UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

DATE: February 14, 2007

SUBJECT: Orthosulfamuron (IR5878) Toxicology Data Evaluation Records.
PC Code: 108209 DP Barcode: D330824 TXR #: 0053612

TO: James Tompkins, RM 25
Herbicide Branch
Registration Division

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

THRU: Richard Loranger, Branch Senior Scientist
Registration Action Branch 2
Health Effects Division (7509 P)

ACTION REQUESTED: The Health Effects Division (HED) was asked to review the submitted toxicology studies conducted with the new active ingredient (a.i.), orthosulfamuron (IR5878). The Data Evaluation Records (DERs) are included for the MRIDs listed in Table 1. DERS for previously reviewed orthosulfamuron subchronic and developmental toxicity studies are also available (TXR # 0052629). The acute toxicity studies were reviewed by the Registration Division (RD).

BACKGROUND: Orthosulfamuron, is a systemic herbicide belonging to the sulfamoylurea class of chemicals. The registrant, Isagro S.p.A. is proposing this new active ingredient for control of various weeds 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.

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Table 1. List of Orthosulfamuron Toxicology Studies

Study Type	MRID #	Studies included in review	Comments
870.3200 28 Day Dermal	46578911	N/A	New DER
870.3800 2-Generation Reproduction	46219033	N/A	Updated Executive Summary Previous TXR# 0052629
870.4100 Chronic Dog	46578987	N/A	New DER
870.4200 Carcinogenicity	46578912	N/A	New DER
870.4300 Combined Chronic/Carcinogenicity	46578913	N/A	New DER
870.5100 Bacterial Gene Mutation (metabolite-IR8181)	46578923	N/A	New DER
870.5100 Bacterial Gene Mutation (metabolite-IR7825)	46578916	N/A	New DER
870.5100 Bacterial Gene Mutation (metabolite-IR7863)	46578919	N/A	New DER
870.5300 Mammalian Gene Mutation (metabolite-IR8181)	46578925	N/A	New DER
870.5300 Mammalian Gene Mutation (metabolite-IR7863)	46578920	N/A	New DER
870.5300 Mammalian Gene Mutation (metabolite-IR7825)	46578914	N/A	New DER
870.5375 Structural Chromosome Aberration (metabolite-IR8181)	46578924	N/A	New DER
870.5375 Structural Chromosome Aberration (metabolite- IR7825)	46578917	N/A	New DER
870.5375 Structural Chromosome Aberration (metabolite-IR7863)	46578921	N/A	New DER
870.5395 *In Vivo* Mammalian Cytogenetics (metabolite-IR8181)	46578926	N/A	New DER
870.7485 General Metabolism	46578905 - 46578910	N/A	New DER
NonGuideline- Potential Effects on Thyroid Function in Rats	46578927	N/A	New DER

DATA EVALUATION RECORD

IR5878 (ORTHOSULFAMURON)

Study Type: §82-2; 28-Day Dermal Toxicity in Rats

Work Assignment No. 3-01-82 B (MRID 46578911)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
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Prepared by
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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, M.S. **Signature:** KJB
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 11/2/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PVShah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 11/8/07

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity - Rat; OPPTS 870.3200 [§82-2]; OECD 410.

PC CODE: 108209 **DP BARCODE:** D330824
TXR#: 0053612

TEST MATERIAL (PURITY): IR5878 (Orthosulfamuron; 98.56% a.i.)

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

CITATION: Chapman, M.J. (2004) IR5878: Toxicity study by dermal administration to CD rats for 4 weeks. Huntingdon Life Sciences Ltd., Cambridgeshire, UK. Laboratory Project ID: IGA/036, January 22, 2004. MRID 46578911. Unpublished.

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

EXECUTIVE SUMMARY - In a 28-day dermal toxicity study (MRID 46578911), Orthosulfamuron (IR5878; 98.56% a.i., Batch #: G009/02) was moistened with deionized water and applied by semi-occlusive dressing to the shaved intact skin of 10 Sprague-Dawley rats/sex/dose at dose levels of 0 or 1000 mg/kg bw/day (limit dose), 6 hours/day for 7 days/week during a 4-week period.

No adverse compound-related effects were observed in mortality, clinical signs, dermal effects, body weight, body weight gain, food consumption, food conversion efficiency, hematology, clinical chemistry, ophthalmology, absolute or relative organ weights, or gross and histological pathology.

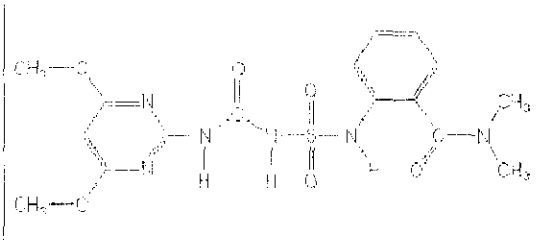
The LOAEL was not determined. The NOAEL is 1000 mg/kg/day (limit dose).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 28-day dermal toxicity study in rats. A LOAEL was not observed; however, the compound was tested at the limit dose.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**		IR5878
	Description:	White powder
	Batch #:	G009/02
	Purity (w/w):	98.56% a.i.
	Stability of compound:	The compound was shown to be stable in deionized water for up to 2 hours at room temperature and up to 8 days at 4EC.
	CAS #:	213464-77-8
	Structure:	

2. **Vehicle** - The compound was moistened with water obtained by reverse osmosis.

3. **Test animals**		
	Species:	Rat
	Strain:	Crl:CD® (SD)IGS BR
	Age and weight at Day 0:	Approximately 9-10 weeks: 321-367 g males; 213-238 g females
	Source:	Charles River (UK) Ltd. (Margate, Kent, England)
	Housing:	Individually in polycarbonate cages with stainless steel mesh lids.
	Diet:	Rat and Mouse No. 1 Maintenance Diet (Special Diets Services Ltd, Witham, Essex, UK), *ad libitum*; except for overnight prior to blood collection.
	Water:	Tap water, *ad libitum*
	Environmental conditions	
	Temperature:	19-23EC
	Humidity:	40-70%
	Air changes:	At least 15/hr
	Photoperiod:	12 hrs light/12 hrs dark
	Acclimation period:	13 days

B. STUDY DESIGN

1. **In-life dates** - Start: 08/19/2003 End: 09/16/2003

2. Animal assignment - Animals were randomly assigned to the test groups noted in Table 1. Cages were rotated around the room each week to minimize any possible spatial gradient of environmental influences.

Table 1. Study design [a]

Dose (mg/kg/day)	Dosing Volume (mL/kg/day)	# of Animals (M/F)
0	2	10/10
1000	2	10/10

a Data were obtained from pages 12 and 13 of the study report.

3. Dose selection rationale – The doses in the study (0 and 1000 mg/kg/day) were selected in conjunction with the sponsor with reference to previous work in rodents that showed IR5878 has low toxicity. Consequently, the limit dose was set to 1000 mg/kg/day.

4. Preparation of dose formulations - The test material formulations were prepared at up to 4 days prior to use and were stored at approximately 4°C. The appropriate amount of the test material was ground using a mortar and pestle and a small amount of deionized water was added to form a viscous paste. The remaining volume of water was added stepwise until the final volume (2 mL/kg) was obtained. The formulations were then mixed with a high shear mixer for approximately 10 seconds. Prior to initiation of treatment, samples were taken from the 1000 mg/kg/day formulations to determine homogeneity and stability. Samples were taken from each formulation prepared for administration in Weeks 1 and 4 to determine achieved concentration.

Results

Homogeneity (range as % C.V.): 0.6-3.25%

Stability (% of time 0):

After storage for 2 hours at room temperature: 101.1%
After storage for 8 days at 4EC: 113.6%

Concentration (% *nominal*): 105.4-110.8

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

5. Preparation and treatment of animal skin - Approximately 24 hours prior to the first application and as needed thereafter, the fur of each test animal was clipped from the back and flanks of the trunk (approximately 10% of the total body surface). The test material (2 mL/kg) was placed on a gauze patch (5 x 5 cm = 25 cm^2) and applied to the treatment area. A cotton wool pad was placed over the patch and secured with surgical tape, and a Tubigrip® dressing was wrapped around the trunk of the animal and secured with surgical tape. The applied quantities of the test substance were based on the most recent individual body weights. The dressings were removed after 6-8 hours and the application areas were cleaned with saline and dabbed dry. The animals were exposed to the test material for 6 hours/day for 7 days/week during a 4-week

period. Animals in the control group were treated with deionized water using the same procedure as described for the treated animals.

6. Statistics - Data were analyzed using the following statistical procedures. Significance for pair-wise comparisons is denoted in the study report and DER at $p<0.05$ and $p<0.01$.

Parameter	Statistical method
Categorical data (including pathology findings)	The proportion of animals was analyzed using Fisher's Exact test for each group versus the control.
Body weight gains and organ weights	If Bartlett's test was significant, a Behrens-Fisher test was used, otherwise a Dunnett's test was used.
Continuous clinical pathology data	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 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 and square-root transformations were applied. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for monotonic trend was applied. If the H1 test was significant, Steel's test was performed instead.

The reviewers consider the statistical analyses appropriate.

C. METHODS

1. Observations

a. Cageside observations - Animals were observed at least twice daily for signs of mortality, moribundity, and clinical signs of toxicity.

b. Clinical examinations - Detailed clinical examinations were conducted daily during Week 1 and twice weekly thereafter at the following times relative to dosing: immediately prior to dosing, immediately following dosing upon returning to the home-cage, on completion of dosing for each group, between 1-2 hours post-dosing, and as late as possible in the working day. Examinations for dermal irritation (erythema and edema) were performed daily using the Draize

method before each administration and at bandage removal. Additionally, a more detailed physical examination was performed weekly.

c. Neurological evaluations - Neurological evaluations were not performed in this study.

2. Body weight - Animals were weighed just prior to initiation of treatment (Week 0), weekly throughout the study, and at necropsy.

3. Food consumption and efficiency - Individual food consumption was recorded weekly, and mean food consumption was reported as g/animal/week. Food conversion efficiency (%) was calculated weekly based on food consumption and body weight gains.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all animals prior to initiation of treatment and during Week 4. The eyes were examined using a 0.5% tropicamide ophthalmic solution and an indirect ophthalmoscope.

5. Hematology and clinical chemistry - Blood for hematology and clinical chemistry tests was collected from the retro-orbital sinus under light isoflurane anesthesia during Week 4 prior to dosing. The following 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 corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
-	Platelet count*	-	*Reticulocyte count*
-	Blood clotting measurements*	X	Erythrocyte morphology
X	(Activated Partial Thromboplastin time)		
	(Clotting time 'Hepato-Quick')		
X	(Prothrombin time)		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
- *Not examined*

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
-	Magnesium	X	Urea*
X	Inorganic phosphorus	X	Total cholesterol*
X	Potassium* (K)	-	Globulins
X	Sodium* (Na)	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (AP)*	X	Total protein*
-	Cholinesterase (ChE)	-	Triglycerides
-	Creatine phosphokinase	-	Serum protein electrophoresis
-	Lactic acid dehydrogenase (LDH)	X	A/G Ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
-	Glutamate dehydrogenase		
-	Sorbitol dehydrogenase*		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
- Not examined

6. **Sacrifice and pathology** - All animals were sacrificed via CO_2 asphyxiation and subjected to a gross pathological examination. The following CHECKED (X) tissues were collected from all animals. In addition, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
-	Tongue	X	Aorta, thoracic*	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*
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*+		OTHER
-	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
-	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin* (treated & untreated)
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	-	Oviducts		
X	Lung*	XX	Uterus w/cervix*+		
X	Nasal cavity*	X	Mammary area*		
X	Pharynx*				
X	Larynx*				

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
- Organ weights required.
+ Weighed together
- Not examined

The eyes were fixed in Davidson's solution. The testes and epididymides were initially fixed in Bouin's solution prior to transfer to 70% industrial methylated spirits. All other organs and tissues collected from all animals, as well as all gross lesions, were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, except for the testes which were stained with a standard periodic acid/Schiff method. Except for the head (including nasal cavity, paranasal sinuses, and nasopharynx), the tissues collected from the control and 1000 mg/kg animals were examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs** - No treatment-related clinical signs were observed in any animal in either sex.

2. **Mortality** - All animals survived to scheduled necropsy.

3. ***Dermal irritation*** - No treatment-related dermal effects were observed in any animal in either sex. In the 1000 mg/kg/day females, very slight to well defined erythema and exfoliation noted was observed in 1-4 animals, but the majority of the observations were in one animal (# 39) and were transient in nature. In the 1000 mg/kg/day males, exfoliation and eschar formation were observed in only one animal (# 19) on Days 15-16 and 18-20. These findings were considered unrelated to treatment.

B. BODY WEIGHT AND WEIGHT GAIN - No adverse treatment-related effects on body weight or body weight gain were observed in either sex. An increase in overall (Weeks 0-4) body weight gain was observed in the 1000 mg/kg females (826%; Table 2); however, this finding was not considered adverse.

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

Parameter	Interval (Weeks)	Dose (mg/kg/day)			
		0	1000	0	1000
		Males		Females	
Body weight	0	342±16.0	344±15.1	228±7.5	223±5.5
	4	396±29.4	399±22.6	259±15.9	262±15.8
Body Weight Gain	0-4	54	54	31	39 (826)

a Data were obtained from Table 1, page 31 of the study report, n=10. Numbers presented parenthetically (calculated by the reviewers) represent percent difference from controls.

C. FOOD CONSUMPTION - No treatment-related effects on mean food intake (g/animal/week) or food conversion efficiency (%) were observed in either sex. Food efficiency was increased by 24% compared to controls in the 1000 mg/kg/day females; however, this finding was not considered to be adverse

D. OPHTHALMOSCOPIC EXAMINATION - No treatment-related ocular lesions were observed in any control or 1000 mg/kg/day animals at Week 4.

E. BLOOD ANALYSES

1. Hematology - No treatment-related effects were observed in any hematological parameter in either sex. In the 1000 mg/kg/day males, increased lymphocytes (820%; $p<0.05$) were observed compared to controls; however, this finding was considered incidental because there were no significant differences in the total numbers of leukocytes or any other hematological parameter.

2. Clinical chemistry - No treatment-related effects were observed in any clinical chemistry parameter in either sex. At 1000 mg/kg/day, minor decreases ($p<0.05$) were noted in potassium levels in the males (95%) and sodium levels in the females (91%), but were not considered to be toxicologically significant.

F. SACRIFICE AND PATHOLOGY

1. Organ weight - No treatment-related effects on absolute or relative (to body) organ weights were observed in either sex. In the 1000 mg/kg/day males, minor increases were observed in absolute (812%; not significant) and relative (812%; $p<0.05$) spleen weight. Due to a lack of corroborative hematological (red blood cell indices) or histopathological effects, these findings were considered unrelated to treatment.

2. Gross pathology - No adverse treatment-related gross lesions were observed in any animal in either sex. Increased incidence of pale areas were observed in the livers of the 1000 mg/kg/day males (6/10 treated vs 1/10 controls). Due to a lack of corroborative histopathological effects in the liver and any other finding indicative of a hepatic effect, these findings were not considered to be toxicologically important.

3. Microscopic pathology - No treatment-related histopathological findings were observed in any animal in either sex. Any lesions noted occurred with similar frequency in the treated and control animals.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that under the conditions of this study, IR 5878 applied at 1000 mg/kg/day (limit dose) did not induce any clear dermal response at the application site or adverse treatment-related systemic effects in rats dermally exposed for 6 hrs/day, 7 days/week for up to 4 weeks.

B. REVIEWER COMMENTS - No adverse compound-related effects were observed in mortality, clinical signs, dermal effects, body weight, body weight gain, food consumption, food conversion efficiency, hematology, clinical chemistry, ophthalmology, absolute or relative organ weights, or gross and microscopic pathology.

The LOAEL was not determined. The NOAEL is 1000 mg/kg/day (limit dose).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 28-day dermal toxicity study in rats. A LOAEL was not observed; however, the compound was tested at the limit dose.

C. STUDY DEFICIENCIES - None

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** [signature]
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 11/12/07
EPA Reviewer: Alan Levy, Ph.D. **Signature:** Alan C. Levy
Registration Action Branch 2, Health Effects Division (7509P) **Date:** JAN. 12, 2007

TXR#: 0053612

UPDATED EXECUTIVE SUMMARY for DATA EVALUATION RECORD, Previous TXR # 0052629

STUDY TYPE: Reproduction and Fertility Effects Study - Rat; OPPTS 870.3800 [§83-4]; OECD 416.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): Orthosulfamuron (IR5878; 98.5% a.i.)

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

CITATION: Marburger, A. (2003) IR5878: two-generation reproduction toxicity study in the rat. RCC Ltd. Toxicology and Environmental Chemistry & Pharmanalytics, CH-4452 Itingen, Switzerland. Laboratory Study No. 841291. October 17, 2003. MRID 46219033. Unpublished.

Marburger, A., C. Knuppe, and D. Nehrbass (2002) IR5878: Preliminary reproduction toxicity study in the rat. RCC Ltd. Toxicology and Environmental Chemistry & Pharmanalytics, CH-4452 Itingen, Switzerland. Laboratory Study No. 841176. July 15, 2002. MRID 46260106. Unpublished.

SPONSOR: ISAGRO S.p.A., Centro Uffici San Siro, Fabbricato D - ala 3, Via Caldera, 21, 1-20153 Milano

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID 46219033), IR5878 (98.5% a.i., batch # G009/02) was administered in the diet to 24 male and 24 female HanBrl:WIST (SPF) rats/dose at concentrations of 0, 350, 1400, or 5600 ppm throughout the study for males and during premating, mating, and gestation for females. Concentrations of the test article in the diet were reduced to 0, 225, 900, and 3600 ppm during lactation of the F_0 and F_1 dams. Dietary concentrations were selected on the basis of a preliminary one-generation study (MRID 46260106). One litter was produced in each generation. Premating doses for the F_0 parental animals were 0, 38.1, 145.3, and 634.5 mg/kg/day, respectively, for males and 0, 47.8, 205.0, and 764.9 mg/kg/day, respectively, for females. Premating doses for the treated F_1 parental animals were 0, 41.2, 164.7, and 711.9 mg/kg/day, respectively, for males and 0, 52.4, 214.8, and 888.3 mg/kg/day, respectively, for females. Due to unrealistically high food

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consumption data, the calculated test item intake in this study is extremely high. This could possibly be due to food spillage of the powdered diet. For a more realistic estimation of the actual test item intake, the study author used values from a concurrent 2-generation reproduction study (RCC 842842) performed with the same rat strain and same diet (although pelleted) to calculate achieved doses. At concentrations of 0, 350, 1400, 5600 ppm, the achieved premating doses for the F_0 parental animals were 0, 22.2, 88.6, and 354.5 mg/kg/day, respectively, for males and 0, 25.6, 102.2, and 408.89 mg/kg/day, respectively, for females. Achieved premating doses for the treated F_1 parental animals were 0, 24.3, 97.0, and 388.1 mg/kg/day, respectively, for males and 0, 27.8, 111.2, and 444.6 mg/kg/day, respectively, for females. These values are comparable to those calculated using a generic conversion (20:1) of ppm to mg/kg/day. F_0 and F_1 parental animals were administered test or control diet for 70 or 120 days, respectively, prior to mating, throughout mating, gestation, and lactation, and until sacrifice. At approximately six weeks of age, the F_1 parental animals were subjected to a Functional Observational Battery (FOB) and tested for motor activity.

No treatment-related deaths or clinical signs of toxicity were observed in any group during the study. No treatment-related adverse effects on body weight, body weight gain, or food consumption were observed during the premating interval in adult animals of either generation or in F_0 and F_1 dams during gestation and lactation. No abnormalities were observed in the FOB. High-dose F_1 males had significantly reduced ($p \leq 0.01$) motor activity at all time points and in total activity. No differences in motor activity were observed between treated and control females.

No treatment-related effects on organ weight and no gross lesions were seen in adults of either generation. High-dose females of both generations had increased incidences and severity of kidney lesions. Tubular mineralization was observed at similar incidence in all groups but the mean severity grade was 1.8 in both the high-dose F_0 and F_1 females compared with 1.0-1.4 in the controls. The incidence (severity) of urothelial hyperplasia was 11/24 (1.3) in both F_0 and F_1 high-dose females compared with 3-4/24 (1.0) for the control groups.

The parental systemic LOAEL was 5600/3600 ppm for males and females (354.5-388.1 and 408.89-444.6 mg/kg/day, respectively), based on kidney lesions in F_0 and F_1 females and decreased motor activity in F_1 males. The parental systemic NOAEL was 1400/900 ppm in males (88.6-97.0 mg/kg/day) and females (102.2-111.2 mg/kg/day).

No treatment-related differences in estrus cycle or estrus cycle length were observed between the treated and control females of either generation and no differences in testicular and epididymal sperm counts, sperm motility, or sperm morphology were found between the treated and control males of either generation. Mating, fertility, and gestation indices, number of days to mating, and gestation length were not affected by treatment of either generation during litter production. Mean day of sexual maturation and body weight at attainment were not affected in the F_1 animals.

The reproductive toxicity LOAEL was not identified. The reproductive toxicity NOAEL is 5600/3600 ppm for males and females (354.5-388.1 and 408.89-444.6 mg/kg/day, respectively).

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No treatment-related differences in estrus cycle or estrus cycle length were observed between the treated and control females of either generation and no differences in testicular and epididymal sperm counts, sperm motility, or sperm morphology were found between the treated and control males of either generation. Mating, fertility, and gestation indices, number of days to mating, and gestation length were not affected by treatment of either generation during litter production. Mean day of sexual maturation and body weight at attainment were not affected in the F_1 animals.

The offspring toxicity LOAEL was not identified. The offspring NOAEL is 5600/3600 ppm for males and females (354.5-388.1 and 408.89-444.6 mg/kg/day, respectively).

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a two-generation reproduction study (OPPTS 870.3800; OECD 416) in rats.

Comments: The DER has been updated to include values from a concurrent 2-generation reproduction (same rat strain and pelleted diet) study that provides a more realistic estimate of test item intake. The calculated (mg/kg/day) theoretical test item intake (based on body weight and food consumption) for this study is extremely high based on unrealistic food consumption data.

DATA EVALUATION RECORD

IR5878 (ORTHOSULFAMURON)

Study Type: §83-1b, Chronic Toxicity Study in Dogs

Work Assignment No. 3-1-82 P (MRID 46578987)

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DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity in Dogs (capsule); OPPTS 870.4100b [§83-1b]; OECD 452.

PC CODE: 108209 **DP BARCODE:** D330824
TXR#: 0053612

TEST MATERIAL (PURITY): IR5878 (Orthosulfamuron; 98.56% a.i.)

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

CITATION: Whittaker, R. (2004) IR5878: toxicity study by oral capsule administration to Beagle dogs for 52 weeks. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project ID.: IGA/022/033476, January 12, 2004. MRID 46578987. Unpublished.

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

EXECUTIVE SUMMARY - In a chronic toxicity study (MRID 46578987), IR5878 (Orthosulfamuron; 98.56% a.i.; Batch No.: G009/02) was administered to 4 beagle dogs/sex/dose in capsules for 52 weeks at doses of 0, 75, 300, or 1000 mg/kg/day.

No treatment-related effects were observed on ophthalmoscopic examination.

One 1000 mg/kg/day female (#992) was treated for 32 weeks, off treatment for 8 weeks, and then sacrificed at Week 40 due to significant weight loss and increases in alkaline phosphatase (ALP) and alanine aminotransferase (ALT) values. The amount of change noted in these enzyme levels was indicative of liver dysfunction which, if continued, would have been considered detrimental to the animal's health. Treatment-related effects were first observed at Week 26. Pigmented Kupffer cells in the liver was the only treatment-related histopathological finding in this animal. All other animals survived until sacrificed.

There were also treatment-related occurrences (# total occurrences/52 weeks) of loss of appetite (7-57) and pale feces (14-16) in 3/4 males and all females. In the 300 mg/kg/day males, body weights were decreased (not significant [NS]) throughout treatment (Weeks 1-52; decr. 6-11%), resulting in decreased (NS) overall (Weeks 0-52) body weight gains (decr. 20%). Additionally,

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at 1000 mg/kg/day, body weights were decreased (NS) in both sexes throughout treatment (2-22%), resulting in decreased overall body weight gains (28-35%; p =< 0.05 for males, NS for females). Treatment-related decreases (8%; p =< 0.05) in overall (Weeks 1-52) mean weekly food consumption were observed in the 1000 mg/kg/day females when compared with controls.

At 1000 mg/kg/day, platelets were increased (p =< 0.05) in males throughout the study (Weeks 0-52; 41-46%), and in females only at Week 26 (38%). Additionally at this dose, decreased (p =< 0.05) hematocrit, hemoglobin, and red blood cell values (12-14%) were observed in the males at Week 52. In the females, decreases (p =< 0.05) in hematocrit and hemoglobin were noted beginning on Week 26 (11-17%), and decreased (p =< 0.05) red blood cell counts were observed throughout the study (11-20%). Increased (NS, except p =< 0.05 in the females at Week 52) reticulocytes were also noted in females beginning on Week 26 (↑54-100%). The changes in red blood cell parameters were indicative of a minor anemia. The observed increases in reticulocytes were associated with an increased cellularity of the bone marrow (sternum) in response to the anemic condition observed in the dogs.

At >= 300 mg/kg/day, alkaline phosphatase was increased throughout the study in both males and females (88-637%); p =< 0.05, except for 300 mg/kg/day on Weeks 13 and 26. Creatinine (>=300 mg/kg/day)was decreased (p =< 0.05) in the males 12-21%). Additionally, at 1000 mg/kg/day, decreases (p =< 0.05) in albumin (17-27%) and total protein (11-19%) were observed in both sexes throughout the study. Except for the effects on ALP levels, most of the effects on clinical chemistry plateaued by Week 26 and remained at that level until study termination.

In females only, treatment-related increases (35%; p =< 0.01) in mean absolute liver weights were observed at 300 mg/kg/day. Liver weights were also significantly (p =< 0.01) increased in the 1000 mg/kg/day males (50%) and females (43%). Upon macroscopic examination, treatment-related hypertrophy of the liver was observed in 1/4 males and 2/4 females at 300 mg/kg/day and in 3/4 males and 3/3 females at 1000 mg/kg/day.

