic changes were observed.

3. **Microscopic pathology**

a. Non-neoplastic - The following lesions were observed in the centrilobular hepatocytes of the males (Table 4): increased ($p \leq 0.001$) incidence of slight to moderate hypertrophy at ≥ 500 mg/kg/day (48-68%) compared to slight in controls (8%); and increased ($p \leq 0.05$) incidence of slight to marked vacuolation at 1000 mg/kg/day (50%), and slight to moderate vacuolation at 500 mg/kg/day (38%), both compared to slight to moderate in controls (18%). Similar changes were not observed in females. Increased ($p \leq 0.01$) splenic white pulp cellularity was noted in the 1000 mg/kg/day females (28%) compared to controls (3%); however, this effect was no longer significant when animals that died prior to scheduled termination were included. No other treatment-related adverse effects were observed during non-neoplastic microscopic pathology.

Table 4. Percent incidence and severity of selected non-neoplastic microscopic lesions in male mice treated with IR5878 for up to 78 weeks [a]

Dose (mg/kg/day)		0	100	500	1000
Centrilobular hepatocyte hypertrophy -	Total	8%	20%	48% ***	68% ***
	Slight	8%	18%	40%	54%
	Moderate	0	2%	8%	14%
Centrilobular hepatocyte vacuolation -	Total	18%	24%	38% *	50% **
	Slight	12%	18%	28%	24%
	Moderate	6%	6%	10%	24%
	Marked	0	0	0	2%

a Data were obtained from Text table 3 on page 28 and Table 10 F on page 147 of the study report. Percent difference from controls was calculated by the reviewers, n=50.
* Significantly different from controls, $p \leq 0.05$.
** Significantly different from controls, $p \leq 0.01$.
*** Significantly different from controls, $p \leq 0.001$.

b. Neoplastic - Neoplasia data from Tables 10 A, B, and C on pages 105-114, Table 10 H on page 162, and pages 175-186 of study report are included as an appendix. No indication of carcinogenic potential was observed.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The LOAEL was 500 mg/kg/day based on increased incidence of centrilobular hepatocyte vacuolation and the consequential effect on lipid metabolism and/or transport in males.

B. REVIEWER COMMENTS: No adverse treatment-related effects were observed on clinical signs, mortality, body weights, body weight gains, food consumption or food conversion efficiency, hematology, or gross pathology.

Absolute (↑12%, $p \leq 0.05$) and relative to body (↑14%, $p \leq 0.05$) liver weights were increased in the 1000 mg/kg/day males after 78 weeks of treatment when compared with controls. An increased ($p \leq 0.001$) incidence of slight to moderate centrilobular hepatocyte hypertrophy was observed at ≥500 mg/kg/day (48-68%) compared to slight hypertrophy in controls (8%). Also, an increased ($p \leq 0.05$) incidence of slight to marked liver vacuolation was observed at 1000 mg/kg/day (50%), and slight to moderate liver vacuolation at 500 mg/kg/day (38%), both compared to slight to moderate in controls (18%). Similar changes were not observed in females. The reviewers noted that the observed increased incidence of centrilobular hepatocyte vacuolation suggested an effect on fat metabolism in 500 and 1000 mg/kg/day males and that this effect was also observed in the corresponding rat carcinogenicity study MRID 46578913 (Huntingdon Life Sciences Report Number AGR 131/033063) with this test compound.

No indication of carcinogenic potential was observed.

The reviewers disagree with the Sponsor's LOAEL at 500 mg/kg/day. Increased incidence of centrilobular hepatocyte vacuolation was mostly slight with moderate vacuolation observed in 10% of the animals. However, centrilobular hepatocyte vacuolation in the 1000 mg/kg/day males is more clearly defined with an increase in incidence as well as severity. This effect coupled with more pronounced increases in liver weights and centrilobular hypertrophy in the 1000 mg/kg/day males provide a weight of evidence sufficient to determine the LOAEL at 1000 mg/kg/day.

The LOAEL is 1000 mg/kg/day, based on increases in absolute and relative to body liver weights, centrilobular hepatocyte hypertrophy, and centrilobular hepatocyte vacuolation in males. The NOAEL is 500 mg/kg/day.

At the doses tested, there was not a treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on increased absolute and relative to body liver weights, and increased incidence of centrilobular hepatocyte hypertrophy and centrilobular hepatocyte vacuolation.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b: OECD 451) for a carcinogenicity study in mice.

C. **STUDY DEFICIENCIES:** The following minor deficiency was observed but does not change the conclusions of this review.

- There were no historical control data provided for this study.

EPA Primary Reviewer: Kelly Schumacher, M.S. **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date**_____
EPA Secondary Reviewer: Alan Levy, Ph.D. **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date**_____
Template version 11/01

TXR#: 0052629

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity Feeding Study - Mice; OPPTS 870.3100 (§82-1a); OECD 408.

PC CODE: 108209 **DP BARCODE:** D306738
SUBMISSION NO.: S755326

TEST MATERIAL (PURITY): IR5878 (Orthosulfamuron, 98.0%)

SYNONYMS: 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)-phenylsulfamoyl] urea; benzamide, 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N-N-dimethyl-

CITATION: Webley, L. (2001). IR5878: Preliminary toxicity study by dietary administration to CD-1 mice for 13 weeks. Huntingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, England PE28 4HS. Study No. AGR 129/012173, August 16, 2001. MRID 46260102. Unpublished.

SPONSOR ISAGRO SpA, Centro Uffici San Siro - Fabbricato D, ala 3, Via Caldera, 21, 20153 Milano - Italy.

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity study (MRID 46260102), IR5878 (98.0% a.i., Batch No. FCF/T/172-00 (ex 20525/03/8) was administered to ten CD-1 mice/sex/dose in the diet at dose levels of 0, 250, 1250, and 6000 ppm (equivalent to 0, 36, 187, and 865 mg/kg bw/day, respectively, for males and to 0, 47, 228, and 1096 mg/kg bw/day, respectively, for females).

There were no compound-related effects on clinical signs, mortality, body weights, clinical chemistry, gross pathology, or histopathology. Compared to controls, slight decreases in food consumption, MCH, and MCV in high-dose females and slight decreases in the lymphocyte count of high-dose males were noted, but these changes were not biologically significant.

The LOAEL was not identified in this study (>865 mg/kg/day for male mice and >1096 mg/kg/day for females). The NOAEL is the highest dose tested in this study (6000 ppm), which was equivalent to 865 mg/kg/day for male mice and 1096 mg/kg/day for females.

Dosing in this study is considered adequate because the highest dose tested is sufficiently close to the limit dose of 1000 mg/kg/day.

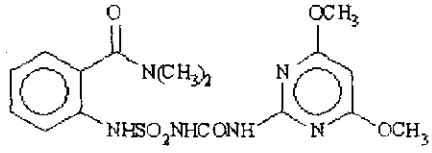
This study is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** IR5878

Description:	White crystalline powder
Lot/Batch #:	Batch No. FCF/T/172-00 (ex 20525/03/8)
Purity:	98.0% a.i.
Compound Stability:	Stable in rodent diet for 15 days at freezing temperature and for 8 days at room temperature
CAS No. of TGAI:	213464-77-8
Structure:	

2. **Vehicle and/or positive control:** The test material was incorporated into the diet. No positive control was used in this study.

3. **Test animals:**

Species:	Mouse	
Strain:	CD-1	
Age/weight at study initiation:	Approximately 40-44 days of age / males: 31-32 g; females: 25-26 g	
Source:	Charles River (UK) Limited, Margate, Kent, England	
Housing:	Housed two of the same sex per cage in polycarbonate cages with stainless steel mesh lids	
Diet:	Rat and Mouse No. 1 Maintenance Diet (Special Diets Services Ltd., Witham, Essex, England), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	40-70%
	Air changes:	≥15 times/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	14 days	

B. STUDY DESIGN:

1. **In life dates:** Start: November 1, 2000; End: January 31-February 1, 2001

2. **Animal assignment:** Animals were randomly assigned to the test groups noted in Table 1. Two males, whose body weights were at the extreme ends of the weight range of the other mice, were replaced by two spare males from the same batch of animals.

TABLE 1: Study design				
Test group	Conc. in diet (ppm)	Dose to animal (mg/kg/day)[a]	# Male	# Female
Control	0	0	10	10
Low	250	♂ (36) ♀ (47)	10	10
Mid	1250	♂ (187) ♀ (228)	10	10
High	6000	♂ (865) ♀ (1096)	10	10

[a]Data from page 39 of MRID 46260102.

3. **Dose selection rationale:** The dose levels were selected based on the results from a four-week oral toxicity study in mice (Report No. AGR126/000096) where animals were administered 0, 50, 250, 1250, or 6000 ppm in the diet. Decreased body weight gains and increased liver weights were observed in males at 6000 ppm, but females were unaffected by treatment at all dose levels tested.

4. **Diet preparation and analysis:** Premix was prepared weekly by mixing appropriate amounts of test substance with Rat and Mouse No. 1 Maintenance Diet. Test diets were then prepared weekly by serially diluting the concentrated premix. Homogeneity and stability of IR5878 in the feed, at concentrations of 50 and 12500 ppm, were tested in an earlier study (Report No. AGR 127/003821); however, the results of this study were not provided in MRID 46260102. During the 90-day study, samples of treated food from each test group were analyzed on weeks 1, 6, and 12 for active ingredient concentration using a solvent extraction method, followed by reverse phase HPLC analysis with external calibration.

Results:

Homogeneity analysis: Homogeneity was confirmed at nominal concentrations of 50 and 12500 ppm in an earlier study (Report No. AGR 127/003821); however, the results of this study were not reported in MRID 46260102.

Stability analysis: Stability of IR5878 in the diet was confirmed for 15 days with freezer storage and for 8 days with storage at room temperature; however, results of the stability analysis were not reported in MRID 46260102.

Concentration analysis: Dietary concentrations were 95-99%, 94-97%, and 93-100% of target concentrations for the low-, mid-, and high-dose groups, respectively.

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

5. **Statistics:** Body weights and organ weights were tested for homogeneity of variance using Bartlett's test. If significant, a Behrens-Fisher test was used for pairwise comparisons; if not significant, Dunnett's test was used. Gross pathological and histopathological findings were analyzed using Fisher's exact test to compare each treated group with controls.

   For clinical pathology data, if 75% of the findings (across all treatment groups) were the same number, then a frequency analysis was conducted. Treatment groups were compared using a Mantel test and pairwise Fisher's Exact tests.

   If Bartlett's test was not significant at the 1% level, parametric analysis was conducted. Then, if the F1 test was not significant at the 1% level, Williams' test was applied, but if it was significant at that level, Dunnett's test was used.

   If Bartlett's test was significant at the 1% level, the data were subjected to transformation to obtain equality of variance and then tested again using Bartlett's test. If still significant, non-parametric analysis was applied. Then, if the H1 test was not significant at the 1% level, Shirley's test was applied, but if it was significant at that level, Steel's test was used.

C. METHODS:

1. **Observations:**

   1a. **Cageside observations:** Animals were inspected twice a day for signs of toxicity and mortality.

   1b. **Clinical examinations:** Clinical observations were conducted weekly.

   1c. **Neurological evaluations:** Neurological evaluations were not conducted.

2. **Body weight:** Animals were weighed prior to initiation of the study, on the first day of dosing, weekly during the dosing period, and before necropsy.

3. **Food consumption and compound intake:** Food consumption for each cage was determined, and mean weekly diet consumption was calculated as g food/animal/week. Food efficiency was calculated on a weekly basis as 100 x (g body weight gain per week/g food consumption per week). Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. **Ophthalmoscopic examination:** No ophthalmoscopic examinations were conducted; however, the eyes were observed during clinical, macropathological, and histopathological examinations.

5. **Hematology and clinical chemistry:** Blood was collected from the retro-orbital sinus of all animals during week 13 for hematology and of all surviving animals at study termination for

clinical chemistry. During sampling, animals were held under isoflurane anesthesia. The CHECKED (X) parameters were examined.

a. Hematology:

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*		
-	(Thromboplastin time)		
-	(Clotting time)		
-	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
- Not examined

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	-	Creatinine*
-	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	-	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
-	Cholinesterase (ChE)	-	Triglycerides
-	Creatine phosphokinase	-	Serum protein electrophoresis
-	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
-	Sorbitol dehydrogenase*		
-	Gamma glutamyl transferase (GGT)*		
-	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
- Not examined

6. **Urinalysis:** Urinalysis was not conducted in this study.

7. **Sacrifice and pathology:** All animals were sacrificed on schedule and were subjected to gross pathological examination; the CHECKED (X) tissues were collected for histological examination. The adrenals, cecum, colon, duodenum, epididymides, esophagus, head, heart, ileum, jejunum, liver, lungs with mainstem bronchi, mandibular and mesenteric lymph nodes, ovaries, prostate, rectum, sciatic nerve, stomach, testes, thymus, thyroid with parathyroids, trachea, urinary bladder, and the uterus with cervix were collected and preserved, but these organs were not examined histologically. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
-	Tongue	-	Aorta*	XX	Brain*+
-	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	-	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	-	*Pituitary**
X	Duodenum*	XX	Spleen*+	-	Eyes (optic nerve)*
X	*Jejunum**	XX	Thymus*+		**GLANDULAR**
X	Ileum*			X	Adrenal gland*+
X	Cecum*		**UROGENITAL**	-	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*	XX	Testes*+		***OTHER***
-	Gall bladder (not rat)*	XX	Epididymides*+	-	Bone (sternum and/or femur)
-	Bile duct (rat)	X	*Prostate**	-	Skeletal muscle
-	Pancreas*	-	Seminal vesicles*	-	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Head (including nasal cavity, paranasal sinuses, nasopharynx)
XX	Lung*	-	Mammary gland*		
X	Nose*	-	Coagulating gland		
X	*Pharynx**	XX	Cervix		
-	Larynx*	-	Vagina		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.
- Not weighed or collected for examination

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs were observed.

2. **Mortality:** One control male and one mid-dose female were sacrificed during week 13 for humane reasons (both suffered eye damage during blood sampling). Otherwise, no unscheduled mortalities occurred during the study.

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weights and total body weight gains are given in Table 2. There were no treatment-related effects on body weights or body weight gains in this study.

TABLE 2. Mean body weights and body weight gains during 90 days of treatment[a]					
Dose (ppm)	Body weights (g ± SD)				Total Weight Gain (g)
	Week -1	Week 1	Week 7	Week 13	
Male					
0	31.0 ± 2.8	34.2 ± 2.5	41.5 ± 4.1	43.6 ± 5.4	12.6
250	30.5 ± 1.9	34.3 ± 1.9	41.0 ± 3.4	43.9 ± 2.9	13.4
1250	30.8 ± 3.0	34.3 ± 3.1	42.3 ± 4.0	44.1 ± 6.9	13.3
6000	31.9 ± 1.7	34.9 ± 1.8	41.7 ± 2.8	42.8 ± 3.6	10.9
Female					
0	25.1 ± 1.7	26.7 ± 1.7	29.4 ± 2.3	30.0 ± 2.5	4.9
250	25.0 ± 0.9	26.6 ± 1.6	30.7 ± 2.1	30.5 ± 2.6	5.5
1250	26.4 ± 2.4	27.5 ± 2.2	30.8 ± 3.3	31.8 ± 4.0	5.4
6000	25.2 ± 1.3	26.7 ± 1.4	30.3 ± 2.2	30.1 ± 2.7	4.9

[a] Data obtained from pages 32-33 in the study report.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Food consumption was comparable between groups for males. In treated females, food consumption was slightly lower than in control females (Table 3).

TABLE 3. Mean food consumption of female mice at selected intervals during 90 days of treatment[a]					
Dose (ppm)	Food consumption (g/week ± SD)				
	Week 1	Week 7	Week 10	Week 13	Weeks 1-13[b]
0	38 ± 5	41 ± 6	45 ± 4	45 ± 3	550 (100)
250	33 ± 3	39 ± 4	40 ± 7	38 ± 5	503 (91)
1250	32 ± 3	39 ± 4	39 ± 4	35 ± 4	499 (91)
6000	34 ± 2	38 ± 6	36 ± 4	37 ± 5	484 (88)

[a] Data obtained from pages 36-37 in the study report.
[b] Results in parentheses are percentages of controls.

