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RPA 203328 **Subchronic Oral Study (82-1a)**

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DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Toxicity [Feeding]-[Rat]

DP BARCODE: D224202
P.C. CODE: 123000
MRID NO.: 43904813

SUBMISSION CODE: S501233
TOX. CHEM. NO.: [New Chemical]

TEST MATERIAL (PURITY): RPA 203328 (99.7%)

CHEMICAL NAME: 2-Methanesulphonyl-4-trifluoromethylbenzoic acid

CITATION: Dange, M. (1995) RPA 203328 (a metabolite of RPA 201772). 28-Day Toxicity Study in the Rat By Dietary Administration. Rhône-Poulenc Agrochimie, Sophia Antipolis; Report No. SA 94097; April 27, 1995. MRID NUMBER: 43904813. (Unpublished)

SPONSOR: Rhône-Poulenc Agrochimie, Lyon, France

EXECUTIVE SUMMARY:

In a 28-day subchronic toxicity study (MRID# 43904813), RPA 203328 (99.7% a.i.) was administered in the diet to male and female Charles River France, Sprague-Dawley rats (10/sex/dose) at dosage levels of 0, 150, 500, 5,000, and 15,000 ppm (0, 11.14,37.57, 376.96 or 1,117.79 mg/kg/day in males and 12.68, 42.70, 421.53 or 1268.73 mg/kg/day in females, respectively) for 28 days.

Among males, a slightly lower urinary pH at 15,000 ppm and minimally higher urinary refractive index at 500 and 15,000 ppm were noted. In the absence of adverse effects on other parameters, these changes were considered as a normal physiological response to ingestion of an acidic compound. There were no compound related adverse effects on survival, clinical signs, body weight, food consumption, clinical chemistry, hematology, and gross or microscopic pathology.

The LOEL is >15,0000 ppm (1,117.79 mg/kg/day in males and 1,268.73 mg/kg/day in females).

The NOEL for both sexes is ≥15,000 ppm.

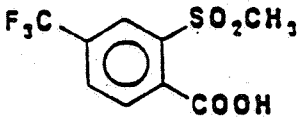
This study is classified acceptable (nonguideline), and does not satisfy any guideline requirement.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 203328
Chemical Name: 2-Methanesulphonyl-4-trifluoromethylbenzoic acid
Synonym: None
Description: A white powder
Batch #: DA938
Purity: 99.7%
Storage: stored in an air-tight, light-resistant container at room temperature
Structure:



2. Vehicle: Dietary admixture

3. Test animals: Rat
Strain: Sprague-Dawley
Age and weight at arrival: Approx. 4 weeks; Males - 240 to 313 g; Females - 175 to 218 g (at dosing)
Source: Charles River France, St. Aubin-les-Elbeuf, France
Housing: Individually in stainless steel cages
Diet: Commercial Rodent Diet Powder AO4C P1 (UAR, Villemoisson-sur-orge, France) ad libitum
Water: Tap water ad libitum
Environmental conditions: Temperature: 22±2°C; Relative Humidity: 55±15%; Air changes: 10-15/hour; Photoperiod: 12 hours light/dark
Acclimation period: Approx. 20 days

B. STUDY DESIGN:

1. In life dates - start: May 10, 1994
end: September 26, 1994

2. Animal assignment

Animals were assigned to the test groups on a weight basis (within 20% of the mean body weight), using a computer generated randomization procedure (see Table 1).

TABLE 1: STUDY DESIGN

Dietary Concentration (ppm)	Male	Female
Control (0)	10	10
150	10	10
500	10	10
5,000	10	10
15,000	10	10

3. Diet preparation and analysis

The test diets were prepared once for each concentration by incorporating the test substance into the diet by dry mixing to provide the required dietary concentrations. Mixtures were stored frozen below -15 °C and allowed to reach room temperature before sampling and use. The homogeneity of the test diet samples from the top, middle, and bottom portions of the mixtures was verified for the 150 and 15,000 ppm concentrations. The concentration analysis was performed on triplicate samples for each concentration. At the end of the study, the stability of the frozen dietary mixtures was analyzed for the 150 and 15,000 ppm concentrations after 26 and 48 days. A second set of samples for the same concentrations, stored at room temperature for a week after being frozen for 3 weeks, was analyzed for stability (Report Appendix L, pages 319-332).