The following microscopic findings were observed in the liver at >= 300 mg/kg/day compared to 0 controls except where noted: i) minimal to moderate hepatocyte hypertrophy (p =< 0.05) in all animals, increasing in severity with dose; ii) slight to moderate pigmented Kupffer cells in 2/4 dogs (both sexes) at 300 mg/kg/day, and in 3/4 males and 2/3 females at 1000 mg/kg/day; iii) minimal to slight centrilobular vascular/perivascular inflammatory cell infiltration in 3/4 males at both doses compared to 1/4 controls; and iv) minimal to slight periportal inflammatory cell infiltration in 2/4 males and 1/4 females at 300 mg/kg/day and 1/4 males and 1/4 females at 1000 mg/kg/day. Additionally at >= 300 mg/kg/day, minimal to slight hemosiderosis was noted in the spleen of the males at 300 (2/4) and 1000 (3/4) mg/kg/day, respectively, compared to 0 controls. In females, the incidence and severity of hemosiderosis were similar between control and treated groups. The reviewers note that hemosiderosis is common in diseases such as hemolytic and pernicious anemias, and chronic infection. Therefore, this effect would be expected in response to a decrease in RBC counts as reported in hematology.

(minimal to slight severity). However, the reviewers were uncertain regarding the toxicological significance of this effect. Additionally at this dose, minimal to slight increased cellularity of the bone marrow (sternum) was also observed in 3/3 females compared to 1/4 controls (none in males). The Sponsor noted that the increased cellularity of the bone marrow was a response to the anemic condition of these dogs noted in hematology, and that the changes in hematology were not caused by direct effects of the test compound on the bone marrow.

The LOAEL is 300 mg/kg/day based on changes observed in body weights, body weight gains, clinical signs of toxicity, food consumption (females), hematology and clinical chemistry, increased liver weights, and histopathological changes in the liver, and bone marrow of the sternum. The NOAEL is 75 mg/kg/day.

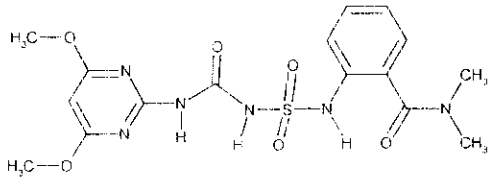
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR5878 (Orthosulfamuron)

Description:	Off white powder
Batch No.:	G009/02
Purity (w/w):	98.56% a.i.
Stability of compound:	Not reported
CAS #:	213464-77-8
Structure:	

2. Vehicle - Gelatin capsules

3. Test animals

Species:	Dog
Strain:	Beagle
Age/mean weights at study initiation:	5 to 6 months old; 7.1-8.9 kg males; 6.6-8.2 kg females
Source:	Harlan Hillcrest Ltd., UK
Housing:	2 of the same sex and dose/pen with underfloor heating: after dog No. 992 was removed from the study (week 32), it's pen-mate was housed with Nos. 996 and 998 for the remainder of the treatment period.
Diet:	Diet A, standard dry pellet diet (Special Diets Services, Ltd.); 400 g/day between 10:00-16:00; dogs fed individually, then returned to group housing [control, low- and mid-dose dogs were offered food for 1-2 hours at about noon. High-dose dogs allowed longer feeding time (amount not stated) and then returned to group housing 2-4 P.M.]
Water:	Tap water, *ad libitum*, except during urine collection
Environmental conditions	
Temperature:	15-24°C
Humidity:	Not reported
Air changes:	Approximately 12/hour
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	At least 4 weeks

B. STUDY DESIGN

1. In life dates - Start: 06/13/02 End: 06/16/03

2. Animal assignment - The dogs were randomly assigned, stratified by body weight, to the test groups shown in Table 1.

Table 1. Study design [a]

Test Group	Dose (mg/kg/day)	# of Animals/Sex
Control	0	4
Low	75	4
Mid	300	4
High	1000	4

a Data were obtained from page 13 of the Study Report.

3. Dose-selection rationale - The Sponsor stated that the dose-selection rationale was based on a 13-week oral toxicity study in Beagle dogs (not provided) where dosages of up to 1000 mg/kg/day were well tolerated. No additional information was provided.

4. Treatment preparation and analysis - Appropriate amounts of test substance, adjusted on the basis of each individual animal's most recently recorded body weight, were weighed in gelatin capsules and stored protected from light at room temperature. The capsules were prepared up to 8 days in advance. Control animals received empty capsules of the same number and size as the 1000 mg/kg/day group, up to 4 capsules/day. The capsules were administered daily one hour before feeding, for 12 months.

5. Statistics - Data were subjected to the statistical procedures listed below. Significance at $p<0.05$ and 0.01 were reported.

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
Organ weights	Analysis of covariance; if significant at 10%, then the treatment comparisons were made on adjusted group means to allow for differences in body weight.
Clinical pathology, pre-treatment data	Student's 't' test
Body weight, food consumption, organ weight, and 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.

A 1000 mg/kg/day female (# 992) that was removed from the study (week 32) and sacrificed moribund (week 40) prior to study termination was excluded from statistical analysis of body weight gains, food consumption, and organ weights.

Assuming that normality of the data was confirmed before parametric testing, the statistical analyses were considered appropriate.

C. METHODS

1. Observations - Animals were inspected at least twice daily for mortality, moribundity, and injury. Detailed clinical observations were recorded immediately before dosing, 30 minutes to 2 hours after dosing, and as late as possible in the work day. These observations were performed daily in Week 1, twice/week during Weeks 2, 3, and 4, once/week during Weeks 5-13, and then once every 2 weeks thereafter. A complete physical examination was conducted on all animals weekly.

2. Body weight - All animals were weighed before feeding, prior to treatment on the day treatment commenced, weekly during treatment, and on the day of necropsy.

3. Food consumption - Food consumption (g) was measured daily for each animal throughout treatment. Weekly food consumption was reported.

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4. Ophthalmoscopic examination - Ophthalmoscopic examinations were conducted on all animals prior to treatment and at Week 52 (binocular indirect ophthalmoscope).

5. Hematology and clinical chemistry - Blood samples for hematology and clinical chemistry analyses were collected before feeding from all animals prior to treatment, and at Weeks 13, 26, and 52. Additionally for animal no. 992F, hematology samples were taken at Weeks 32, 34, 35, and 40; and clinical chemistry samples were taken at Weeks 32, 34, 35, 36, 37, and 40. Blood smears were prepared for all samples. 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 corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count* (PLT)	X	Reticulocyte count
	Blood clotting measurements*		
X	(Activated partial thromboplastin time)		
-	(Clotting time)		
X	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100
- 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 *
-	Cholinesterase (ChE)	-	Triglycerides
X	Creatine phosphokinase	-	Serum protein electrophores
-	Lactic acid dehydrogenase (LDH)	X	Albumin/Globulin
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyltransferase (GGT)*		
-	Glutamate dehydrogenase		

* Recommended for chronic studies based on Guideline 870.4100
\- Not examined

6. Urinalysis - Urine samples were collected from all animals prior to treatment, and during Weeks 13, 26, and 52. Samples were collected overnight (approximately 16 hours) while each individual animal was in a metabolism cage without food or water. The CHECKED (X) parameters were examined.

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

* Recommended for chronic studies based on Guideline 870.4100
\- Not examined

7. Sacrifice and pathology - All animals (including #992F) were sacrificed via exsanguination under pentobarbitone anesthesia, weighed, and subjected to a gross pathological examination. The following CHECKED (X) tissues were collected and examined microscopically in all animals. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
-	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (with optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroids*[a]
X	Rectum*	X	Urinary bladder*	XX	Thyroid*[a]
XX	Liver*++	XX	Testes*+		OTHER
X	Gall bladder*	XX	Epididymides*+	X	Bone (sternum and femur)
X	Pancreas*	XX	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	XX	Uterus(with cervix)*+	X	All gross lesions and masses*
X	Lung*++	X	Mammary gland*		
X	Nasal structures*	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Required for chronic studies based on Guideline 870.4100.
\+ Organ weight required in chronic studies.
++ Organ weight required if inhalation route.
a Thyroid and parathyroids were weighed together.
\- Not taken

Samples to be examined microscopically were fixed in 10% neutral buffered formalin (or Davidson's fixative for the eye, and Bouin's fixative for testes and epididymides), prepared routinely, and stained with hematoxylin and eosin. Gross lesions from all animals were also examined. Findings were reported as present or assigned a severity grade.

II. RESULTS

A. OBSERVATIONS

1. Mortality - One 1000 mg/kg/day female (#992) was sacrificed at Week 40 due to significant weight loss and increases in alkaline phosphatase (ALP) and alanine aminotransferase (ALT) values. The severity of change noted in these enzyme levels was indicative of liver dysfunction which would not have been expected to be tolerated if treatment had continued. Treatment-related effects were first observed at Week 26 and lasted to Week 32 when treatment was ended. Pigmented Kupffer cells in the liver was the only treatment-related histopathological finding in this animal. All other animals survived until sacrificed.

2. Clinical signs of toxicity- The following treatment-related clinical signs of toxicity were observed (# total occurrences/52 weeks) in 3/4 males and all females (including the animal terminated at Week 40) at 1000 mg/kg/day: pale feces (14-16) and loss of appetite (7-57) in males and females. These effects were not observed in the controls or remaining treatment groups.

B. BODY WEIGHT AND WEIGHT GAIN - At 300 mg/kg/day, there was a slight decrease (NS) in group mean body weight gain (controls = 6.1 kg, 300 = 4.9; individual dog gains over 52 weeks, controls = 7.1, 6.0, 5.7 and 5.4; 300 = 6.1, 4.6., 4.6 and 4.1). Female weight gains at 300 mg/kg/day were similar to controls (0=7.5, 5.4, 4.3 and 4.3 kg; 300=5.9, 5.1, 4.8 and4.5 kg) over the treatment period. At 1000 mg/kg/day, there were decreases in body weight gain in males (controls = 7.1, 6.0, 5.7 and 5.4; 1000 mg/kg/day = 5.3, 5.1, 3.9 and 3.1) and in females (controls = 5.7, 5.4, 4.3 and 4.3; 1000 mg/kg/day = 4.2, 3.3 and 2.2).

Decreased overall body weight gains were also observed in the 75 mg/kg/day males; individual body weight gains over 52 weeks were: controls = 7.1, 6.0, 5.7 and 5.4 kg; 75 mg/kg/day = 5.9, 5.7, 4.2 and 4.1 kg. Therefore, this finding was not considered adverse. Body weights and body weight gains in the 75 mg/kg/day female group were comparable to the controls.

Table 2. Selected mean body weights and body weight gains (kg) in dogs treated with IR5878 in capsules for up to 1 year .[a]

WEEKS ON STUDY	MALES				FEMALES			
	DOSE (mg/kg/day)				DOSE (mg/kg/day)			
	0	75	300	1000	0	75	300	1000
0 BW	8.0	8.2	7.6	8.0	7.4	7.5	7.5	7.0(↓5)
1 BW	8.5	8.6	8.0(↓6)	8.5	7.8	7.9	7.9	7.2(↓8)
13 BW	12.1	11.6(↓4)	10.9(↓10)	10.4(↓14)	10.5	10.7	10.6	8.5(↓19)
26 BW	13.3	12.5(↓6)	12.0(↓10)	11.5(↓14)	11.6	12.0	11.8	10.0(↓14)
39 BW	14.0	12.8(↓9)	12.4(↓11)	12.1(↓14)	12.2	12.8	12.3	10.2(↓16)
52 BW	14.1	13.2(↓6)	12.5(↓11)	12.3(↓13)	12.3	13.3	12.5	10.2(↓17)
0-13 BWG	4.1	3.4(↓17)	3.3(↓20)	2.4(↓41)	3.1	3.2	3.1	1.5(↓52)
13-26 BWG	1.2	0.9	1.1	1.1	1.1	1.3	1.2	1.5
26-39 BWG	0.7	0.3	0.4	0.6	0.6	0.8	0.5	0.2
39-52 BWG	0.1	0.4	0.1	0.2	0.1	0.5	0.2	0.0
0-52 BWG	6.1	5.0(↓18)	4.9(↓20)	4.3*(↓30)	4.9	5.8	5.0	3.2(↓35)

Data extracted from table 1, pages 44-47.
Number in () % from control (↓ = decrease)
NOTE: No standard deviations provided in the report.

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C. FOOD CONSUMPTION - Treatment-related decreases (↓8%; $p \leq 0.05$) in overall (Weeks 1-52) mean weekly food consumption were observed in the 1000 mg/kg/day females when compared with controls. It was stated that two of the four animals (#992, removed from the study at Week 32, and #994) were fed moistened food to improve palatability of the dry diet. Food consumption in the remaining treatment groups was comparable to controls.

D. OPHTHALMOSCOPIC EXAMINATION - No treatment-related effect was observed during ophthalmoscopic examination.

E. BLOOD ANALYSES

1. Hematology - Hematology data are discussed below and presented in Table 3. At 1000 mg/kg/day, platelets were increased ($p \leq 0.05$) in males throughout the study (Weeks 0-52; ↑41-46%), and in females only at Week 26 (↑38%; Table 3). Additionally at this dose, decreased ($p \leq 0.05$) hematocrit (↓13%), hemoglobin (↓12%), and red blood cell (↓14%) values were observed in the males at Week 52. In the females, decreases ($p \leq 0.05$) in hematocrit (↓11-16%) and hemoglobin (↓14-17%) were noted beginning on Week 26, and decreased ($p \leq 0.05$) red blood cell counts were observed throughout the study (↓11-20%). Increased (NS, except $p \leq 0.05$ in the females at Week 52) reticulocytes were also noted in females beginning on Week 26 (↑54-100%). The changes in red blood cell parameters were indicative of a minor anemia. The observed increases in reticulocytes were associated with an increased cellularity of the bone marrow (sternum) in response to the anemic condition observed in the dogs.

At 300 mg/kg/day, platelets were increased in males throughout the study, reaching statistical significance at Week 26 (↑19%; $p \leq 0.01$), and reticulocytes were increased (NS) in the males beginning on Week 13 (↑49-85%). The values for these parameters were similar to those observed prior to dosing and were considered incidental. Decreases in red blood cell counts were observed in females throughout the study, reaching statistical significance only at Week 26 (↓9%; $p \leq 0.05$). These findings were minor and not considered adverse. All other differences from controls were considered to be sporadic and/or minor.

Table 3. Selected mean (±SD) hematology values in dogs treated with IR5878 for up to 1 year [a].

Parameter		Dose (mg/kg/day)			
		0	75	300	1000
Males					
Platelets ($x10^9$/L)	Pre-test	310±79.7	324±43.6	371±32.0	380±40.7
	Week 13	307±33.3	326±25.4	377±88.7 (123)	448±33.6** (146)
	Week 26	293±19.0	313±16.7	349±19.5* (119)	413±51.8** (141)
	Week 52	296±22.7	332±23.3	364±27.0 (123)	429±84.2** (145)
Hematocrit (L/L)	Pre-test	0.393±0.0045	0.399±0.0306	0.401±0.0065	0.386±0.0240
	Week 52	0.492±0.0161	0.484±0.0268	0.474±0.0301	0.430±0.0154** (113)
Hemoglobin (g/dL)	Pre-test	12.7±0.14	12.8±0.99	13.0±0.28	12.3±0.74
	Week 52	16.4±0.57	16.3±0.89	16.2±1.15	14.4±0.66** (112)
Red blood cell ($x10^{12}$/L)	Pre-test	5.64±0.158	5.84±0.411	5.84±0.138	5.74±0.260
	Week 52	7.10±0.247	7.05±0.523	6.71±0.336	6.13±0.194** (114)
Females					
Platelets ($x10^9$/L)	Pre-test	352±53.1	353±54.6	334±77.7	302±92.8
	Week 13	326±38.3	335±41.2	350±79.3	417±100.2 (128)
	Week 26	331±51.7	328±25.4	363±76.8	456±95.2* (138)
	Week 52	375±119.0	370±35.6	369±67.3	491±146.8 (131)
Hematocrit (L/L)	Pre-test	0.402±0.0138	0.394±0.0251	0.403±0.0155	0.404±0.0235
	Week 13	0.427±0.0310	0.417±0.0206	0.428±0.0207	0.401±0.0153
	Week 26	0.458±0.0165	0.443±0.0436	0.425±0.0217	0.383±0.0146** (116)
	Week 52	0.496±0.0491	0.497±0.0242	0.474±0.0121	0.440±0.0106* (111)
Hemoglobin (g/dL)	Pre-test	12.9±0.39	12.8±0.81	13.0±0.60	13.0±0.94
	Week 13	14.4±0.90	14.2±0.91	14.7±0.69	13.6±0.63
	Week 26	15.7±0.59	15.1±1.49	14.8±0.72	13.1±0.48** (117)
	Week 52	16.9±1.70	16.8±0.57	16.0±0.49	14.6±0.36* (114)
Red blood cell ($x10^{12}$/L)	Pre-test	5.92±0.312	5.79±0.411	5.95±0.414	5.90±0.412
	Week 13	6.48±0.575	6.30±0.379	6.34±0.499	5.74±0.116* (111)
	Week 26	6.98±0.325	6.61±0.515	6.34±0.454* (19)	5.56±0.139** (120)
	Week 52	7.40±0.601	7.18±0.423	6.79±0.384	6.16±0.245** (117)
Reticulocytes (%)	Pre-test	0.81±0.042	0.91±0.159	0.69±0.144	0.82±0.270
	Week 26	0.57±0.137	0.54±0.174	0.51±0.073	0.88±0.553 (154)
	Week 52	0.87±0.397	1.08±0.186	0.60±0.247	1.74±0.461** (1100)

a Data were obtained from Table 3 on pages 52-75. Percent differences from controls (calculated by reviewers) *are included in parentheses; n=4 except for 1000 mg/kg/day females, where n=3 after Week 26.*

* Statistically different from controls, $p \leq 0.05$.

** Statistically different from controls, $p \leq 0.01$.

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2. Clinical chemistry - Clinical chemistry data are presented in Tables 4a and b. At ≥300 mg/kg/day, alkaline phosphatase was increased throughout the study in both males (↑88-637%; p≤0.05) and females (↑103-475%; p≤0.05, except for 300 mg/kg/day on Weeks 13 and 26), and creatinine was decreased (p≤0.05) in the males (↓12-21%). Additionally at 1000 mg/kg/day, decreases (p≤0.05) in albumin (↓17-27%) and total protein (↓11-19%) were observed in both sexes throughout the study. These findings were considered treatment-related. Calcium levels (group mean and individual dog values) for males and females primarily at weeks 26 and 52 at 1000 mg/kg/day were lower than controls. Although there was statistical significance, the differences from controls were relatively small.

Other parameters and/or doses showed sporadic and/or not dose related differences from respective control values; these were not considered to be of toxicological significance.

Table 4a. Selected mean (±SD) clinical chemistry values in male dogs treated with IR5878 for up to 1 year [a].

Parameter		Dose (mg/kg/day)			
		0	75	300	1000
ALP (U/L)	Pre-dose	161±72.8	183±54.2	177±72.0	149±31.2
	Week 13	121±43.4	147±57.8	227±58.5* (↑88)	598±259.9** (↑394)
	Week 26	96±22.6	121±63.2	261±63.2** (↑172)	657±207.1** (↑584)
	Week 52	79±13.5	96±46.8	296±89.2** (↑275)	582±167.2** (↑637)
Creatinine (µmol/L)	Pre-dose	58±4.1	56±6.2	56±4.3	57±5.9
	Week 13	73±4.2	67±4.6	64±5.4* (↓12)	60±2.8** (↓18)
	Week 26	82±6.8	75±6.1	68±6.7** (↓17)	67±4.1** (↓18)
	Week 52	84±3.5	78±8.1	71±7.8* (↓15)	66±5.2** (↓21)
Albumin (g/L)	Pre-dose	25±0.6	26±2.9	25±2.8	23±0.5
	Week 13	29±0.6	29±1.7	27±2.2	23±2.6** (↓21)
	Week 26	30±0.5	30±1.7	26±1.7** (↓13)	22±1.7** (↓27)
	Week 52	30±1.5	32±1.3	28±3.7	24±1.5** (↓20)
Total protein (g/L)	Pre-dose	53±1.0	54±3.0	52±1.4	51±1.7
	Week 13	53±1.8	54±2.2	51±3.6	46±3.9** (↓13)
	Week 26	54±1.9	55±3.6	50±2.2	44±3.0** (↓19)
	Week 52	60±1.6	57±2.1	55±5.3	51±3.0** (↓15)
Calcium (mmol/L)	Pre-dose	3.10±0.123	3.21±0.196	3.12±0.125	3.12±0.153
	Week 13	3.03±0.048	2.97±0.078	2.92±0.077	2.80±0.123** (↓8)
	Week 26	2.94±0.043	2.92±0.123	2.75±0.093* (↓6)	2.75±0.116* (↓6)
	Week 52	2.78±0.049	2.81±0.017	2.66±0.116	2.61±0.085* (↓6)

a Data were obtained from Table 4 on pages 80-91. Percent differences from controls (calculated by reviewers) are included in parentheses; n=4.

* Statistically different from controls, p≤0.05.

** Statistically different from controls, p≤0.01.

Table 4b. Selected mean (±SD) clinical chemistry values in female dogs treated with IR5878 for up to 1 year [a].

Parameter		Dose (mg/kg/day)			
		0	75	300	1000
ALP (U/L)	Pre-dose	140±58.7	158±29.8	144±29.5	122±25.2
	Week 13	115±43.2	148±46.9	234±82.9 (↑103)	512±217.2** (↑345)
	Week 26	89±26.3	140±60.9	269±79.4 (↑202)	512±229.3** (↑475)
	Week 52	71±17.8	122±66.8	299±120.9* (↑321)	301±151.8* (↑324)
Albumin (g/L)	Pre-dose	26±2.2	25±1.3	25±0.8	25±1.3
	Week 13	30±1.4	31±0.6	29±1.9	25±1.9** (↓17)
	Week 26	32±2.2	31±0.0	28±0.6** (↓13)	26±1.7** (↓19)
	Week 52	32±2.4	31±1.0	30±1.3	25±2.5** (↓22)
Total protein (g/L)	Pre-dose	52±22	54±2.1	53±1.0	53±1.3
	Week 13	56±5.0	56±2.4	52±2.6	50±3.3* (↓11)
	Week 26	57±5.9	56±1.3	50±1.0* (↓12)	50±2.5* (↓12)
	Week 52	60±4.1	59±2.9	56±2.2	52±4.0* (↓13)
AST (U/L)	Pre-dose	37±3.9	35±1.2	35±5.3	36±16.9
	Week 13	46±10.7	39±3.3	33±5.1* (↓28)	29±4.1** (↓37)
	Week 26	49±10.4	47±10.3	34±12.1	33±3.3* (↓33)
	Week 52	48±6.7	42±5.7	34±5.4	39±16.3
Calcium (mmol/L)	Pre-dose	3.11±0.098	3.09±0.127	3.11±0.083	3.08±0.095
	Week 13	3.02±0.096	2.97±0.024	2.98±0.057	2.86±0.067** (↓5)
	Week 26	3.01±0.094	2.83±0.055* (↓6)	2.80±0.070** (↓7)	2.83±0.112** (↓6)
	Week 52	2.82±0.044	2.76±0.042	2.75±0.041	2.62±0.078** (↓7)

a Data were obtained from Table 4 on pages 80-91. Percent differences from controls (calculated by reviewers) are included in parentheses; n=4 except for 1000 mg/kg/day females, where n=3 after Week 26.

* Statistically different from controls, $p \leq 0.05$.

** Statistically different from controls, $p \leq 0.01$.

F. URINALYSIS - Urinary pH was decreased ($p \leq 0.05$) in the males on Week 13 at 300 (pH 5.8) and 1000 (pH 5.6) mg/kg/day compared to controls (pH 6.7), and on Week 52 at 1000 mg/kg/day (pH 5.6) compared to controls (pH 6.8). Urinary protein was decreased (NS) in the 1000 mg/kg/day males throughout treatment (↓26-40%). Urinary protein was also decreased (NS) in the ≥ 75 mg/kg/day females on Week 26 (↓44-56%), but dose-dependency was not observed. No other findings were observed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - At ≥300 mg/kg/day, treatment-related increases ($p \leq 0.01$) in absolute (↑13-50; NS for the 300 mg/kg/day males) liver weights were noted in both sexes (Table 5).

Additionally at ≥300 mg/kg/day, decreases in absolute heart (↓15-16%; $p \leq 0.01$) and spleen (↓25-39%; NS at 300 mg/kg/day) weights were observed in the males; however, similar findings were not observed in females, and there were no corroborating histopathological findings. Therefore, these findings were considered incidental.

At 1000 mg/kg/day, decreases ($p \leq 0.05$) in absolute prostate (↓45%) weight was observed in the males, and decreased ($p \leq 0.05$) brain (↓10%) and increased ($p \leq 0.01$) kidney (↑18%) weights were observed in the females. However, these findings were confined to a single sex and there were no corroborating histopathological findings. Therefore, these findings were considered incidental. All other organ weights were similar to controls.

Table 5. Selected mean (±SD) absolute (g) organ weights (%) in dogs treated with IR5878 for up to 1 year[a]

Organ	Dose (mg/kg/day)			
	0	75	300	1000
Males				
Terminal body weight (kg)	14.0±0.6	13.0±0.3	12.4±1.0 (↓11)	12.3±1.0 (↓12)
Liver	412±19	437±36	467±43 (↑13)	617±104** (↑50)
Heart	116±9	108±8	98±2** (↓15)	97±7** (↓16)
Spleen	120±30b	128±12	90±35 (↓25)c	73±10* (↓39)
Prostate	10±1.5	9±2.7	10±2.5	6±1.9* (↓60)
Females				
Terminal body weight (kg)	12.1±0.8	13.3±2.2	12.4±4.1	10.0±1.2 (↓17)
Liver	359±18	409±52	491±56 (↑35)**	479±22 (↑33)**
Brain	88±5.3	85±1.7	83±6.8	79±4.8* (↓10)
Kidneys	52±2.6	51±5.0	55±2.9	58±4.9 (↑12)

a Data were obtained from Table 6 on pages 100-105. Percent differences from controls (calculated by reviewers) are included in parentheses; n=4 except for 1000 mg/kg/day females, where n=3 after Week 26.

b Individual spleen weights (g): b = 76, 125, 137 and 143; c = 55, 66, 113 and 126 (Report page 225)

* Statistically different from controls, $p \leq 0.05$.

** Statistically different from controls, $p \leq 0.01$.

2. Gross pathology - Treatment-related hypertrophy of the liver was observed in 1/4 males and 2/4 females at 300 mg/kg/day and in 3/4 males and 3/3 females at 1000 mg/kg/day. There were no other treatment-related macroscopic findings.