2. **Compound consumption:** Time-weighted average compound intakes are shown in Table 1.

3. **Food efficiency:** Compared to controls, food efficiency was marginally decreased in high-dose males.

D. OPHTHALMOSCOPIC EXAMINATION: Ophthalmoscopic examinations were not conducted in this study.

E. BLOOD ANALYSES:

1. **Hematology:** Selected hematology parameters are presented in Table 4. In males, a marginal decrease in lymphocytes was seen in the high-dose group compared to controls; however, this effect was not seen in treated females or in the low- or mid-dose male groups. In females, the mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) values were slightly decreased in all treated groups compared to controls; no change in MCH or MCV was found in males.

TABLE 4. Selected hematology findings in mice following 90 days of treatment with IR5878[a]

	Male			Female		
Dose (ppm)	Lymphocyte count (10^9/L ± SD)	MCH (pg ± SD)	MCV (fL ± SD)	Lymphocyte count (10^9/L ± SD)	MCH (pg ± SD)	MCV (fL ± SD)
0	7.5 ± 1.8	16.4 ± 0.7	51.3 ± 1.5	3.1 ± 1.6	17.0 ± 0.5	54.6 ± 1.8
250	6.1 ± 1.7	15.6 ± 0.5	49.4 ± 1.5	2.5 ± 1.1	16.3 ± 0.4 **	52.2 ± 1.3 **
1250	5.8 ± 2.3	15.9 ± 0.5	50.1 ± 2.3	3.0 ± 1.0	16.4 ± 0.7 **	51.8 ± 2.2 **
6000	4.7 ± 2.7 **	15.9 ± 0.8	50.9 ± 2.0	3.5 ± 1.2	16.4 ± 0.4 **	52.2 ± 1.5 **

[a] Data obtained from pages 40-42 in the study report.
** $p < 0.01$

2. **Clinical chemistry:** There were no treatment-related effects on clinical chemistry in this study.

F. URINALYSIS: Urinalysis was not conducted in this study.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Absolute and relative liver weights were marginally increased in high-dose males compared to controls, but only the change in liver weights relative to body weights was statistically significant. Female liver weights were unaffected by treatment. Absolute heart weights were slightly decreased, compared to controls, in high-dose females only. Statistically significant decreases in mid-dose male thymus weights and low-dose female uterus/cervix weights were not attributed to treatment since similar increases were not seen at higher dose levels.

TABLE 5. Absolute and relative liver weights (mean ± SD) in mice following 90 days of treatment with IR5878[a]						
Dose (ppm)	Male			Female		
	Body weight (g)	Liver (g)	Liver/body (g liver/100 g)	Body weight (g)	Liver (g)	Liver/body (g liver/ 100 g)
0	42.2 ± 3.8	1.9 ± 0.4	4.5 ± 0.6	29.6 ± 2.4	1.5 ± 0.3	5.2 ± 0.6
250	43.0 ± 3.1	1.9 ± 0.3	4.5 ± 0.5	29.9 ± 2.4	1.5 ± 0.2	4.9 ± 0.4
1250	43.4 ± 6.4	2.1 ± 0.3	4.8 ± 0.5	30.8 ± 4.2	1.5 ± 0.2	5.0 ± 0.5
6000	42.2 ± 2.9	2.2 ± 0.3	5.1 ± 0.5* (114)[b]	29.4 ± 2.7	1.6 ± 0.2	5.4 ± 0.6

[a] Data obtained from pages 48-53 in the study report.
[b] Results in parentheses are percent of control calculated by the reviewer.
* Significantly different from controls, $p \leq 0.05$

2. **Gross pathology:** There were no treatment-related changes observed. Although several animals (one low-dose, two mid-dose, and two high-dose males, plus one control and three low-dose females) had abnormal findings in the lymph nodes, these effects appeared sporadic and not related to dose.

3. **Microscopic pathology:** There were no treatment-related changes observed. Of the animals with abnormal gross pathological findings in the lymph nodes, three (one mid-dose male, one high-dose male, and one low-dose female) had reactive histiocytosis; however, these effects were sporadic and not considered to have been related to dosing.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study authors concluded that, in male CD-1 mice, dietary exposure to IR5878 near the limit dose caused slight changes in body weight gains, food conversion efficiency, and liver weights. The increase in male liver weights was considered minimal, adaptive, and not toxicologically significant. In females, the limit dose of IR5878 was associated with lower food consumption, but the investigators did not consider this effect toxicologically significant since no change in body weights or body weight gains was seen. Therefore, the study authors determined that the NOAEL was 6000 ppm, which is equivalent to 865 mg/kg/day for male mice and to 1096 mg/kg/day for female mice.

B. **REVIEWER COMMENTS:** The reviewer concurs with the study authors' conclusion that dietary exposure to IR5878 for 90 days caused minimal, non-toxicologically significant changes in male and female CD-1 mice. These changes include slightly decreased food consumption by high-dose females, with no changes in body weights or body weight gains compared to controls. Also, compared to controls, mean MCH and MCV values were marginally decreased in all treated female groups, and mean lymphocyte counts were slightly decreased in high-dose males; however, these decreases were not biologically significant. Absolute and relative male liver weights were slightly increased compared to controls, but

only the change in relative liver weights of the high-dose animals reached statistical significance. Furthermore, no treatment-related changes were seen in clinical chemistry, gross pathology, or histopathology to suggest liver or other toxicity.

The LOAEL was not identified in this study (>865 mg/kg/day for males and >1096 mg/kg/day for females). The NOAEL is the highest dose tested in this study (6000 ppm), which was equivalent to 865 mg/kg/day for males and 1096 mg/kg/day for females. Dosing in this study is considered adequate because the highest dose tested is sufficiently close to the limit dose of 1000 mg/kg/day.

This study is **Acceptable/Guideline** and **satisfies** the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse.

C. **STUDY DEFICIENCIES:** Examinations of the following parameters/organs, which are recommended by the guidelines for a 90-day oral toxicity study in rodents, were not performed: blood clotting potential, creatinine, urinalysis, and gall bladder. Additionally, adrenal gland weights, which are required by the guidelines, were not reported. Although not all of the guideline recommendations and requirements were addressed in this study, this does not invalidate the study results.

DATA EVALUATION RECORD

IR5878 (ORTHOSULFAMURON)

Study Type: §85-1; Metabolism Study in Rats

Work Assignment No. 3-1-82 A (MRIDs 46578905 through 46578910)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
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Primary Reviewer:
Ronnie J. Bever Jr, Ph.D. Signature: _____
Date: _____

Secondary Reviewer:
Michael E. Viana, Ph.D., D.A.B.T. Signature: _____
Date: _____

Program Manager:
Mary L. Menetrez, Ph.D. Signature: _____
Date: _____

Quality Assurance:
Steven Brecher, Ph.D. Signature: _____
Date: _____

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey **Signature:** _____
Reregistration Branch 2, Health Effects Division (7509C) **Date**_____
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:**_____
Registration Action Branch 1, Health Effects Division (7509C) **Date**_____
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 (§85-1); OECD 417.

PC CODE: 108209 **DP BARCODE:** D319264
TXR#: 0053612

TEST MATERIAL (RADIOCHEMICAL PURITY): IR5878 (Orthosulfamuron; >97%)

SYNONYM: 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]amino]sulfonyl]amino]-N,N-dimethylbenzamide

CITATION: Triolo, A. (2000) ^{14}C-IR-5878: preliminary blood pharmacokinetics, excretion and tissue distribution study in the rat after single oral administration. LCG-RBM, Istituto di Ricerche Biomediche, Colleretto Giacosa, Italy. Laboratory No.: R06100, September 4, 2000. MRID 46578905. Unpublished.

Castoldi, F.C. and G. Pizzingrilli (2000) Profiling of radiolabelled metabolites of [^{14}C-U-phenyl] IR5878 in urine and faeces of rats after single oral administration. Isagro Ricerca Srl, Biochemistry & Toxicology Department, Novara, Italy. Laboratory Study No.: ABT.00.15, October 25, 2000. MRID 46578906. Unpublished.

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EXECUTIVE SUMMARY: In a rat metabolism study (MRIDs 46578905 through 46578910), [^{14}C-U-phenyl] IR5878 (Lot No. 182) or [^{14}C-5-pyrimidinyl] IR5878 (Lot No. 180) in 0.5% aqueous carboxymethycellulose (radiochemical purity >97%) was administered by gavage to Sprague Dawley rats. In the preliminary study, 2 rats/sex received a single 250 mg/kg dose. In the main study, 4 rats/sex/dose received a single dose of 5 or 1000 mg/kg or 14 daily doses at 5 mg/kg (non-radioactive) followed by a single radioactive dose (5 mg/kg) on day 15. Additionally, a biliary excretion study was performed where 4 males and 7 females received a single dose at 5 mg/kg. Pharmacokinetic analyses of the absorption and distribution were performed, including blood kinetics, along with identification of the metabolites in the excreta.

Absorption was rapid in all groups, regardless of sex, dose, or number of doses. T_{max} values were 12 min for the 5 mg/kg repeated dose group, 24 min-1 h for the single 5 mg/kg dose group, and 1-4 h for the single 1000 mg/kg dose group. Following a single 5 mg/kg dose of [^{14}C-U-phenyl] IR5878, 76-82% of the dose was absorbed and found in the urine/cage wash, bile, and carcass, indicating extensive absorption. The half-life (8-48 h) was similar regardless of sex, dose, or number of doses (8.9-13.3 h), with the exception of the females treated with a single 5 mg/kg dose of [^{14}C-5-pyrimidinyl] IR5878 (16.7 h).

Within 12 h of administration of the radiolabeled dose (5 or 1000 mg/kg single dose or multiple 5 mg/kg doses), approximately half of the dose was excreted, and excretion was almost complete at 72 h post-dose.

At 72 h post dose, overall recovery of the radioactive dose from both sexes was 92-100%. The majority of the dose was recovered in the feces (43-73%); 18-46% of the dose was found in the urine; and cage wash accounted for 1-5% dose. Minimal radioactivity was detected in the carcass and GI tract (<0.8% dose). In a preliminary study, <0.02% of the radioactive dose was isolated in the expired air; therefore, this route of excretion was not analyzed. A difference in the excretion profile was generally not noted based on sex, dose, or number of doses.

Regardless of sex, dose, or number of doses, the distribution of radioactivity in tissues was similar. Comparison of the concentrations of radioactivity in tissues on the basis of radiolabel was not possible due to differing sampling times and/or differing T_{max} while a time course of tissue distribution was not performed. Excluding the GI tract, concentrations of radioactivity were highest in the liver, kidney, lung, and whole blood, with the lung having the lowest

concentrations. At 5 mg/kg (single or multiple doses), these tissue concentrations were 1.74-23.83 µg equiv./g vs 2.41-5.10 µg equiv./g in whole blood. At 1000 mg/kg in all treated rats, these tissue concentrations were 161-435 µg equiv./g vs 305-495 µg equiv./g in whole blood. As relatively little radioactivity remained in the carcass at 72 h post-dose (≤0.70% dose), bioaccumulation is not suspected under the test conditions.

HPLC and HPLC-MS analyses were used to identify parent and a total of 10 metabolites in excreta from rats treated with [^{14}C] IR5878. Six to 7 metabolites were identified after treatment with each radiolabeled compound, and 3 of these metabolites were common to treatment with both radiolabeled compounds. Parent and identified metabolites in excreta accounted for 71-86% dose in all animals, and overall recovery was 95-99% dose. Unidentified compounds accounted for 1-16% dose, but no single compound accounted for ≥5% dose.

The parent was found in 5 mg/kg animals (single and multiple doses) at 1-6% dose and in 1000 mg/kg animals at 33-56% dose. The predominant metabolite was 1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea (O-desm IR5878). It was found at 53-64% dose in all animals treated at 5 mg/kg and at 14-20% dose in all animals treated at 1000 mg/kg. This compound was found in similar quantities in the urine and feces. 2-Sulfoamino-N,N-dimethylbenzamide (DBS acid) was a primary metabolite in all animals treated with [^{14}C-U-phenyl] IR5878 and was found at 8-12% dose. This compound was found primarily in the feces. In all animals treated with [^{14}C-5-pyrimidinyl] IR5878, a fraction was isolated (primarily in feces) that contained (4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)urea (Pyr-O-Glucur DOP urea) and 1-(4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea (Pyr-O-Glucur IR5878). This fraction represented 9-18% dose. Additionally, 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(methylcarbamoyl)phenyl-sulfamoyl]urea (N-desm IR5878) was found at 5-8% dose in the urine of animals treated with 1000 mg/kg [^{14}C-5-pyrimidinyl] IR5878. All other identified metabolites each accounted for <5% dose. Identification of IR5878 metabolites indicates that metabolism is mainly occurring through O- and/or N-demethylations. Additionally, hydrolytic cleavage of the sulfamoylurea linkage yields DOP urea, and hydroxylation of the pyrimidinyl ring occurs followed by glucuronic acid or sulfate conjugation.

The metabolic profile of the liver, kidney, and bile was also evaluated. In the liver and kidneys of all animals treated with [^{14}C-U-phenyl] IR5878, the parent and O-desm IR5878 were found in the highest concentrations. In the liver and kidneys of all animals treated with [^{14}C-5-pyrimidinyl] IR5878, O-desm IR5878 and an unidentified fraction were generally found in the highest concentrations, as well as 2-amino-N,N-dimethylbenzamide (DB amine) in the male kidney. In the bile, O-desm IR5878 and an unidentified fraction were found in the highest concentration. Other identified compounds (same as found in the excreta) were not detected in the liver, kidney, or bile or were generally found at relatively low concentrations.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

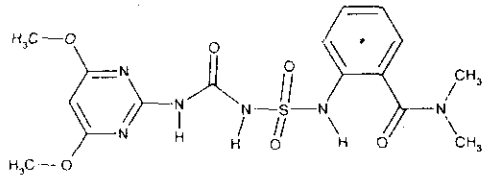
COMPLIANCE: Signed and dated Quality Assurance and Data Confidentiality statements were provided. A signed and dated GLP Compliance statement was included that indicated that the study conforms to the GLP standards of Italy or the United Kingdom but not of the USA. The preliminary blood pharmacokinetics study (MRID 46578905) included a GLP Compliance statement which indicated that the study was not carried out according to GLP guidelines.

I. MATERIALS AND METHODS

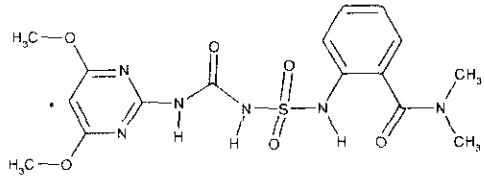
A. MATERIALS:

1. Test compound:

Radiolabeled test material 1: [^{14}C-U-phenyl] IR5878

Radiochemical purity:	>97%
Specific Activity:	5.700 MBq/mg
Lot No.:	182
Structure:	

Radiolabeled test material 2: [^{14}C-5-pyrimidinyl] IR5878

Radiochemical purity:	>97%
Specific Activity:	4.152 MBq/mg
Lot No.:	180
Structure:	

Non-radiolabeled test material: IR5878

Description:	Solid
Batch Nos.:	FCF/T/168-00 (EX20525/03/9); FCT/T/191-01
Purity:	>98%
Contaminants:	Not provided
CAS # of TGAI:	213464-77-8

2. Vehicle and/or positive control: 0.5% aqueous carboxymethylcellulose

3. Test animals (main study):

Species:	Rat
Strain:	Sprague Dawley
Age and weight at dosing:	Approximately 6-11 weeks and 157-268 g
Source:	Charles River (UK) Limited
Housing:	Individually in all-glass metabolism cages (for excreta collection) or in polypropylene and stainless steel cages with wire mesh floors for other studies
Diet:	SDS Rat and Mouse Maintenance Diet No. 1 (Special Diets Services, Stepfield, Witham, Essex, England), *ad libitum*
Water:	Tap water, *ad libitum*

Environmental conditions:	**Temperature:**	18-28°C
	Humidity:	27-75%
	Air changes:	Not reported
	Photoperiod:	12 h light/12 h dark
Acclimation period:	Not reported, but 5 days in preliminary study	

4. Preparation of dosing solutions: The radioactive concentration was determined in an aliquot of [^{14}C] IR5878 in acetonitrile, then diluted with non-radiolabeled compound in a mortar. The acetonitrile was removed under nitrogen and the residue was ground with 0.5% aqueous carboxymethylcellulose. Methanol was used as the solvent in the preliminary study. A magnetic stirrer was used to keep the formulations in suspension.