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

Analyses for homogeneity of the 150 and 15,000 ppm diet formulations showed that the mean concentration of RPA 203328 in the top, middle and bottom portions of the samples was within 5% of the intended concentration (range: 95%-97% and 95%-99% at 150 and 15,000 ppm, respectively; Report SA 94097, Appendix L, page 328).

The stability analyses of the 150 and 150,000 ppm diet samples revealed that the test compound was stable in the diet for up to 7 weeks when stored frozen (range:

95%-96% and 97-99% after 3 and 7 weeks frozen storage, respectively) and for 4 weeks, when stored at room temperature for 1 week after being frozen for 3 weeks (range: 93% and 95%, at 150 and 15,000 ppm, respectively; Report SA 94097, Appendix L, page 329).

The concentration analyses of the test diets showed that the percents of the intended RPA 203328 concentration in each of the test diet formulations were within ±10% (95%, 93%, 110%, and 96% for the 150, 500, 5,000 and 15,000 ppm groups, respectively; Report SA 94097, Appendix L, page 330).

The dose levels selected for this study were based on the results of acute and subchronic toxicity studies conducted earlier by the sponsor. However, no details were provided.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

Clinical pathology and organ weight - Bartlett's test was conducted for homogeneity of variances between groups followed by ANOVA and Dunnett's test for significant differences. If Bartlett's test revealed heterogeneous variances, then modified t-test was employed to identify significant differences.

Body weight and food consumption - Bartlett's test was conducted for homogeneity of variances; significant differences were analyzed by ANOVA and Dunnett's test. If Bartlett's test revealed heterogeneous variances, then the significant differences were assessed by Kruskal-Wallis's one way analysis of variance and the Mann-Whitney's test.

Differential leukocyte counts - Both absolute count and percentages were determined for neutrophils and lymphocytes (Report page 17).

Means and standard deviations were calculated for each sex separately for each group at each time period.

C. METHODS:

1. Observations:

Animals were observed at twice daily for clinical signs of toxicity, mortality and moribundity. Detailed physical examinations were performed at least weekly during the dosing period.

2. Body weight

Animals were weighed prior to the beginning of treatment, on the first day of dosing, then once weekly thereafter and before necropsy.

3. Food consumption and compound intake

Food consumption per cage was determined weekly and is presented as g food/animal/day.

4. Ophthalmoscopic examination

The report stated that ophthalmoscopic examination was conducted on all animals prior to start of the study. Eyes of control and high-dose animals were reexamined during Weeks 2 and 4 using an indirect ophthalmoscope following instillation of an atropinic agent.

5. Clinical Pathology:

On study days 23, 24 and 25, blood was collected under anesthesia from the retro-orbital venous plexus of all rats (fasted overnight) for hematology and clinical chemistry analysis. On each day an equal number of rats from each group were sampled. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	-	Neutrophils (Neut)
X	Hemoglobin (HGB)*	-	Monocytes (Mono)
X	Leukocyte count (WBC)*	-	Eosinophils (Eos)
X	Erythrocyte count (RBC)*	-	Basophils (Baso)
x	Platelet count* (PLT	-	Fibrinogen (Fib)
	Blood clotting measurements*	X	Leukocyte differential count*
-	(Activated partial thromboplastin time) (APPT)	X	Mean corpuscular HGB (MCH)
-	(Thromboplastin time)	X	Mean corpusc. HGB conc.(MCHC)
-	(Clotting time and potential)	X	Mean corpusc. volume (MCV)
X	(Prothrombin time) (PT)	X	Reticulocyte count (RET)
		-	Erythroblast count (EBL)
		-	Lymphocytes (Lympho)

* Required for subchronic studies based on Subdivision F Guidelines
"-" = not examined