3. Microscopic pathology - The following microscopic findings were observed in the liver at ≥300 mg/kg/day compared to 0 controls except where noted (Table 6): i) minimal to moderate hepatocyte hypertrophy ($p \leq 0.05$) in all animals, increasing in severity with dose; ii) slight to moderate pigmented Kupffer cells in 2/4 dogs (both sexes) at 300 mg/kg/day, and in 3/4 males and 2/3 females at 1000 mg/kg/day; iii) minimal to slight centrilobular vascular/perivascular inflammatory cell infiltration in 3/4 males at both doses compared to 1/4 controls; and iv) minimal to slight periportal inflammatory cell infiltration in 2/4 males and 1/4 females at 300 mg/kg/day and 1/4 males and 1/4 females at 1000 mg/kg/day. Additionally at ≥300 mg/kg/day, minimal to slight hemosiderosis was noted in the spleen in the males at 300 (2/4) and 1000 (3/4) mg/kg/day compared to 0 controls.

At 1000 mg/kg/day, minimal to moderate cortical vacuolation of the zona fasciculata/reticularis of the adrenal glands was observed in all animals compared to 1/4 control males and 3/4 females (minimal to slight severity). Additionally at this dose, minimal to slight increased cellularity of the bone marrow (sternum) was also observed in 3/3 females compared to 1/4 controls. The Sponsor noted that the increased cellularity of the bone marrow was a response to the anemic condition of these dogs noted in hematology, and that the changes in hematology were not caused by direct effects of the test compound on the bone marrow.

At 1000 mg/kg/day, minimal epithelial hyperplasia and/or minimal inflammatory cell infiltration/epithelial ulceration of the gall bladder was observed in 2/4 males. The Sponsor noted that one of these males also had the highest ALT and AST values pre-dose which may have indicated slight damage to the gallbladder prior to treatment; therefore, the reviewers do not consider this finding treatment-related.

Table 6. Selected microscopic findings (# affected) in male and female dogs treated with IR5878 for up to 1 year[a]

Finding		Dose (mg/kg/day)							
		Males				Females			
		0	75	300	1000	0	75	300	1000
Liver: hepatocyte hypertrophy	total	0	0	4*	4*	0	0	4*	3*
	minimal	0	0	1	0	0	0	2	0
	slight	0	0	3	2	0	0	2	3
	moder.	0	0	0	2	0	0	0	0
pigmented Kupffer cells-total		0	0	2	3	0	1	2	2
	slight	0	0	2	2	0	1	2	2
	moder	0	0	0	1	0	0	0	0
centrilobular vascular/ perivascular inflammatory cell infiltration	total	1	2	3	3	2	2	1	1
	minimal	1	2	2	2	1	2	1	0
	slight	0	0	1	1	0	0	0	1
	moder.	0	0	0	0	1	0	0	0
periportal inflammatory cell infiltration	total	0	0	2	1	0	1	1	1
	minimal	0	0	2	0	0	1	1	0
	slight	0	0	0	1	0	0	0	1
Spleen: hemosiderosis	total	0	1	2	3	2	3	4	1
	minimal	0	0	1	0	0	1	2	0
	slight	0	1	1	3	1	1	2	1
	moder.	0	0	0	0	1	1	0	0
Adrenals: zona fasciculata/reticularis, cortical vacuolation	total	1	3	2	4	3	1	1	3
	minimal	1	3	2	2	1	1	1	1
	slight	0	0	0	0	2	0	0	1
	moder.	0	0	0	2	0	0	0	1
Sternum: marrow, increased cellularity	total	0	0	0	0	1	1	1	3
	minimal	0	0	0	0	0	1	1	1
	slight	0	0	0	0	1	0	0	2

a Data were obtained from Table 8 on pages 109-121; n=4, except for 1000 mg/kg/day females, where n=3 after week 26.

* Statistically different from controls, $p \leq 0.05$.

moder. = moderate

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - It was concluded that the LOAEL was 300 mg/kg/day, based upon changes observed in hematology and clinical chemistry, increased liver weights, and histopathological changes in the liver, adrenal glands, gall bladder, and bone marrow of the sternum.

B. REVIEWER COMMENTS - No treatment-related effects were observed on ophthalmoscopic examination.

One 1000 mg/kg/day female (#992) was sacrificed moribund at Week 40 (after 32 weeks of treatment) due to significant weight loss and increases in alkaline phosphatase (ALP) and alanine aminotransferase (ALT) values. The severity of change noted in these enzyme levels was indicative of liver dysfunction which, if continued, would have been considered deleterious to the animal's health. Treatment-related effects were first observed at Week 26. Pigmented Kupffer cells in the liver was the only treatment-related histopathological finding in this animal. All other animals survived until sacrificed.

There were also treatment-related occurrences (# total occurrences/52 weeks) of loss of appetite (7-57) and pale feces (14-16) in 3/4 males and all females. In the 300 mg/kg/day males, body weights were decreased throughout treatment, resulting in decreased overall (Weeks 0-52) body weight gains (no decrease in females). Therefore, this finding was considered treatment-related. At 1000 mg/kg/day, body weights were decreased in both sexes throughout treatment, resulting in decreased overall body weight gains. Treatment-related decreases in overall (Weeks 1-52) mean weekly food consumption were observed in the 1000 mg/kg/day females when compared with controls.

At 1000 mg/kg/day, platelets were increased in males and females (not stat. sig. at all intervals in females) throughout the study. Additionally at this dose, decreased hematocrit, hemoglobin, and red blood cell values were observed in the males at Week 52. In the females, decreases in hematocrit and hemoglobin were noted beginning on Week 26, and decreased red blood cell counts were observed throughout the study. Increased reticulocytes were also noted in females beginning on Week 26. The changes in red blood cell parameters were indicative of a minor anemia. The observed increases in reticulocytes were considered to have been associated with an increased cellularity of the bone marrow (sternum) in response to the anemic condition observed in the dogs.

At ≥ 300 mg/kg/day, alkaline phosphatase was increased throughout the study (up to 637%) in both sexes, and creatinine was decreased 12-17% in the males. Additionally at 1000 mg/kg/day, decreases in albumin and total protein were observed in both sexes throughout the study. These findings were considered treatment-related. Except for the effects on ALP levels, most of the effects on clinical chemistry plateaued by Week 26 and remained at that level until study termination, possibly indicating a recovery or saturation effect.

Treatment-related increases in mean absolute liver weights were observed in the 300 mg/kg/day females ($p \leq 0.01$). Liver weights were also significantly increased in both sexes at 1000 mg/kg/day. Upon macroscopic examination, treatment-related hypertrophy of the liver was observed in 1/4 males and 2/4 females at 300 mg/kg/day and in 3/4 males and 3/3 females at 1000 mg/kg/day.

The following microscopic findings were observed in the liver at ≥300 mg/kg/day compared to 0 controls except where noted: i) minimal to moderate hepatocyte hypertrophy ($p \leq 0.05$) in all animals, increasing in severity with dose; ii) slight to moderate pigmented Kupffer cells in 2/4 dogs (both sexes) at 300 mg/kg/day, and in 3/4 males and 2/3 females at 1000 mg/kg/day; iii) minimal to slight centrilobular vascular/perivascular inflammatory cell infiltration in 3/4 males at both doses compared to 1/4 controls; and iv) minimal to slight periportal inflammatory cell infiltration in 2/4 males and 1/4 females at 300 mg/kg/day and 1/4 males and 1/4 females at 1000 mg/kg/day. Additionally at ≥300 mg/kg/day, minimal to slight hemosiderosis was noted in the spleen in males at 300 (2/4) and 1000 (3/4) mg/kg/day, compared to 0 controls. The reviewers note that hemosiderosis is common in diseases such as hemolytic and pernicious anemias, and chronic infection. Therefore, this effect would be expected in response to a decrease in RBC counts as reported in hematology.

At 1000 mg/kg/day, minimal to moderate cortical vacuolation of the zona fasciculata/reticularis of the adrenal glands was observed in all animals compared to 1/4 control males and 3/4 females (minimal to slight severity), but changes in this parameter are of questionable toxicological significance. Additionally at this dose, minimal to slight increased cellularity of the bone marrow (sternum) was also observed in 3/3 females compared to 1/4 controls. The Sponsor noted that the increased cellularity of the bone marrow was a response to the anemic condition of these dogs, and that the changes in hematology were not caused by direct effects of the test compound on the bone marrow.

The LOAEL is 300 mg/kg/day based on changes observed in body weights, body weight gains, clinical signs of toxicity, food consumption (females), hematology and clinical chemistry, increased liver weights, and histopathological changes in the liver, and bone marrow of the sternum. The NOAEL is 75 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

C. **STUDY DEFICIENCIES** - No deficiency was noted.

DATA EVALUATION RECORD

IR5878 (ORTHOSULFAMURON)

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

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

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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.

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Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date** 11/8/07

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**: D330824
TXR#: 0053612

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

SYNONYM:
2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N,N-dimethylbenzamide; 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]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 Orthosulfamuron [IR5878; 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.

The LOAEL is 500 mg/kg/day, based on increased incidences of centrilobular hepatocyte hypertrophy, and centrilobular hepatocyte vacuolation in males. The NOAEL is 100 mg/kg/day.

At the doses tested, there were no treatment-related increases 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 observed in treated animals.

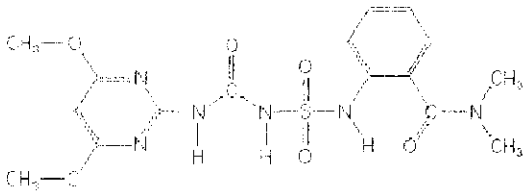
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

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 (MRID 46260102) 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 method
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≤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≤0.05) and a decrease in percent monocytes (↓26%; p≤0.05) were observed in the 1000 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≤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. 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 134/033063) with this test compound.
No indication of carcinogenic potential was observed.

The LOAEL is 500 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 100 mg/kg/day.

At the doses tested, there were no treatment-related increases 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 observed in treated animals.

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.

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)

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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.

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Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 11/8/07

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:** D330824
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; 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea

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), 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.

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%

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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, hepatotoxicity, and nephrotoxicity in both sexes. The NOAEL is 5 mg/kg/day.

After 2 years, an increased ($p\leq0.05$) 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 suggest increased elimination of T4 and possible stimulation of the thyroid 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

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.

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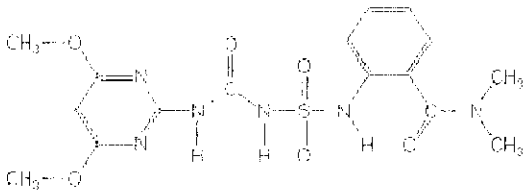
I. MATERIALS AND METHODS

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 (MRID 46260103) 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 (high and low) 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, and 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 method
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 were determined individually over ten 6-minute intervals.

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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 as well as 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.
- Not examined

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.
- Not examined

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*		
X	Pharynx*				
X	Larynx*				

* 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
\- Not examined

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.

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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 the carcinogenicity phase animals. In the toxicity phase (52 weeks), body weight gains for treated males were 97-104% of the control values and for females, the percents of the control values were 90, 107, 91, and 89% for the 1, 5, 500, and 1000 mg/kg/day groups. 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≤0.05

** Significantly different from controls; p≤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 spleen weights 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≤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≤0.05
** Significantly different from controls; p≤0.01

2. Gross pathology - 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≤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≤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≤0.05) from the controls

3. Microscopic pathology

a. Non-neoplastic - At Week 52 (Table 5a), increased (p≤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≤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≤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≤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≤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≤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≤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≤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≤0.05
** Significantly different from controls; p≤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 (no adenomas or carcinomas reported in female groups). 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≤0.01) in the 1000 mg/kg/day males (↑12%). Increased (p≤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≤0.01) were observed in relative kidney weights in the 1000 mg/kg/day males (↑7%).

At 2 years, blood urea was increased (p≤0.01) by 29% in the 1000 mg/kg/day females. Relative to body kidney weights were increased (p≤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≤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≤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≤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 suggest an increased elimination of T4 and possible stimulation of the thyroid 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.

The LOAEL is 500 mg/kg/day, based on decreased body weight gains, hepatotoxicity, and 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. The thyroid effects were secondary to metabolic induction of the liver and were not considered relevant to humans because of reasons stated above.

C. **STUDY DEFICIENCIES** - 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.

DATA EVALUATION RECORD

IR8181; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 3-01-82 K (MRID 46578923)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by
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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: Lisa Austin, Ph.D. **Signature:** [signature]
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 1/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 2/6/07

Template version 11/01

TXR#: 0053612

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Bacterial Gene Mutation (*Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i.)

SYNONYMS: 1-[2-(dimethylcarbamoyl)phenylsulfamoyl]-3-(4-hydroxy-6-methoxy-2-pyrimidinyl)urea

CITATION: Krüeger, I. (2004) Reverse Mutation Assay using Bacteria (*Salmonella Typhimurium*) with IR8181. BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany. Laboratory Study No.: 040382. April 28, 2004. MRID 46578923. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In two independent reverse gene mutation assays in bacteria (MRID 46578923), *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i., Batch # 30072/85) in dimethylsulfoxide at concentrations of 0, 31.6, 100, 316, 1000, 2500, or 5000 µg/plate. Both trials were performed in the presence and absence of S9-activation (derived from the livers of male Wistar rats induced with phenobarbital/β-naphthoflavone). The standard plate incorporation method was performed in Trial 1 and the pre-incubation method was used in Trial 2. Standard strain-specific mutagens served as positive controls.

IR8181 was tested up to the limit dose (5000 µg/plate). It was not noted if precipitation of the test material was observed at any concentration in the presence or absence of S9. Although all plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9, slight cytotoxicity was observed in TA 102 at 5000 µg/plate (+S9) in Trial 2. No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced the appropriate response in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR8181; a metabolite of IR5878

Description:	Fine, white powder
Batch #:	30072/85
Purity (w/w):	97.02% a.i.
CAS # of TGAI:	Not provided
Structure:	Not provided
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control materials

Negative - The untreated medium served as the negative control.
Solvent - DMSO (100 µL/plate)
Positive

Non-activation		
Sodium azide (NaN_3; in deionized water)	10 µg/plate	TA100, TA1535
4-Nitro-o-phenylene-diamine (4-NOPD; in DMSO)	40 µg/plate	TA1537
	10 µg/plate	TA98
Methyl methanesulfonate (MMS; in deionized water)	1 µg/plate	TA102
Activation		
2-Aminoanthracene (2-AA; in DMSO)	2.5 µg/plate	TA98, TA100 TA1535, TA1537
	10 µg/plate	TA102

3. Activation - The S9 fraction was derived from male Wistar rats (Prepared at BSL Bioservice GmbH. Age, weight, and supplier were not reported)

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			None		Other		

The S9 fraction was prepared in the laboratory and stored at ≤-75 °C until used. It was stated that the protein concentration of the batch was 36 mg/mL. The efficacy of the S9 fraction was verified in the *S. typhimurium* assay. It was reported that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of approximately 0.75 mg/mL in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 5 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4).

ml

4. Test organisms - *S. typhimurium* and *E. coli* strains

	TA97	X	TA98	X	TA100	X	TA102		WP2 (pKM101)
X	TA1535	X	TA1537		TA1538		WP2 *uvr*A		

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	X	Yes		No

5. Test compound concentrations used

Preliminary cytotoxicity assay - A preliminary cytotoxicity assay was performed using tester strains TA98 and TA100 at concentrations of 3.16, 10, 31.6, 100, 316, 1000, 2500, or 5000 µg/plate (±S9).

Mutagenicity assay - All tester strains

Non-activated conditions: 31.6, 100, 316, 1000, 2500, or 5000 µg/plate (Trial 1 and Trial 2)

Activated conditions: 31.6, 100, 316, 1000, 2500, or 5000 µg/plate (Trial 1 and Trial 2)

All concentrations of the test article and positive and negative controls were plated in triplicate, both in the presence and absence of S9-activation, for each tester strain (*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537).

B. TEST PERFORMANCE

1. Type of assay

x standard plate test (Trial 1)
x pre-incubation (60 minutes; Trial 2)
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test
__ other

2. Protocol - Two independent mutagenicity trials were conducted both in the presence and absence of S9-activation. Prior to plating, inocula of the tester strains were cultured in nutrient broth for 10 hours at 37°C. The standard plate incorporation method was used in Trial 1. Bacteria (0.1 mL); test compound, solvent, or positive control (0.1 mL); and 0.5 mL of S9 mix (for tests requiring metabolic activation) or buffer were added to 2 mL of melted top agar supplemented with L-histidine (10.5 mg/L) and D-biotin (12.2 mg/L). The top agar components were thoroughly mixed and poured into triplicate plates containing solidified minimal agar. In Trial 2, the pre-incubation method was used. The bacteria; test material, solvent, or positive control; and S9 mix or buffer (same quantities as listed above) were first mixed together and incubated for 60 minutes at 37°C. After pre-incubation, 2 mL top agar was added and the mixture was poured into triplicate plates containing solidified minimal agar. After solidification of the top agar, the plates were inverted and incubated for 48 hours at 37°C in the dark. After incubation, the plates were scored for number of revertant colonies by an automated colony

counter (Protocol, Meintrup DWS Laborgerate GmbH). If precipitation of the test item interfered with automatic counting then manual counting by hand was performed. TA1535 and TA1537 were also manually counted. The plates were also checked for cytotoxicity (thinning of the background lawn and/or reduced number of revertants).

3. **Statistical analysis** - Statistical analysis of the data was not performed.

4. **Evaluation criteria**

Assay validity - The assay was considered valid if the following criteria were met:

- Regular background growth was observed in the negative and solvent controls.
- The spontaneous reversion rates of all strains in the negative and solvent controls (-S9) were within the historical control ranges.
- The positive controls should induce significant increases in revertant colonies.
- Typical responses to crystal violet and ampicillin were demonstrated.

Positive result - The test article was considered to be positive for mutagenicity if the following criteria were met:

- The number of revertants/plate exceeded the threshold of 2x the solvent controls in strains TA100 and TA102, and 3x the solvent controls in strains TA98, TA1535, and TA1537.
- A biologically relevant positive response for at least one of the dose groups in at least one tester strain (±S9) was observed.
- A dose dependent increase in the number of revertant colonies occurs.

II. REPORTED RESULTS

The test material formulations were not analyzed for actual concentration.

A. PRELIMINARY CYTOTOXICITY ASSAY - In the preliminary cytotoxicity assay, strains TA98 and TA100 were exposed to concentrations of 3.16, 10, 31.6, 100, 316, 1000, 2500, or 5000 µg/plate (±S9). No evidence of cytotoxicity was observed at any concentration in either strain with or without S9. Based on the results of this assay, concentrations of 31.6, 100, 316, 1000, 2500, or 5000 µg/plate (±S9) were chosen for Trial 1 of the mutagenicity assay.

B. MUTAGENICITY ASSAY - The results of the mutagenicity trials were presented on pages 24-33. As this Study Report did not provide a summary table of results, and the results of this assay were negative, pages 26 and 31 are included as an Attachment to this DER. It was not noted if precipitation of the test material was observed at any concentration in the presence or absence of S9. Although all plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9, slight cytotoxicity was observed in TA 102 at 5000 µg/plate (+S9) in Trial 2. No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that under the conditions of this study, IR8181 (a metabolite of IR5878) did not induce mutations in *S. typhimurium* strains TA98, TA100, TA102, TA1535, or TA1537 when tested up to the limit dose (5000 µg/plate) in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - It was not noted if precipitation of the test material was observed at any concentration in the presence or absence of S9. All plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9. Evidence of slight cytotoxicity was observed in TA 102 at 5000 µg/plate (+S9) in the pre-incubation method. No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiencies were noted, but do not change the results of this DER:

- The age, weight, and source of the animals used for the S9 fraction were not provided.
- The test material formulations were not analyzed for actual concentrations.
- It was not noted if precipitation of the test material was observed.

ATTACHMENT

The following attachment contains the data for strain TA1535 (Trials 1 and 2) from pages 26 and 31 of MRID 46578923.

Tester Strain: TA 1535 Experiment: 1

Treatment	Dose	Plate count	Mean	SD	Plate count	Mean	SD	Mutation factor	
A. dest.		14 20 7	**14**	6.5	9 9 8	**9**	0.6	**0.9**	**0.7**
DMSO		14 15 17	**15**	1.5	9 10 16	**12**	3.8	**1.0**	**1.0**
Test Item	31.6 µg	15 14 16	**15**	1.0	12 8 18	**13**	5.0	**1.0**	**1.1**
Test Item	100 µg	13 22 15	**17**	4.7	11 14 10	**12**	2.1	**1.1**	**1.0**
Test Item	316 µg	7 14 19	**13**	6.0	10 20 16	**15**	5.0	**0.9**	**1.3**
Test Item	1000 µg	17 15 9	**14**	4.2	12 15 14	**14**	1.5	**0.9**	**1.2**
Test Item	2500 µg	11 13 15	**13**	2.0	12 14 11	**12**	1.5	**0.8**	**1.1**
Test Item	5000 µg	14 21 8	**14**	6.5	8 8 10	**9**	1.2	**0.9**	**0.7**
Sodium azide	10 µg	1086 1131 1256	**1158**	88.1	/ / /	**/**	/	**75.5**	/
2-AA	2.5 µg	/ / /	/	/	154 140 218	**171**	41.6	/	**14.6**

SD: Standard deviation
2-AA: 2-aminoanthracene

$$\text{Mutation factor (rounded value)} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

Tester Strain: TA 1535 Experiment 2

Treatment	Dose/plate	REVERTANT COLONIES PER PLATE						MUTATION FACTOR	
		Without activation			With activation				
		Counts	Mean	SD	Counts	Mean	SD	S9	S30
A. dest		18 21 31	**23**	6.8	11 12 18	**14**	3.8	**1.5**	**0.7**
DMSO		15 13 18	**15**	2.5	20 14 22	**19**	4.2	**1.0**	**1.0**
Test Item	31.6 µg	18 21 19	**19**	1.5	14 17 16	**16**	1.5	**1.3**	**0.8**
Test Item	100 µg	26 9 20	**18**	8.6	18 11 11	**13**	4.0	**1.2**	**0.7**
Test Item	316 µg	4 17 20	**14**	8.5	10 10 9	**10**	0.6	**0.9**	**0.5**
Test Item	1000 µg	10 22 12	**15**	6.4	25 18 21	**21**	3.5	**1.0**	**1.1**
Test Item	2500 µg	15 24 18	**19**	4.6	13 13 19	**15**	3.5	**1.2**	**0.8**
Test Item	5000 µg	10 8 15	**11**	3.6	14 13 17	**15**	2.1	**0.7**	**0.8**
Sodium azide	10 µg	1157 1123 1194	**1158**	35.5	/ / /	**/**	/	**75.5**	**/**
2-AA	2.5 µg	/ / /	**/**	/	206 194 197	**199**	6.2	**/**	**10.7**

SD: Standard deviation
2-AA: 2-aminoanthracene

$$\text{Mutation factor (rounded value)} = \frac{\text{mean revertants (test item)}}{\text{mean revertants (solvent control)}}$$

DATA EVALUATION RECORD

IR7825; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 3-01-82 F (MRID 46578916)

Prepared for
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U.S. Environmental Protection Agency
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Prepared by
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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.

78

EPA Reviewer: Lisa Austin, Ph.D. **Signature:** Lisa Austin
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 1/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 3/6/07

Template version 11/01

TXR#: 0053612

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** (*Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR7825 (a metabolite of IR5878; Orthosulfamuron; 99.3%)

SYNONYMS: None

CITATION: Poth, A. (2003) *Salmonella Typhimurium* Reverse Mutation Assay with IR7825. RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Laboratory Study No.: 778802, July 14, 2003. MRID 46578916. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In two independent reverse gene mutation assays in bacteria (MRID 46578916), *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to IR7825 (a metabolite of IR5878; Orthosulfamuron; 99.3% a.i., Batch # FCF/T/198-01 [ex 20687/38]) in dimethylsulfoxide at concentrations of 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 1) and 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 2). Both trials were performed in the presence and absence of S9-activation (derived from the livers of male Wistar rats induced with phenobarbital/β-naphthoflavone). The standard plate incorporation method was performed in Trial 1 and the pre-incubation method was used in Trial 2. Standard strain-specific mutagens served as positive controls.

IR7825 was tested up to the limit dose (5000 µg/plate). No precipitation of the test material was observed at any concentration in the presence and absence of S9. All plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9. However, evidence of cytotoxicity (reduced number of revertants) was observed in the following strains: (i) TA102 at ≥2500 µg/plate (+S9, both trials) and at ≥1000 and 5000 µg/plate (-S9, Trials 1 and 2, respectively); (ii) TA1535 at 5000 µg/plate (+S9, Trial 2) and at

≥2500 µg/plate (-S9, Trial 2); and (iii) TA1537 at 5000 µg/plate (+S9, Trial 1) and at 5000 µg/plate (-S9, Trial 2). No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR7825; a metabolite of IR5878

Description:	White solid
Batch #:	FCF/T/198-01 (ex 20687/38)
Purity (w/w):	99.3% a.i.
CAS # of TGAI:	Not provided
Structure:	Not provided
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control materials

Negative - The untreated medium served as the negative control.
Solvent - DMSO (100 µL/plate)
Positive

Non-activation		
Sodium azide (NaN_3; in deionized water)	10 µg/plate	TA100, TA1535
4-Nitro-o-phenylene-diamine (4-NOPD; in DMSO)	50 µg/plate	TA1537
	10 µg/plate	TA98
Methyl methanesulfonate (MMS; in deionized water)	4 µg/plate	TA102
Activation		
2-Aminoanthracene (2-AA; in DMSO)	2.5 µg/plate	TA98, TA100 TA1535, TA1537
	10 µg/plate	TA102

3. Activation - The S9 fraction was derived from male Wistar rats (8-12 weeks old, weighing 220-320 g; supplier not reported)

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			None		Other		

The S9 fraction was prepared in the laboratory and stored at -80 °C until used. It was stated that the protein concentration of the batches was 35.1-36.1 mg/mL. It was not reported if the efficacy of the batches was checked before use. It was reported that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 15% (v/v) in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 5 mM NADP in 100mM sodium-ortho-phosphate buffer (pH 7.4).

4. **Test organisms** - *S. typhimurium* and *E. coli* strains

	TA97	X	TA98	X	TA100	X	TA102		WP2 (pKM101)
X	TA1535	X	TA1537		TA1538		WP2 *uvr*A		

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	X	Yes		No

5. **Test compound concentrations used**

Preliminary cytotoxicity assay - A preliminary cytotoxicity assay was performed using tester strains TA98 and TA100 at concentrations of 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (±S9).

Mutagenicity assay - All tester strains

Non-activated conditions: 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 1)
10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 2)

Activated conditions: 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 1)
10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 2)

All concentrations of the test article and positive and negative controls were plated in triplicate, both in the presence and absence of S9-activation, for each tester strain (*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537).

B. TEST PERFORMANCE

1. **Type of assay**

x standard plate test (Trial 1)
x pre-incubation (60 minutes; Trial 2)
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test
__ other

2. **Protocol** - Two independent mutagenicity trials were conducted both in the presence and absence of S9-activation. Prior to plating, inocula of the tester strains were cultured in nutrient broth in a shaking water bath for 4 hours at 37°C. The standard plate incorporation method was used in Trial 1. Bacteria (0.1 mL); test compound, solvent, or positive control (0.1 mL); and 0.5 mL of S9 mix (for tests requiring metabolic activation) or S9 mix substitution buffer were added to 2 mL of melted top agar supplemented with L-histidine (10.5 mg/L) and D-biotin (12.2 mg/L). The top agar components were thoroughly mixed and poured into triplicate plates containing solidified minimal agar. In Trial 2, the preincubation method was used. The bacteria; test material, solvent, or positive control; and S9 mix or buffer (same quantities as listed above) were first mixed together and incubated for 60 minutes at 37°C. After preincubation, 2 mL top agar was added and the mixture was poured into triplicate plates containing solidified minimal agar.

After solidification of the top agar, the plates were inverted and incubated for 48 hours at 37°C in the dark. After incubation, the plates were scored for number of revertant colonies by an automated colony counter (Artek Systems Corporation). The plates were also checked for cytotoxicity (thinning of the background lawn and/or reduced number of revertants).

3. **Statistical analysis** - Statistical analysis of the data was not performed.

4. **Evaluation criteria**

Assay validity - The assay was considered valid if the following criteria were met:

- Regular background growth observed in the negative and solvent controls.
- The spontaneous reversion rates of all strains in the negative and solvent controls were within the historical control ranges.
- The positive controls should induce significant increases in revertant colonies.

Positive result - The test article was considered to be positive for mutagenicity if any of the following criteria were met:

- The number of revertants/plate exceeded the threshold of 2x the solvent controls in strains TA98, TA100, and TA102, and 3x the solvent controls in strains TA1535 and TA1537.
- A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.
- An increase that exceeds the threshold at one concentration is considered biologically relevant if it is reproducible.
- A dose dependent increase in the number of revertant colonies below the threshold is considered biologically relevant if it is reproducible and the colony counts exceed the historical control range.

II. REPORTED RESULTS

The test material formulations were not analyzed for actual concentration.

A. PRELIMINARY CYTOTOXICITY ASSAY - In the preliminary cytotoxicity assay, strains TA98 and TA100 were exposed to concentrations of 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (±S9). The background lawn growth was normal. No evidence of precipitation or cytotoxicity was observed at any concentration in all strains with or without S9. Based on the results of this assay, concentrations of 33, 100, 333, 1000, 2500, or 5000 µg/plate (±S9) were chosen for Trial 1 of the mutagenicity assay.

B. MUTAGENICITY ASSAY - The results of the mutagenicity trials were presented in Appendices 12.2 and 12.3 of the study report on pages 25-34, and were summarized in Appendix 12.4 on page 35. As the results of these assays were negative, only the summary table (Appendix 12.4) is included as an Attachment to this DER. No precipitation of the test material was observed at any concentration in the presence or absence of S9. Although all plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the

presence and absence of S9, evidence of cytotoxicity (reduced number of revertants) was observed in the following strains: (i) TA102 at ≥2500 µg/plate (+S9, both trials) and at ≥1000 and 5000 µg/plate (-S9, Trials 1 and 2, respectively); (ii) TA1535 at 5000 µg/plate (+S9, Trial 1) and at ≥2500 µg/plate (-S9, Trial 2); and (iii) TA1537 at 5000 µg/plate (+S9, Trial 1) and at 5000 µg/plate (-S9, Trial 2). No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that under the conditions of this study, IR7825 (a metabolite of IR5878) did not induce mutations in *S. typhimurium* strains TA98, TA100, TA102, TA1535, or TA1537 when tested up to the limit dose (5000 µg/plate) in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - No precipitation of the test material was observed at any concentration in the presence and absence of S9. All plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9. However, evidence of cytotoxicity (reduced number of revertants) was observed in strains TA102 at ≥2500 µg/plate (+S9, both trials) and at ≥1000 and 5000 µg/plate (-S9, Trials 1 and 2, respectively); (ii) TA1535 at 5000 µg/plate (+S9, Trial 1) and at ≥2500 µg/plate (-S9, Trial 2); and (iii) TA1537 at 5000 µg/plate (+S9, Trial 1) and at 5000 µg/plate (-S9, Trial 2). No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiencies were noted, but do not change the results of this DER:

- The source of the animals used for the S9 fraction was not provided.
- The test material formulations were not analyzed for actual concentrations.

ATTACHMENT

The following attachment contains the summary data table from page 35 of MRID 46578916.

Test item: IR7825

S9 mix from: Rat liver (Batch R 121202 and R 070203)

without S9 mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative control	12	20	6	12	28	42	146	179	203	239
Solvent control	14	20	8	11	32	44	152	153	223	246
Positive control#	1347	1045	60	67	248	326	722	1419	1379	838
10	/	22	/	8	/	39	/	152	/	234
33	14	24	5	11	32	35	133	174	174	252
100	15	19	8	9	29	50	131	173	176	236
333	11	22	6	14	29	37	144	159	181	184
1000	16	24	6	8	32	28	145	152	99	172
2500	11	8	4	8	29	32	131	125	67	140
5000	8	7	6	5	22	28	90	92	0	42

with S9 Mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative control	11	16	14	22	24	58	221	276	161	179
Solvent control	15	15	17	20	41	49	160	223	170	215
Positive control##	247	122	129	206	1580	1500	1384	1653	1190	814
10	/	16	/	21	/	55	/	193	/	204
33	15	12	13	19	41	54	129	243	160	220
100	13	13	9	23	35	49	161	233	202	199
333	22	13	12	23	37	55	163	247	221	137
1000	14	13	13	27	33	46	154	186	90	114
2500	12	16	12	18	30	47	93	210	64	74
5000	6	7	7	15	28	40	89	127	14	11

Sodium azide (10.0 µg/plate) strains TA 1535 and TA 100
4-nitro-o-phenylene-diamine strains TA 1537 (50 µg/plate) and TA 98 (10.0 µg/plate)
Methyl methane sulfonate (4 µL/plate) strain TA 102

2-aminoanthracene (2.5 µg/plate) strains TA 1535, TA 1537, TA 98, and TA 100
2-aminoanthracene (10.0 µg/plate) strain TA 102

DATA EVALUATION RECORD

IR7863; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 3-01-82 H (MRID 46578919)

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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Date: 12/23/05

Disclaimer

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

EPA Reviewer: Lisa Austin, Ph.D. **Signature:** Lisa Austin
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 4/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P V Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 3/16/07

Template version 11/01

TXR#: 0053612

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Bacterial Gene Mutation (*Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 108209 **DP BARCODE**: D330824

TEST MATERIAL (PURITY): IR7863 (a metabolite of IR5878; Orthosulfamuron; 97.8% a.i.)

SYNONYMS: Not provided

CITATION: Sokolowski, M. (2003) *Salmonella Typhimurium* Reverse Mutation Assay with IR7863. RCC, Cytotest Cell Research GmbH, Rossdorf, Germany. Laboratory Study No.: 795502, September 19, 2003. MRID 46578919. Unpublished.

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

EXECUTIVE SUMMARY - In two independent reverse gene mutation assays in bacteria (MRID 46578919), *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to IR7863 (a metabolite of IR5878; Orthosulfamuron; 97.8% a.i., Batch # 20687/50) in deionized water at concentrations of 0, 33, 100, 333, 1000, 2500, or 5000 µg/plate. Both trials were performed in the presence and absence of S9-activation (derived from the livers of male Wistar rats induced with phenobarbital/β-naphthoflavone). The standard plate incorporation method was performed in Trial 1 and the pre-incubation method was used in Trial 2. Standard strain-specific mutagens served as positive controls.

IR7863 was tested up to the limit dose (5000 µg/plate). No precipitation of the test material was observed at any concentration in the presence or absence of S9. All plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9. Evidence of slight cytotoxicity (reduced number of revertants) was observed in strain TA1537 at 5000 µg/plate (+S9) in the plate incorporation and –S9 in the pre-incubation tests. No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR7863; a metabolite of IR5878

Description:	White solid
Batch #:	20687/50
Purity (w/w):	97.8% a.i.
CAS # of TGAI:	Not provided
Structure:	Not provided
Solvent used:	Deionized water

2. Control materials

Negative - The untreated medium served as the negative control.
Solvent - DI water (100 µL/plate)
Positive

Non-activation

Sodium azide (NaN_3; in deionized water)	10 µg/plate	TA100, TA1535
4-Nitro-o-phenylene-diamine (4-NOPD; in DMSO)	50 µg/plate	TA1537
	10 µg/plate	TA98
Methyl methanesulfonate (MMS; in deionized water)	4 µL/plate	TA102

Activation

2-Aminoanthracene (2-AA; in DMSO)	2.5 µg/plate	TA98, TA100 TA1535, TA1537
	10 µg/plate	TA102

3. Activation - The S9 fraction was derived from male Wistar rats (8-12 weeks old, weighing 220-320 g; supplier not reported)

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			None		Other		

The S9 fraction was prepared in the laboratory and stored at -80 °C until used. It was stated that the protein concentration of the batch was 30.8 mg/mL. It was not reported if the efficacy of the batch was checked before use. It was reported that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 15% (v/v) in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 5 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4).

4. Test organisms - *S. typhimurium* and *E. coli* strains

	TA97	X	TA98	X	TA100	X	TA102		WP2 (pKM101)
X	TA1535	X	TA1537		TA1538		WP2 *uvr*A		

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	X	Yes		No

5. Test compound concentrations used

Preliminary cytotoxicity assay - A preliminary cytotoxicity assay was performed using tester strains TA98 and TA100 at concentrations of 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (±S9).

Mutagenicity assay - All tester strains

Non-activated conditions: 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 1 and Trial 2)

Activated conditions: 33, 100, 333, 1000, 2500, or 5000 µg/plate (Trial 1 and Trial 2)

All concentrations of the test article and positive and negative controls were plated in triplicate, both in the presence and absence of S9-activation, for each tester strain (*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537).

B. TEST PERFORMANCE

1. Type of assay

x standard plate test (Trial 1)
x pre-incubation (60 minutes; Trial 2)
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test
__ other

2. Protocol - Two independent mutagenicity trials were conducted both in the presence and absence of S9-activation. Prior to plating, inocula of the tester strains were cultured in nutrient broth in a shaking water bath for 4 hours at 37°C. The standard plate incorporation method was used in Trial 1. Bacteria (0.1 mL); test compound, solvent, or positive control (0.1 mL); and 0.5 mL of S9 mix (for tests requiring metabolic activation) or buffer were added to 2 mL of melted top agar supplemented with L-histidine (10.5 mg/L) and D-biotin (12.2 mg/L). The top agar components were thoroughly mixed and poured into triplicate plates containing solidified minimal agar. In Trial 2, the pre-incubation method was used. The bacteria; test material, solvent, or positive control; and S9 mix or buffer (same quantities as listed above) were first mixed together and incubated for 60 minutes at 37°C. After pre-incubation, 2 mL top agar was added and the mixture was poured into triplicate plates containing solidified minimal agar. After solidification of the top agar, the plates were inverted and incubated for 48 hours at 37°C in the dark. After incubation, the plates were scored for number of revertant colonies by an automated

colony counter (Artek Systems Corporation). The plates were also checked for cytotoxicity (thinning of the background lawn and/or reduced number of revertants).

3. **Statistical analysis** - Statistical analysis of the data was not performed.

4. **Evaluation criteria**

Assay validity - The assay was considered valid if the following criteria were met:

- Regular background growth was observed in the negative and solvent controls.
- The spontaneous reversion rates of all strains in the negative and solvent controls were within the historical control ranges.
- The positive controls should induce marked increases in revertant colonies.

Positive result - The test article was considered to be positive for mutagenicity if the following criteria were met:

- The number of revertants/plate exceeded the threshold of 2x the solvent controls in strains TA98, TA100, and TA102, or 3x the solvent controls in strains TA1535 and TA1537.
- A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.
- An increase that exceeds the threshold at one concentration is considered biologically relevant if it is reproducible.
- A dose dependent increase in the number of revertant colonies below the threshold is considered biologically relevant if it is reproducible and the colony counts exceed the historical control range.

II. REPORTED RESULTS

The test material formulations were not analyzed for actual concentration.

A. PRELIMINARY CYTOTOXICITY ASSAY - In the preliminary cytotoxicity assay, strains TA98 and TA100 were exposed to concentrations of 0, 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (±S9). No evidence of cytotoxicity was observed at any concentration in either strain with or without S9. Based on the results of this assay, concentrations of 0, 33, 100, 333, 1000, 2500, or 5000 µg/plate (±S9) were chosen for Trial 1 of the mutagenicity assay.

B. MUTAGENICITY ASSAY - The results of the mutagenicity trials were presented in Annex 12.2 and 12.3 of the study report on pages 24-33, and were summarized in Annex 12.4 on page 34. As the results of these assays were negative, only the summary table (Annex 12.4) is included as an Attachment to this DER. No precipitation of the test material was observed at any concentration in the presence or absence of S9. Although all plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9, evidence of slight cytotoxicity (reduced number of revertants) was observed in strain TA1537 at 5000 µg/plate (+S9) in the plate incorporation and –S9 in the pre-incubation tests. No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that under the conditions of this study, IR7863 (a metabolite of IR5878) did not induce mutations in *S. typhimurium* strains TA98, TA100, TA102, TA1535, or TA1537 when tested up to the limit dose (5000 µg/plate) in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - No precipitation of the test material was observed at any concentration in the presence or absence of S9. All plates exposed to the test material exhibited normal background lawn growth at up to 5000 µg/plate in the presence and absence of S9. Evidence of slight cytotoxicity (reduced number of revertants) was observed in strain TA1537 at 5000 µg/plate (+S9) in the plate incorporation and –S9 in the pre-incubation tests. No marked increases in the mean number of revertants/plate were observed in any strain under any test condition in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiencies were noted, but do not change the results of this DER:

- The source of the animals used for the S9 fraction was not provided.
- The test material formulations were not analyzed for actual concentrations.

ATTACHMENT

The following attachment contains the summary data from page 34 of MRID 46578919.

12.4 Summary of Results

Test item: IR7863

S9 mix from: Rat liver (Batch 210203)

without S9 mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative control	9	12	6	8	26	38	147	180	182	160
Solvent control	13	12	5	9	27	35	162	180	181	203
Positive control*	764	1317	82	113	207	555	531	525	1020	965
33	12	10	4	6	26	33	148	182	168	153
100	10	12	3	8	24	38	167	188	161	166
333	9	11	8	6	23	41	168	153	135	186
1000	9	7	5	6	21	38	167	162	131	164
2500	6	10	5	8	25	41	160	188	171	196
5000	8	9	5	3	27	32	158	163	213	225

with S9 Mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative control	12	13	6	10	33	49	186	199	196	202
Solvent control	11	11	5	7	29	38	200	195	179	228
Positive control**	276	275	302	343	607	1630	565	550	950	1056
33	7	10	7	7	33	40	231	206	204	208
100	8	11	4	10	34	45	225	210	191	289
333	8	10	3	8	30	47	184	192	200	277
1000	13	11	4	7	20	45	214	220	157	186
2500	10	14	3	9	34	40	195	227	147	241
5000	8	10	2	9	32	40	217	209	165	256

* Sodium azide (10.0 µg/plate) strains TA 1535 and TA 100
4-nitro-o-phenylene-diamine strains TA 1537 (50 µg/plate) and TA 98 (10.0 µg/plate)
Methyl methane sulfonate (4 µL/plate) strain TA 102

** 2-aminoanthracene (2.5 µg/plate) strains TA 1535, TA 1537, TA 98, and TA 100
2-aminoanthracene (10.0 µg/plate) strain TA 102

DATA EVALUATION RECORD

IR8181; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; Mouse Lymphoma Cell/Mammalian Activation Gene Forward Mutation Assay (L5178Y TK+/-)

Work Assignment No. 3-01-82 M (MRID 46578925)

Prepared for
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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.

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EPA Reviewer: Lisa Austin, Ph.D. **Signature:** Lisa Austin
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 1/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 2/6/07

Template version 11/01

TXR#: 0053612

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells Gene Mutation Assay in L5178Y Mouse Lymphoma Cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i.)

SYNONYMS: 1-[2-(dimethylcarbamoyl)phenylsulfamoyl]-3-(4-hydroxy-6-methoxy-2-pyrimidinyl)urea

CITATION: Krüeger, I. (2004) *In Vitro* Mammalian Cell Gene Mutation Assay (Thymidine Kinase Locus/TK+/-) in Mouse Lymphoma L5178Y Cells with IR8181. BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany. Laboratory Study No.: 040384. MRID 46578925. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In two independent trials of a mammalian cell gene mutation assay at the TK+/- locus (MRID 46578925), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i., Batch # 30072/85) in cell culture media for 4 hours at concentrations of 0, 40, 80, 156, 312.5, 625, 1250, 2500, or 5000 µg/mL (Trial 1, +/-S9) or 0, 125, 250, 500, 1000, 2000, 3000, 4000, or 5000 µg/mL (Trial 2, +S9), or for 24 hours at concentrations of 0, 40, 80, 156, 312.5, 625, 1250, 2500, or 5000 µg/mL (Trial 2, -S9 only). The S9-activation was derived from the livers of male Wistar rats induced with phenobarbital/β-naphthoflavone. Standard mutagens served as positive controls.

IR8181 (a metabolite of IR5878) was tested up to the limit dose (5000 µg/plate). It was not noted if precipitation of the test material was observed at any concentration in the presence or absence of S9. No evidence of cytotoxicity was observed in Trial 1 (4-hour treatment, +/-S9) or Trial 2 (4-hour treatment, +S9). Evidence of slight cytotoxicity (decreased relative suspension

growth, 32%) was observed in Trial 2 (24-hour treatment, -S9) at 5000 µg/mL. No marked increase in mutant frequency was observed in either trial. The positive controls induced the expected response. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR8181; a metabolite of IR5878

Description:	Fine, white powder
Batch #:	30072/85
Purity (w/w):	97.02% a.i.
CAS # of TGAI:	Not provided
Structure:	Not provided
Solvent used:	Cell culture media

2. Control materials

Negative - The untreated medium served as the negative control.
Solvent - Cell culture media
Positive

Non-activation - Methyl methanesulfonate (MMS, in normal saline; Final concentration: 10 µg/mL)
Ethyl methanesulfonate (EMS, in medium; Final concentration: 200 µg/mL and 700 µg/mL)

Activation - Benzo[a]pyrene (B[a]P, in 1% DMSO; Final concentration: 1.5 µg/mL and 2.5 µg/mL)

3. Activation - The S9 fraction was derived from male Wistar rats (age, weight, and supplier not reported)

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared in the laboratory and stored at ≤-75 °C until used. It was stated that the protein concentration of the batch was 36 mg/mL. The efficacy of the S9 fraction was verified in the *S. typhimurium* assay. It was reported that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 0.75 mg/mL in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 4 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4).

4. Test cells - Mammalian cells in culture

X	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		others (list)

Media: Culture medium - RPMI 1640 medium, supplemented with 3% horse serum (7.5% during the 24 hour treatment), 100 U penicillin, 100 µg/mL streptomycin, and 220 µg/mL sodium-pyruvate.
Selection medium - culture medium supplemented with 3 µg/mL trifluorothymidine (TFT).

Properly maintained?	X	Yes		No
Periodically checked for mycoplasma contamination?	X	Yes		Not reported

Periodically checked for karyotype stability?	[X] Yes	[ ] Not reported
Periodically "cleansed" against high spontaneous background?	[X] Yes	[ ] Not reported

5. Locus examined

	X	**Thymidine kinase (TK)**		**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na^+/K^+ ATPase**
Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	trifluorothymidine (TFT,3 µg/mL)				

6. Test compound concentrations used

a. Preliminary cytotoxicity assay - A preliminary cytotoxicity assay was performed under the conditions as described for the mutagenicity assay without activation at concentrations:

39, 78, 156, 312.5, 625, 1250, 2500, or 5000 µg/mL (-S9; 4 hours)

b. Mutagenicity assays

Non-activated: 40, 80, 156, 312.5, 625, 1250, 2500, or 5000 µg/mL (Trial 1, 4 hours and Trial 2, 24 hours)

Activated: 40, 80, 156, 312.5, 625, 1250, 2500, or 5000 µg/mL (Trial 1, 4 hours)
125, 250, 500, 1000, 2000, 3000, 4000, or 5000 µg/mL (Trial 2, 4 hours)

B. TEST PERFORMANCE

1. Cell treatment

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 or 24 hours (non-activated) and 4 hours (activated).

b. After washing, cells were cultured for 68 (4 hour treatment) or 48 (24 hour treatment) hours (expression period) before cell selection.

c. After expression, 2000 cells/well (4 plates/group, 384 wells/group) were cultured for 11-14 days in selection medium to determine numbers of mutants and 1.6 cells/well (2 plates/group, 192 wells/group) were cultured for 6 days without selective agent to determine cloning efficiency.

2. Statistical methods - Statistical analysis of the data were not performed.

3. Evaluation criteria

a. Assay validity – As stated in the study report, the assay was considered valid if the following criteria were met:

- At least three out of four plates from the selective portion of the experiment were analyzable.
- The absolute cloning efficiency of the negative and/or solvent controls was >50%.
- The spontaneous mutant frequency of the negative and/or solvent controls was within the performing laboratory historical control range (30-165 mutants per 10^6 cells).
- The positive controls (EMS, MMS and B[a]P) induced significant (≥2-fold) increases in the mutant frequencies.

b. Positive result - The test article was considered mutagenic if the following criteria were met:

- A clear, concentration-related increase in the mutant frequency
- A biologically relevant positive response (≥2-fold increase above the spontaneous mutant frequency of the concurrent solvent/negative controls) for at least one of the test points
- An increased occurrence of small colonies compared to large colonies in addition to an increase in mutant frequency

II. REPORTED RESULTS

Dose formulations were not analyzed for actual concentrations. It was reported that the pH value was 7.1 at 5000 µg/mL. The reviewers assume that pH was similar to this value for the other test concentrations. It was not noted if precipitation of the test material was observed at any concentration in the presence or absence of S9.

A. PRELIMINARY CYTOTOXICITY ASSAY - In the preliminary cytotoxicity test, cells were exposed to IR8181 for 4 hours at concentrations of 39, 78, 156, 312.5, 625, 1250, 2500, or 5000 µg/mL in the absence of S9. No excessive cytotoxicity (based on % relative suspension growth) was observed at any dose after 4 hours treatment. Based on these results, doses of 40, 80, 156, 312.5, 625, 1250, 2500, or 5000 µg/mL were selected for Trial 1 (+/-S9) and Trial 2 (-S9), and doses of 125, 250, 500, 1000, 2000, 3000, 4000, or 5000 µg/mL were selected for Trial 2 (+S9) of the mutagenicity assays.

B. MUTAGENICITY ASSAY - The results of the mutagenicity assays were presented in Study Report Tables 2-13 on pages 26-33. As this Study Report did not provide a summary table of results, and the results of this assay were negative, Tables 3-4 and 6-7 are included as an Attachment to this DER.

No evidence of cytotoxicity was observed in Trial 1 (4-hour treatment, ±S9) or Trial 2 (4-hour treatment, +S9). Evidence of slight cytotoxicity (decreased relative suspension growth) was observed in Trial 2 (24-hour treatment, -S9) at 5000 µg/mL. No positive response was observed in Trial 1. In Trial 2 (+S9), a slight increase in mutation frequency was observed at 125 µg/mL, however, this increase was within the range of historical control data and not considered biologically relevant. The incidence of large and small colonies was comparable to controls at all concentrations in both trial ±S9. The positive controls (EMS and MMS, -S9 and B[a]P, +S9) induced significant increases in mutation frequency in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigator concluded that IR8181 (a metabolite of IR5878) did not induce mutation at the TK+/- locus of L5178Y mouse lymphoma cells at up to 5000 µg/mL (limit dose) in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - IR8181 (a metabolite of IR5878) was tested up to the limit dose (5000 µg/plate). Evidence of slight cytotoxicity (decreased relative suspension growth) was observed in Trial 2 (24-hour treatment, -S9) at 5000 µg/mL. No evidence of cytotoxicity was observed in Trial 1 (4-hour treatment, ±S9) or Trial 2 (4-hour treatment, +S9). No marked increase in mutation frequency was observed in either trial. The positive controls induced the expected response. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiencies were noted, but do not affect the conclusions of this DER:

- The dose formulations were not analyzed for actual concentrations.
- It was not noted if precipitation of the test material was observed at any concentration in the presence or absence of S9.
- The source, age, and weight of the animals used for the S9 fraction were not provided

ATTACHMENT

The following attachment contains Tables 3-4 and 6-7 from pages 27 and 29 of MRID 46578925

Table 3: Mutagenicity Data, **with** metabolic activation

Test Group	Concentration [µg/mL]	Cloning Efficiency (CE)			Mutagenicity Data						
		Plate 1[e]	Plate 2[e]	RCE [%][f]	Number of cultures / 96 wells					Mutants/ 10^6 cells[g]	Mutation factor
					Plate 1[e]	Plate 2[e]	Plate 3[e]	Plate 4[e]	Mean		
S1	0	76	84	100.00	21	27	18	14	20.00	104.31	
S2		86	88	100.00	19	17	15	17	17.00	65.87	
3	40	82	77	95.21	24	25	17	16	20.50	109.13	1.28
4	80	85	83	100.60	17	21	19	19	19.00	84.85	1.00
5	156	85	82	100.00	13	19	16	14	15.50	69.10	0.81
6	312.5	85	88	103.59	18	18	23	17	19.00	76.28	0.90
7	625	80	79	95.21	24	18	21	13	19.00	100.19	1.18
8	1250	76	79	92.81	14	24	11	22	17.75	99.33	1.17
9	2500	82	83	98.80	22	23	23	21	22.25	107.53	1.26
10	5000	76	77	91.62	9	18	14	20	15.25	86.82	1.02
B[a]P	1.50	76	80	93.41	40	46	46	36	42.00	274.97	3.23

S: Solvent control
e: Number of cultures with cell growth
f: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
g: Mutation frequency = {-ln [negative cultures/total wells (selective medium)] / -ln [negative cultures/total wells (non selective medium)]} x800

Table 4: Colony Sizing, **with** metabolic activation

Test Group	Concentration [µg/mL]	Wells with at least 1 colony	Large colonies	Small colonies	Quotient Large/Small
S1	0	80	58	22	2.64
S2		68	50	18	2.78
8	1250	71	51	20	2.55
9	2500	89	66	23	2.87
10	5000	61	38	23	1.65
B[a]P	1.50	168	90	78	1.15

S: Solvent control

Table 6: Mutagenicity Data, **without** metabolic activation

Test Group	Concentration [µg/mL]	Cloning Efficiency (CE)			Mutagenicity Data						
		Plate 1[e]	Plate 2[e]	RCE [%][f]	Number of cultures / 96 wells					Mutants/ 10^6 cells[g]	Mutation factor
					Plate 1[e]	Plate 2[e]	Plate 3[e]	Plate 4[e]	Mean		
S1	0	82	74	100.00	18	9	16	12	13.75	73.88	
S2		89	92	100.00	17	16	22	23	19.50	63.52	
3	40	83	84	99.11	22	14	22	13	17.75	80.23	1.17
4	80	81	86	99.11	16	16	11	19	15.50	69.10	1.01
5	156	84	81	97.92	20	14	15	17	16.50	76.91	1.12
6	312.2	83	85	99.70	16	22	20	20	19.50	87.35	1.27
7	625	82	85	99.11	21	19	16	13	17.25	77.73	1.13
8	1250	84	82	98.52	16	16	13	17	15.50	70.46	1.03
9	2500	83	85	99.70	14	20	27	14	18.75	83.60	1.22
10	5000	83	88	101.48	18	19	19	15	17.75	73.91	1.08
EMS	700	58	58	68.84	83	87	84	83	84.25	1813.19	26.39
MMS	10	67	67	79.53	67	66	59	61	63.25	718.73	10.46

S: Solvent control
e: Number of cultures with cell growth
f: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
g: Mutation frequency = {-ln [negative cultures/total wells (selective medium)] / -ln [negative cultures/total wells (non selective medium)]}x800

Table 7: Colony Sizing, **without** metabolic activation

Test Group	Concentration [µg/mL]	Wells with at least 1 colony	Large colonies	Small colonies	Quotient Large/Small
S1	0	55	44	11	4.00
S2		78	55	23	2.39
8	1250	62	44	18	2.44
9	2500	75	60	15	4.00
10	5000	71	59	12	4.92
MMS	10	253	107	146	0.73

S: Solvent control

DATA EVALUATION RECORD

IR7863; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; Mouse Lymphoma Cell/Mammalian Activation Gene Forward Mutation Assay (L5178Y TK+/-)

Work Assignment No. 3-01-82 I (MRID 46578920)

Prepared for
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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.

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Registration Action Branch 2, Health Effects Division (7509C) **Date:** 1/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 3/6/07

Template version 11/01

TXR#: 0053612

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells Gene Mutation Assay in L5178Y Mouse Lymphoma Cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR7863 (a metabolite of IR5878; Orthosulfamuron; 97.8% a.i.)

SYNONYMS: Not provided

CITATION: Poth, A. (2003) Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells with IR7863. RCC, Cytotest Cell Research GmbH, Rossdorf, Germany. Laboratory Study No.: 795503, October 27, 2003. MRID 46578920. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In two independent trials of a mammalian cell gene mutation assay at the TK+/- locus (MRID 46578920), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to IR7863 (a metabolite of IR5878; Orthosulfamuron; 97.8% a.i., Batch # 20687/50) in deionized water at concentrations of 0, 84.4, 168.8, 337.5, 675.0, 1350, or 2700 µg/mL for 4 hours (Trial 1, +/-S9) or for 24 hours (Trial 2, -S9 only). The S9 fraction was derived from the livers of male Wistar rats induced with phenobarbital/β-naphthoflavone. Cyclophosphamide (CPA) and methyl methanesulphonate (MMS) served as positive controls in the presence and absence of S9, respectively.

IR7863 (a metabolite of IR5878) was tested up to the limit of solubility (2700 µg/mL, ±S9). No evidence of cytotoxicity was observed in Trial 1 (4-hour treatment, +/-S9) or Trial 2 (24-hour treatment, -S9). No marked increase in mutation frequency was observed at any concentration in either trial (±S9).The positive controls induced the expected response. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR7863; a metabolite of IR5878

Description:	White solid
Batch #:	20687/50
Purity (w/w):	97.8% a.i.
CAS # of TGAI:	Not provided
Structure:	Not provided
Solvent used:	Deionized water (DI water)

2. Control materials

Negative - The untreated medium served as the negative control.
Solvent - DI water
Positive

Non-activation - Methyl methanesulphonate (MMS, in medium; Final concentration: 13 µg/mL)

Activation - Cyclophosphamide (CPA, in medium; Final concentration: 3 µg/mL)

3. Activation - The S9 fraction was derived from male Wistar rats (8-12 weeks old, weighing 220-320 g; supplier not reported)

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared in the laboratory and stored at -80 °C until used. It was stated that the protein concentration of the batch was 26.2 mg/mL. It was not reported if the efficacy of the batch was checked before use. It was reported that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 0.75 mg/mL in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 4 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4).

4. Test cells - Mammalian cells in culture

X	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		others (list)

Media: Culture medium - RPMI 1640 medium, supplemented with 3% horse serum (15% during the 24 hour treatment), 100 U penicillin, 100 µg/mL streptomycin, 220 µg/mL sodium-pyruvate, and 1.25 U/mL amphotericin. Selection medium - culture medium supplemented with 5 µg/mL trifluorothymidine (TFT).

Properly maintained?	X	Yes		No
Periodically checked for mycoplasma contamination?	X	Yes		Not reported
Periodically checked for karyotype stability?	X	Yes		Not reported

Periodically "cleansed" against high spontaneous background? [X] Yes [ ] Not reported

5. **Locus examined**

	X	**Thymidine kinase (TK)**		**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na^+/K^+ ATPase**
Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	trifluorothymidine (TFT, 5 µg/mL)				

6. **Test compound concentrations used**

a. Preliminary cytotoxicity assay - It was stated that the highest dose in the preliminary test was limited by the solubility of the test material in DI water.
21.1, 42.2, 84.4, 168.8, 337.5, 675.0, 1350, or 2700 µg/mL (+/-S9; 4 hours)
21.1, 42.2, 84.4, 168.8, 337.5, 675.0, 1350, or 2700 µg/mL (-S9; 24 hours)

b. Mutagenicity assays
Non-activated: 84.4, 168.8, 337.5, 675.0, 1350, or 2700 µg/mL (Trial 1, 4 hours and Trial 2, 24 hours)

Activated: 84.4, 168.8, 337.5, 675.0, 1350, or 2700 µg/mL (Trial 1, 4 hours)

Note: At day 4, the 84.4 µg/mL treated cultures were not continued since only a minimum of 4 concentrations were required.

B. TEST PERFORMANCE

1. **Cell treatment**

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 or 24 hours (non-activated) and 4 hours (activated).

b. After washing, cells were cultured for 68 (4 hour treatment) or 48 (24 hour treatment) hours (expression period) before cell selection.

c. After expression, 4000 cells/well (2 plates/group, 192 wells/group) were cultured for 10-15 days in selection medium to determine numbers of mutants and 2.5 cells/well (2 plates/group, 192 wells/group) were cultured for 10-15 days without selective agent to determine cloning efficiency. Colonies were counted manually.

2. **Statistical methods** - Statistical analysis was not performed on the mutation frequency data. The survival rate and viability was determined based on the Poisson distribution method.

3. **Evaluation criteria**

a. Assay validity - The assay was considered valid if the following criteria were met:

- Both plates from either the survival or selective portion of the experiment were analyzable.
- The absolute cloning efficiency 2 of the negative and/or solvent controls was >50%.
- The spontaneous mutant frequency of the negative and/or solvent controls was within the historical control range (39-202, 4 hours; 33-192, 24 hours)
- The positive controls (MMS and CPA) induced ≥2-fold increases in the mutant frequencies. The values of the cloning efficiencies and the relative total growth are greater than 10% of the concurrent vehicle control group.

b. Positive result - The test article was considered mutagenic if either of the following criteria were met:

- A reproducible, concentration-related increase in the mutant frequency
- A reproducible positive response (≥2-fold increase above the spontaneous mutant frequency of the concurrent solvent controls) was observed for at least one of the test points.

II. REPORTED RESULTS

Dose formulations were not analyzed for actual concentrations. Addition of the test material had no effect on the pH or osmolality of the test cultures. No precipitation of the test material was observed in either trial.

A. PRELIMINARY CYTOTOXICITY ASSAY - In the preliminary cytotoxicity test, cells were treated with the test material for 4 hours (±S9) and 24 hours (-S9) at concentrations ranging from 21.1-2700 ug/mL. Excessive cytotoxicity (% relative survival less than 10%) was not observed at any dose after 4 hours treatment (±S9) or after 24 hours treatment (-S9). Based on these results, doses of 84.4, 168.8, 337.5, 675.0, 1350, or 2700 µg/mL (+/-S9) were selected for Trial 1 of the mutagenicity assay.

B. MUTAGENICITY ASSAY - The results of the mutagenicity assays were presented in Study Report Tables IV to XV (section 12.2) on pages 28-41 and were summarized in Table I on page 14. As the results of this assay were negative, Table I is included as an Attachment to this DER.

No evidence of cytotoxicity was observed in Trial 1 (4-hour treatment, ±S9) or Trial 2 (24-hour treatment, -S9). No positive response was observed in either culture in Trial 1 or Trial 2. The positive controls (MMS, -S9 and CPA, +S9) induced significant increases in mutation frequency in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that IR7863 (a metabolite of IR5878) did not induce mutation at the TK+/- locus of L5178Y mouse lymphoma cells at up to 2700 µg/mL in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - IR7863 (a metabolite of IR5878) was tested up to the limit of solubility (2700 µg/mL, ±S9). No marked increase in mutation frequency was observed at any concentration in either trial (±S9). The positive controls induced the expected response. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiency was noted, but does not affect the conclusions of this DER:

- The dose formulations were not analyzed for actual concentrations.

ATTACHMENT

The following attachment contains Table 1 from page 14 of MRID 46578920

Table I: Summary of results

	conc. µg per mL	S9 mix	relative cloning efficiency 1	relative total growth	mutant colonies/ 10^6 cells	induction factor	relative cloning efficiency 1	relative total growth	mutant colonies/ 10^6 cells	induction factor
Column	1	2	3	4	5	6	7	8	9	10
Experiment I			**culture I**				**culture II**			
Neg. contr. with medium		-	100.0	100.0	131		100.0	100.0	129	
Solvent contr. with water		-	100.0	100.0	123	1.0	100.0	100.0	165	1.0
Pos. control with MMS	13.0	-	88.3	87.9	506	3.9	95.6	59.9	460	3.6
Test item	84.4	-	132.6	culture was not continued#			95.3	culture was not continued#		
Test item	168.8	-	101.6	205.5	76	0.6	106.9	89.4	126	0.7
Test item	337.5	-	91.1	203.4	69	0.6	83.1	124.9	176	1.0
Test item	675.0	-	96.9	113.2	127	1.0	86.9	98.0	176	1.0
Test item	1350.0	-	103.3	159.9	100	0.8	84.3	76.9	140	0.8
Test item	2700.0	-	95.4	168.8	106	0.9	72.8	91.2	155	0.8
Neg. contr. with medium		+	100.0	100.0	124		100.0	100.0	128	
Solvent contr. with water		+	100.0	100.0	126	1.0	100.0	100.0	166	1.0
Pos. control with CPA	3.0	+	38.4	31.7	798	6.4	33.3	50.4	1098	8.6
Test item	84.4	+	72.6	culture was not continued#			84.5	culture was not continued#		
Test item	168.8	+	68.3	66.6	110	0.9	83.2	117.7	103	0.8
Test item	337.5	+	77.2	70.8	142	1.1	84.5	147.3	123	0.7
Test item	675.0	+	98.1	99.4	160	1.3	130.6	134.9	133	0.8
Test item	1350.0	+	66.3	104.3	134	1.1	73.6	158.9	146	0.9
Test item	2700.0	+	100.0	91.2	75	0.6	101.8	170.5	149	0.9
Experiment II			**culture I**				**culture II**			
Neg. control with medium		-	100.0	100.0	113		100.0	100.0	87	
Solvent control with water		-	100.0	100.0	111	1.0	100.0	100.0	102	1.0
Solv. control with MMS	13.0	-	43.9	32.8	705	6.2	33.2	19.4	1264	14.6
Test item	84.4	-	144.9	culture was not continued#			117.1	culture was not continued#		
Test item	168.8	-	98.1	111.1	115	1.0	85.7	98.3	80	0.8
Test item	327.5	-	96.2	111.3	97	0.9	96.5	81.5	102	1.0
Test item	675.0	-	94.4	126.3	89	0.8	107.9	103.8	79	0.8
Test item	1350.0	-	98.1	103.1	105	0.9	100.0	85.9	109	1.1
Test item	2700.0	-	89.4	86.6	155	1.4	103.8	90.7	102	1.0

culture was not continued since a minimum of four concentrations is required by the guidelines

DATA EVALUATION RECORD

IR7825; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; Mouse Lymphoma Cell/Mammalian Activation Gene Forward Mutation Assay (L5178Y $TK^{+/-}$)

Work Assignment No. 3-01-82 E (MRID 46578914)

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.

15

EPA Reviewer: Lisa Austin, Ph.D. **Signature:**
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Work Assignment Manager: P.V. Shah, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 2/6/07

Template version 11/01

TXR#: 0053612

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells in Culture Gene Mutation Assay in L5178Y Mouse Lymphoma Cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR7825; a metabolite of IR5878; Orthosulfamuron; 99.3%)

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

CITATION: Wollny, H. (2003) Cell Mutation Assay at the Thymidine Kinase Locus (TK +/-) in Mouse Lymphoma L5178Y Cells with IR7825. RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Laboratory Study No.: 778803, June 24, 2003. MRID 46578914. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In two independent trials of an *in vitro* mammalian cell gene mutation assay at the $TK^{+/-}$ locus (MRID 46578914), L5178Y mouse lymphoma cells were exposed to IR7825 (a metabolite of IR5878; Orthosulfamuron; 99.3% a.i., Batch # FCF/T/198-01 [ex 20687/38]) in dimethylsulfoxide for 4 hours at concentrations of 10.9, 21.9, 43.8, 87.5, 175, or 350 µg/mL (Trial 1) in the presence and absence of S9-activation (derived from the livers of male Wistar rats induced with phenobarbital/β-naphthoflavone) or for 24 hours at the same concentrations (Trial 2, -S9 only). Methyl methanesulphonate (MMS) and 20-methylcholanthrene (3-MC) served as positive controls in the absence and presence of S9, respectively.

IR7825 (a metabolite of IR5878) was tested up to the limit of solubility (350 µg/mL). No evidence of cytotoxicity was observed in Trial 1 (4-hour treatment) with or without S9-activation. Moderate cytotoxicity (relative cloning efficiency, 43.1-45.2%; and relative total growth, 23.1-34.1%) was observed in the second culture of Trial 2 (24-hour treatment) at ≥175 µg/mL. No biologically-relevant positive response was observed in either trial. The positive

controls induced the expected response. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR7825; a metabolite of IR5878

Description:	White solid
Batch #:	FCF/T/198-01 (ex 20687/38)
Purity (w/w):	99.3% a.i.
CAS # of TGAI:	Not provided
Structure:	Not reported
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control materials

Negative - The untreated medium served as the negative control.
Solvent - DMSO (0.5% v/v)
Positive

Non-activation - Methyl methanesulphonate (MMS, in medium; Final concentration: 13 µg/mL)

Activation - 3-Methylcholanthrene (3-MC, in DMSO; Final concentration: 3 µg/mL)

3. Activation - The S9 fraction was derived from male Wistar rats (8-12 weeks old, weighing 220-320 g; supplier not reported)

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared in the laboratory and stored at -80°C until used. It was stated that the protein concentration of the batch was 34.6 mg/mL. It was not reported if the efficacy of the batch was checked before use. It was reported that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 0.75 mg/mL in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 4 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4)

4. Test cells - Mammalian cells in culture

X	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		others (list)

Media: Culture medium - RPMI 1640 medium, supplemented with 3% horse serum (15% during the 24 hour treatment), 100 U penicillin, 100 µg/mL streptomycin, 220 µg/mL sodium-pyruvate, and 1.25 U/mL amphotericin. Selection medium - culture medium supplemented with 5 µg/mL trifluorothymidine (TFT).

Properly maintained?	X	Yes		No
Periodically checked for mycoplasma contamination?	X	Yes		Not reported
Periodically checked for karyotype stability?	X	Yes		Not reported
Periodically "cleansed" against high spontaneous background?	X	Yes		Not reported

5. Locus examined

	X	**Thymidine kinase (TK)**		**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na^+/K^+ ATPase**
Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	trifluorothymidine (TFT, 5 µg/mL)				

6. Test compound concentrations used

a. Preliminary cytotoxicity assay - It was stated that the highest dose in the preliminary test was limited by the solubility of the test material in DMSO.

2.7, 5.5, 10.9, 21.9, 43.8, 87.5, 175, or 350 µg/mL (+/-S9; 4 hours)
2.7, 5.5, 10.9, 21.9, 43.8, 87.5, 175, or 350 µg/mL (-S9; 24 hours)

b. Mutagenicity assays

Non-activated: 10.9, 21.9, 43.8, 87.5, 175, or 350 µg/mL (Trial 1, 4 hours and Trial 2, 24 hours)

Activated: 10.9, 21.9, 43.8, 87.5, 175, or 350 µg/mL (Trial 1, 4 hours)

Note: The 10.9 ug/mL cultures were not continued since only a minimum of four concentrations were required.

B. TEST PERFORMANCE

1. Cell treatment

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 or 24 hours (non-activated) and 4 hours (activated).

b. After washing, cells were cultured for 68 (4 hour treatment) or 48 (24 hour treatment) hours (expression period) before cell selection.

c. After expression, 4000 cells/well (2 plates/group, 192 wells/group) were cultured for 10-15 days in selection medium to determine numbers of mutants; and 2.5 cells/well (2 plates/group, 192 wells/group) were cultured for 10-15 days without selective agent to determine cloning efficiency. Colonies were counted manually.

2. **Statistical methods** – The survival rate and viability were determined based on the Poisson distribution. Statistical analysis of the mutation frequency data was not performed.

3. **Evaluation criteria**

a. **Assay validity** - The assay was considered valid if the following criteria were met:
- Both plates from either the survival or selective portion of the experiment were analyzable.
- The absolute cloning efficiency 2 of the negative and/or solvent controls was >50%.
- The spontaneous mutant frequency of the negative and/or solvent controls was within the historical control range (39-202, 4 hours; 33-192, 24 hours) of the performing laboratory.
- The positive controls (MMS and 3-MC) induced significant (≥2-fold) increases in the mutant frequencies. The values of the cloning efficiencies and the relative total growth are greater than 10% of the concurrent solvent control group.

b. **Positive result** - The test article was considered mutagenic if either of the following criteria were met:
- A reproducible, concentration-related increase in the mutant frequency.
- A reproducible positive response (≥2-fold increase above the spontaneous mutant frequency of the concurrent solvent controls) for at least one of the test points.

II. REPORTED RESULTS

Dose formulations were not analyzed for actual concentrations. Addition of the test material had no effect on the pH or osmolality of the test cultures without metabolic activation. It was stated in the study report that precipitation was observed at >350 ug/mL.

A. **PRELIMINARY CYTOTOXICITY ASSAY** - In the preliminary cytotoxicity test, cells were exposed to the test material at concentrations ranging from 2.5 to 350 ug/mL for 4 hours (±S9) or 24 hours (-S9). No precipitation or excessive cytotoxicity (% relative survival less than 10%) was observed at any dose after either treatment. Based on these results, doses of 10.9, 21.9, 43.8, 87.5, 175, or 350 µg/mL (+/-S9) were selected for Trial 1 of the mutagenicity assay.

B. **MUTAGENICITY ASSAY** - The results of the mutagenicity assays were presented in Study Report Tables 4-15 on pages 28-42. As the results of this assay were negative, Tables 6 and 12 are included as an Attachment to this DER.

The test material was tested up to the limit of solubility in DMSO (350 µg/mL). No evidence of cytotoxicity was observed in Trial 1 (4-hour treatment) with or without S9-activation. Moderate cytotoxicity (relative cloning efficiency, 43.1-45.2%; and relative total growth, 23.1-34.1%) was

observed in the second culture of Trial 2 at ≥175 µg/mL. No positive response was observed in either culture in Trial 1 in the presence or absence of S9. In the first culture of Trial 2 (-S9), the number of mutants per 10^6 cells were greater than 2x the solvent control at ≥175 µg/mL (180 and 186 treated vs 78 control). However, this finding was considered not to be biologically relevant as the number of mutants was not 2x the negative control, the finding was not reproducible, and the values were within the historical control range. The positive controls (MMS, -S9 and 3-MC, +S9) induced a ≥2-fold increase in mutation frequency in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that IR7825 (a metabolite of IR5878) did not induce mutation at the $TK^{+/-}$ locus of L5178Y mouse lymphoma cells in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - IR7825 (a metabolite of IR5878) was tested up to the limit of solubility (350 µg/mL). No biologically-relevant positive response was observed in either trial. The positive controls induced the expected response. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiencies were noted, but do not affect the conclusions of this DER:

- The dose formulations were not analyzed for actual concentrations.
- The source of the animals used for the S9 fraction was not provided.
- Flagging statement was not provided.

ATTACHMENT

The following attachment contains Tables 6 and 12 from pages 30 and 38 of MRID 46578914

Table VI: Mutagenicity data (part 2); experiment I; culture i

			number of empty wells found after plating in TFT medium*								mean		mutant	small	large
			calculated for plate 1			calculated for plate 2			mean	mean	of all		colonies	mutant	mutant
	conc. µg	S9	large	small		large	small		large	small	empty	standard	per	colonies/	colonies/
	per mL	mix	colonies	colonies	sum.	colonies	colonies	sum.	colonies	colonies	wells	deviation	10^6 cells	10^6 cells	10^6 cells
Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Neg. contr. with medium		-	85	75	64	90	77	71	88	76	67.5	4.95	86	61	24
Solvent control with DMSO		-	90	78	72	89	78	71	90	78	71.5	0.71	71	53	18
Pos. control with MMS	13.0	-	86	35	25	87	35	26	87	35	25.5	0.71	440	398	41
Test item	10.9	-	culture was not continued#												
Test item	21.9	-	91	73	68	93	76	73	92	75	70.5	3.54	83	71	12
Test item	43.8	-	91	74	69	92	73	69	92	74	69.0	0.00	94	80	14
Test item	87.5	-	92	77	73	90	81	75	91	79	74.0	1.41	59	46	13
Test item	175.0	-	95	85	84	91	83	78	93	84	81.0	4.24	43	34	8
Test item	350.0	-	95	75	74	95	79	78	95	77	76.0	2.83	71	68	3
Neg. contr. with medium		+	91	69	64	90	65	59	91	67	61.5	3.54	114	98	16
Solvent control with DMSO		+	88	68	60	91	66	61	90	67	60.5	0.71	135	113	22
Pos. control with 3-MC	3.0	+	85	23	12	81	26	11	83	25	11.5	0.71	956	864	92
Test item	10.9	+	culture was not continued#												
Test item	21.9	+	92	68	64	92	65	61	92	67	62.5	2.12	178	159	18
Test item	43.8	+	91	58	53	89	58	51	90	58	52.0	1.41	176	156	20
Test item	87.5	+	93	68	65	90	59	53	92	64	59.0	8.49	159	142	17
Test item	175.0	+	92	75	71	91	68	63	92	72	67.0	5.66	116	99	16
Test item	350.0	+	92	66	62	90	63	57	91	65	59.5	3.54	163	143	19

* 96 wells minus number of wells containing large or small colonies

\# culture was not continued since only a minimum of four concentrations is required by the guidelines

Table XII: Mutagenicity data (part 2); experiment II; culture I

			number of empty wells per plate found after plating in TFT medium*								mean		mutant	small	large
	conc.	S9	calculated for plate 1			calculated for plate 2			mean	mean	of all		colonies	mutant	mutant
	µg/mL	mix	large	small		large	small		large	small	empty	standard	per	colonies/	colonies/
			colonies	colonies	sum.	colonies	colonies	sum.	colonies	colonies	wells	deviation	10^6 cells	10^6 cells	10^6 cells
Column	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Neg. contr.		-	94	64	62	87	63	54	91	64	58.0	5.66	111	97	14
Solvent contr. with DMSO		-	95	71	70	95	69	68	95	70	69.0	1.41	78	76	3
Pos. control with MMS	13.0	-	85	15	4	85	18	7	85	17	5.5	2.12	1424	1332	92
Test item	10.9	-	culture was not continued#												
Test item	21.9	-	91	56	51	92	60	56	92	58	53.5	3.54	153	140	13
Test item	43.8	-	91	75	70	92	73	69	92	74	69.5	0.71	104	88	16
Test item	87.5	-	93	63	60	92	65	61	93	64	60.5	0.71	116	106	10
Test item	175.0	-	92	67	63	92	62	58	92	65	60.5	3.54	180	162	17
Test item	350.0	-	95	60	59	94	65	63	95	63	61.0	2.83	186	180	7

* 96 wells minus number of wells containing large or small colonies

\# culture was not continued since only a minimum of four concentrations is required by the guidelines

DATA EVALUATION RECORD

IR8181; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; *In Vitro* Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes

Work Assignment No. 3-01-82 L (MRID 46578924)

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.

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Work Assignment Manager: P.V. Shah, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 2/6/07

TXR#: 0053612

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cytogenetics (Chromosomal Aberration assay in Human Peripheral Blood Lymphocytes) OPPTS 870.5375 [§84-2]; OECD 473.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i.)

SYNONYMS: 1-[2-(dimethylcarbamoyl)phenylsulfamoyl]-3-(4-hydroxy-6-methoxy-2-pyrimidinyl)urea

CITATION: Becker, T. (2004) *In vitro* mammalian chromosome aberration test in human lymphocytes with IR8181. BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany. Laboratory Study No.: 040383, July 13, 2004. MRID 46578924. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In a mammalian cell cytogenetics assay (chromosome aberration; MRID 46578924), duplicate lymphocyte cultures, prepared from human blood, were exposed to IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i., Batch # 30072/85) in culture medium at concentrations of 0, 78, 156, 313, 625, 1250, 2500, or 5000 µg/mL (limit dose) in the presence and absence of S9-activation for 4 hours with a 20 hour recovery period. Cyclophosphamide and ethylmethane sulfonate served as the positive controls in the presence and absence of S9, respectively.

IR8181 was tested up to the limit dose (5000 µg/mL, +/-S9). Precipitation was not observed at any concentration ±S9. Evidence of cytotoxicity (reduced relative mitotic index) was observed at 5000 µg/mL (+S9). Increases (exceeding the historical control range) in the mean percent aberrant cells (excluding gaps) were noted at 1250 and 2500 µg/mL (+S9; 5.0-7.0 treated vs 4.0 control) and at ≥1250 µg/mL (-S9; 4.5-11.0 treated vs 3.0 control). Polyploidy was not observed at any concentration ±S9. The positive controls induced the appropriate response. **There was evidence of chromosome aberrations induced over background in the presence and absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the Guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR8181 (a metabolite of IR5878)

Description:	White, odorless powder
Batch #:	30072/85
Purity:	97.02% a.i.
Stability:	Not reported
CAS # of TGAI:	Not available
Structure:	Not provided
Solvent used:	Culture medium (RPMI 1640)

2. Control materials

Negative control: Untreated medium served as the negative control.
Solvent control: None
Positive controls
Non-activation: Ethylmethane sulfonate (EMS, 600 µg/mL in nutrient medium)
Activation: Cyclophosphamide (CPA, 7.5 µg/mL in nutrient medium)

3. Activation - The S9 fraction was derived from male Wistar rats (age, weight, and supplier were not provided):

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared in the laboratory and stored at ≤-75 °C until used. It was stated that the protein concentration of the batch was 34 mg/mL. It was stated that the efficacy of the batch was checked before use (*Salmonella typhimurium* assay). It was stated that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 0.75 mg/mL in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 4 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4).

4. Test cells - Human blood samples were obtained by venipuncture in heparinized tubes within 4 hours of culture initiation from one female donor (not receiving medication).

	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells	X	Human lymphocytes

Media: The culture medium consisted of serum-free RPMI-1640 medium supplemented with 0.05 mL heparin, 0.50 mL phytohemagglutinin and an antibiotic solution (concentrations not reported).

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination? **Not applicable**		Yes		No
Periodically checked for karyotype stability? **Not applicable**		Yes		No

Whole blood cultures were established within 4 hours of collection by placing 0.9 mL blood in 8.5 mL culture medium. To stimulate the lymphocytes to divide, 0.5 mL of phytohemagglutinin (concentration not reported) was added, and the cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2.

5. Test compound concentrations used:

Preliminary cytotoxicity: (Duplicate cultures)	Non-activated and Activated conditions:	78, 156, 313, 625, 1250, 2500, or 5000 µg/mL for 4 hours with an 20 hour recovery period
Cytogenetic assay: (Duplicate cultures)	Non-activated and Activated conditions:	156, 313, 625, 1250, 2500, or 5000 µg/mL for 4 hours with an 20 hour recovery period

B. TEST PERFORMANCE

1. **Preliminary cytotoxicity assay** - A preliminary cytotoxicity test was performed using concentrations of 78, 156, 313, 625, 1250, 2500, or 5000 µg/mL (limit dose) following the same procedures described below. The mitotic index was used as an indicator of cytotoxic effects. The mitotic index was determined from the count of 1000 cells per culture. Based on the results of the preliminary test, concentrations of 156, 313, 625, 1250, 2500, or 5000 µg/mL were chosen for the mutagenicity assay.

2. **Cytogenetic assay** - Approximately 48 hours after culture establishment, the culture medium was replaced with serum-free medium containing the test material. Additionally, 50 µL/mL of the S9 mix was added to the cultures requiring activation. Concurrent negative and positive controls were performed. The treatment medium was removed by centrifugation after 4 hours of exposure, and the cells were washed twice by resuspension in phosphate buffered saline (PBS). After washing, the cells were resuspended in fresh medium for the 20 hour recovery period. It was stated that due to the positive result observed after 4 hours of treatment, a long-time (24 hour) exposure was not performed.

a. Cell exposure time (hrs)

	Test material		Negative control		Positive control	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Non-activated	4	NA	4	NA	4	NA
Activated	4	NA	4	NA	4	NA

b. Spindle inhibition

Inhibitor use (concentration): Colcemid (0.2 µg/mL)

Administration time: At least 2 hours (prior to cell harvest)

c. Cell harvest time after termination of treatment (hrs)	Test material		Negative control		Positive control	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Non-activated	20	NA	20	NA	20	NA
Activated	20	NA	20	NA	20	NA

NA - Not applicable

d. Details of slide preparation - After cell division was arrested, the cultures were centrifuged, the supernatant was removed, and the cells were resuspended in 5 mL hypotonic KCl (0.4%) for 20 minutes at 37°C. The cultures were again centrifuged, the supernatant was removed, and the cells were fixed in methanol/glacial acetic acid (3:1, v/v). The lymphocytes were dropped onto clean microscope slides. The slides were stained with Giemsa, rinsed, air-dried, and mounted with coverslips (assumed by reviewers).

e. Metaphase analysis - The aberration percentages, both including and excluding gaps, were recorded.

No. of cells examined per dose: 200 (100/replicate) cells in the treated, negative, and positive controls were scored for structural aberrations; 1000 cells for mitotic index; and 100 metaphase cells/culture were scored for numerical aberrations.

Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes, polyploidy		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria

Assay validity - The assay was considered valid if the following criteria were met:
- The number of chromosomal aberrations in the negative and/or solvent controls was within the historical control range (0.0-4.0%).
- The positive controls induced biologically relevant increases in the frequencies of structural aberrations.

Positive result - The test article was considered to be mutagenic if either of the following criteria were met:
- A clear and dose-related increase in the number of cells with aberrations.
- A biologically relevant response for at least one dose group, which exceeds the negative and/or solvent historical control range (0.0-4.0%).

g. Statistical methods - Statistical analysis of the data was not performed.

II. REPORTED RESULTS

The dose formulations were not analyzed for actual concentrations. No precipitation of the test material was observed at any concentration in the presence or absence of S9. It was not reported if addition of the test material had any significant effect on the osmolality or pH of the culture media.

A. PRELIMINARY CYTOTOXICITY ASSAY - In the preliminary cytotoxicity test, cytotoxicity (as indicated by a decrease in relative mitotic index) was limited to 5000 µg/mL in the presence of S9 (relative mitotic index 50%). Based on these results, concentrations of 156, 313, 625, 1250, 2500, or 5000 µg/mL were selected for the cytogenetic assay.

B. CYTOGENETIC ASSAY - The results of the cytogenetic assay were presented in the Study Report Tables 2-4 on pages 24-26, and were summarized in Table 5 on page 27.

The test material was tested up to the limit dose (5000 µg/mL, ±S9). Concentrations of 156, 313, 625, 1250, 2500, or 5000 µg/mL were tested for 4 hours (with a 20 hour recovery period) in the presence and absence of S9 activation. The concentrations evaluated for aberrant cells were 1250, 2500, or 5000 µg/mL. Evidence of cytotoxicity (reduced relative mitotic index) was observed 5000 µg/mL (+S9, 50%, Table 1). The mean percent aberrant cells in the negative controls (4.0, +S9 and 3.0, -S9, Table 2) were within the historical control range (0-4%). In the presence of S9, increases in the mean percent aberrant cells (excluding gaps) were noted at 1250 and 2500 µg/mL (5.0-7.0 treated vs 4.0 control). The percent aberrant cells at 5000 µg/mL (+S9) was within the historical control range (3.5%); however, this was likely due to the markedly decreased mitotic index (50%). In the absence of S9, increases in the mean percent aberrant cells (excluding gaps) were noted at ≥1250 µg/mL (4.5-11.0%). Numerical aberrations were not observed at any concentration ±S9. The positive controls induced marked increases in mean percent aberrant cells (28.0 and 14.5, in the presence and absence of S9, respectively).

Table 1. Summary of mitotic index data in human lymphocytes treated with IR8181 for 4 hours with a 20 hour recovery period.[a]

Concentration (µg/mL)	Relative Mitotic Index (%)	
	Without S9	With S9
0	100	100
1250	74	74
2500	77	57
5000	58	50
CPA [c]	74	34

a Data were obtained from Tables 2 and 5 on pages 24 and 27 of the study report, MRID 46578924.

b Historical control range for mean % aberrant cells is 0-4%.

c Cyclophasphamide (7.5 µg/mL) in the presence of S9 and EMS (600 µg/mL) in the absence of S9

TABLE 2. Cytogenetic assay with IR8181 using four-hour exposure[a]

Treatment (µg/mL)	Gaps	Chromatid Aberrations[b]			Chromosome Aberrations[c]			Exchanges	Other[d]	Aberrant Metaphases (%)[e]
		b	f	d	ib	if	id			
Without S9-mix, 4-hour treatment, 20-hour post-treatment harvest (200 cells/treatment)										
0	1	4	1	0	0	0	0	0	0	3.0
IR8181										
1250	2	12	6	0	2	0	0	0	0	7.5
2500	1	4	3	2	1	0	0	0	0	4.5
5000	11	10	11	1	0	2	0	0	0	11.0
EMS(600)	11	17	5	3	1	3	0	0	0	14.5
With S9-mix, 4-hour treatment, 20-hour post-treatment harvest (200 cells/treatment)										
0	2	8	0	1	0	0	0	1	0	4.0
IR8181										
1250	7	5	3	1	0	1	0	0	0	5.0
2500	3	7	6	0	0	0	0	1	0	7.0
5000	6	4	1	1	1	0	0	0	0	3.5
CPA (7.5)	10	52	5	3	6	0	0	14	6	28.0

[a] Data summarized from Tables 3 and 4 pages 25 and 26 in Study Report, MRID 46578924.
[b] Chromatid: b = breaks, f = fragments, d = deletions
[c] Chromosome: ib = breaks, if = fragments, id = deletions
[d] Other = sum of metaphases with multiple aberrations, multiple aberration with exchanges and/or chromosome disintegration
[e] Percentage of metaphases with structural aberrations excluding gaps
EMS = ethyl methanesulfonate
CPA = cyclophosphamide

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that IR8181 (a metabolite of IR5878) did induce chromosome aberrations in cultured human peripheral blood lymphocytes at ≥1250 µg/mL in the presence and absence of S9.

B. REVIEWER COMMENTS - The test material was tested up to the limit dose (5000 µg/mL, ±S9). Precipitation was not observed at any concentration ±S9. Evidence of cytotoxicity (reduced relative mitotic index) was observed at 5000 µg/mL (+S9). Increases (exceeding the historical control range, 0-4%) in the mean percent aberrant cells (excluding gaps) were noted at 1250 and 2500 µg/mL (+S9) and at ≥1250 µg/mL (-S9). Numerical aberrations were not observed at any concentration ±S9. The positive controls induced the appropriate response in the presence and absence of S9. **There was evidence of chromosome aberrations induced over background in the presence and absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the Guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data.

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

- The dose formulations were not analyzed for actual concentrations of test substance.
- The age, weight, and supplier of the rats used for the S9 fraction were not provided.
- Effects on the osmolality and pH of the medium by addition of the test material were not reported.

DATA EVALUATION RECORD

IR7825; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; *In Vitro* Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes

Work Assignment No. 3-01-82 G (MRID 46578917)

Prepared for

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

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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: Lisa Austin, Ph.D. **Signature:** Lisa Austin
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 1/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 2/6/07
Template version 11/01

TXR#: 0053612

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cytogenetics (Chromosomal Aberration assay in Human Peripheral Blood Lymphocytes) OPPTS 870.5375 [§84-2]; OECD 473.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR7825 (a metabolite of IR5878; Orthosulfamuron; 99.3% a.i., Batch # FCF/T/198-01 [ex 20687/38])

SYNONYMS: Not provided

CITATION: Schulz, M. (2003) Chromosome Aberration Test in Human Lymphocytes *In Vitro* with IR7825. RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Laboratory Study No.: 778804, July 17, 2003. MRID 46578917. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In two independent trials of a mammalian cell cytogenetics assay (chromosome aberration; MRID 46578917), lymphocyte cultures were prepared from human peripheral blood and exposed to IR7825 (a metabolite of IR5878; Orthosulfamuron; 99.3% a.i., Batch # FCF/T/198-01 [ex 20687/38]) in dimethylsulfoxide (DMSO) at concentrations of 2.3, 4.0, 7.0, 12.2, 21.3, 37.3, 65.3, 114.3, 200, or 350 µg/mL for 4 hours with an 18 hour recovery period (Trial 1, +/-S9); 2.3, 4.0, 7.0, 12.2, 21.3, 37.3, 65.3, 114.3, 200, or 350 µg/mL for 22 hours with no recovery period (Trial 1, -S9); 37.3, 65.3, 114.3, 200, or 350 µg/mL for 46 hours with no recovery period (Trial 2, -S9); or 37.3, 65.3, 114.3, 200, or 350 µg/mL for 4 hours with a 42 hour recovery period (Trial 2, +S9).

IR7825 was tested up to the limit of solubility in DMSO (350 µg/mL). Cytotoxicity was only observed in Trial 2 at 350 µg/mL in the 46 hour exposure in the absence of S9 (mitotic index was 32% of controls). There were no treatment-related increases in the percent aberrant cells (excluding gaps) noted in either trial in the presence or absence of S9 at any exposure period. An increase in polyploidy was only observed in Trial 1 at 200 ug/mL in the 4 hour exposure in the presence of S9 (0.8% vs 0/0.2% in the negative/solvent controls, respectively). The positive controls induced the appropriate response in both trials in the presence and absence of S9. **There was no evidence of chromosome structural or numerical aberrations induced over background in the presence or absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the Guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR7825 (a metabolite of IR5878)

Description: White solid
Batch #: FCF/T/198-01 (ex 20687/38)
Purity: 99.3% a.i.
Stability: Not reported
CAS # of TGAI: Not provided
Structure: Not provided
Solvent used: Dimethylsulfoxide (DMSO)

2. Control materials

Negative control: Untreated medium served as the negative control.
Solvent control: DMSO (0.5 %/mL)
Positive controls
Non-activation: Ethylmethane sulfonate (EMS, 440-550 µg/mL in nutrient medium)
Activation: Cyclophosphamide (CPA, 15-22.5 µg/mL in nutrient medium)

3. Activation - The S9 fraction was derived from male Wistar rats (8-12 weeks old, weighing 220-320 g; supplier not reported):

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared in the laboratory and stored at -80 °C until used. It was stated that the protein concentration of the batches was 26.2-34.6 mg/mL. It was not reported if the efficacy of the batches was checked before use. It was stated that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 0.75 mg/mL in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 4 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4).

4. Test cells - Human blood samples were obtained by venipuncture in heparinized tubes within 24 hours of culture initiation from one male donor (not receiving medication).

	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells	X	Human lymphocytes

Media: The complete culture medium consisted of modified Eagle medium/Ham's F-12 medium supplemented with 10% (v/v) fetal calf serum (FCS), 3 µg/mL phytohemagglutinin,25,000 U/mL heparin, 10,000 units/mL penicillin, and 10,000 µg/mL streptomycin.

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination? **Not applicable**		Yes		No
Periodically checked for karyotype stability? **Not applicable**		Yes		No

Whole blood cultures were established by placing 0.9 mL blood in 9.05 mL complete culture medium. To stimulate the lymphocytes to divide, 0.05 mL of phytohemagglutinin (3 µg/mL) was added, and the cultures were incubated at 37°C in a humidified atmosphere with 15% CO_2.

5. Test compound concentrations used: Since the preliminary cultures fulfilled the requirements for cytogenic evaluation, the preliminary test was designated as Trial 1.

Non-activated conditions:	Preliminary cytotoxicity: (Duplicate cultures)	2.3, 4.0, 7.0, 12.2, 21.3, 37.3, 65.3, 114.3, 200, or 350 µg/mL for 4 or 22 hours with an 18 and 0 hour recovery period, respectively
	Mutagenicity assay (Duplicate cultures)	2.3, 4.0, 7.0, 12.2, 21.3, 37.3, 65.3, 114.3, 200, or 350 µg/mL for 4 or 22 hours with an 18 and 0 hour recovery period, respectively (Trial 1)
		37.3, 65.3, 114.3, 200, or 350 µg/mL for 46 hours with no recovery period (Trial 2)
Activated conditions:	Preliminary cytotoxicity: (Duplicate cultures)	2.3, 4.0, 7.0, 12.2, 21.3, 37.3, 65.3, 114.3, 200, or 350 µg/mL for 4 hours with an 18 hour recovery period
	Mutagenicity assay: (Duplicate cultures)	2.3, 4.0, 7.0, 12.2, 21.3, 37.3, 65.3, 114.3, 200, or 350 µg/mL for 4 hours with an 18 hour recovery period (Trial 1)
		37.3, 65.3, 114.3, 200, or 350 µg/mL for 4 hours with a 42 hour recovery period (Trial 2)

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay - A preliminary cytotoxicity test was performed following the same procedure as described below. It was stated that the highest concentration used (350 µg/mL, ±S9) was based on the solubility of the test material in DMSO. Since the preliminary cultures fulfilled the requirements for cytogenic evaluation, the preliminary test was designated as Trial 1 of the cytogenetic assay.

2. Cytogenetic assay - Approximately 50-80 hours after culture establishment, the culture medium was replaced with serum-free medium (for treatment with S9) or with complete medium with 10% FCS (for treatment without S9), containing the test material. Additionally, 50 µL/mL of the S9 mix was added to the cultures requiring activation. Concurrent solvent, negative, and positive controls were performed. In Trial 1 (±S9) and Trial 2 (+S9), the treatment medium was removed by centrifugation after 4 hours of exposure, and the cells were washed twice by resuspension in 'saline G' (composed of the following [per liter]: 8000 mg NaCl, 400 mg KCl, 1100 mg glucose, 290 mg sodium phosphate buffer, and 150 mg KH_2PO_4 at pH 7.2). After washing, the cells were resuspended in complete medium for the 18 or 42 hour recovery period (Trial 1 ±S9 and Trial 2 +S9, respectively). For the 22 and 46 hour exposure periods in the absence of S9 (Trial 1 and 2, respectively), the cultures remained in the treatment medium until slide preparation (no recovery period).

a. Cell exposure time (hrs)	Test material		Solvent control		Positive control	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Non-activated	4 or 22	46	4 or 22	46	4 or 22	46
Activated	4	4	4	4	4	4

b. Spindle inhibition

Inhibitor use (concentration): Colcemid (0.2 µg/mL)

Administration time: 3 hours (prior to cell harvest)

c. Cell harvest time after termination of treatment (hrs)	Test material		Solvent control		Positive control	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Non-activated	18 or 0	0	18 or 0	0	18 or 0	0
Activated	18	42	18	42	18	42

d. Details of slide preparation - After cell division was arrested, the cultures were centrifuged, the supernatant was removed, and the cells were resuspended in 5 mL hypotonic KCl (0.0375 M) for 20 minutes at 37°C. The cultures were again centrifuged, the supernatant was removed, and the cells were fixed in methanol/glacial acetic acid (3:1, v/v). The lymphocytes were dropped onto clean microscope slides (at least 2 slides/group). The slides were stained with Giemsa or according to the Fluorescent plus Giemsa technique, rinsed, air-dried, and mounted with coverslips (assumed by reviewers).

e. Metaphase analysis - The aberration percentages, both including and excluding gaps, were recorded.

No. of cells examined per dose: 200 (100/replicate) cells in the treated, negative, solvent, and positive controls were scored for structural aberrations; 1000 cells for mitotic index; and 250 metaphase cells were scored for numerical aberrations.

Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes, polyploidy and endoreduplication		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria

Assay validity - The assay was considered valid if the following criteria were met:

- The number of chromosomal aberrations in the negative and/or solvent controls was within the performing laboratory historical control range (0.0-4.0%).
- The positive controls induced statistically significant increases in the frequencies of aberrations.

Positive result - The test article was considered to be mutagenic if the following criteria were met:

- A statistically significant or concentration-related increase in the number of structural aberrations was observed, and the increase exceeded the historical control range (0.0-4.0%).

g. Statistical methods - The data were analyzed using the Fisher-Exact test. Significance was defined at $p \leq 0.05$.

II. REPORTED RESULTS

The dose formulations were not analyzed for actual concentrations. Addition of the test material had no significant effect on the osmolality or pH of the culture media.

A. PRELIMINARY CYTOTOXICITY ASSAY - The results of the preliminary cytotoxicity test were designated as Trial 1 of the cytogenetic assay and are reported below.

B. CYTOGENETIC ASSAY - The results of the cytogenetic assays were presented in the Study Report Tables 5-13 on pages 27-36, and were summarized in Table 1 on pages 13-14. As the results of these assays were negative, a copy of Table 1 is included as an Attachment to this DER.

The test material was tested up to the limit of solubility in DMSO (350 µg/mL). In Trial 1, concentrations of 2.3, 4.0, 7.0, 12.2, 21.3, 37.3, 65.3, 114.3, 200, or 350 µg/mL were tested in the presence and absence of S9-activation. In Trial 2, concentrations of 37.3, 65.3, 114.3, 200, or 350 µg/mL were tested in the presence and absence of S9-activation. There was no evidence of precipitation at any concentration for any exposure time (±S9) in either trial. In Trial 1, the concentrations evaluated for aberrant cells were 114.3, 200, and 350 µg/mL in the 4 hour (±S9) and 22 hour (-S9) exposures. In Trial 2, the concentrations evaluated for aberrant cells were 200 and 350 µg/mL for the 46 hour exposure (-S9), and 114.3, 200, and 350 µg/mL for the 4 hour exposure (+S9). Cytotoxicity (as evidenced by reduced mitotic index) was limited to Trial 2 at 350 µg/mL in the 46 hour exposure in the absence of S9 (mitotic index was 32% of controls). There were no significant increases in the percent aberrant cells (excluding gaps) noted in either trial in the presence or absence of S9 at any exposure period. An increase in polyploidy was only observed in Trial 1 at 200 ug/mL in the 4 hour exposure in the presence of S9 (0.8% compared to 0/0.2% in the negative/solvent controls, respectively). The positive controls induced increases ($p \leq 0.05$) in percent aberrant cells (excluding gaps) in all trials in the presence and absence of S9.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that IR7825 (a metabolite of IR5878) did not induced chromosome aberrations in cultured human peripheral blood lymphocytes in the presence or absence of S9.

B. REVIEWER COMMENTS - The test material was tested up to the limit of solubility in DMSO (350 µg/mL). Cytotoxicity was only observed in Trial 2 at 350 µg/mL in the 46 hour exposure in the absence of S9 (mitotic index was 32% of controls). There were no treatment-related increases in the percent aberrant cells (excluding gaps) noted in either trial in the presence or absence of S9 at any exposure period. An increase in polyploidy was only observed in Trial 1 at 200 ug/mL in the 4 hour exposure in the presence of S9 (0.8% vs 0/0.2% in the negative/solvent controls, respectively). The positive controls induced the appropriate response in both trials in the presence and absence of S9. **There was no evidence of chromosome structural or numerical aberrations induced over background in the presence or absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the Guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data.

C. **STUDY DEFICIENCIES** - The following minor deficiency was noted, but does not alter the conclusions of this review:

- The dose formulations were not analyzed for actual concentrations of test substance.

ATTACHMENT

The following Attachment contains the cytogenetic assay summary Table 1 from pages 13-14 of MRID 46578917

RCC-CCR Study Number 778804 Report
IR7825

Table 1: Summary of results of the chromosomal aberration study with IR7825

Exp.	Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	excl. gaps*	with exchanges
Exposure period 4 hrs without S9 mix							
I	22 hrs	negative control	0.4	100	2.5	2.0	0.5
		solvent control[1]	0.0	100	2.5	0.5	0.0
		positive control[2]	0.0	107	9.5	**8.0[S]**	0.5
		114.3	0.0	100	2.0	2.0	0.0
		200.0	0.4	86	2.5	1.5	0.0
		350.0	0.2	91	2.5	1.0	0.0
Exposure period 22 hrs without S9 mix							
I	22 hrs	negative control	0.0	100	1.0	1.0	0.0
		solvent control[1]	0.8	100	3.5	1.5	0.0
		positive control[2]	0.2	81	13.5	**11.5[S]**	1.5
		114.3	0.0	86	2.0	1.0	0.0
		200.0	0.0	83	3.0	2.5	0.5
		350.0	0.2	91	1.5	1.5	0.0
Exposure period 46 hrs without S9 mix							
II	46 hrs	negative control	0.0	100	2.5	2.0	0.0
		solvent control[1]	0.0	100	4.0	2.5	0.5
		positive control[3]	0.0	34	24.5	**22.0[S]**	9.0
		200.0	0.0	71	0.5	0.5	0.0
		350.0	0.0	32	3.0	3.0	0.0

* inclusive cells carrying exchanges

S aberration frequency statistically significant higher than corresponding control values

1 DMSO 0.5 % (v/v)

2 EMS 550 µg/mL

3 EMS 440 µg/mL

RCC-CCR Study Number 778804 Report
IR7825

Table 1 cont.: Summary of results of the chromosomal aberration study with IR7825

Exp.	Preparation interval	Test item concentration *in µg/mL*	Polyploid cells *in %*	Mitotic indices *in %* of control	Aberrant cells in % incl. gaps*	excl. gaps*	with exchanges
Exposure period 4 hrs with S9 mix							
I	22 hrs	negative control	0.0	100	1.0	1.0	0.0
		solvent control[1]	0.2	100	2.0	1.5	0.0
		positive control[2]	0.0	37	16.5	**13.0**[s]	2.5
		114.3	0.0	84	3.5	2.0	0.5
		200.0	0.8	70	4.5	2.0	0.0
		350.0	0.0	77	3.5	3.0	0.0
II	46 hrs	negative control	0.0	100	0.0	0.0	0.0
		solvent control[1]	0.0	100	2.0	2.0	0.0
		positive control[3]	0.0	46	12.0	**12.0**[s]	3.0
		114.3	0.2	67	2.0	0.5	0.0
		200.0	0.2	97	3.5	3.0	0.5
		350.0	0.0	92	2.0	0.0	0.0

* inclusive cells carrying exchanges

[s] aberration frequency statistically significant higher than corresponding control values

[1] DMSO 0.5 % (v/v)

[2] CPA 22.5 µg/mL

[3] CPA 15.0 µg/mL

DATA EVALUATION RECORD

IR7863; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; *In Vitro* Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes

Work Assignment No. 3-01-82 J (MRID 46578921)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by
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Quality Assurance:
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Date: 12/27/05

Disclaimer

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

EPA Reviewer: Lisa Austin, Ph.D. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 1/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 2/6/07

TXR#: 0053612

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cytogenetics (Chromosomal Aberration assay in Human Peripheral Blood Lymphocytes) OPPTS 870.5375 [§84-2]; OECD 473.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR7863 (a metabolite of IR5878; Orthosulfamuron; 97.8% a.i.)

SYNONYMS: Not provided

CITATION: Schulz, M. (2003) Chromosome Aberration Test in Human Lymphocytes *In Vitro* with IR7863. RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Laboratory Study No.: 795504, November 10, 2003. MRID 46578921. Unpublished.

SPONSOR: ISAGRO SpA, Milano, Italy

EXECUTIVE SUMMARY - In two independent trials of a mammalian cell cytogenetics assay (chromosome aberration; MRID 46578921), lymphocyte cultures were prepared from human peripheral blood and exposed to IR7863 (a metabolite of IR5878; Orthosulfamuron; 97.8% a.i., Batch # 20687/50) in deionized water at concentrations of 0, 17.6, 30.7, 53.7, 94.0, 164.5, 287.9, 503.8, 881.6, 1542.9, or 2700 µg/mL for 4 hours with an 18 hour recovery period (Trial 1, +/-S9); 0, 17.6, 30.7, 53.7, 94.0, 164.5, 287.9, 503.8, 881.6, 1542.9, or 2700 µg/mL for 22 hours with no recovery period (Trial 1, -S9); 0, 503.8, 881.6, 1542.9, or 2700 µg/mL for 46 hours with no recovery period (Trial 2, -S9); or 0, 503.8, 881.6, 1542.9, or 2700 µg/mL for 4 hours with a 42 hour recovery period (Trial 2, +S9). Cyclophosphamide and ethylmethane sulfonate served as the positive controls in the presence and absence of S9, respectively.

IR7863 was tested up to the limit dose (2700 µg/mL, +/-S9; approximately 10 mM). No evidence of cytotoxicity (reduced mitotic index) was observed at any exposure time in the presence or absence of S9 in either trial. There were no treatment-related increases in the percent aberrant cells (excluding gaps) or polyploidy noted in either trial in the presence or absence of S9 at any exposure period. The positive controls induced the appropriate response. **There was *no* evidence of chromosome aberrations induced over background in the presence or absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the Guideline requirement (OPPTS

870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR7863 (a metabolite of IR5878)

Description:	White solid
Batch #:	20687/50
Purity:	97.8% a.i.
Stability:	It was stated that the test material was stable in water for approximately 4 hours.
CAS # of TGAI:	Not provided
Structure:	Not provided
Solvent used:	Deionized water

2. Control materials

Negative control: Untreated medium served as the negative control.
Solvent control: Deionized water (0.5 %/mL)
Positive controls

Non-activation: Ethylmethane sulfonate (EMS, 440/550 µg/mL in nutrient medium for the 46 and 22-hour exposures, respectively)
Activation: Cyclophosphamide (CPA, 15-22.5 µg/mL in nutrient medium for the 46 and 22-hour exposures, respectively)

3. Activation - The S9 fraction was derived from male Wistar rats (8-12 weeks old, weighing 220-320 g; supplied by RCC Ltd; Biotechnology and Animal Breeding Division, Füllinsdorf):

X	induced		Aroclor 1254	X	Rat	X	Liver
	non-induced	X	Phenobarbital		Mouse		Lung
		X	β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was prepared in the laboratory and stored at -80 °C until used. It was stated that the protein concentration of the batch was 26.2 mg/mL. It was not reported if the efficacy of the batch was checked before use. It was stated that an appropriate quantity of S9 fraction was mixed with the following cofactors to give a final protein concentration of 0.75 mg/mL in the cultures: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 4 mM NADP in 100 mM sodium-ortho-phosphate buffer (pH 7.4).

4. Test cells - Human blood samples were obtained by venipuncture in heparinized tubes within 24 hours of culture initiation from one male donor (not receiving medication).

	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells	X	Human lymphocytes

Media: The complete culture medium consisted of modified Eagle medium/Ham's F-12 medium supplemented with 10% (v/v) fetal calf serum (FCS), 3 ug/mL phytohemaglutinin, 25,000 U/mL heparin, HEPES (10 mM), 10,000 units/mL penicillin, and 10,000 µg/mL streptomycin.

Properly maintained?		X	Yes	No
Periodically checked for Mycoplasma contamination?	**Not applicable**		Yes	No

Periodically checked for karyotype stability? **Not applicable** ☐ Yes ☐ No

Whole blood cultures were established within 24 hours of collection by placing 0.9 mL blood in 9.05 mL complete culture medium. To stimulate the lymphocytes to divide, 0.05 mL of phytohemagglutinin (3 µg/mL) was added, and the cultures were incubated at 37°C in a humidified atmosphere with 5.5% CO_2.

5. Test compound concentrations used: Since the preliminary cultures fulfilled the requirements for cytogenic evaluation, the preliminary test was designated as Trial 1.

Non-activated conditions:	Preliminary cytotoxicity: (Duplicate cultures)	17.6, 30.7, 53.7, 94.0, 164.5, 287.9, 503.8, 881.6, 1542.9, or 2700 µg/mL for 4 or 22 hours with an 18 and 0 hour recovery period, respectively
	Mutagenicity assay: (Duplicate cultures)	17.6, 30.7, 53.7, 94.0, 164.5, 287.9, 503.8, 881.6, 1542.9, or 2700 µg/mL for 4 or 22 hours with an 18 and 0 hour recovery period, respectively (Trial 1)
		503.8, 881.6, 1542.9, or 2700 µg/mL for 46 hours with no recovery period (Trial 2)
Activated conditions:	Preliminary cytotoxicity: (Duplicate cultures)	17.6, 30.7, 53.7, 94.0, 164.5, 287.9, 503.8, 881.6, 1542.9, or 2700 µg/mL for 4 hours with an 18 hour recovery period
	Mutagenicity assay: (Duplicate cultures)	17.6, 30.7, 53.7, 94.0, 164.5, 287.9, 503.8, 881.6, 1542.9, or 2700 µg/mL for 4 hours with an 18 hour recovery period (Trial 1)
		503.8, 881.6, 1542.9, or 2700 µg/mL for 4 hours with a 42 hour recovery period (Trial 2)

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay - A preliminary cytotoxicity test was performed following the same procedure as described below. The highest concentration used (2700 µg/mL, ±S9) was the limit dose (approximately 10 mM). Since the preliminary cultures fulfilled the requirements for cytogenic evaluation, the preliminary test was designated as Trial 1 of the cytogenetic assay.

2. Cytogenetic assay -

a. Cell exposure time (hrs)

	Test material		Solvent control		Positive control	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Non-activated	4 or 22	46	4 or 22	46	4 or 22	46
Activated	4	4	4	4	4	4

b. Spindle inhibition

Inhibitor use (concentration): Colcemid (0.2 µg/mL)

Administration time: 3 hours (prior to cell harvest)

c. Cell harvest time after termination of treatment (hrs)

Test material		Solvent control		Positive control	
Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2

Non-activated	18 or 0	0	18 or 0	0	18 or 0	0
Activated	18	42	18	42	18	42

d. Details of slide preparation – Approximately 50-80 hours after culture establishment, the culture medium was replaced with serum-free medium (for treatment with S9) or with complete medium with 10% FCS (for treatment without S9), containing the test material. Additionally, 50 µL/mL of the S9 mix was added to the cultures requiring activation. Concurrent solvent, negative, and positive controls were performed. In Trial 1 (±S9) and Trial 2 (+S9), the treatment medium was removed by centrifugation after 4 hours of exposure, and the cells were washed twice by resuspension in 'saline G' (composed of the following [per liter]: 8000 mg NaCl, 400 mg KCl, 1100 mg glucose, 290 mg sodium phosphate buffer, and 150 mg KH_2PO_4 at pH 7.2). After washing, the cells were resuspended in complete medium for the 18 or 42 hour recovery period (Trial 1 ±S9 and Trial 2 +S9, respectively). For the 22 and 46 hour exposure periods in the absence of S9 (Trial 1 and 2, respectively), the cultures remained in the treatment medium until slide preparation (no recovery period).

After cell division was arrested, the cultures were centrifuged, the supernatant was removed, and the cells were resuspended in 5 mL hypotonic KCl (0.0375 M) for 20 minutes at 37°C. The cultures were again centrifuged, the supernatant was removed, and the cells were fixed in methanol/glacial acetic acid (3:1, v/v). The lymphocytes were dropped onto clean microscope slides (at least 2 slides/group). The slides were stained with Giemsa or according to the Fluorescent plus Giemsa technique, rinsed, air-dried, and mounted with coverslips (assumed by reviewers).

e. Metaphase analysis - The aberration percentages, both including and excluding gaps, were recorded.

No. of cells examined per dose: 200 (100/replicate) cells in the treated, negative, solvent, and positive controls were scored for structural aberrations; 1000 cells for mitotic index; and 250 metaphase cells were scored for numerical aberrations.

Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes, polyploidy and endoreduplication		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria

Assay validity - The assay was considered valid if the following criteria were met:

- The number of chromosomal aberrations in the negative and/or solvent controls was within the performing laboratory historical control range (0.0-4.0%).
- The positive controls induced statistically significant increases in the frequencies of aberrations.

Positive result - The test article was considered to be mutagenic if the following criteria were met:

- A statistically significant or concentration-related increase in the number of structural aberrations (excluding gaps) was observed, and the increase exceeded the historical control range (0.0-4.0%).

g. Statistical methods - The data were analyzed using Fisher-Exact test. Significance was defined at $p \leq 0.05$.

II. REPORTED RESULTS

The dose formulations were not analyzed for actual concentrations. No precipitation of the test material was observed at any concentration in the presence or absence of S9 in either trial. Addition of the test material had no significant effect on the osmolality or pH of the culture media.

A. PRELIMINARY CYTOTOXICITY ASSAY - The results of the preliminary cytotoxicity test were designated as Trial 1 of the cytogenetic assay and are reported below.

B. CYTOGENETIC ASSAY - The results of the cytogenetic assays were presented in the Study Report Tables 5-14 on pages 27-36, and were summarized in Table 1 on pages 13-14. As the results of these assays were negative, a copy of Table 1 is included as an Attachment to this DER.

The test material was tested up to the limit dose (2700 µg/mL; approximately 10 mM). In Trial 1, concentrations of 17.6, 30.7, 53.7, 94.0, 164.5, 287.9, 503.8, 881.6, 1542.9, or 2700 µg/mL were tested in the presence and absence of S9-activation. In Trial 2, concentrations of 503.8, 881.6, 1542.9, or 2700 µg/mL were tested in the presence and absence of S9-activation. In Trial 1, the concentrations evaluated for aberrant cells were 881.6, 1542.9, or 2700 µg/mL in the 4 hour (±S9) and 22 hour (-S9) exposures. In Trial 2, the concentrations evaluated for aberrant cells were 2700 µg/mL for the 46 hour exposure (-S9), and 881.6, 1542.9, or 2700 µg/mL for the 4 hour exposure (+S9). No evidence of cytotoxicity (reduced mitotic index) was observed at any exposure time in the presence or absence of S9 in either trial. There were no significant increases in the percent aberrant cells (excluding gaps) or polyploidy noted in either trial in the presence or absence of S9 at any exposure period. The positive controls induced increases ($p \leq 0.05$) in percent aberrant cells (excluding gaps) in all trials in the presence and absence of S9.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that IR7863 (a metabolite of IR5878) did not induce chromosome aberrations in cultured human peripheral blood lymphocytes at up to the limit dose (2700 µg/mL; approximately 10 mM) in the presence or absence of S9.

B. REVIEWER COMMENTS - The test material was tested up to the limit dose (2700 µg/mL; approximately 10 mM). No evidence of cytotoxicity (reduced mitotic index) was observed at any exposure time in the presence or absence of S9 in either trial. There were no treatment-related increases in the percent aberrant cells (excluding gaps) or polyploidy noted in either trial in the presence or absence of S9 at any exposure period. The positive controls induced the appropriate response in both trials in the presence and absence of S9. **There was no evidence of chromosome aberrations induced over background in the presence or absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the Guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data.

C. STUDY DEFICIENCIES - The following minor deficiency was noted, but does not alter the conclusions of this review:

- The dose formulations were not analyzed for actual concentrations of test substance.

ATTACHMENT

The following Attachment contains the cytogenetic assay summary Table 1 from pages 13-14 of MRID 46578921

Table 1: Summary of results of the chromosomal aberration study with IR7863

Exp.	Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	excl. gaps*	with exchanges
Exposure period 4 hrs without S9 mix							
I	22 hrs	negative control	0.1	100.0	1.5	1.0	0.0
		solvent control[1]	0.0	100.0	1.5	1.5	0.0
		positive control[2]	0.0	103.6	9.0	**8.0[S]**	0.5
		881.6	0.1	102.3	1.0	0.5	0.0
		1542.9	0.2	98.7	2.0	1.5	0.5
		2700.0	0.0	112.8	1.0	1.0	0.0
Exposure period 22 hrs without S9 mix							
I	22 hrs	negative control	0.0	100.0	0.5	0.5	0.0
		solvent control[1]	0.2	100.0	1.5	0.5	0.0
		positive control[2]	0.0	50.9	13.5	**11.5[S]**	6.0
		881.6	0.0	83.6	3.5	2.0	0.0
		1542.9	0.1	115.0	0.0	0.0	0.0
		2700.0	0.0	88.1	0.0	0.0	0.0
Exposure period 46 hrs without S9 mix							
II	46 hrs	negative control	0.0	100.0	2.0	2.0	0.0
		solvent control[1]	0.0	100.0	0.0	0.0	0.0
		positive control[3]	0.0	16.8	15.5	**14.0[S]**	6.0
		2700.0	0.0	105.7	4.5	**2.0[S]**	0.0

* inclusive cells carrying exchanges

S aberration frequency statistically significant higher than corresponding control values

1 deionised water

2 EMS 550 µg/mL

3 EMS 440 µg/mL

Table 1 cont.: Summary of results of the chromosomal aberration study with IR7863

Exp	Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Mitotic Indices in % of control	Aberrant cells in % incl. gaps*	excl. gaps*	with exchanges
Exposure period 4 hrs with S9 mix							
I	22 hrs	negative control	0.0	100.0	0.5	0.0	0.0
		solvent control[1]	0.0	100.0	1.0	0.5	0.5
		positive control[2]	0.0	46.3	13.5	12.5[§]	1.0
		881.6	0.0	89.6	0.5	0.5	0.0
		1542.9	0.0	84.4	2.0	0.5	0.0
		2700.0	0.1	88.4	1.0	1.0	0.0
II	46 hrs	negative control	0.0	100.0	2.0	1.5	0.0
		solvent control[1]	0.0	100.0	1.5	1.5	0.0
		positive control[3]	0.0	19.1	9.5	9.5[§]	2.5
		881.6	0.0	81.7	3.5	2.5	0.0
		1542.9	0.0	91.1	0.5	0.5	0.0
		2700.0	0.0	96.5	0.5	0.5	0.0

* inclusive cells carrying exchanges

§ aberration frequency statistically significant higher than corresponding control values

1 deionised water

2 CPA 22.5 µg/mL

3 CPA 15.0 µg/mL

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DATA EVALUATION RECORD

IR8181; A METABOLITE of IR5878 (ORTHOSULFAMURON)

Study Type: §84-2; *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice

Work Assignment No. 3-01-82 N (MRID 46578926)

Prepared for
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U.S. Environmental Protection Agency
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Prepared by
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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.

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EPA Reviewer: Lisa Austin, Ph.D. **Signature:** Lisa Austin
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 1/11/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 2/6/07

TXR#: 0053612

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

PC CODE: 108209 **DP BARCODE:** D330824

TEST MATERIAL (PURITY): IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i.)

SYNONYMS: 1-[2-(dimethylcarbamoyl)phenylsulfamoyl]-3-(4-hydroxy-6-methoxy-2-pyrimidinyl)urea

CITATION: Krüger, I. (2004) Mammalian Micronucleus Test of Murine Bone Marrow Cells with IR8181. BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany. Laboratory Study No.: 041175, August 23, 2004. MRID 46578926. Unpublished.

SPONSOR: ISAGRO SpA, Italy

EXECUTIVE SUMMARY - In a bone marrow micronucleus assay (MRID 46578926), 5 NMRI mice/sex/dose/sacrifice time were treated once via intraperitoneal injection (20 mL/kg) with IR8181 (a metabolite of IR5878; Orthosulfamuron; 97.02% a.i., Batch # 30072/85) in cotton seed oil at doses of 0 or 2000 mg/kg (limit dose). Bone marrow was harvested at 24 or 48 hours post-dosing. Cyclophosphamide (40 mg/kg; i.p.) served as the positive control during the 24 hour post-dosing harvest.

IR8181 was tested up to the limit dose (2000 mg/kg). There were no mortalities or clinical signs of toxicity observed in any animal. Evidence of bone marrow toxicity was not observed at either harvest time. No treatment-related increases in MPCEs were observed at 24 or 48 hours post-dosing. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow compared to controls.**

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The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IR8181 (a metabolite of IR5878)

Description: White, odorless powder
Batch #: 30072/85
Purity: 97.02% a.i.
Stability: Not reported
CAS # of TGAI: Not available
Structure: Not provided
Vehicle used: Cotton seed oil

2. Control materials

Negative: Cotton seed oil
Vehicle: Cotton seed oil (20 mL/kg; i.p.)
Positive control: Cyclophosphamide (CPA; in 0.9%NaCl at 40 mg/kg; 10 mL/kg via i.p)

3. Test animals

Species: Mouse
Strain: NMRI
Age/Weight at study initiation: At least 7 weeks/ 29-34 g males and 24-32 g females
Source: Harlan Winkelmann
Number of animals used per sex/dose/harvest time: 5
Properly maintained? Yes

4. Test compound administration

	Dose levels	Final volume	Route
Preliminary	2000 mg/kg	20 mL/kg	i.p.
Main study	2000 mg/kg	20 mL/kg	i.p.

B. TEST PERFORMANCE

1. Treatment and sampling times

a. Test compound and vehicle control

Dosing:	X	Once		Twice (24 hrs apart)				Other (describe)		
Sampling after last treatment:		6 hrs		12 hrs	X	24 hrs	X	48 hrs		72 hrs
Other: (describe)										

b. *Positive control*

Dosing:	X	Once		Twice (24 hrs apart)				Other (describe)		
Sampling after last treatment:		6 hrs		12 hrs	X	24 hrs		48 hrs		72 hrs
Other: (describe)										

2. Tissues and cells examined

Bone marrow or other (list)	Bone marrow
No. of polychromatic erythrocytes (PCE) examined per animal	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined/animal	Not reported
For determination of marrow toxicity, the ratio of immature (PCE) to mature (NCE) erythrocytes was determined by examining at least 200 erythrocytes per animal.	

3. Details of slide preparation - All animals were sacrificed via cervical dislocation. Immediately after sacrifice, both femurs were excised, and the heads were removed. The marrow was flushed from the femurs using fetal calf serum in a 5 mL syringe. The marrow was centrifuged, and the supernatant was discarded. The marrow was then resuspended in serum. Bone marrow smears (at least one slide/animal) were prepared on glass slides and allowed to air-dry. The slides were fixed stained with May-Grünwald/Giemsa. The slides were coded prior to evaluation.

4. Evaluation criteria

a. Assay validity - The assay was considered valid if the following criteria were met:
- The incidence of micronucleated polychromatic erythrocytes (MPCEs) in the vehicle controls was within the historical control range (0.01-0.32).
- The incidence of MPCEs in the positive control was significantly ($p \leq 0.05$) increased compared to the vehicle control.
- At the beginning of the study, the weight variation of the animals should not exceed ±20% of the mean weight for each sex.

b. Positive result - The test article was considered to be mutagenic if there was either a positive dose-response trend and/or a statistically significant increase in the number of MPCEs at one or more dose levels compared to the controls.

5. Statistical methods - The frequency of MPCEs at all doses at each harvest time was evaluated using a Mann-Whitney test. Significance was denoted at $p \leq 0.05$. The reviewers consider the statistical methods to be acceptable.

II. REPORTED RESULTS

The dose formulations were not analyzed for actual concentrations.

A. PRELIMINARY TOXICITY ASSAY - A range-finding study was performed using 3 mice/sex at a dose of 2000 mg/kg. No clinical signs of toxicity were noted during the 3-day observation period after dosing. Based on the results of this study, a dose of 2000 mg/kg (limit dose) was chosen for the mutagenicity assay.

B. MICRONUCLEUS ASSAY - The results of the micronucleus assays were reported in Tables 2-8 on pages 22-27 of the study report. As the results of this assay were negative, copies of Tables 7 and 8 are included as an Attachment to this DER. There were no mortalities or clinical signs of toxicity observed in any animal. No evidence of cytotoxicity to the marrow (decreased relative PCE) was noted at any dose or time point. No treatment-related increase in the percentage of cells with micronuclei was observed at 24 or 48 hours post-dosing. The increase ($p \leq 0.05$) noted in the females at 24 hours post-dosing (0.19%) was considered unrelated to treatment as it fell within the historical control range for females (0.01-0.26%). The positive control induced an increase ($p < 0.01$) in the percentage of cells with micronuclei (1.94-3.36 treated vs 0.08-0.11 controls) in both sexes at 24 hours post-dosing.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigators concluded that IR8181 did not induce micronuclei in polychromatic erythrocytes of the bone marrow of male or female mice at 2000 mg/kg (limit dose). No evidence of bone marrow toxicity (decreased relative PCE) was observed at 24 or 48 hours post-dosing.

B. REVIEWER COMMENTS - IR8181 was tested up to the limit dose (2000 mg/kg). There were no mortalities or clinical signs of toxicity observed in any animal. Evidence of bone marrow toxicity was not observed at either harvest time. No treatment-related increases in MPCEs were observed at 24 or 48 hours post-dosing. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow compared to controls.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

C. STUDY DEFICIENCIES - The following minor deficiency was noted, but does not change the conclusions of this DER:

- The dose formulations were not analyzed for actual concentrations.

ATTACHMENT

The following attachment contains Tables 7 and 8 from page 27 of MRID 46578926.

Table 7: Percentage of Cells with Micronuclei

Dose Group	concentration [mg/kg bw]	preparation time [h]	male [%]	female [%]
NC	0	24	0.08	0.11
1	2000	24	0.09	0.19
CPA	40	24	3.36	1.94
NC	0	48	0.06	0.13
1	2000	48	0.09	0.18

NC: Negative Control
CPA: Cyclophosphamide

Table 8: Statistical significance at the 5% level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

Negative Control versus Test Group	Preparation time [h]	Significance male	Significance female	p-value male	p-value female
2000 mg/kg bw	24	-	(+)	0.8413	0.0159
2000 mg/kg bw	48	-	-	0.4206	1.0000
CPA 40 mg/kg bw	24	+	+	0.0079	0.0079

\+ significant
(+) statistically significant but biologically not relevant
\- not significant
CPA: Cyclophosphamide

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
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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.

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EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:**
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Work Assignment Manager: P.V. Shah, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 1/18/07

Template version 11/01

DATA EVALUATION RECORD

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

D330824

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.

McCombe, W.S. and L. Gedik (2002) The disposition of [^{14}C]-IR-5878 in the rat following single and repeated oral administration. Inveresk Research, Tranent, Scotland. Laboratory Project No.: 201385, July 9, 2002. MRID 46578907. Unpublished.

Rizzo, F., K. Mainolfi, and G. Pizzingrilli (2002) Profiling of radiolabelled metabolites of [^{14}C-U-phenyl] IR5878 in urine and faeces of rats following single and repeated oral administrations. Isagro Ricerca Srl, Environmental Chemistry, Novara, Italy. Laboratory Study No.: MEF.01.13, October 4, 2002. MRID 46578908. Unpublished.

Kidd, G.G. and L. Gedik (2002) The disposition of [^{14}C-5-Pyrimidinyl]-IR5878 in the rat following single and repeated oral administration. Inveresk Research, Tranent, Scotland. Laboratory Project No.: 202771, November 12, 2002. MRID 46578909. Unpublished.

Rizzo, F., K. Mainolfi, and G. Pizzingrilli (2003) Profiling of radiolabelled metabolites of [^{14}C-5-pyrimidinyl]IR5878 in urine and faeces of rats following

single and repeated oral administrations. Isagro Ricerca Srl, Environmental Chemistry, Novara, Italy. Laboratory Study No.: MEF.02.15, February 13, 2003. MRID 46578910. Unpublished.

SPONSOR: Isagro SpA, Centro Uffici S. Siro - Fabbricato D, ala 3, Via Caldera, 21 Milano, Italy

EXECUTIVE SUMMARY: In a series of rat metabolism studies (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 contrasting sampling times and/or T_{max}; 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.

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

The parent was found in the 5 mg/kg animals (single and multiple doses) at 1-6% dose and in the 1000 mg/kg animals at 33-56% dose in urine and feces. 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/day and at 14-20% dose in all animals treated at 1000 mg/kg; it 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 primarily in the feces at 8-12% dose. 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 mainly occurs 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.

1. MATERIALS AND METHODS

A. MATERIALS:

1. Test compound:

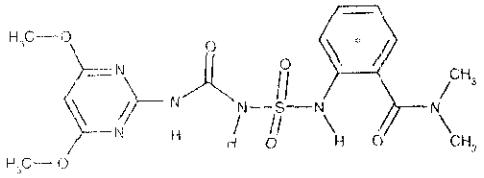
Radiolabeled test material 1: [^{14}C-U-phenyl] IR5878

Radiochemical purity: ≥97%

Specific Activity: 5.700 MBq/mg

Lot No.: 182

Structure:



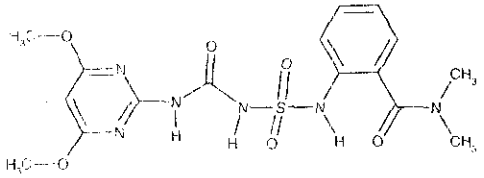
Radiolabeled test material 2: [^{14}C-5-pyrimidinyl] IR5878

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		

Single low dose	5	Male: 4.49-5.06 Female: 4.61-5.06		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	4/sex	
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.

(table continues next page)

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	23.27±4.81	28.41±7.11	7.44±0.90	9.63±2.27	25.32±0.48	31.58±11.61
0-12 h	28.83±3.11	35.45±7.34	14.36±1.92	18.81±2.33	28.79±1.29	37.86±9.34
0-72 h	30.34±3.35	37.93±7.67	17.65±2.11	23.28±3.71	31.52±2.45	40.69±9.21
Feces: 0-12 h	37.79±5.39	20.76±10.50	55.76±4.29	40.48±17.95	31.42±8.23	13.46±12.31
0-24 h	60.36±4.27	41.62±10.35	62.07±5.74	60.97±5.81	54.79±3.70	34.82±8.11
0-72 h	66.38±2.82	52.93±13.12	72.89±3.33	66.27±4.77	62.76±1.81	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±2.37	96.91±1.82
[^{14}C-5-pyrimidinyl] IR5878						
Urine: 0-6 h	25.25±3.76	39.67±3.34	8.56±1.35	21.98±3.00	22.29±5.48	30.60±12.97
0-12 h	28.42±3.37	43.87±3.78	11.33±2.83	33.96±3.49	27.77±5.48	32.89±13.85
0-72 h	29.45±3.36	45.88±4.64	31.15±4.11	41.37±4.65	28.91±3.34	34.64±13.95
Feces: 0-12 h	16.27±14.5 1	10.59±8.71	20.87±14.02	4.58±4.63	26.32±7.79	15.39±17.53
0-24 h	61.22±3.72	39.49±5.70	51.18±17.26	44.76±7.82	58.28±6.46	47.11±13.79
0-72 h	63.09±3.72	42.60±5.61	61.71±7.03	51.38±5.04	61.62±4.93	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	93.26±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). Tmax 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 [14C-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 [14C-U-phenyl] IR5878 treated males (419x) and females (334x) and in the [14C-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	12.86	5595.59	4299.67	13.14	13.78
[14C-5-pyrimidinyl] IR5878						
C_{max} (µg equiv./g)	1.990	2.025	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	16.7-	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 [14C] IR5878 on Day 15.

2. Tissue distribution: 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 [14C-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 [14C-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.

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Comparison of the concentrations of radioactivity in tissues on the basis of radiolabel was not possible due to differing sampling times and/or differing Tmax. 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 MRID46578909.
b Animals received 14 daily doses of IR5878 (5 mg/kg/day) followed by 5 mg/kg of [14C] IR5878 on Day 15.
c. [14C-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 [14C-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.

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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 Tier 1 study.

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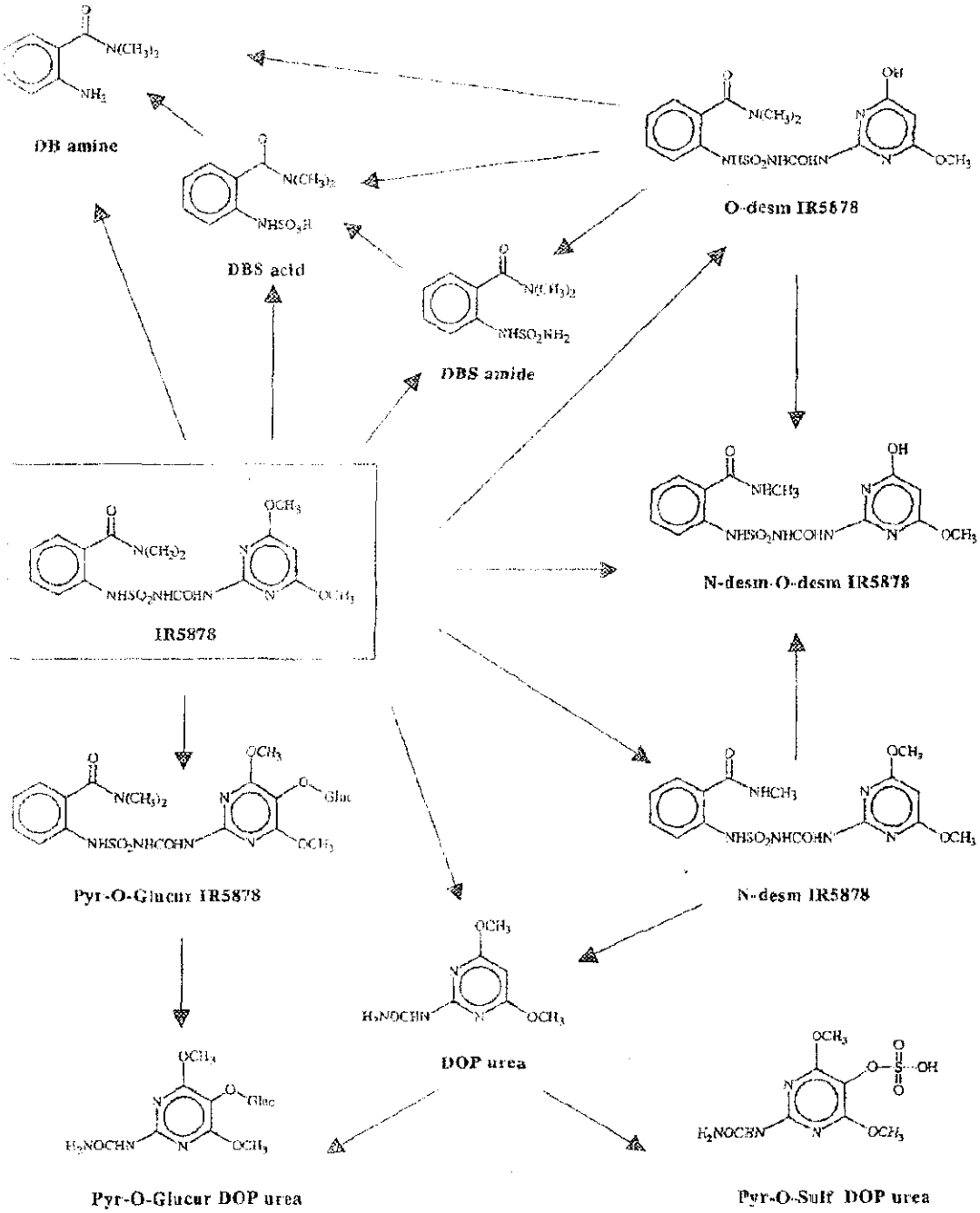
APPENDIX

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


DB amine
DBS acid
DBS amide
O-desm IR5878
IR5878
N-desm-O-desm IR5878
Pyr-O-Glucur IR5878
N-desm IR5878
DOP urea
Pyr-O-Glucur DOP urea
Pyr-O-Sulf DOP urea

17. CHEMICAL NAME OF COMPOUNDS

Code	Chemical name *(common name)*
M1f	(4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)urea *Pyr-O-Glucur DOP urea*
M1g	1-(4,6-dimethoxy-5-O-glucuronidyl pyrimidin-2-yl)-3-[2-(dimethylcarbamoyl) phenylsulfamoyl]urea *Pyr-O-Glucur IR5878*
M2	(4,6-dimethoxy-5-sulfate pyrimidin-2-yl)urea *Pyr-O-Sulf DOP urea*
M6	1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(methylcarbamoyl)phenylsulfamoyl]urea *(N-desm-O-desm IR5878)*
M7	1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(methyl carbamoyl)phenylsulfamoyl]urea *(N-desm IR5878)*
M8	1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl] urea *(O-desm IR5878)*
M9	1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea ***(IR5878)***
M10	(4,6-dimethoxy pyrimidin-2-yl)urea *(DOP urea)*

17. CHEMICAL NAME OF COMPOUNDS

Code	IUPAC name *(common name)*
C7	1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea ***(IR5878)***
C3	2-sulfoamino-N,N-dimethylbenzamide *(DBS acid)*
C4	1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(methylcarbamoyl)phenylsulfamoyl]urea *(N-desm-O-desm IR5878)*
C5	1-(4,6-dimethoxy pyrimidin-2-yl)-3-[2-(methyl carbamoyl)phenyl sulfamoyl]urea (*N-desm* IR5878)
C6	1-(4-methoxy-6-hydroxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea *(O-desm IR5878)*
C8	2-sulfamoylamino-N,N-dimethylbenzamide *(DBS amide)*
C9	2-amino-N,N-dimethylbenzamide *(DB amine)*

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
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Office of Pesticide Programs
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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.

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EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** Karlyn
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 11/12/07
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 11/14/07

Template version 11/01

DATA EVALUATION RECORD

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

PC CODE: 108209 **DP BARCODE:** D330824
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.

14(b)

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 the propylthiouracil group there was 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. 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 suggest 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.

With a disruption in thyroid homeostasis, there is typically a reduction in both circulating serum T4 and T3 and a subsequent increase in TSH. In this study, there were transient decreases in concentrations of T3 observed in the 5 and 1000 mg/kg/day groups (Day 30), and no reductions in T4 concentrations. TSH levels at Day 90 in the 1000 mg/kg/day group were slightly increased (↑58%); however, TSH levels were comparable to controls at all other time points.

Common measured parameters that indicate disruption of thyroid homeostasis include but are not limited to increases in thyroid weight and histological indication of cellular hypertrophy and hyperplasia. There were enlarged thyroids noted in the study at 5 and 1000 mg/kg/day, but there were no treatment-related effects observed on thyroid/parathyroid weights; liver weights were increased at 1000 mg/kg/day. At 1000 mg/kg/day, there were increased incidences of centrilobular hepatocyte hypertrophy and thyroid follicular cell hypertrophy observed.

There were several increases in liver enzymes and activities observed in males at 1000 mg/kg/day. These included the following: (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%). Pharmacokinetic data in the study, revealed the following differences (not compared to negative controls: (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%). The increase in liver enzymes/activities and pharmacokinetic data

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suggest that orthosulfamuron may enhance thyroid hormone metabolism and clearance via induction of liver microsomal enzymes.

The results of the clearance test and perchlorate discharge assay study along with the thyroid data (increased organ weight and histopathology) in the combined chronic carcinogenicity study are characteristic of increases in thyroid growth. Thus, it is plausible that exposure to orthosulfamuron may cause thyroid tumors via perturbation of thyroid-pituitary functioning due to enhanced hepatic clearance of thyroxin. However, the thyroid hormone data, which are critical to delineating a sequence of key events leading to tumor formation, are inadequate. Therefore, the available data do not clearly support the proposed mode of action.

The following deficiencies were noted in the study:

- Alterations in thyroid hormones are typically seen at early time points, with decreases observed in circulating levels of T4 and T3 and consequent increases in TSH. It should be noted, that in general, hepatic microsomal enzyme inducers appear to affect T3 less than T4, and thus, T4 and TSH tend to be more reliable indicators of altered pituitary-thyroid homeostasis. However, in this thyroid study, there were only small increases in TSH, seen only at Day 90, and transient decreases in T3, seen only at Day 30. Decreases in T3 were observed at 1000 mg/kg/day and similar decreases were seen at the non-tumorigenic dose of 5 mg/kg/day. Additionally, there were no decreases in T4 observed.

- The phenobarbital (positive control) group did not effectively alter hormone levels, and when examining the concentrations of thyroid hormones in the plasma, was not sampled with the negative controls and treated groups on Day 30; the critical time frame where changes in thyroid hormones are expected to occur. Suggested time points for the study are 14 days (early), 30-50 days (mid), and 90 days (late), including measurements of positive control at all time points.

- 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.

- There was no dose response concordance with the tumor response observed in the chronic/carcinogenicity study in the rat. Tumors were observed at 500 and 1000 mg kg/day, while the thyroid study investigated effects at 5 and 1000 mg/kg/day. It is recommended that 3 doses be used for the study, and that these doses correspond with the

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observed tumor response. In this case, 250, 500, and 1000 mg/kg/day are recommended doses for identification of a clear dose response.

It is recommended that the registrant meet with the Agency prior to conducting a repeat thyroid study.

This study is classified as unacceptable/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

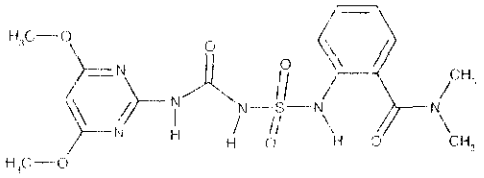
Purity (w/w): 98.79% a.i.

Vehicle Diet

Stability of compound: The test substance was stable in the diet for up to 15 days at 21EC (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:	19-24EC
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 (21EC) 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 4EC. 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#0.05, p#0.01, and p#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#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#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#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 4EC 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 :Ci/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 :Ci) 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 :Ci/mL (equivalent to a dose of 1.12 :Ci 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 4EC. Frozen livers were thawed by standing in ice-cold isotonic Tris buffer (pH 7.4) containing 0.25M sucrose. Microsomal sub-cellular 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 -75EC 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 (91-8%), resulting in significantly decreased (913%; $p \leq 0.05$) 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 (98-

12%) during treatment (Weeks 12 and 13), resulting in significantly decreased body weight gain for Weeks 0-12 (913%). 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
1	115	118	119	112	118
3	231	235	228 (91)	226	227
6	306	307	290 (95)	300	296
12	370	367	340 (98)	357	340 (98)
13	380	377	357 (96)	---	333 (912)
Overall (Week 0-12) Gain	255	249	221* (913)	245	222 (913)***

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#0.05
*** Significantly different from controls; p<0.001

C. FOOD CONSUMPTION: Food consumption was slightly decreased (91-9%) 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 (95%; p≤0.05). In the rats treated with propylthiouracil, food intake was decreased (923-24%; significance not denoted) during Weeks 12 and 13, resulting in decreased (98%; p≤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 (91)	155	152
6	161	161	147 (99)	156	153
12	152	154	142 (97)	150	117 (923)
13	148	143	140 (95)	136	112 (924)
Overall (Weeks 1-13) Gain	155	154	147* (95)	150	143* (98)

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#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 (858%) compared to negative controls, with an even greater increase in the phenobarbital group (8148%). 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 (920-23%; 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 (nmol/L)	1.80 ± 0.354	1.73 ± 0.185	1.77 ± 0.213	1.85 ± 0.253
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
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* (923)	1.37 ± 0.188* (920)	---
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* (858)	16.6 ± 5.36* (8148)

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 (94-19%); (ii) decreased AUC_{72} values

(911%); (iii) increased systemic clearance (812%); (iv) increased volume of the central compartment (V_c; 812%); and (v) increased volume at steady state (V_{ss}; 89%). 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 (929-46%) and AUC_{72} values (941%), increased V_c (875%) and V_{ss} (869%) values, and significantly ($p \leq 0.001$) increased systemic clearance (870%).

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
		5 mg/kg/day	1000 mg/kg/day	75 mg/kg/day
1	2.05 ± 0.29	2.09 ± 0.36	1.96 ± 0.10 (94)	1.21 ± 0.17 (941)
2	1.59 ± 0.22	1.62 ± 0.27	1.53 ± 0.09 (94)	0.99 ± 0.13 (938)
4	1.23 ± 0.17	1.24 ± 0.20	1.17 ± 0.09 (95)	0.79 ± 0.12 (936)
6	0.99 ± 0.21	1.11 ± 0.17	1.03 ± 0.08	0.67 ± 0.09 (932)
8	1.01 ± 0.14	0.96 ± 0.17	0.82 ± 0.06 (919)	0.56 ± 0.09 (945)
12	0.77 ± 0.10	0.78 ± 0.09	0.64 ± 0.06 (917)	0.46 ± 0.07 (940)
24	0.52 ± 0.06	0.52 ± 0.08	0.45 ± 0.03 (913)	0.28 ± 0.03 (946)
36	0.29 ± 0.04	0.31 ± 0.04	0.25 ± 0.03 (97)	0.16 ± 0.02 (941)
48	0.19 ± 0.02	0.19 ± 0.03	0.17 ± 0.02 (911)	0.11 ± 0.01 (942)
72	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01 (914)	0.05 ± 0.00 (929)

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) pharmacokinetic 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 (911)	19.63 ± 2.23 (941)
k (hour^{-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 (812)	4.86 ± 0.51*** (870)
V_c (mL)	72.68 ± 11.43	70.98 ± 11.84	81.43 ± 5.49 (812)	126.96 ± 20.03 (875)
V_{ss} (mL)	66.29 ± 10.74	65.12 ± 11.54	72.20 ± 4.98 (89)	111.90 ± 16.91 (869)

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: ln2/(mean rate constant); n = 6.
*** Significantly different from the negative control group at p≤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 (8154-

315%); (ii) decreased radioactivity in the thyroid on a per weight basis (975-93%) and as a percent of the total dose (924-69%); (iii) increased radioactivity in whole blood on a per weight basis (812-46%) and as a percent of the total dose (830%; perchlorate group only); and thus (iv) decreased thyroid:whole blood ratio (975-95%).

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 (963-68%) and increased radioactivity in whole blood (832-38%), resulting in a decreased thyroid:whole blood ratio (978%) 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*(930)	0.0200 ± 0.0036	0.0558 ± 0.0124***(8154)
perchlorate	0.0162 ± 0.0052	0.0128 ± 0.0020*(921)	0.0177 ± 0.0031	0.0672 ± 0.0079***(8315)
Radioactivity				
Thyroid, % dose/g				
saline	430 ± 236	487 ± 128	602 ± 85**(840)	107 ± 23***(975)
perchlorate	461 ± 133	541 ± 150	651 ± 70**(841)	33.9 ± 14.9***(993)
Thyroid, total % dose				
saline	7.82 ± 1.73	7.43 ± 2.39	11.9 ± 2.22***(852)	5.98 ± 1.64 **(924)
perchlorate	7.28 ± 2.39	6.91 ± 2.08	11.6 ± 2.97***(859)	2.24 ± 0.92 **(969)
Whole blood, % dose/g				
saline	0.272 ± 0.035	0.289 ± 0.054	0.273 ± 0.053	0.305 ± 0.046***(812)
perchlorate	0.276 ± 0.018	0.285 ± 0.043	0.288 ± 0.058	0.404 ± 0.043***(846)
Whole blood, total % dose				
saline	7.34 ± 0.64	7.69 ± 1.24	6.83 ± 1.16	6.95 ± 0.81* (95)
perchlorate	7.40 ± 2.37	7.32 ± 0.78	7.16 ± 0.66	9.59 ± 0.99* (830)
Thyroid:whole blood ratio				
saline	1397	1653	2217**(859)	349***(975)
perchlorate	1616	1843	2287**(842)	78***(995)

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 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*** (968)
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*** (963)
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** (832)
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*** (838)
Thyroid whole blood ratio				
saline	1397	1653	2217	349
perchlorate	1616	1843	2287	78*** (978)

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 reviewer) are included in parentheses. n = 6

* Perchlorate subgroup significantly different from saline subgroup at p≤0.05

** Perchlorate subgroup significantly different from saline subgroup at p≤0.01

*** Perchlorate subgroup significantly different from saline subgroup at p≤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 (810%; p≤0.01) compared to negative controls, and an increase of an even greater magnitude was observed in the phenobarbital group (821%; p≤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** (810)	16.28 ± 1.30*** (821)
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≤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 (832%); (ii) concentration of cytochrome P450 (828-69%); (iii) activity of PROD (82958-4020%); and (iv) activity of thyroxine UDP-GT (864-115%). 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** (832)	18.4 ± 1.7*** (857)
Cytochrome P-450				
nmoles/mg protein	0.537 ± 0.085	0.556 ± 0.047	0.690 ± 0.049** (828)	1.565 ± 0.136*** (8191)
nmoles/g liver	6.31 ± 1.50	6.92 ± 0.73	10.64 ± 1.81** (869)	28.89 ± 4.81*** (8358)
PROI				
nmoles/min/mg protein	0.012 ± 0.001	0.010 ± 0.00	0.367 ± 0.096* (82958)	1.638 ± 0.245*** (813550)
nmoles/min/g liver	0.138 ± 0.015	0.121 ± 0.014	5.686 ± 1.831** (84020)	30.208 ± 5.917*** (821790)
Thyroxine UDP-GT [b]				
pmoles/min/mg protein	0.325 ± 0.099	0.269 ± 0.097	0.533 ± 0.096** (864)	0.375 ± 0.083 (815)
pmoles/min/g liver	3.753 ± 1.090	3.311 ± 1.047	8.070 ± 0.864** (8115)	6.968 ± 1.990*** (886)

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#0.05

** Significantly different from controls; p#0.01

*** Significantly different from controls; p#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. <u>REVIEWER COMMENTS</u>:

With a disruption in thyroid homeostasis, there is typically a reduction in both circulating serum T4 and T3 and a subsequent increase in TSH. In this study, there were transient decreases in concentrations of T3 observed in the 5 and 1000 mg/kg/day groups (Day 30), and no reductions in T4 concentrations. TSH levels at Day 90 in the 1000 mg/kg/day group were slightly increased (↑58%); however, TSH levels were comparable to controls at all other time points.

Common measured parameters that indicate disruption of thyroid homeostasis include but are not limited to increases in thyroid weight and histological indication of cellular hypertrophy and hyperplasia. There were enlarged thyroids noted in the study at 5 and 1000 mg/kg/day, but there were no treatment-related effects observed on thyroid/parathyroid weights; liver weights were increased at 1000 mg/kg/day. At 1000 mg/kg/day, there were increased incidences of centrilobular hepatocyte hypertrophy and thyroid follicular cell hypertrophy observed.

There were several increases in liver enzymes and activities observed in males at 1000 mg/kg/day. These included the following: (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%). Pharmacokinetic data in the study, revealed the following differences (not compared to negative controls: (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%). The increase in liver enzymes/activities and pharmacokinetic data suggests that orthosulfamuron may enhance thyroid hormone metabolism and clearance via induction of liver microsomal enzymes.

The results of the clearance test and perchlorate discharge assay study along with the thyroid data (increased organ weight and histopathology) in the combined chronic carcinogenicity study are characteristic of increases in thyroid growth. Thus, it is plausible that exposure to orthosulfamuron may cause thyroid tumors via perturbation of thyroid-pituitary functioning due to enhanced hepatic clearance of thyroxin. However, the thyroid hormone data, which are critical to delineating a sequence of key events leading to tumor formation, are inadequate. Therefore, the available data do not clearly support the proposed mode of action.

The following deficiencies were noted in the study:

- Alterations in thyroid hormones are typically seen at early time points, with decreases observed in circulating levels of T4 and T3 and consequent increases in TSH. It should be noted, that in general, hepatic microsomal enzyme inducers appear to affect T3 less than T4, and thus, T4 and TSH tend to be more reliable indicators of altered pituitary-thyroid homeostasis. However, in this thyroid study, there were only small increases in TSH, seen only at Day 90, and transient decreases in T3, seen only at Day 30. Decreases in T3 were observed at 1000 mg/kg/day and similar decreases were seen at the non-tumorigenic dose of 5 mg/kg/day. Additionally, there were no decreases in T4 observed.

- The phenobarbital (positive control) group did not effectively alter hormone levels, and when examining the concentrations of thyroid hormones in the plasma, was not sampled with the negative controls and treated groups on Day 30; the critical time frame where changes in thyroid hormones are expected to occur. Suggested time points for the study are 14 days (early), 30-50 days (mid), and 90 days (late), including measurements of positive control at all time points.

- 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.

- There was no dose response concordance with the tumor response observed in the chronic/carcinogenicity study in the rat. Tumors were observed at 500 and 1000 mg/kg/day, while the thyroid study investigated effects at 5 and 1000 mg/kg/day. It is recommended that 3 doses be used for the study, and that these doses correspond with the observed tumor response. In this case, 250, 500, and 1000 mg/kg/day are recommended doses for identification of a clear dose response.

It is recommended that the registrant meet with the Agency prior to conducting a repeat thyroid study.

ATTACHMENT

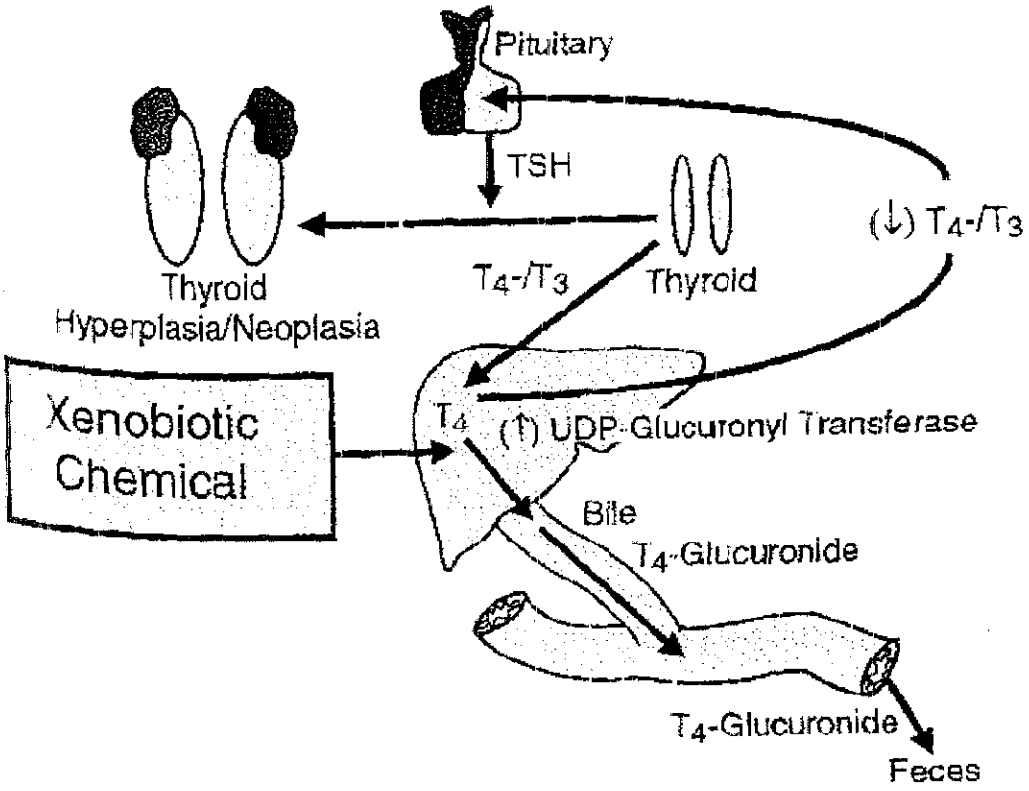


Figure 21-22. Hepatic microsomal enzyme induction by the chronic administration of xenobiotic chemicals,leading to thyroid follicular cell hyperplasia and neoplasia.

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

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