B. STUDY DESIGN AND METHODS

1. Group arrangements: It was not specified that the animals were randomly assigned to the test groups noted in Table 1. The first experimental start date was May 9, 2000. The last in-life experimental completion date was May 24, 2002.

Table 1. Dose groups for [^{14}C] IR5878 rat metabolism study [a]

Dose Group	Nominal dose (mg/kg)	Actual dose range (mg/kg)	# Animals per dose group per radiolabel [b]	Comments
Preliminary Study Single mid dose	250	Not reported	2/sex	Excretion Kinetics and Tissue Distribution: Cage wash, urine, and feces were collected for up to 168 h post-dose. Expired air was collected for up to 24 h post-dose. Blood, plasma, and tissue samples were collected at 168 h post-dose, and samples were assayed for radioactivity.
			2/sex	Blood Kinetics: Whole blood was sampled for up to 72 h post-dose, and samples were assayed for radioactivity.
Single low dose	5	Male: 4.67-5.05 Female: 4.70-5.10	4/sex	Excretion Kinetics: Cage wash, urine, and feces were collected for up to 72 h post-dose, assayed for radioactivity, and used for metabolite identification/characterization.
Single high dose	1000	Male: 1005-1068 Female: 988-1050		
Multiple low dose [c]	5	Male: 4.67-5.25 Female: 4.24-5.22		
Single low dose	5	Male: 4.46-5.08 Female: 4.66-5.08	4/sex	Blood Kinetics: Whole blood was sampled for up to 72 h post-dose, and assayed for radioactivity.
Single high dose	1000	Male: 983-1040 Female: 993-1064		
Multiple low dose [c]	5	Male: 4.96-5.13 Female: 5.01-5.14		

(table continues next page)

Dose Group	Nominal dose (mg/kg)	Actual dose range (mg/kg)	# Animals per dose group per radiolabel[b]	Comments
Single low dose	5	Male: 4.49-5.06 Female: 4.61-5.06	4/sex	Tissue Distribution: Animals were killed between 12 minutes and 2 h post-radiolabeled dose. Blood, organ, and tissue samples were collected and radioassayed.
Single high dose	1000	Male: 929-1003 Female: 938-1008		
Multiple low dose[c]	5	Male: 4.69-5.02 Female: 4.86-5.06		
Single low dose	5	Male: 3.98-4.14 Female: 4.04-5.26	4 male 7 female	Biliary Elimination: Bile, urine, and feces were collected for up to 72 h post-dose, and samples were assayed for radioactivity.

a Data were obtained from MRID 46578909 on pages 21, 22, 73, and 74; MRID 46578907 on pages 23, 25, 81, and 82; and MRID 46578905 on page 19.

b Both [^{14}C-U-phenyl] IR5878 and [^{14}C-5-pyrimidinyl] IR5878 were tested for each dose group except in the preliminary and biliary elimination studies where only [^{14}C-U-phenyl] IR5878 was tested.

c Animals were treated once a day for 14 days with non-radiolabeled IR5878 (5 mg/kg) and with [^{14}C] IR5878 (5 mg/kg) on the fifteenth day.

2. Dosing and sample collection: Except for the repeated dose group, animals received a single gavage dose of [^{14}C] IR5878 at a nominal concentration of 5 or 1000 mg/kg in a 10 mL/kg volume. Animals in the repeated dose group were treated once a day by gavage for 14 days with non-radiolabeled IR5878 (5 mg/kg) and then with [^{14}C] IR5878 (5 mg/kg) on the fifteenth day. In the preliminary study, animals were fasted for approximately 16 h prior to dosing and for 6 h following dosing. It was not stated if the other animals were fasted prior to dosing in the other studies. The actual administered radioactive dose was determined gravimetrically and is reported in Table 1, along with the number of animals treated. The average actual dose of [^{14}C-U-phenyl] IR5878 administered was 10.0 µCi/mg at 5 mg/kg, 0.095 µCi/mg at 250 mg/kg and 0.053 µCi/mg at 1000 mg/kg. The average actual dose of [^{14}C-5-pyrimidinyl] IR5878 administered was 15.3 µCi/mg at 5 mg/kg and 0.092 µCi/mg at 1000 mg/kg.

a. Pharmacokinetic studies: All excretory samples were collected from individual animals. Collection of excreta from animal groups were facilitated by housing in metabolism cages suitable for the separate collection of urine and feces.

In the preliminary study (250 mg/kg), samples of whole blood were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 h. Plasma samples were prepared from blood collected at sacrifice. Urine was collected at 0-8 h, 8-24 h, daily to 96 h, and 96-168 h post-dose. Feces and cage wash (water:ethanol, 1:1) were collected daily to 96 h, and 96-168 h post-dose. Expired carbon dioxide was collected in two carbosorb bottles (100 mL) in series at 0-4, 4-8, and 8-24 h. Treated animals were sacrificed at 168 h post-dose, but controls were sacrificed at 24 h following vehicle administration. The blood, brain, liver, lungs, kidneys, stomach wall, intestine wall, spleen, muscle, femur, brown fat, testes, and ovaries were collected, and the remaining carcass was kept. Feces were homogenized with water and combusted. Tissues were homogenized and solubilized with Soluene-350 before analysis by liquid scintillation counting (LSC). Carcasses were dissolved in concentrated nitric acid, neutralized with NaOH after dissolution, bleached

with 30% H_2O_2, and analyzed with LSC. Whole blood was added to Soluene-350:propanol-2 (1:1) mixture, bleached with hydrogen peroxide, and analyzed by LSC. Plasma, urine, cage wash, and carbosorb samples were analyzed directly by LSC.

The following methods were used in studies other than the preliminary study. In the biliary excretion study, rats were anesthetized with a mixture of isofluorane in oxygen/nitrous oxide, and their bile ducts and duodenum were cannulated. Thus, bile was collected, and artificial bile salts were infused into the duodenum. After a 4-5 day recovery, animals were dosed. Carbonate solutions were used during the processing of samples. Ammonium carbonate (1 M at pH 9) was used with samples from animals treated with [^{14}C-5-pyrimidinyl] IR5878, and 10% sodium carbonate (pH 8) was used with samples from animals treated with [^{14}C-U-phenyl] IR5878. Bile (from the [^{14}C-U-phenyl] IR5878-treated rats only) and urine (all rats) were collected into containers containing a carbonate solution and cooled by solid CO_2 at 0-6, 6-12, and 12-24 h, and at 24 h intervals up to 72 h post-dose. Feces (all rats) were collected into containers cooled by dry ice at 0-12 h and at 24 h intervals up to 72 h post-dose. Cages were washed at the time of each feces collection, and the washes were retained. Blood samples were collected from the tail vein at 0.1, 0.2, 0.4, 1, 2, 4, 6, 8, 24, 48, and 72 h post-dose. Animals were sacrificed by CO_2 narcosis at 72 h post-dose for all animals except the tissue distribution study. In the tissue distribution study, [^{14}C-U-phenyl] IR5878-treated animal groups were sacrificed at the following times post-dose: single 5 mg/kg dose group at 1 h, single 1000 mg/kg dose group at 2 h, and repeated dose group at 1 h after the final dose. [^{14}C-5-pyrimidinyl] IR5878-treated animal groups were sacrificed at the following times post-dose: single 5 mg/kg dose group at 30 min, single 1000 mg/kg dose group at 2 h, and repeated dose group at 12 min after the final dose. The brain, liver, lungs, kidneys, GI tract, spleen, muscle, bone, brown fat, subcutaneous fat, and heart were collected; and the remaining carcass was also saved. Samples of bile, urine, and cage wash were analyzed directly with LSC. Feces and minced carcasses were homogenized separately in an approximately equal volume of carbonate solution. The GI tract from [^{14}C-5-pyrimidinyl] IR5878-treated animals was also homogenized in ammonium carbonate. Other tissues and the carcass were finely chopped/minced. Samples other than bile, urine, and cage wash were combusted and subsequently analyzed by LSC. Samples were allowed to stabilize with regard to light and temperature prior to LSC analysis. All radioassays were performed in duplicate. A limit of reliable measurement was set at 30 dpm above background, and representative blank samples were run.

b. <u>Metabolite studies</u>: In the preliminary study, urine and feces were each pooled by dose group, sex, and time interval. Urine samples collected at 0-8 and 8-24 h and fecal samples collected at 0-24 and 24-48 h were analyzed. Fecal samples were extracted with acetonitrile:33 mM $NaHCO_3$ (1:1) and concentrated prior to analysis. Extraction efficiency was 92-96%. The profile of the radiolabelled compounds was obtained by HPLC and TLC. Co-chromatography with reference compounds and HPLC-MS were used to identify compounds.

In the other studies, urine and feces were each pooled by dose groups, sex, and time interval. Liver and kidney samples were each pooled by sex. Urine and fecal samples collected at the following time intervals were analyzed: urine (0-6, 6-12, 12-24 h); feces (0-12, 12-24, 24-48 h). Additionally, in the 1000 mg/kg [^{14}C-5-pyrimidinyl] IR5878-treated rats, urine collected at 24-48

h and feces collected at 48-72 h were also analyzed. Tissue and fecal samples were extracted with acetonitrile:33 mM $NaHCO_3$ (1:1) prior to analysis and concentrated. For each rat, less than 4% of the administered dose remained in the fecal residues, and <0.75% remained in the tissue residue. Bile samples collected at 6 h were pooled by sex. The profile of the radiolabelled compounds was obtained by HPLC and TLC. Additionally, HPLC-MS was used to identify compounds. Enzymatic hydrolysis with glucuronidase or sulfatase was also performed to aid in compound identification of samples from the [^{14}C-5-pyrimidinyl] IR5878-treated rats.

3. Statistics: Statistical analysis was not performed. Pharmacokinetic parameters were calculated using non-compartmental analysis with the Pharm-NCA program version 1.4e (Innaphase, Champs-sur-Marne, France).

II. RESULTS

A. PHARMACOKINETIC STUDIES

1. Preliminary study: In combined sexes, total mean recovery was 100%: 34% was accounted for in urine and cage wash, 66% in feces, and <0.02% in expired air and carcass (Table 2). Most of the radioactivity (96.85% dose) was excreted in the feces and urine within 48 h post-dose. The C_{max} was 238 µg equiv./mL at a t_{max} of 0.31h; the elimination half-life was 14.4 h. Tissue levels were 0.00 µg equiv./g in all tissues that were examined. As almost none of the radioactive dose was isolated in the expired air, air was not sampled in the main studies.

Table 2. Recovery (% of administered dose; 0-168 h) of radioactivity in tissues and excreta of rats following treatment with [^{14}C-U-phenyl] IR5878.[a]

Matrix	250 mg/kg (Single dose) Males and Females; n=4
Urine	32.01±9.00
Feces	66.10±11.78
Cage wash	2.09±0.55
Expired air	0.02±0.01
Carcass	0.00±0.00
Total	100.22±7.61

a Data (mean±SD; n=4 [males and females combined]) were obtained from pages 40-43 of MRID 46578905.

2. Absorption and excretion: In the main studies within 12 h of administration of the radiolabeled dose (5 or 1000 mg/kg single dose or multiple 5 mg/kg doses), approximately half of the dose was excreted: 51-68% of the [^{14}C-U-phenyl] IR5878 dose and 38-54% of the [^{14}C-5-pyrimidinyl] IR5878 dose (Table 3a). At 72 h post-dose, overall recovery of the radioactive dose from both sexes was 92-100%. The majority of the dose was recovered in the feces within 72 h post-dose (51-73%), with the exception of the females treated with a single 5 mg/kg dose of [^{14}C-

5-pyrimidinyl] IR5878 (43% in feces and 46% in urine). Excretion in the urine accounted for 18-46% of the dose, and cage wash accounted for 1-5% dose. In all groups, minimal radioactivity was detected in the carcass and GI tract after 72 h (<0.8% dose). In general, there were no differences in the excretion profile based on sex, dose, or number of doses. In the single dose treated animals (5 and 1000 mg/kg groups), less radioactivity was found in the feces of the [^{14}C-5-pyrimidinyl] IR5878 treated animals at 12 h post-dose than the animals treated with [^{14}C-U-phenyl] IR5878; however, these values became more similar by 72 h post-dose.

Table 3a. Recovery (% of administered dose) of radioactivity in tissues and excreta of rats following treatment with [^{14}C] IR5878.[a]

Matrix	5 mg/kg (Single dose)		1000 mg/kg (Single dose)		5 mg/kg (Repeated dose[b])	
	Males	Females	Males	Females	Males	Females
[^{14}C-U-phenyl] IR5878						
Urine:						
0-6 h					25.32±0.48	
0-12 h					28.79±1.29	
0-72 h	23.27±4.81	28.41±7.11	7.44±0.90	9.63±2.27	31.52±2.45	31.58±11.61
	28.83±3.11	35.45±7.34	14.36±1.92	18.81±2.33		37.86±9.34
	30.34±3.35	37.93±7.67	17.65±2.11	23.28±3.71		40.69±9.21
Feces:						
0-12 h		20.76±10.50			31.42±8.23	
0-24 h		41.62±10.35			54.79±3.70	
0-72 h	37.79±5.39		53.76±4.29	40.48±17.95		13.46±12.31
	60.36±4.27	52.93±13.12	67.67±5.74	60.97±5.81	62.76±1.81	34.82±8.11
	66.38±2.82		72.89±3.33	66.27±4.77		50.73±5.68
Cage wash	2.65±0.66	3.19±2.58	1.15±0.14	2.18±0.80	1.84±0.71	4.60±4.14
GI Tract	0.04±0.03	0.06±0.05	0.03±0.02	0.04±0.02	0.07±0.04	0.08±0.03
Carcass	0.14±0.06	0.33±0.07	0.12±0.03	0.06±0.06	0.00±0.01	0.70±1.37
Total	99.59±1.91	94.45±6.97	91.85±1.16	91.84±2.67	96.19±2.57	96.91±1.82
[^{14}C-5-pyrimidinyl] IR5878						
Urine:						
0-6 h					22.29±5.48	
0-12 h					27.77±3.48	
0-72 h	25.25±3.76	39.67±3.34	8.56±1.35	21.98±3.00		30.60±12.97
	28.42±3.37	43.87±3.78	21.33±2.87	33.96±3.49	28.91±3.34	32.89±13.85
	29.45±3.36	45.88±4.64	31.15±4.11	41.37±4.65		34.64±13.95
Feces:						
0-12 h					26.32±7.79	
0-24 h					58.28±6.46	
0-72 h	16.27±14.51	10.59±8.71	20.87±14.02	4.58±4.63		15.39±17.53
	61.22±3.72	39.49±5.70	51.18±11.76	44.76±7.82	61.62±4.91	47.11±13.79
	63.09±3.72	42.60±5.61	61.71±7.05	51.38±5.04		54.46±10.39
Cage wash	2.07±0.99	4.13±2.79	4.10±0.62	3.43±0.47	2.03±0.46	4.75±2.23

GI Tract	0.02±0.01	0.05±0.01	0.19±0.22	0.06±0.04	0.02±0.00	0.08±0.04
Carcass	0.25±0.13	0.70±0.93	0.50±0.10	0.29±0.06	0.14±0.02	0.26±0.12
Total	94.88±4.27	93.36±1.37	97.65±3.07	96.53±2.10	92.71±1.29	94.18±1.58

a Data (mean±SD; n=4) were obtained from pages 36-41 of MRID 46578907 and pages 32-37 of MRID 46578909.

b Animals received 14 daily doses of IR5878 (5 mg/kg/day) followed by 5 mg/kg of [^{14}C] IR5878 on Day 15.

Following a single 5 mg/kg dose of [^{14}C-U-phenyl] IR5878 to bile-duct cannulated rats, 76-82% of the dose was absorbed and found in the urine/cage wash, bile, and carcass, indicating extensive absorption (Table 3b).

Table 3b. Recovery (% of administered dose) of radioactivity in bile, tissues, and excreta of rats following treatment with a single dose of 5 mg/kg [^{14}C-U-phenyl] IR5878.[a]

Matrix		Males	Females
Urine:	**0-6 h**	30.64±5.91	29.98±4.22
	0-12 h	37.14±4.37	34.64±3.15
	0-72 h	39.64±4.84	36.58±3.10
Feces:	**0-12 h**	4.11±6.97	5.40±5.79
	0-24 h	13.21±3.14	13.24±7.61
	0-72 h	17.05±3.64	17.47±6.75
Bile:	**0-6 h**	36.50±6.92	33.57±8.79
	0-72 h	38.37±6.71	36.36±8.60
Cage wash		4.11±2.04	3.28±4.18
GI Tract		0.16±0.09	0.06±0.07
Carcass		0.07±0.06	0.08±0.16
Total		99.40±2.82	93.79±4.89

a Data (mean±SD; n=4) were obtained from pages 61-62 of MRID 46578907.

Absorption was rapid in all groups, regardless of sex, dose, or number of doses (Table 3c). T_{max} values were 12 min for the 5 mg/kg repeated dose group (3.2-7.5 µg equiv./g), 24 min-1 h for the single 5 mg/kg dose group (2.0-2.4 µg equiv./g), and 1-4 h for the single 1000 mg/kg dose group (285-582 µg equiv./g). The elimination half-life (8-48 h) was similar regardless of sex, dose, or number of doses (8.9-13.3 h), with the exception of the females treated with a single 5 mg/kg dose of [^{14}C-5-pyrimidinyl] IR5878 (16.7 h). Absorption was not limited by dose in this test but was not proportional to dose either. An increase of 200x dose resulted in an increase AUC in the [^{14}C-U-phenyl] IR5878 treated males (419x) and females (334x) and in the [^{14}C-5-pyrimidinyl] IR5878 treated males (953x) and females (607x). The Sponsor stated that the increase AUC in high dose animals indicates a saturation of clearance pathways for whole blood.

Table 3c. Pharmacokinetic parameters in whole blood of rats following treatment with [^{14}C] IR5878.[a]

Parameter	5 mg/kg (Single dose)		1000 mg/kg (Single dose)		5 mg/kg (Repeated dose[b])	
	Males	Females	Males	Females	Males	Females
[^{14}C-U-phenyl] IR5878						
C_{max} (µg equiv./g)	2.328	2.411	305.02	285.09	3.203	6.322
T_{max} (h)	1 h	1 h	4 h	2 h	12 min	12 min
$T_{½}$ (8-48 h)	10.89	11.09	10.88	11.87	9.27	10.85
AUC	13.35	12.89	5595.59	4299.67	13.14	13.78
[^{14}C-5-pyrimidinyl] IR5878						
C_{max} (µg equiv./g)	1.990	2.035	582.21	481.72	2.364	7.545
T_{max} (h)	24 min	1 h	2 h	1 h	12 min	12 min
$T_{½}$ (8-48 h)	13.29	16.74	8.90	9.23	10.66	13.23
AUC	9.49	9.01	9045.40	5467.07	8.89	11.94

a Data (mean±SD; n=4) were obtained from page 48 of MRID 46578907 and page 44 of MRID 46578909.
b Animals received 14 daily doses of IR5878 (5 mg/kg/day) followed by 5 mg/kg of [^{14}C] IR5878 on Day 15.

2. <u>Tissue distribution</u>: The distribution of radioactivity in tissues was similar, regardless of sex, dose, or number of doses (Table 4). Excluding the GI tract, concentrations of radioactivity were highest in the liver, kidney, lung, and whole blood, with the lung having the lowest concentrations. At 5 mg/kg (single or multiple doses) in the [^{14}C-U-phenyl] IR5878 treated rats, these tissue concentrations were 1.74-12.22 µg equiv./g vs 2.41-3.12 µg equiv./g in whole blood. At 5 mg/kg (single or multiple doses) in the [^{14}C-5-pyrimidinyl] IR5878 treated rats, these tissue concentrations were 2.19-23.83 µg equiv./g vs 3.03-5.10 µg equiv./g in whole blood. At 1000 mg/kg in all treated rats, these tissue concentrations were 161-435 µg equiv./g vs 305-495 µg equiv./g in whole blood

Comparison of the concentrations of radioactivity in tissues on the basis of radiolabel was not possible due to differing sampling times and/or differing T_{max}. Time courses of radioactivity distribution in tissues were not performed; however, relatively little radioactivity remained in the carcass at 72 h post-dose (≤0.70% dose). Therefore, bioaccumulation is not suspected.

Table 4. Concentration of radioactivity (µg equiv./g) in selected tissues/organs of rats following treatment with [^{14}C] IR5878. [a]

Tissue	5 mg/kg (Single dose)		1000 mg/kg (Single dose)		5 mg/kg (Repeated dose [b])	
	Males	Females	Males	Females	Males	Females
[^{14}C-U-phenyl] IR5878 (rats sacrificed at various times post-dose [c])						
Liver	11.18±1.39	9.30±0.20	266.90±157.52	282.88±24.18	12.05±2.23	12.00±1.90
Kidney	8.46±1.40	3.51±0.97	406.75±299.04	259.78±33.20	12.22±1.96	3.71±0.85
Lung	1.74±0.36	1.75±0.42	161.37±102.69	223.31±33.36	1.95±0.39	2.45±0.46
Whole Blood	2.86±0.76	2.41±0.50	305.06±32.50	311.81±48.45	2.99±0.83	3.12±0.38
[^{14}C-5-pyrimidinyl] IR5878 (rats sacrificed at various times post-dose [d])						
Liver	18.72±1.89	23.83±1.78	434.68±22.47	404.20±35.02	9.90±2.89	15.47±10.23
Kidney	4.72±0.58	9.18±6.59	394.05±67.48	339.60±32.92	4.19±1.52	6.32±4.22
Lung	2.74±0.08	3.07±0.78	320.19±11.15	314.13±74.94	2.19±0.82	3.53±1.83
Whole Blood	4.04±0.61	5.10±1.68	494.61±24.59	464.95±76.26	3.03±0.81	4.92±3.38

a Data (mean±SD; n=4) were obtained from pages 49-59 of MRID 46578907 and page 45-56 of MRID 46578906.

b *Animals received 14 daily doses of IR5878 (5 mg/kg/day) followed by 5 mg/kg of* [^{14}C] IR5878 on Day 15.

c [^{14}C-U-Phenyl] IR5878-treated animal groups were sacrificed at the following times post-dose: single 5 mg/kg dose group at 1 h, single 1000 mg/kg dose group at 2 h, and repeated dose group at 1 h after the final dose.

d [^{14}C-5-Pyrimidinyl] IR5878-treated animal groups were sacrificed at the following times post-dose: single 5 mg/kg dose group at 30 min, single 1000 mg/kg dose group at 2 h, and repeated dose group at 12 min after the final dose.

B. METABOLITE CHARACTERIZATION STUDIES: HPLC and HPLC-MS analyses identified parent and a total of 10 metabolites in excreta from rats treated with [^{14}C] IR5878. Six to 7 metabolites were identified after treatment with each radiolabeled compound, and 3 of these metabolites were common to treatment with both radiolabeled compounds. Parent and identified metabolites in excreta accounted for 71-86% dose in all animals, and overall recovery was 95-99% dose (Table 5). Unidentified compounds accounted for 1-16% dose, but no single compound accounted for ≥5% dose.

The parent was found in 5 mg/kg animals (single and multiple doses) at 1-6% dose and in 1000 mg/kg animals at 33-56% dose. The predominant metabolite was 1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea (O-desm IR5878). It was found at 53-64% dose in all animals treated at 5 mg/kg and at 14-20% dose in all animals treated at 1000 mg/kg. This compound was found in similar quantities in the urine and feces.

2-Sulfoamino-N,N-dimethylbenzamide (DBS acid) was a primary metabolite in all animals treated with [^{14}C-U-phenyl] IR5878 and was found at 8-12% dose. This compound was found primarily in the feces. In all animals treated with [^{14}C-5-pyrimidinyl] IR5878, a polar fraction was isolated (primarily in feces) that contained (4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)urea (Pyr-O-Glucur DOP urea) and 1-(4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea (Pyr-O-Glucur IR5878). This fraction represented 9-18% dose. Additionally, 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(methylcarbamoyl)phenyl-sulfamoyl]urea (N-desm IR5878) was found at 5-8% dose in the urine of animals treated with 1000 mg/kg [^{14}C-5-pyrimidinyl] IR5878. All other identified metabolites each accounted for <5% dose. IUPAC names for the identified compounds and the proposed metabolic pathways are included as an appendix to this DER.

The metabolic profiles of the liver, kidney, and bile were also evaluated. In the liver and kidneys of all animals treated with [^{14}C-U-phenyl] IR5878, parent and O-desm IR5878 were found in the highest concentrations. In the liver and kidneys of all animals treated with [^{14}C-5-pyrimidinyl] IR5878, parent, O-desm IR5878, and an unidentified fraction were generally found in the highest concentrations, as well as 2-amino-N,N-dimethylbenzamide (DB amine) in the male kidney. In the bile, O-desm IR5878 and an unidentified fraction were found in the highest concentration. Other identified compounds (same as found in the excreta) were not detected in the liver, kidney, or bile or were generally found at relatively low concentrations.

Table 5. Metabolite profile (% dose) in excreta of rats treated with [^{14}C] IR5878. [a]

Compound	5 mg/kg (Single dose)		1000 mg/kg (Single dose)		5 mg/kg (Repeated dose) [b]	
	Males	Females	Males	Females	Males	Females
[^{14}C-U-phenyl] IR5878						
Parent	0.78	3.73	46.78	56.19	0.66	6.37
C6	59.85	57.17	14.11	14.73	53.38	53.82
C3	12.28	10.57	8.96	7.77	9.29	8.97
Other identified metabolites [c]	5.13	4.27	8.68	3.13	7.67	4.63
Total identified	78.04	75.74	78.53	81.82	71.00	73.79
Unknown C1	10.49	7.75	6.47	3.43	12.68	7.19
Total unidentified [d]	11.75	8.98	9.68	4.97	15.61	9.82
Total analyzed	**89.79**	**84.72**	**88.21**	**86.79**	**86.61**	**83.61**
Unanalyzed excreta	4.47	10.74	10.79	12.17	7.40	10.91
Fecal residue	1.92	1.50	0.34	0.38	0.97	1.82
Tissues [e]	0.18	0.39	0.15	0.10	0.07	0.78
Total unanalyzed	**6.57**	**12.63**	**11.28**	**12.65**	**8.44**	**13.51**
Total accounted for	**96.36**	**97.35**	**99.49**	**99.44**	**95.05**	**97.12**
[^{14}C-5-pyrimidinyl] IR5878						
Parent	0.81	6.30	33.25	50.68	0.99	3.56
M8	64.30	59.21	20.46	16.73	56.23	59.52
M1	16.09	10.65	16.29	8.98	18.38	11.82
M7	ND	ND	5.17	7.54	ND	ND
Other identified metabolites [c]	4.64	5.24	6.48	2.51	5.50	3.82
Total identified	85.84	81.40	81.65	86.44	81.10	78.72
Total unidentified [d]	0.92	1.26	6.77	1.94	1.23	2.89
Total analyzed	**86.76**	**82.66**	**88.42**	**88.38**	**82.33**	**81.61**
Unanalyzed excreta	7.83	12.33	8.07	7.44	9.96	11.73
Fecal residue	1.80	1.67	0.76	0.76	2.58	2.22
Tissues [e]	0.27	0.75	0.69	0.35	0.16	0.34
Total unanalyzed	**9.90**	**14.75**	**9.52**	**8.55**	**12.70**	**14.29**
Total accounted for	**96.66**	**97.41**	**97.94**	**96.93**	**95.03**	**95.90**

a Data were obtained from pages 43, 51-54, and 67-69 MRID 46578908 and 47, 57-62, and 75-77 of MRID 46578910 (n=3-4).

b Animals received 14 daily doses of IR5878 (5 mg/kg/day) followed by 5 mg/kg of [^{14}C] IR5878 on Day 15.

c Other metabolites were identified, but each of these metabolites represents less than 5% of the administered dose, except that C4 was present at 5.63% dose in males of the [^{14}C-U-phenyl] IR5878 repeated dose study.

d All unidentified metabolites accounted for less than 5% dose each, and included C1, C2, M3, M4, and M5. Unknown C1 consisted of at least 7 compounds.

e From Table 3a in this DER, Carcass and GI Tract were summed.

ND Less than the limit of quantitation

C1 Unknown consisting of at least 7 compounds

C3 2-sulfoamino-N,N-dimethylbenzamide

C6/M8 1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea

M1 composed primarily of M1f and M1g

M1f (4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)urea

M1g 1-(4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea

M7 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(methylcarbamoyl)phenylsulfamoyl]urea

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The compound was excreted mainly in the feces, and bile was an important route of elimination. Excretion was nearly complete in the urine within 24 h post-dose and in feces within 48 h post-dose. Excretion profile was similar regardless of sex, dose, or number of doses. The only tissues containing significant radioactivity were the liver, kidneys, and GI tract. The metabolic profile was also similar regardless of sex, dose, or number of doses.

B. REVIEWER COMMENTS: Absorption was rapid in all groups, regardless of sex, dose, or number of doses. T_{max} values were 12 min for the 5 mg/kg repeated dose group (3.2-7.5 µg equiv./g), 24 min-1 h for the single 5 mg/kg dose group (2.0-2.4 µg equiv./g), and 1-4 h for the single 1000 mg/kg dose group (285-582 µg equiv./g). Following a single 5 mg/kg dose of [^{14}C-U-phenyl] IR5878, 76-82% of the dose was absorbed and found in the urine/cage wash, bile, and carcass, indicating extensive absorption. The elimination half-life (8-48 h) was similar regardless of sex, dose, or number of doses (8.9-13.3 h), with the exception of the females treated with a single 5 mg/kg dose of [^{14}C-5-pyrimidinyl] IR5878 (16.7 h).

Within 12 h of administration of the radiolabeled dose (5 or 1000 mg/kg single dose or multiple 5 mg/kg doses), approximately half of the dose was excreted: 51-68% of the [^{14}C-U-phenyl] IR5878 dose and 38-54% of the [^{14}C-5-pyrimidinyl] IR5878 dose. At 72 h post-dose, overall recovery of the radioactive dose from both sexes was 92-100%. The majority of the dose was recovered in the feces within 72 h post-dose (51-73%), with the exception of the females treated with a single 5 mg/kg dose of [^{14}C-5-pyrimidinyl] IR5878 (43% in feces and 46% in urine). Excretion in the urine accounted for 18-46% of the dose, and cage wash accounted for 1-5% dose. In all groups, minimal radioactivity was detected in the carcass and GI tract after 72 h (<0.8% dose). In a preliminary study, <0.02% of the radioactive dose was isolated in the expired air; therefore, this route of excretion was not analyzed. In general, no difference in the excretion profile was noted based on sex, dose, or number of doses.

Regardless of sex, dose, or number of doses, the distribution of radioactivity in tissues was similar. Comparison of the concentrations of radioactivity in tissues on the basis of radiolabel was not possible due to differing sampling times and/or differing T_{max}. Excluding the GI tract, concentrations of radioactivity were highest in the liver, kidney, lung, and whole blood, with the lung having the lowest concentrations. At 5 mg/kg (single or multiple doses), these tissue concentrations were 1.74-23.83 µg equiv./g vs 2.41-5.10 µg equiv./g in whole blood. At 1000 mg/kg in all treated rats, these tissue concentrations were 161-435 µg equiv./g vs 305-495 µg equiv./g in whole blood. Time courses of radioactivity distribution in tissues were not performed; however, relatively little radioactivity remained in the carcass at 72 h post-dose (≤0.70% dose). Therefore, bioaccumulation is not suspected.

HPLC and HPLC-MS analyses identified parent and a total of 10 metabolites in excreta from rats treated with [^{14}C] IR5878. Six to 7 metabolites were identified after treatment with each radiolabeled compound, and 3 of these metabolites were common to treatment with both radiolabeled compounds. Parent and identified metabolites in excreta accounted for 71-86%

dose in all animals, and overall recovery was 95-99% dose. Unidentified compounds accounted for 1-16% dose, but no single compound accounted for ≥5% dose.

The parent was found in 5 mg/kg animals (single and multiple doses) at 1-6% dose and in 1000 mg/kg animals at 33-56% dose. The predominant metabolite was O-desm IR5878. It was found at 53-64% dose in all animals treated at 5 mg/kg and at 14-20% dose in all animals treated at 1000 mg/kg. This compound was found in similar quantities in the urine and feces. DBS acid was a primary metabolite in all animals treated with [^{14}C-U-phenyl] IR5878 and was found at 8-12% dose. This compound was found primarily in the feces. In all animals treated with [^{14}C-5-pyrimidinyl] IR5878, a polar fraction was isolated (primarily in feces) that contained Pyr-O-Glucur DOP urea and Pyr-O-Glucur IR5878. This fraction represented 9-18% dose. Additionally, N-desm IR5878 was found at 5-8% dose in the urine of animals treated with 1000 mg/kg [^{14}C-5-pyrimidinyl] IR5878. All other identified metabolites each accounted for <5% dose. Identification of IR5878 metabolites indicates that metabolism is mainly occurring through O- and/or N-demethylations. Additionally, hydrolytic cleavage of the sulfamoylurea linkage yields DOP urea, and hydroxylation of the pyrimidinyl ring occurs followed by glucuronic acid or sulfate conjugation.

The metabolic profile of the liver, kidney, and bile was also evaluated. In the liver and kidneys of all animals treated with [^{14}C-U-phenyl] IR5878, the parent and O-desm IR5878 were found in the highest concentrations. In the liver and kidneys of all animals treated with [^{14}C-5-pyrimidinyl] IR5878, O-desm IR5878 and an unidentified fraction were generally found in the highest concentrations, as well as 2-amino-N,N-dimethylbenzamide (DB amine) in the male kidney. In the bile, O-desm IR5878 and an unidentified fraction were found in the highest concentration. Other identified compounds (same as found in the excreta) were not detected in the liver, kidney, or bile or were generally found at relatively low concentrations.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

C. STUDY DEFICIENCIES: No deficiency was noted for a Tier 1 study.

APPENDIX

The following are pages 15 and 47 from MRID 46578910 and page 43 from MRID 46578908

Cancer Assessment Rev...

Page _____ is not included in this copy.

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DATA EVALUATION RECORD

(IR5878) ORTHOSULFAMURON

Study Type: Non-guideline; Potential Effects on Thyroid Function in Rats

Work Assignment No. 3-01-82 O (MRID 46578927)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date**_____
Work Assignment Manager: P.V. Shah **Signature:**_____
Registration Action Branch 1, Health Effects Division (7509C) **Date**_____
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Potential Effects on Thyroid Function - Rats

PC CODE: 108209 **DP BARCODE:** D319264
TXR#: 0053612

TEST MATERIAL (PURITY): Orthosulfamuron (IR5878) technical; (98.79% a.i.)

SYNONYM: 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea

CITATION: Taylor, L.M. (2004) IR5878: investigation of the potential effects on thyroid function in male rats using the perchlorate discharge and thyroxine clearance tests. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Study No.: IGA 037/043176, November 24, 2004. MRID 46578927. Unpublished.

SPONSOR: ISAGRO S.p.A., Centro Uffici San Siro, Fabbricato D-ala 3, Via Caldera 21, I-20153 Milano, Italy

EXECUTIVE SUMMARY: The purpose of this non-guideline study (MRID 46578927) was to examine the effects of oral administration of orthosulfamuron (IR5878; Batch# G 009/02; 98.79% a.i.) on thyroid function in rats, through potential direct pathways (using the perchlorate discharge test) or indirect mechanisms [determined by: (i) thyroxine pharmacokinetics using ^{125}I clearance; (ii) plasma thyroid hormone levels; and (iii) organ weights and histopathology of the liver and thyroid].

The test substance was administered in the diet to 28 male Han Wistar rats/dose at doses of 0, 5, and 1000 mg/kg for up to 90 days. Out of the 28 rats/group placed on study, 6 rats/group were used for each of the three categories of parameters listed above. Propylthiouracil (200 mg/kg) was included as a positive control for the perchlorate discharge test and was administered daily via oral gavage to 15 rats for the final 16 days prior to termination on Day 90. Phenobarbital (75 mg/kg) was included as a positive control for the remaining parameters and was administered daily via oral gavage to 15 rats for 14 days prior to termination of Day 90.

Minor decreases in weekly and overall body weights and food consumption were observed at 1000 mg/kg.

Potential direct effects of the test substance on the thyroid were examined using the perchlorate discharge assay. The positive control, propylthiouracil, acts as an inhibitor of the thyroid peroxidases responsible for the iodide organification necessary for T3/T4 synthesis. The results from the propylthiouracil group in this study were consistent with this blocked uptake of iodide, including decreased radioactive iodide in the thyroid, increased radioactive iodide in whole blood, and thus, decreased thyroid:whole blood ratio of radioactive iodide. Several significant ($p<=0.05$) differences from negative controls were noted in the groups treated with the test substance. However, none of these changes were considered to be toxicologically relevant because the direction of the change was opposite of that which would indicate prevention of iodide organification.

In contrast to the mechanism of propylthiouracil, perchlorate (ClO_4^-) exerts a direct affect on the thyroid by acting as a competitive inhibitor of iodide transport from the circulation into the follicular cells, thus limiting T3/T4 synthesis. Comparison of the perchlorate and saline subgroups within each group revealed no effects of perchlorate on thyroid weight or radioactive iodide in the thyroid or whole blood in the groups treated with 0, 5, or 1000 mg/kg orthosulfamuron, indicating no blockage of iodide uptake into the thyroid due to the test substance. The only differences between the perchlorate and saline subgroups were noted in the propylthiouracil group, with decreased radioactivity in the thyroid and increased radioactivity in whole blood, resulting in a decreased thyroid:whole blood ratio in the perchlorate subgroups compared to the saline subgroups. These findings confirm that perchlorate, as a competitive inhibitor of iodide transport, displaced the free iodide present in the thyroid. The levels of free iodide were higher in this group because the process of organification of free iodide was inhibited by propylthiouracil.

In the liver, the following findings were observed. Liver weights were increased at 1000 mg/kg compared to negative controls, and an increase of an even greater magnitude was observed in the phenobarbital group. Minimal centrilobular hepatocyte hypertrophy was observed at 1000 mg/kg (4/6 rats) compared to negative controls (0/6 rats). In the phenobarbital group, both the severity (slight) and incidence (6/6 rats) of this finding was increased compared to the 1000 mg/kg group. Concentrations of microsomal protein and cytochrome P450 and the activities of PROD and UDP-GT were increased ($p<=0.05$) at 1000 mg/kg compared to negative controls. For each of these liver enzymes, the concentration/activity was increased ($p<=0.01$) in the phenobarbital group to a greater extent than the 1000 mg/kg group.

Induction of UDP-GT results in increased elimination of T4, which is supported by the pharmacokinetic data. At 1000 mg/kg/day, ^{125}I-T4 exhibited slightly increased clearance compared to negative controls, indicated by decreased whole blood concentrations of radioactivity and AUC_{72} values and increased systemic clearance, V_c, V_{ss}, and V_z. The positive

control group responded similarly, only with a greater magnitude difference from negative controls.

The increased elimination of T4 activates a negative feedback loop to stimulate the pituitary to release TSH. In this study, TSH levels were increased at 1000 mg/kg on Day 90, with an even greater increase in the phenobarbital group. TSH acts on the thyroid follicular cells to produce T3/T4; when this process is prolonged, it results in hypertrophy, hyperplasia, and eventually, tumor formation. Although thyroid/parathyroid weights were comparable to controls in this study, enlarged thyroids were noted in the 5 mg/kg (1/6 rats) and 1000 mg/kg (2/6 rats) compared to 0/6 negative controls and 4/6 phenobarbital treated rats. Minimal follicular cell hypertrophy was noted at 1000 mg/kg (4/6 rats) compared to negative controls (1/6 rats). In the phenobarbital group, minimal to slight follicular cell hypertrophy was observed in all (6/6) rats.

In conclusion, there was no direct effect of the test substance on the thyroid, as indicated by the perchlorate assay. The increased organ weight, hypertrophy, and induction of UDP-GT in the liver and the increased TSH and enlarged thyroids and follicular cell hypertrophy support an indirect effect of the test substance on the thyroid.

This study is classified as **acceptable/non-guideline**.

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

I. INTRODUCTION AND OBJECTIVES: Xenobiotics can affect the thyroid in a number of ways. They can affect the thyroid directly by: (i) inhibiting iodine uptake into the thyroid, thus reducing hormone production; (ii) inhibiting hormone synthesis; or (iii) blocking hormone release. Chemicals can also affect the thyroid via indirect mechanisms by: (i) inducing microsomal enzymes, thus leading to increased clearance of thyroid hormones from the systemic circulation; or by (ii) inhibiting the conversion of thyroxine (T4) to tri-iodothyronine (T3). When the thyroid is affected (either by direct or indirect pathways), the pituitary is stimulated, via a negative feedback loop, to produce more thyroid stimulating hormone (TSH) which acts on the thyroid follicular cells. When this process is prolonged, it results in thyroid follicular hypertrophy, hyperplasia, and eventually, tumor formation. However, indirect effects on the thyroid via induction of microsomal enzymes are not expected to be of a concern to human health because of the longer half life and greater binding capacity of T4 in humans.

In previous studies (i.e., MRID 46578913), the test substance induced thyroid hypertrophy in male rats following chronic treatment. Thus, the purpose of this study was to examine the effects of oral administration of the test substance on thyroid function in rats, through potential direct pathways (using the perchlorate discharge test) or indirect mechanisms (determined by thyroxine pharmacokinetics, plasma thyroid hormone levels, and histopathology of the liver and thyroid). The perchlorate discharge test uses perchlorate, a competitive inhibitor of thyroidal iodine transport, to displace accumulated ^{125}I from the thyroid. Propylthiouracil was used as a positive

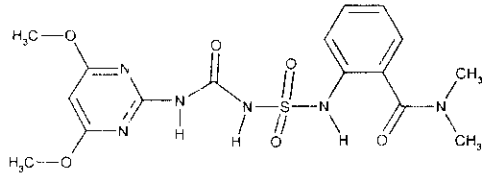
control in the perchlorate discharge assay because it is an inhibitor of the thyroid peroxidases responsible for iodide organification, and therefore acts directly on the thyroid. Phenobarbital was used as a positive control for the parameters examining indirect effects because it is a known inducer of hepatic drug-metabolizing enzymes and enhances clearance via increased glucuronidation and biliary excretion of conjugated T4.

II. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Orthosulfamuron (IR5878) technical

Description:	White powder
Lot/Batch #:	G 009/02
Purity (w/w):	98.79% a.i.
Vehicle	Diet
Stability of compound:	The test substance was stable in the diet for up to 15 days at 21°C (MRID 46578913)
CAS #:	213464-77-8
Structure:	



2. Positive controls

a) Sodium phenobarbital

Description:	White powder
Lot/Batch #:	112K2500
Purity (w/w):	>99% a.i.
Vehicle	Water

b) Propylthiouracil

Description:	White powder
Lot/Batch #:	A015991201
Purity (w/w):	98% a.i.
Vehicle	Water

4. Test animals

Species:	Rat
Strain:	Han Wistar
Age/body weight range at study initiation:	41 ± 2 days; 104-139 g

Source:	Harlan UK Ltd., Bicester, England
Housing:	Up to 5/cage in suspended cages with wire mesh floors
Diet:	Ground SDS Rat and Mouse Maintenance Diet No. 1, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	19-24°C
Humidity:	40-86%
Air changes:	Not provided
Photoperiod:	Not provided
Acclimation period:	6 days

B. STUDY DESIGN

1. In life dates: Start: 05/05/04 End: 08/03/04

2. Animal assignment/dose levels: The animals were randomly assigned to the test groups shown in Table 1.

Table 1. Study design [a]

Group	Treatment	Nominal Dose (mg/kg/day)	Achieved Dose (mg/kg/day)	Total (#) [b]	Thyroid hormones, Organ weights, & Liver enzymes (#)	T4 clearance (#)	Perchlorate (#) [c]
1	Control	0	0	28	6	6	12
2	Low dose	5	5.1	28	6	6	12
3	High dose	1000	1010	28	6	6	12
4	Phenobarbital [d]	75	NA	15	6	6	0
5	Propylthiouracil [e]	200	NA	15	0	0	12

a Data were obtained from pages 17, 21 through 24, and 47 of the study report.
b Out of these 28 rats, 2/dose were not used in the T4 clearance tests and 2/dose were not used in the perchlorate assay for Groups 1-3. In Groups 4 and 5, 3 rats/dose were not used for the perchlorate assay. However, these extra rats were included in body weight or food consumption data.
c After 92 days of treatment, 24 hours after the last dose of propylthiouracil, all 12 rats were dosed (i.p.) with ^{125}I, followed 6 hours later by potassium perchlorate to 6 rats/group and saline to the remaining 6 rats/group.
d Included as a positive control for all parameters except for the perchlorate discharge assay.
e Included as a positive control for the perchlorate discharge test.
NA Not applicable

3. Dose-selection rationale: The dose levels selected for this study were based on findings in a concurrently submitted chronic toxicity/carcinogenicity study (MRID 46578913), in which the NOAEL was 5 mg/kg/day, and effects on the thyroid (including adenomas) were noted at 500 and 1000 mg/kg/day.

4. Dosage preparation and analysis: For each of the two groups treated with IR 5878, a pre-mix was prepared by mixing the appropriate amount of the test substance with diet. The required dietary concentrations were prepared weekly by diluting and mixing the appropriate pre-mix with additional diet. Dietary concentrations were adjusted weekly for the first four weeks and every two weeks thereafter. Homogeneity (top, middle, bottom) and concentration of the test substance in the diet were determined for both dose groups at the beginning (Week 1) and end (Week 13) of the study. Stability at 5 and 30,000 ppm was confirmed for up to 15 days at room temperature (21°C) in a concurrently submitted combined chronic toxicity/oncogenicity study in rats (MRID 46578913).

Results

Homogeneity (coefficient of variation): 0.74-1.06%

Stability (% initial): 96-101%

Concentration (% nominal): 99-107%

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

Positive controls - Phenobarbital solutions were prepared weekly by dissolving the appropriate amount of sodium phenobarbital in sterile water to achieve a concentration of 15 mg/mL and storing at 4°C. Propylthiouracil formulations were prepared daily by suspending the appropriate amount of propylthiouracil in sterile water to achieve a concentration of 40 mg/mL.

5. Dose administration: Groups 1 through 3 were administered the test substance in the diet at doses of 0, 5, or 1000 mg/kg/day for 13 weeks. Beginning at Week 12, Group 4 animals were administered phenobarbital daily via oral gavage for 14 consecutive days, and Group 5 animals were administered propylthiouracil daily via oral gavage for 16 consecutive days. The dose volume of phenobarbital or propylthiouracil administered to each animal (5 mL/kg) was adjusted based on the most recently recorded body weight. Animals treated with phenobarbital or propylthiouracil were fed the basal diet (same batch as Groups 1-3).

6. Statistics: Data were analyzed using the following statistical procedures. Significance for pair-wise comparisons is denoted in the study report and DER at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$. The statistical methods were considered appropriate.

Parameter	Statistical test
Body weight Food consumption Thyroid hormones	1) If ≥75% of the data across all groups were the same value, then a frequency analysis was applied. Low and high dose groups were compared using a Mantel test for trend in proportions, and each dose group was compared with the control group using pair-wise Fisher's Exact test. 2) If <75% of the data across all groups were the same value, Bartlett's test for homogeneity of variance was applied. a) If Bartlett's test was not significant at 1%, then parametric analyses were applied. If the F1 test for dose-response monotonicity was not significant at 1%, then Williams' test for trend was applied. If the F1 test was significant, then Dunnett's test was applied instead. b) If Bartlett's test was significant at 1%, then logarithmic transformations were applied. If Bartlett's test was still significant, then non-parametric tests were applied. Except for pre-dose data, Shirley's test was used to test for a dose-related trend. 3) For pre-dose data, analysis of variance (if parametric) or Kruskal-Wallis (if non-parametric) tests were applied to the data. If significant ($p \leq 0.05$), then pair-wise comparisons with the controls were performed using Student's t-test or Wilcoxon Rank Sum test, respectively. 4) For all parameters except for the thyroid hormone data on Day 76, the control group was compared with each positive control group using Student's t-tests or Wilcoxon Rank Sum test.
Organ weights	Analysis of covariance (ANCOVA), using terminal body weight as the covariate, instead of ANOVA, where the relationship between organ weight and body weight was considered significant at $p \leq 0.01$.
Thyroxine clearance	Logarithmic transformation followed by ANOVA then pair-wise comparisons with the controls using Student's t-test, as necessary
Perchlorate discharge	Logarithmic transformation followed by ANOVA to determine any differences among treatment groups, saline or perchlorate subgroups, and their interactions as factors. To confirm the effect of perchlorate on the thyroid, pair-wise comparisons between the subgroups were performed separately for each treatment group using Student's t-test.
Thyroid weight	Same statistical methods used for the perchlorate discharge data, except that ANCOVA, instead of ANOVA, was used if the relationship between body weight and thyroid weight was significant ($p \leq 0.05$).

C. METHODS

1. Observations: All rats were examined a least twice daily for clinical signs of toxicity. Additionally during Weeks 12 and 13 in the animals receiving phenobartital or propylthiouracil via oral gavage (positive controls), checks for clinical signs of toxicity were conducted: pre-dose; immediately after each dose; at the end of dosing the complete group; approximately 1-2 hours after dosing; and as late as possible during the working day.

2. **Body weight:** Each rat was weighed before treatment, on the day treatment commenced, and weekly thereafter. Additionally, rats used for thyroid and liver histopathology were weighed at necropsy; animals used for thyroxine clearance tests were weighed prior to thyroxine administration and again at termination; and rats used for perchlorate discharge investigations were weighed prior to sodium ^{125}iodide administration.

3. **Food consumption:** Food consumption was recorded at weekly intervals from the start of treatment. Mean daily food consumption (g/rat/day) was calculated by subtracting the amount of food remaining from the total amount of food offered in each cage and dividing that difference by the number of animal days (where "animal day" corresponds to a single day for each animal alive for a whole day).

4. **Measurement of thyroid hormones:** Prior to treatment and on Day 90, blood samples were collected under general anesthesia from the orbital sinus from 6 animals/group from Groups 1through 4 to measure plasma levels of tri-iodothyronine (T3), reverse (inactive) tri-iodothyronine (rT3), thyroxine (T4) and thyroid stimulating hormone (TSH). Additionally, these hormones were measured in Groups 1-3 on Day 30 and in the phenobarbital group on Day 76.

5. **Thyroxine clearance test**

a. Administration of ^{125}I-Thyroxine - On the day of administration, (^{125}I)Thyroxine (specific activity 150 µCi/µg and radiochemical purity >93% measured via HPLC) was diluted with 0.9% saline to a final volume of 14 mL to provide a solution with a nominal concentration of 43 µCi/mL (actual concentration 41.88 µCi/mL). Four hours after the final dose of phenobarbital on Day 90 of treatment, a fixed volume of ^{125}I-Thyroxine (0.4 mL equivalent to 16.75 µCi) was administered to 6 rats/group as a bolus intravenous injection into a lateral tail vein.

b. Sample collection - Samples of whole-blood (*ca* 300 µL) were collected from a tail vein (not the same vein used for dose administration) at 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours after the administration of ^{125}I-Thyroxine. After collection of the 72-hour blood sample, animals were killed by cervical dislocation (not under general anaesthesia) and the carcasses discarded. The blood samples were stored at approximately 4°C until measurement of radioactivity.

6. **Perchlorate discharge test**

a. Dose preparation - Sodium ^{125}iodide was diluted in physiological saline (0.9% w/v) to obtain a solution containing approximately 2 µCi/mL. Potassium perchlorate was dissolved in physiological saline using a magnetic stirrer to obtain a 10 mg/mL solution.

b. Dose administration - Upon completion of 92 days of treatment, 24 hours after the last dose of propylthiouracil, all remaining rats (14 animals/group) were dosed intraperitoneally with sodium ^{125}iodide (about 1 µCi) in 0.5 mL saline solution. Six hours later, potassium perchlorate was administered intraperitoneally to 6 animals from each group at a dose volume of 2 mL/kg

bodyweight in order to obtain a dose level of 20 mg/kg. A further six animals from each group received 0.9% (w/v) saline at the same dose volume.

c. Dose quantification - The actual dose administered to all animals was 2.24 µCi/mL (equivalent to a dose of 1.12 µCi to each rat).

d. Terminal procedures - Exactly two and a half minutes after intraperitoneal injection of potassium perchlorate solution or saline, each animal was anesthetized with isoflurane and a blood sample was collected from the vena cava. Duplicate weighed aliquots (0.5 mL) of each blood sample were then taken for scintillation analysis. Immediately after blood sampling, each animal was killed by cervical dislocation, and the thyroid gland from each rat was removed, rinsed in ice-cold saline, blotted dry, weighed, and taken for measurement of radioactivity *in toto*. The carcasses were discarded without further investigation.

7. **Necropsy and tissue collection** - On Day 91, the rats not used for terminal metabolic studies (i.e, the 6/group used to examine thyroid hormone levels) were killed by carbon dioxide asphyxiation and subjected to gross necropsy. The liver and thyroid were removed and weighed (the thyroids for each animal were weighed together). Sections of the liver were collected, preserved in 10% neutral buffered formalin, and examined microscopically. A portion of the remaining liver was removed and snap frozen in liquid nitrogen for enzyme assays.

8. **Liver enzyme assays** - All preparation stages involved in the isolation of subcellular fractions were conducted in an environment maintained at approximately 4°C. Frozen livers were thawed by standing in ice-cold isotonic Tris buffer (pH 7.4) containing 0.25M sucrose. Microsomal subcellular fractions were prepared by differential centrifugation of liver homogenates using standard techniques. The microsomal pellet was suspended in the buffer, such that 1 mL of the suspension was approximately equivalent to 300 mg pooled liver. The microsomes were divided into aliquots and stored at approximately -75°C until enzyme assays were conducted.

Microsomal protein concentrations were determined by the method of Lowry *et al.* (1951) and the cytochrome P450 concentration determined by the method of Rutton *et al.* (1987). 7-Pentoxyresorufin O-dependentylase (PROD) activity was assayed essentially by the method of Lubet *et al.* (1985) and thyroxine UDP-glucuronosyltransferase (UDP-GT) activity was assayed by a method based on that of Visser *et al.* (1993).

III. RESULTS

A. OBSERVATIONS

1. **Mortality**: All animals survived to scheduled termination.

2. Clinical signs of toxicity: It was stated that clinical signs characteristic of treatment with phenobarbital, including abnormal gait and underactivity, were noted in the Group 4 animals throughout the treatment period. However, these data were not presented in the study report clinical signs table (Table 1 on page 44), and none of the parameters listed in that table showed any relationship with dose.

B. BODY WEIGHT: Beginning at Week 3, minor decreases in body weights were observed at 1000 mg/kg compared to controls (↓1-8%), resulting in significantly decreased (↓13%; $p \leq 0.05$) body weight gain for Weeks 0-12 (Table 2). As expected for animals receiving propylthiouracil, body weight gain was comparable to controls prior to treatment and lower than controls (↓8-12%) during treatment (Weeks 12 and 13), resulting in significantly decreased body weight gain for Weeks 0-12 (↓13%). Body weights in the 5 mg/kg and phenobarbital groups were either comparable to controls or not significantly decreased.

Table 2. Mean body weight and cumulative body weight gains (g) in male rats treated for up to 13 weeks with orthosulfamuron.[a]

Study week	Orthosulfamuron			Phenobarbital	Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg	200 mg/kg
0	115	118	119	112	118
3	231	235	228 (↓1)	226	227
6	306	307	290 (↓5)	300	296
12	370	367	340 (↓8)	357	340 (↓8)
13	380	377	357 (↓6)	---	333 (↓12)
Overall (Weeks 0-12) Gain	255	249	221* (↓13)	245	222 (↓13)***

a Data were obtained from page 30, Table 2 on page 45, and Appendix 3 on pages 81-88 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n = 28

--- Animals were killed prior to body weight measurement during Week 13 (Day 91).

* Significantly different from controls; $p \leq 0.05$

*** Significantly different from controls; $p < 0.001$

C. FOOD CONSUMPTION: Food consumption was slightly decreased (↓1-9%) throughout the study at 1000 mg/kg (Table 3). Although significance for weekly mean food consumption was not denoted in Table 3 on page 46 of the study report, it was stated that these minor decreases were significant. Furthermore in these animals, food consumption for the overall (Weeks 1-13) treatment period was decreased (↓5%; $p \leq 0.05$). In the rats treated with propylthiouracil, food intake was decreased (↓23-24%; significance not denoted) during Weeks 12 and 13, resulting in decreased (↓8%; $p \leq 0.05$) food consumption for the overall study. Food consumption in the 5 mg/kg and phenobarbital groups were comparable to controls throughout the study.

Table 3. Mean food consumption (g) in male rats treated for up to 13 weeks with orthosulfamuron. [a]

Study week	Orthosulfamuron			Phenobarbital	Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg	200 mg/kg
1	158	159	157 (↓1)	155	152
8	161	161	147 (↓9)	156	153
12	152	154	142 (↓7)	150	117 (↓23)
13	148	143	140 (↓5)	136	112 (↓24)
Overall (Weeks 1-13) Gain	155	154	147* (↓5)	150	143* (↓8)

a Data were obtained from page 30 and Table 3 on page 46 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
n = 7 cages for orthosulfamuron groups, 4 cages for the phenobarbital group, and 3 cages for the propylthiouracil group.
* Significantly different from controls; $p \leq 0.05$

D. ACHIEVED INTAKE: Mean achieved test substance intake values for the overall study are included in Table 1 of this DER.

E. THYROID HORMONE CONCENTRATIONS: Mean thyroid hormone concentrations are presented in Table 4. On Day 90, TSH levels were increased at 1000 mg/kg (↑58%) compared to negative controls, with an even greater increase in the phenobarbital group (↑148%). In the text of the study report, it was stated that these increases were statistically significant; however, no symbols or p-values were indicated in the summary tables. There were no other differences in thyroid hormones that could be attributed to treatment.

On Day 30, concentrations of T3 were significantly decreased (↓20-23%; p-value not indicated) in the 5 and 1000 mg/kg groups compared to negative controls (note that a positive control phenobarbital groups was not included at this time point). However, the decrease in T3 was transient, in that concentrations at Day 90 were comparable to controls. Concentrations of T3 and TSH at all other time points and concentrations of rT3 and T4 at all time points were comparable to controls.

Table 4. Mean (± SD) thyroid hormone levels in male rats treated for up to 13 weeks with orthosulfamuron.[a]

Thyroid hormone	Orthosulfamuron			Phenobarbital
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg
Pre-treatment				
rev T3 (nmol/L)	0.13 ± 0.054	0.14 ± 0.036	0.13 ± 0.036	0.13 ± 0.018
T3 total (nmo /L)	1.80 ± 0.354	1.73 ± 0.185	1.77 ± 0.213	1.85 ± 0.251
T4 total (nmo /L)	52 ± 11.4	45 ± 8.5	49 ± 7.7	45 ± 8.3
TSH (ng/mL)	5.8 ± 0.88	6.1 ± 1.37	6.7 ± 1.03	6.1 ± 0.91
Day 30				
rev T3 (nmol/L)	0.24 ± 0.010	0.34 ± 0.105	0.24 ± 0.027	---
T3 total (nmol/L)	1.71 ± 0.416	1.31 ± 0.097* (↓23)	1.37 ± 0.188* (↓20)	---
T4 total (nmol/L)	66 ± 9.0	62 ± 17.2	57 ± 9.8	---
TSH (ng/mL)	14.7 ± 10.62	12.6 ± 2.70	16.2 ± 3.34	---
Day 76				
rev T3 (nmol/L)	---	---	---	0.25 ± 0.075
T3 total (nmol/L)	---	---	---	1.85 ± 0.316
T4 total (nmol/L)	---	---	---	50 ± 5.8
TSH (ng/mL)	---	---	---	7.8 ± 1.19
Day 90				
rev T3 (nmol/L)	0.18 ± 0.035	0.21 ± 0.057	0.18 ± 0.023	0.19 ± 0.062
T3 total (nmol/L)	1.71 ± 0.252	1.75 ± 0.128	1.78 ± 0.203	1.82 ± 0.179
T4 total (nmol/L)	48 ± 2.9	50 ± 2.6	47 ± 2.6	47 ± 4.1
TSH (ng/mL)	6.7 ± 0.80	7.6 ± 0.94	10.6 ± 3.22* (↑58)	16.6 ± 5.36* (↑148)

a Data were obtained from Table 5 on pages 48-51 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n = 6.

* Reported to be significantly different from controls on pages 31-32 of the study report; p-value not stated

F. THYROXINE CLEARANCE TEST: Time course data for ^{125}I-Thyroxine concentrations in whole blood following a single intravenous dose are included in Table 5a. Pharmacokinetic parameters derived from these data are presented in Table 5b. In the 1000 mg/kg group, the following slight differences (not significant) compared to negative controls were noted: (i) decreased whole blood concentrations of radioactivity (↓4-19%); (ii) decreased AUC_{72} values (↓11%); (iii) increased systemic clearance (↑12%); (iv) increased volume of the central compartment (V_c; ↑12%); and (v) increased volume at steady state (V_{ss}; ↑9%). It was stated that the volume during the terminal phase (V_z) was also increased at this dose; however, these data were not presented in the summary table. Values for k^{-1} and $T_{1/2}$ at this dose were comparable to

negative controls, and the 5 mg/kg group was comparable to negative controls for concentrations of radioactivity throughout the time course and for all derived pharmacokinetic parameters.

The phenobarbital (positive control) group responded as expected with decreased whole blood concentrations of radioactivity (↓29-46%) and AUC_{72} values (↓41%), increased V_c (↑75%) and V_{ss} (↑69%) values, and significantly ($p \leq 0.001$) increased systemic clearance (↑70%).

Table 5a. Mean (± SD) concentrations of radioactivity in whole-blood after administration of single intravenous doses of ^{125}I-Thyroxine in male rats. [a]

Time (Hours)	Control	Orthosulfamuron		Phenobarbital 75 mg/kg/day
		5 mg/kg/day	1000 mg/kg/day	
1	2.05 ± 0.29	2.09 ± 0.36	1.96 ± 0.10 (↓4)	1.21 ± 0.17 (↓41)
2	1.59 ± 0.22	1.62 ± 0.27	1.53 ± 0.09 (↓4)	0.99 ± 0.13 (↓38)
4	1.23 ± 0.17	1.24 ± 0.20	1.17 ± 0.09 (↓5)	0.79 ± 0.12 (↓36)
6	0.99 ± 0.21	1.11 ± 0.17	1.03 ± 0.08	0.67 ± 0.09 (↓32)
8	1.01 ± 0.14	0.96 ± 0.17	0.82 ± 0.06 (↓19)	0.56 ± 0.09 (↓45)
12	0.77 ± 0.10	0.78 ± 0.09	0.64 ± 0.06 (↓17)	0.46 ± 0.07 (↓40)
24	0.52 ± 0.06	0.52 ± 0.08	0.45 ± 0.03 (↓13)	0.28 ± 0.03 (↓46)
36	0.29 ± 0.04	0.31 ± 0.04	0.25 ± 0.03 (↓7)	0.16 ± 0.02 (↓41)
48	0.19 ± 0.02	0.19 ± 0.03	0.17 ± 0.02 (↓11)	0.11 ± 0.01 (↓42)
72	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01 (↓14)	0.05 ± 0.00 (↓29)

a Data were obtained from Table 6 on page 52 of the study report. n = 6. Percent differences from negative controls are included in parentheses.

Table 5b. Mean (± SD) pharmacokinectic parameters of whole-blood radioactivity after administration of single intravenous does of ^{125}I-Thyroxine in male rats. [a]

Parameter	Control	Orthosulfamuron		Phenobarbital
		5 mg/kg/day	1000 mg/kg/day	75 mg.kg.day
AUC_{72} (%dose h/mL)	33.50 ± 3.98	34.10 ± 4.61	29.66 ± 1.95 (↓11)	19.63 ± 2.23 (↓41)
k (hours $^{-1}$)	0.0396 ± 0.0023	0.0400 ± 0.0031	0.0395 ± 0.0034	0.0386 ± 0.0029
$T_{1/2}$ (hours) [b]	17.5	17.3	17.5	18.0
Cl (mL/hour)	2.86 ± 0.35	2.82 ± 0.38	3.21 ± 0.23 (↑12)	4.86 ± 0.51*** (↑70)
V_c (mL)	72.68 ± 11.43	70.98 ± 11.84	81.43 ± 5.49 (↑12)	126.96 ± 20.03 (↑75)
V_{ss} (mL)	66.29 ± 10.74	65.12 ± 11.54	72.20 ± 4.98 (↑9)	111.90 ± 16.91 (↑69)

a Data were obtained from Table 7 on page 53 of the study report. Percent differences from negative controls are included in parentheses.

b Calculated for ln2/(mean rate constant); n = 6.

*** Significantly different from the negative control group at $p \leq 0.001$. Significance level was not denoted in Table 7 on page 53, but was found in the text of the study report on page 32.

G. PERCHLORATE DISCHARGE TEST: There was no evidence of a direct effect of the test substance on the thyroid. In the perchlorate discharge assay, propylthiouracil exhibited the expected results as a positive control for direct effects on the thyroid with the following differences ($p \leq 0.05$) from negative controls (Table 6a): (i) increased thyroid weight (↑154-315%); (ii) decreased radioactivity in the thyroid on a per weight basis (↓75-93%) and as a percent of the total dose (↓24-69%); (iii) increased radioactivity in whole blood on a per weight basis (↑12-46%) and as a percent of the total dose (↑30%; perchlorate group only); and thus (iv) decreased thyroid:whole blood ratio (↓75-95%).

Several significant ($p \leq 0.05$) differences from negative controls were noted in the groups treated with the test substance. However, none of these changes were considered to be toxicologically relevant because the direction of the change was opposite of that which would indicate prevention of iodide organification.

Comparison of the perchlorate and saline subgroups within each group revealed no effects of perchlorate on thyroid weight or radioactive iodide in the thyroid or whole blood in the groups treated with orthosulfamuron or in the negative controls, indicating no blockage of iodide uptake into the thyroid due to the test substance (Table 6b). The only differences between the perchlorate and saline subgroups were noted in the propylthiouracil group, with decreased radioactivity in the thyroid (↓63-68%) and increased radioactivity in whole blood (↑32-38%), resulting in a decreased thyroid:whole blood ratio (↓78%) in the perchlorate subgroups compared to the saline subgroups. These findings confirm that perchlorate, as a competitive inhibitor of iodide transport, displaced the free iodide present in the thyroid. The levels of free iodide were higher in this group because the process of organification of free iodide was inhibited by propylthiouracil.

Table 6a. Mean (± SD) thyroid weights and radioactivity in thyroid and whole blood in male rats treated for 13 weeks with orthosulfamuron. [a]

Parameter	Orthosulfamuron			Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	200 mg/kg
Thyroid weight (g)				
saline	0.0220 ± 0.0099	0.0154 ± 0.0042*(↓30)	0.0200 ± 0.0036	0.0558 ± 0.0124***(↑154)
perchlorate	0.0162 ± 0.0052	0.0128 ± 0.0020*(↓21)	0.0177 ± 0.0031	0.0672 ± 0.0079***(↑315)
Radioactivity				
Thyroid, % dose/g				
saline	430 ± 236	487 ± 128	602 ± 85**(↑40)	107 ± 23***(↓75)
perchlorate	461 ± 133	541 ± 150	651 ± 70**(↑41)	33.9 ± 14.9***(↓93)
Thyroid, total % dose				
saline	7.82 ± 1.73	7.43 ± 2.39	11.9 ± 2.22***(↑52)	5.98 ± 1.64 **(↓24)
perchlorate	7.28 ± 2.39	6.91 ± 2.08	11.6 ± 2.97***(↑59)	2.24 ± 0.92 **(↓69)
Whole blood, % dose/g				
saline	0.272 ± 0.035	0.289 ± 0.054	0.273 ± 0.053	0.305 ± 0.046***(↑12)
perchlorate	0.276 ± 0.018	0.285 ± 0.043	0.288 ± 0.058	0.404 ± 0.043***(↑46)
Whole blood, total % dose				
saline	7.34 ± 0.64	7.69 ± 1.24	6.83 ± 1.16	6.95 ± 0.81* (↓5)
perchlorate	7.40 ± 0.37	7.32 ± 0.78	7.16 ± 0.66	9.59 ± 0.99* (↑30)
Thyroid:whole blood ratio				
saline	1397	1653	2217**(↑59)	349***(↓75)
perchlorate	1616	1843	2287**(↑42)	78***(↓95)

a Data were obtained from Tables 8 and 9 on pages 54-55 of the study report. Percent differences from negative controls (calculated by reviewers) are included in parentheses. n = 6.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

*** Significantly different from controls; $p \leq 0.001$

Table 6b. Mean (± SD) thyroid weights and radioactivity in thyroid and whole blood in male rats treated for 13 weeks with orthosulfamuron. [a]

Parameter	Orthosulfamuron			Propylthiouracil
	0 mg/kg	5 mg/kg	1000 mg/kg	200 mg/kg
Thyroid weight (g)				
saline	0.0220 ± 0.0099	0.0154 ± 0.0042	0.0200 ± 0.0036	0.0558 ± 0.0124
perchlorate	0.0162 ± 0.0052	0.0128 ± 0.0020	0.0177 ± 0.0031	0.0672 ± 0.0079
Radioactivity				
Thyroid, % dose/g				
saline	430 ± 236	487 ± 128	602 ± 85	107 ± 23
perchlorate	461 ± 133	541 ± 150	651 ± 70	33.9 ± 14.9*** (↓68)
Thyroid, total % dose				
saline	7.82 ± 1.73	7.43 ± 2.39	11.9 ± 2.22	5.98 ± 1.64
perchlorate	7.28 ± 2.39	6.91 ± 2.08	11.6 ± 2.97	2.24 ± 0.92*** (↓63)
Whole blood, % dose/g				
saline	0.272 ± 0.035	0.289 ± 0.054	0.273 ± 0.053	0.305 ± 0.046
perchlorate	0.276 ± 0.018	0.285 ± 0.043	0.288 ± 0.058	0.404 ± 0.043** (↑32)
Whole blood, total % dose				
saline	7.34 ± 0.64	7.69 ± 1.24	6.83 ± 1.16	6.95 ± 0.81
perchlorate	7.40 ± 0.37	7.32 ± 0.78	7.16 ± 0.66	9.59 ± 0.99*** (↑38)
Thyroid:whole blood ratio				
saline	1397	1653	2217	349
perchlorate	1616	1843	2287	78*** (↓78)

a Data were obtained from Tables 8 and 9 on pages 54-55 of the study report. Percent differences of perchlorate subgroup from saline subgroup (calculated by reviewers) are included in parentheses. n = 6.
* Perchlorate subgroup significantly different from saline subgroup at $p \leq 0.05$
** Perchlorate subgroup significantly different from saline subgroup at $p \leq 0.01$
*** Perchlorate subgroup significantly different from saline subgroup at $p \leq 0.001$

H. SACRIFICE AND PATHOLOGY

1. Macroscopic pathology - Enlarged thyroids were noted in the 5 mg/kg (1/6 rats) and 1000 mg/kg (2/6 rats) compared to 0/6 negative controls and 4/6 phenobarbital treated rats. No other macroscopic findings could be attributed to treatment.

2. Organ weights - Selected absolute organ weight data are presented in Table 7. Liver weights were increased at 1000 mg/kg (↑10%; $p \leq 0.01$) compared to negative controls, and an increase of an even greater magnitude was observed in the phenobarbital group (↑21%; $p \leq 0.001$). There were no treatment-related effects on thyroid/parathyroid weights or any other organs measured.

Table 7. Mean (± SD) organ weights (g) in male rats treated for 13 weeks with orthosulfamuron [a]

Parameter	Orthosulfamuron			Phenobarbital
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg
Terminal body weight	372.4 ± 40.2	379.6 ± 38.4	336.3 ± 31.1	352.1 ± 14.0
Liver weight	13.44 ± 1.61	12.81 ± 1.80	14.74 ± 1.32** (↑10)	16.28 ± 1.30*** (↑21)
Thyroids + parathyroids	0.022 ± 0.003	0.023 ± 0.006	0.022 ± 0.004	0.025 ± 0.005

a Data were obtained from Table 10 on page 56 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n = 6.
** Significantly different from controls; p≤0.01
*** Significantly different from controls; p≤0.001

3. Microscopic pathology - Increased incidences of centrilobular hepatocyte hypertrophy were observed in the1000 mg/kg (4/6 rats, not significant; minimal severity) and phenobarbital (6/6 rats, p≤0.01; slight severity) groups compared to negative controls (0/6 rats; Table 8). Thus, both the severity and incidence of this finding were increased in the phenobarbital group compared to the 1000 mg/kg group.

Similarly, increased incidences of minimal to slight follicular cell hypertrophy in the thyroid were observed in the1000 mg/kg (4/6 rats, not significant; minimal) and phenobarbital (6/6 rats, p≤0.05; minimal to slight) groups compared to negative controls (1/6 rats). Thus, both the severity and incidence of this finding were increased in the phenobarbital group compared to the 1000 mg/kg group. There were no other microscopic findings which could be attributed to treatment.

Table 8. Microscopic findings in male rats treated for 13 weeks with orthosulfamuron. [a]

Microscopic finding	Orthosulfamuron			Phenobarbital
	0 mg/kg	5 mg/kg	1000 mg/kg	75 mg/kg
Liver, centrilobular hepatocyte hypertrophy				
Minimal	0	0	4	0
Slight	0	0	0	6
Total	0	0	4	6**
Thyroid, follicular cell hypertrophy				
Minimal	1	0	4	4
Slight	0	0	0	2
Total	1	0	4	6*

a Data were obtained from page 37 of the study report. n = 6.
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

3. Liver enzymes - The following liver enzymes were increased ($p \leq 0.05$) at 1000 mg/kg/day compared to negative controls, when expressed per mass of protein and/or per mass of liver (Table 9): (i) microsomal protein (↑32%); (ii) concentration of cytochrome P450 (↑28-69%); (iii) activity of PROD (↑2958-4020%); and (iv) activity of thyroxine UDP-GT (↑64-115%). With the exception of thyroxine UDP-GT, the concentration/activity of each of these liver enzymes was increased ($p \leq 0.01$) in the phenobarbital group to a greater extent than the 1000 mg/kg/day group. There were no other treatment-related effects on liver enzymes.

Table 9. Mean (± SD) liver enzyme concentrations or activities in male rats treated for 13 weeks with orthosulfamuron.[a]

Parameter	Orthosulfamuron (mg/kg)			Phenobarbital
	0	5	1000	75 mg/kg
Microsomal protein (mg/g liver)	11.7 ± 1.3	12.5 ± 1.1	15.4 ± 1.8** (↑32)	18.4 ± 1.7*** (↑57)
Cytochrome P450				
nmoles/mg protein	0.537 ± 0.085	0.556 ± 0.047	0.690 ± 0.049** (↑28)	1.565 ± 0.136*** (↑191)
nmoles/g liver	6.31 ± 1.50	6.92 ± 0.73	10.64 ± 1.81** (↑69)	28.89 ± 4.81*** (↑358)
PROD[b]				
nmoles/min/mg protein	0.012 ± 0.001	0.010 ± 0.001	0.367 ± 0.096* (↑2958)	1.638 ± 0.245** (↑13550)
nmoles/min/g liver	0.138 ± 0.015	0.121 ± 0.014	5.686 ± 1.831** (↑4020)	30.208 ± 5.917*** (↑21790)
Thyroxine UDP-GT[c]				
pmoles/min/mg protein	0.325 ± 0.099	0.269 ± 0.097	0.533 ± 0.096** (↑64)	0.375 ± 0.083 (↑15)
pmoles/min/g liver	3.753 ± 1.090	3.311 ± 1.047	8.070 ± 0.864** (↑115)	6.968 ± 1.990*** (↑86)

a Data were obtained from Tables 13 through 16 on pages 59-62 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n = 6.
b 7-Pentoxyresorufin O-depentylase
c UDP-glucuronosyltransferase
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$
*** Significantly different from controls; $p \leq 0.001$

IV. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: When the test substance was administered in the diet at 1000 mg/kg/day, the levels of TSH, the clearance of ^{125}I-T4, and levels of liver microsomal protein, cytochrome P450, and of UDP-GT activity were greater than in control rats, although these differences were not statistically significant. Furthermore, these parameters were not elevated to the levels observed after administration of phenobarbital, with the exception of thyroxine UDP-GT which was increased higher at 1000 mg/kg/day than in the phenobarbital group. The dose response pattern for these changes is basically consistent with the relationship

between thyroid follicular hypertrophy seen in this present study and in the preceding combined chronic toxicology/carcinogenicity study in rats.

These data, along with the lack of any differences between the negative control and IR5878 treated groups in the perchlorate discharge test, support an indirect mechanism of action on the thyroid. This results from the induction of the UDP-GT responsible for T4 metabolism, leading to increased T4 clearance and thyroid hypertrophy. Although the test substance induces thyroid metabolism, it is not as potent an inducer as phenobarbital when dosed under the conditions of this study. Such a mechanism would not be considered to be relevant to man.

B. REVIEWER COMMENTS: Minor decreases in weekly and overall body weights and food consumption were observed at 1000 mg/kg. In the animals receiving propylthiouracil during Weeks 12 and 13, body weights and food consumption were decreased, although body weight gain was comparable to controls prior to treatment. Additionally, it was stated that clinical signs characteristic of treatment with phenobarbital, including abnormal gait and underactivity were noted in these positive control animals throughout the treatment period.

Potential direct effects of the test substance on the thyroid were examined using the perchlorate discharge assay. The positive control, propylthiouracil, acts as an inhibitor of the thyroid peroxidases responsible for the iodide organification necessary for T3/T4 synthesis. The results from the propylthiouracil group in this study were consistent with this blocked uptake of iodide, including decreased radioactive iodide in the thyroid, increased radioactive iodide in whole blood, and thus, decreased thyroid:whole blood ratio of radioactive iodide. The increased thyroid weights in animals in this assay can be explained by the continued stimulation of TSH on the follicular cells in response to low circulating levels of T4/T3. Several significant ($p \leq 0.05$) differences from negative controls were noted in the groups treated with the test substance. However, none of these changes were considered to be toxicologically relevant because the direction of the change was opposite of that which would indicate prevention of iodide organification.

In contrast to the mechanism of propylthiouracil, perchlorate (ClO_4^-) exerts a direct affect on the thyroid by acting as a competitive inhibitor of iodide transport from the circulation into the follicular cells, thus limiting T3/T4 synthesis. Comparison of the perchlorate and saline subgroups within each group revealed no effects of perchlorate on thyroid weight or radioactive iodide in the thyroid or whole blood in the groups treated with 0, 5, or 1000 mg/kg orthosulfamuron, indicating no blockage of iodide uptake into the thyroid due to the test substance. The only differences between the perchlorate and saline subgroups were noted in the propylthiouracil group, with decreased radioactivity in the thyroid and increased radioactivity in whole blood, resulting in a decreased thyroid:whole blood ratio in the perchlorate subgroups compared to the saline subgroups. These findings confirm that perchlorate, as a competitive inhibitor of iodide transport, displaced the free iodide present in the thyroid. The levels of free

iodide were higher in this group because the process of organification of free iodide was inhibited by propylthiouracil.

In the liver, the following findings were observed. Liver weights were increased at 1000 mg/kg compared to negative controls, and an increase of an even greater magnitude was observed in the phenobarbital group. Minimal centrilobular hepatocyte hypertrophy was observed at 1000 mg/kg (4/6 rats) compared to negative controls (0/6 rats). In the phenobarbital group, both the severity (slight) and incidence (6/6 rats) of this finding was increased compared to the 1000 mg/kg group. Concentrations of microsomal protein and cytochrome P450 and the activities of PROD and UDP-GT were increased ($p \leq 0.05$) at 1000 mg/kg. For each of these liver enzymes, the concentration/activity was increased ($p \leq 0.01$) in the phenobarbital group to a greater extent than the 1000 mg/kg group.

Induction of UDP-GT results in increased elimination of T4, which is supported by the pharmacokinetic data. At 1000 mg/kg/day, ^{125}I-T4 exhibited slightly increased clearance compared to negative controls, indicated by decreased whole blood concentrations of radioactivity and AUC_{72} values and increased systemic clearance, V_c, V_{ss}, and V_z. The positive control group responded similarly, only with a greater magnitude difference from negative controls.

The increased elimination of T4 activates the negative feedback loop to stimulate the pituitary to release TSH. In this study, TSH levels were increased at 1000 mg/kg on Day 90, with an even greater increase in the phenobarbital group. TSH acts on the thyroid follicular cells to produce T3/T4; when this process is prolonged, it results in hypertrophy, hyperplasia, and eventually, tumor formation. Although thyroid/parathyroid weights were comparable to controls in this study, enlarged thyroids were noted in the 5 mg/kg (1/6 rats) and 1000 mg/kg (2/6 rats) compared to 0/6 negative controls and 4/6 phenobarbital treated rats. Minimal follicular cell hypertrophy was noted at 1000 mg/kg (4/6 rats) compared to negative controls (1/6 rats). In the phenobarbital group, minimal to slight follicular cell hypertrophy was observed in all (6/6) rats.

In conclusion, there was no direct effect of the test substance on the thyroid, as indicated by the perchlorate assay. The increased organ weight, hypertrophy, and induction of UDP-GT in the liver and the increased TSH and enlarged thyroids and follicular cell hypertrophy support an indirect effect of the test substance on the thyroid (See Attachment to this DER).

This study is classified as **acceptable/non-guideline**.

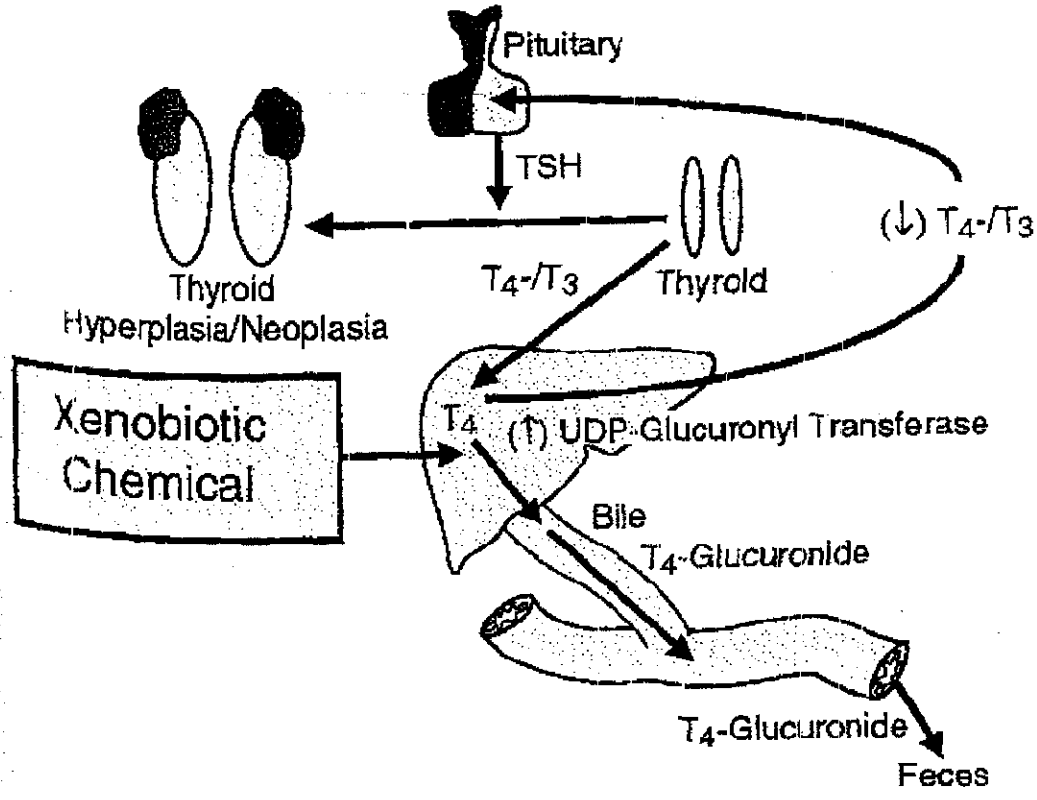
C. **STUDY DEFICIENCIES:** The following deficiencies were noted but do not alter the conclusions of this DER:

- When examining the concentrations of thyroid hormones in the plasma, the phenobarbital (positive control) group was not sampled with the negative controls and treated groups on Day 30, and the orthosulfamuron groups and negative controls were not sampled with the treated groups on Day 76. Thus, the data for Day 76 were of no value, because the validity of the assay could not be evaluated by comparing the positive control with a negative control. The data for Day 30 were helpful in comparing treated groups with a negative control, but were limited in that the decreases could not be compared with a positive control.

- Although the text in the study report mentioned statistically significant findings, significance was not indicated in the summary tables for measurements of thyroid hormones.

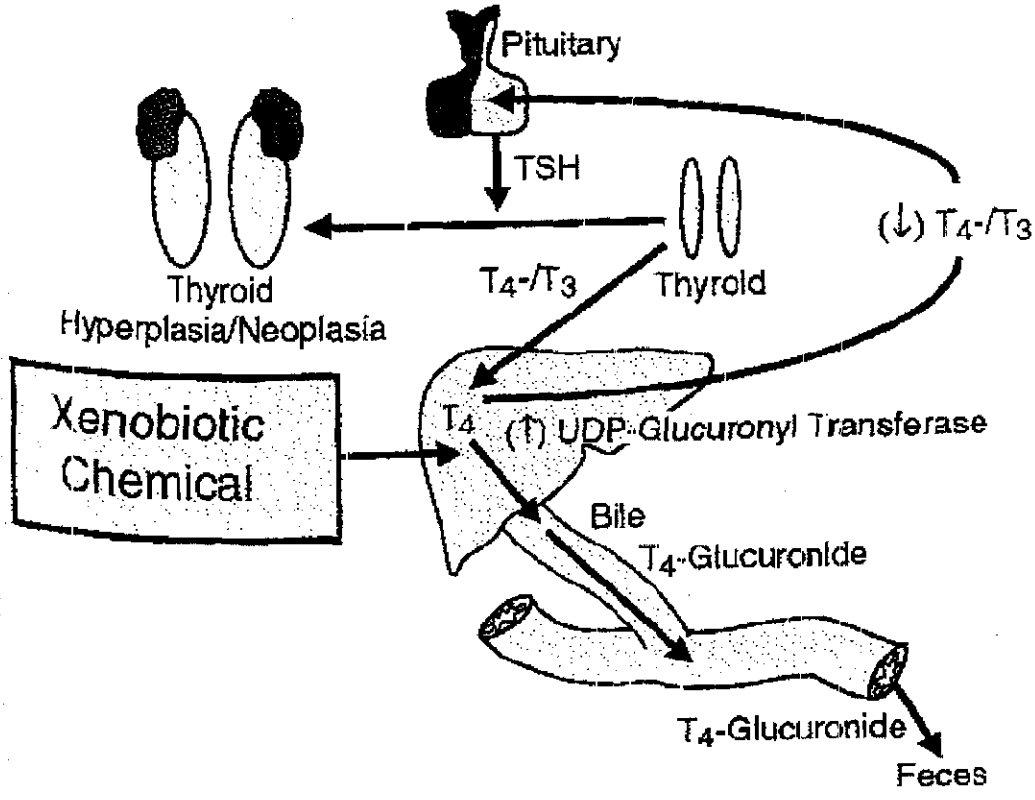
ATTACHMENT

Figure 21-22. Hepatic microsomal enzyme induction by the chronic administration of xenobiotic chemicals, leading to thyroid follicular cell hyperplasia and neoplasia.



Obtained from *Casarett & Doull's Toxicology: The Basic Science of Poisons*. Curtis D. Klassen. 6th edition, p. 729.

Figure 21-22. Hepatic microsomal enzyme induction by the chronic administration of xenobiotic chemicals, leading to thyroid follicular cell hyperplasia and neoplasia.



Obtained from *Casarett & Doull's Toxicology: The Basic Science of Poisons*. Curtis D. Klassen. 6th edition, p. 729.

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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No. 0054345

MEMORANDUM

DATE: August 30, 2006

SUBJECT: **Orthosulfamuron:** Qualitative Risk Assessment Based On Male Han Wistar (HsdBrl Han:Wist) Rat Carcinogenicity Dietary Study

P.C. Code: 108209

TO: Karlyn J. Bailey, Toxicologist
Registration Action Branch 2
Health Effects Division (7509P)

FROM: Lori L. Brunsman, Statistician
Science Information Management Branch
Health Effects Division (7509P)

THROUGH: Jess Rowland, Branch Chief
Science Information Management Branch
Health Effects Division (7509P)

BACKGROUND

A combined chronic toxicity/carcinogenicity study in Han Wistar rats was conducted by Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, for ISAGRO S.p.A., Centro Uffici San Siro, Milano, Italy, and dated July 20, 2004 (Laboratory Project ID No. AGR/131/033063, MRID No. 46578913).

The study design allocated groups of 50 rats per sex to dose levels of 0, 1, 5, 500 or 1000 mg/kg/day (mean achieved doses of 0, 1.0, 5.1, 510.8 or 1026.0 for males; 0, 1.0, 5.2, 520.3 or 1046.5 for females) of Orthosulfamuron for 104 weeks. An additional 20 rats per sex per dose were designated for interim sacrifice at week 52. There were no compound-related tumors in the females so only analyses of the males are presented in this document.

ANALYSES

Survival Analyses

There were no statistically significant incremental changes in mortality with increasing doses of Orthosulfamuron in male rats (Table 1).

Tumor Analyses

Male rats had statistically significant trends in thyroid follicular cell adenomas, and adenomas and carcinomas combined, both at $p < 0.01$. There were statistically significant pair-wise comparisons of the 500 mg/kg/day dose group with the controls for thyroid follicular cell adenomas, and adenomas and carcinomas combined, both at $p < 0.05$. There were also statistically significant pair-wise comparisons of the 1000 mg/kg/day dose group with the controls for thyroid follicular cell adenomas, and adenomas and carcinomas combined, both at $p < 0.01$. The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test for pair-wise comparisons and the *ad hoc* Exact Test for trend since there were no statistically significant trends for mortality (Table 2).

Table 1. Orthosulfamuron – Han Wistar Rat Study (MRID 46578913)

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

	Weeks					
Dose (mg/kg/day)	1-26	27-52	52[i]	53-78	79-106[f]	Total
0	0/70	0/70	20/70	3/50	11/47	14/50 (28)
1	0/70	0/70	20/70	6/50	5/44	11/50 (22)
5	0/70	0/70	20/70	2/50	8/48	10/50 (20)
500	0/70	0/70	20/70	1/50	7/49	8/50 (16)
1000	0/70	0/70	20/70	5/50	6/45	11/50 (22)

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

[i]Interim sacrifice at weeks 52-53.
[f]Final sacrifice at weeks 104-106.

( )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 2. Orthosulfamuron – Han Wistar Rat Study (MRID 46578913)

Male Thyroid Follicular Cell Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (mg/kg/day)				
	0	1	5	500	1000
Adenomas (%)	1/50 (2)	2/50 (4)	1/50 (2)	7[a]/50 (14)	10/49 (20)
p =	0.00006**	0.50000	0.75253	0.02972*	0.00349**
Carcinomas (%)	0/50 (0)	1/50 (2)	1[b]/50 (2)	0/50 (0)	0/49 (0)
p =	0.3223	0.5000	0.5000	1.0000	1.0000
Combined (%)	1/50 (2)	3/50 (6)	2/50 (4)	7/50 (14)	10/49 (20)
p =	0.00030**	0.30865	0.50000	0.02972*	0.00349**

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 70, dose 500 mg/kg/day.
[b]First carcinoma observed at week 91, dose 5 mg/kg/day.

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 $p < 0.01$.

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TUMORS

9/20/09 CARC MTG - ORTHOSULFAMURON

RAT - THYROID FOLLICULAR CELL TUMORS - MALES

TREATMENT-RELATED? Yes, 500+1000

DOSE	0	1	5	500	1000 MKD	H.C
Adenomas	1/50**	2/50	1/50	7/50*	10/49**	0 - 12.5% } LAB
%	2	4	2	14	20	7.51% }
						1.67-12.73% - CRL
CARCINOMAS	0/50	1/50	1/50	0/50	0/49	1.67 - 3.64% CRL
%	0	2	2	0	0	
COMBINED	1/50**	3/50	2/50	7/50*	10/49**	
%	2	6	4	14	20	

- 7 HC
- Benign

Female RATS — NO TREATMENT-RELATED TUMORS

Mice - Both sexes

Adequacy of Dosing:

RATS: Adequate, not excessive (limit dose)
BW, good survival

Mice: Adequate, not excessive
limit dose

HED Cancer Assessment Review Committee Meeting

ORTHOSULFAMURON
September 20, 2006

CARC Members	Signature
Lori Brunsman, Statistician	
William Burnam, Chair	
Marion Copley	
Vicki Dellarco	
Kit Farwell	
Abdallah Khasawinah	
Jessica Kidwell, Executive Secretary	
Nancy McCarroll	
Tim McMahon	
John Pletcher, Consulting Pathologist	
Esther Rinde	
Jess Rowland	
Linda Taylor	
Yin-Tak Woo	

Other Attendees	Signature
Karlyn Bailey (presenter)	
Kevin Crofton (RTP)	Conference call

13544

R139258

Chemical: Orthosulfamuron

PC Code:
108209

HED File Code: 21210 CARC Briefing Package
Memo Date: 9/7/2006
File ID: 00000000
Accession #: 000-00-0115

HED Records Reference Center
1/31/2007