If significant hematological changes were observed, then femoral bone marrow smears from all animals at sacrifice were prepared, stained with May-Grunwald Giemsa and examined microscopically.

b. Clinical Chemistry

	ELECTROLYTES
X	Calcium* (Ca)
X	Chloride* (Cl)
-	Magnesium
X	Inorganic Phosphorus* IP)
X	Potassium* (K)
X	Sodium* (Na)
	ENZYMES
X	Alkaline phosphatase (ALP)
X	Serum alanine amino-transferase (also SGPT or ALT)*
X	Serum aspartate amino-transferase (also SGOT or AST))*
-	Cholinesterase (ChE)
-	Creatine phosphokinase (CPK)
-	Lactate dehydrogenase (LDH)
-	Gamma glutamyl transferase (GGT)
-	Gamma glutamyl transpeptidase (GTP)

	OTHER
X	Albumin*
-	Albumin-globulin ratio (A/G)
-	Phospholipids (PL)
X	Blood urea nitrogen* (BUN)
X	Total Cholesterol (T.Cho)
X	Globulins
X	Glucose* (GLU)
X	Total bilirubin (T. Bil)
-	Direct bilirubin (D. Bil)
X	Creatinine* (Cre)
X	Total protein (TP)*
X	Triglycerides (TG)
-	Serum protein Fractionation

* Required for subchronic studies based on Subdivision F Guidelines
"-" = not examined

6. Urinalysis*

Urinalysis was conducted on study days 29 through 32. Fresh urine was collected from all surviving animals in all groups after overnight fasting. The CHECKED (X) parameters were examined.

-	Appearance	X	Glucose
X	Volume	X	Ketones
-	Specific gravity	X	Bilirubin
X	Refractive Index	X	Blood
X	pH	-	Nitrate
X	Sediment (microscopic)	X	Urobilinogen
X	Protein		
-	Sodium		
-	Potassium		
-	Na/K ratio		
-	Osmolarity		

* Not required for subchronic studies
"-" = not examined

7. Sacrifice and Pathology

All surviving animals from all dose groups that were fasted overnight and sacrificed on study days 29 through 32 were subjected to gross pathological examination; animals found dead were also subjected to necropsy. The CHECKED (X) tissues were collected for histological examination. Histopathological examination was conducted on all tissues of both sexes from the control and 15,000 ppm groups. In addition, the following were examined from all animals in the 150, 500, and 5,000 ppm groups: liver, kidney and lungs. At terminal sacrifice, the (XX) selected organs (adrenals, kidney, liver, spleen, heart, brain, pituitary, thymus, thyroid, epididymis, prostate, uterus, ovary, testis) were weighed and the weights were expressed as absolute and relative to body weights.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Optic nerve
X	Esophagus*	X	Bone marrow*	X	Sciatic nerve*
X	Stomach*	X	Lymph nodes*	X	Spinal cord (3 levels)^{T}
X	Duodenum*	XX	Spleen*		
X	Jejunum*	XX	Thymus*	XX	Pituitary*
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+	XX	Adrenal gland*
X	Rectum*	X	Urinary bladder*	X	Harderian gland^{T}
X	Anus	XX	Testes*$^{+}$	X	Mammary gland^{T}
XX	Liver*$^{+}$	XX	Epididymides	X	Parathyroids*
-	Gall bladder*	XX	Prostate*	XX	Thyroids*
X	Pancreas*	X	Seminal vesicle		
		XX	Ovaries*		OTHER
	RESPIRATORY	XX	Uterus*	X	Aorta
X	Trachea*	X	Vagina	X	Sternum
X	Lung*			X	Femur
-	Nose			X	Skeletal muscle
-	Pharynx			X	Skin
X	Larynx			X	Eye
				X	Mesenteric lymph node
				X	Submaxilllary lymph node
					All abnormal tissue*

* Required for subchronic studies based on Subdivision F Guidelines

$^{+}$ Organ weight required in subchronic and chronic studies.

T = required only when toxicity or target organ

"-" = not examined

II. RESULTS

A. Observations :

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

2. Mortality - No mortalities were noted.

B. Body weight and weight gain:

There were no treatment-related effects on body weight or body weight gain observed in either sex at all dose levels. In females, decreases (≥11%) in body weight gain were noted at 500 and 15,000 ppm over the entire twenty-eight day treatment period. These decrease primarily resulted from slightly lower body weights from days 22-28 and 8-28, respectively (Report page 39). Because of lack of dose response, these findings were not considered to be treatment-related. Although the males had slightly lower initial body weights, the mean body gain over the same period was higher (5%) at 15,000 ppm. Table 2 summarizes the body weights and body weight changes for the selected time intervals during the study.

Table 2
Body Weights and Body Weight Changes in Rats Treated with RPA 20332 for Twenty-eight Days[a]

Parameter	Dosage Levels (ppm)									
	Males					Females				
Body weight (g):	0	150	500	5,000	15,000	0	150	500	5,000	15,000
Day 1	283	280	278	283	280	198	202	202	195	199
Day 28	427	443	439	440	426	265	265	262	260	260
Body Weight Change (g): Day 1-28	144	163	161	157	146	67	63	60	65	61
% of control value[b]	-	113	112	109	101	-	94	89	97	91

a Extracted from Tables 2 and 3 (pages 38 and 39) of the study no. SA 94097.
b Calculated by the reviewers

C. Food consumption and compound intake :

1. Food consumption - There were no significant differences between the treated and control groups in mean daily food consumption.

2. Compound consumption - The average daily consumption of RPA 203328 in mg/kg/day, based on the target concentrations, was 11.14, 37.57, 376.96 or 1117.79 in males and 12.68, 42.70, 421.53 or 1268.73 in females at 150, 500, 5,000 and 15,000 ppm, respectively (Report Table 5, page 18).

D. Ophthalmology: No treatment-related changes were observed.

E. Clinical Pathology: There were no treatment-related changes in the hematology or clinical chemistry parameters measured.

F. Urinalysis: At 15,000 ppm, urinary pH in males was slightly lower (11%) compared to that of controls (pH: 6.50±0.24 vs 7.28±0.51 in controls). In addition, the mean urinary refractive index at 500 and 15,000 ppm in males was slightly higher (+0.3% and +0.4%, respectively) compared to control values (1.35±0.0050 and 1.35±0.0043, respectively, versus 1.35±0.0020 in controls). These resulted from only few individual values being higher than the control values. No other compound-related effects were noted.

G. Sacrifice and Pathology:

There were no treatment-related changes in organ weights as well as gross necropsy examinations of the animals. No neoplastic or nonneoplastic lesions were observed during microscopic examinations.

IV. DISCUSSION

A. Reviewer's interpretation of study results: The analytical chemistry data indicate that the concentration, stability and homogeneity of RPA 203328 were within acceptable limits and the animals received appropriate dosages of the test compound.

Dietary administration of RPA 203328 slightly lowered the male urinary pH at 15,000 ppm (-11%) and minimally elevated (3-4%) the urinary refractive index at 500 and 15,000 ppm.

In the absence of adverse effects in other urinary parameters and/or changes in the organ weight and pathology, one may speculate that these changes are indicative of a physiological response resulting from the ingestion of an acidic compound. The evaluation of body weight and body weight gain data indicated that the body weight gain in females at 500 and 15,000 ppm was lower (≥11%) than the controls over the entire treatment period. This decrease was not accompanied by decrease in food consumption (g/rat/day). There were no adverse effects seen on the survival, clinical signs, ophthalmology, body weight, food consumption, hematology, clinical chemistry, and gross and microscopic pathology. Thus, in the absence of any significant toxicity to animals the decrease in body weight in females at 15,000 ppm was not considered to be biologically significant.

The LOEL is >15,0000 ppm (1,117.79 mg/kg/day in males and 1,268.73 mg/kg/day in females).

The NOEL for both sexes is ≥15,000 ppm.

B. Study deficiencies: No study deficiencies were noted: