Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test(OECD TG422) -Data Sheet-

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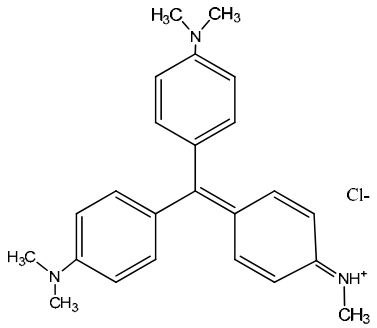
The data sheet was reviewed and created by Hazard-Data Evaluation Committee of National Institute of Technology and Evaluation in fiscal year 2011 based on the study report obtained by Ministry of Economy, Trade and Industry.

Test substance

MITI No. : 5-1969
CAS No. : 8004-87-3
Chemical name : C.I. Basic Violet 1
Molecular weight : 393.95
Molecular formula : $C_{24}H_{28}ClN_3$
Structural formula :



Appearance : Dark yellow-green to dark green crystal (nub)
Solubility : Soluble in ethanol and chloroform, slightly soluble in glycerin, insoluble in ethyl ether
Purity : Unknown

Experimental Method

Test animals : Crl:CD (SD) male and female rats, 10 weeks old at initiation of dosing
Number of animals : M; 7 rats/group (control and high dose groups of main study)
+5 rats/group (control and high dose groups of recovery).
12 rats/group (low and middle dose groups of main study)
F; 12 rats/group (all groups of main study)
+ 5 rats/group (control and high dose groups of recovery).
Dosing period : M; 42days
F; 41-48days (from 14 days before mating to day 4 of lactation)
Administration : oral gavage
Vehicle : 0.5%w/v Methylcellulose aqueous solution (suspended)
Dosing volume : 5 mL/kg
Dose level : 0, 1.6, 8, 40 mg/kg/day

Rationale for dose selection:

In the 14-day range finding study with 5 males and 5 females at dose levels of 0, 40, 120, 400 mg/kg/day, following findings were noticed.[2)]

400: Found dead or killed in extremis

120: Found dead or killed in extremis

40: Decrease in food consumption, suppressed body weight gain, changes in hematological and blood chemical parameters suggesting hepatic and renal disorders (increases in WBC, Neutrophil (MF), Monocyte and Lymphocyte, prolonged PT and APTT, increases in creatinine, inorganic phosphorus and α2-globulin(M), increasing tendency of ALAT and decreasing tendency of CPK(F))

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Results

Dose (mg/kg/day)	1.6	8	40
Repeated dose toxicity			
Mortality	M; 0/12 F; 0/12	M; 0/12 F; 0/12	M; 4/12 (day 9, day 11 (3 animals)) F; 5/12 (day 20 (2animals), GD 6, 18, 21)
Clinical signs	Test article-colored feces	Test article-colored feces	Found dead or killed in extremis Decreased spontaneous activity, prone position, bradypnea, abnormal respiratory tones, hypothermia, abnormal gait, soft stool, emaciation, abdominal distention, dirty around anus, soiled perineal region, external genital bleeding, test article-colored feces survivals soft stool, dirty around anus (MF), external genital bleeding (F)
FOB	NE	NE	Found dead or killed in extremis Bradypnea, prone position, decrease in spontaneous activity, incomplete eyelid opening, abnormal gait survivals no effect
Body weight	NE	NE	BW↓ and BW gain↓(M), BW gain↓(F)
Food consumption	NE	NE	FC↓(MF)
Urinalysis (M only)	NE	NE	NE
Hematology	NE	NE	PLT↑(MF)

Blood chemistry	NE	NE	<u>Killed in extremis</u> AST↑, ALT↑(M), CPK↑(MF) , BUN↑(MF) <u>survivals</u> TP↓, Alb↑, alpha 1-glb↓, A/G↑(M), BUN ↑(M), BUN↑ tendency (F)
Organ weight	NE	NE	NE
Necropsy	NE	Light violet aqueous content in stomach and cecum (M3/12)	<u>Found dead and killed in extremis(M;4/12,F;5/12)</u> Light violet aqueous content and discoloration of mucus membrane in all of the alimentary tract containing oral cavity, subcutaneous tissues and uterus (sporadically noticed in M and F), hydrothorax in thoracic cavity, (M 1/4, F 2/5), small thymus (M 3/4, F 2/5), small spleen(M 1/4, F 2/5), edema in thymus (M 1/4), reddish urine in gallbladder (M 1/4), red discoloration in mucosa of the bladder (M1/4), red discoloration of testis (M1/4), red discoloration of adipose tissue around the testis (M1/4), dilatation of stomach (F4/5), enlargement of adrenal (F4/5), gas retention in stomach (F2/5), dark red viscous retention in vagina (F2/5), dilatation of cecum (F1/5), gas retention in cecum (F1/5) <u>survivals</u> Light violet aqueous content in alimentary tract (MF)

Histopathology	NE	NE	<u>Found dead or killed in extremis</u> Trachea; desquamation of epithelium and inflammatory cell infiltration of mucosa (M1/4, F2/5) Glandular stomach; atrophy of epithelial cell (M1/4, F1/5) Small/large intestine; hypertrophy of epithelial cell (sporadically observed in M and F) Liver; hypertrophy of centrilobular hepatocyte (M3/4, F3/5), necrosis (M1/4, F2/5), vacuolation(M1/4) Adrenal; hypertrophy of zona fasciculata (M2/4, F5/5) Bone marrow; deficient erythropoiesis and granulopoiesis (M3/4, F2/5) Spleen; atrophy of follicle/marginal zone (M2/4, F5/5) and periarterial lymphatic sheath (M3/4, F 5/5) Thymus; atrophy (M4/4, F3/5) /necrosis of lymphocyte(M 3/4, F 4/5) Lymph node; atrophy of follicle / paracortex (M 3/4, F5/5) Spinal cord /fourth ventricle /testis /urinary bladder; hemorrhage or hemorrhagic infarction (M1/4) Vagina; hemorrhage(F2/5), mucoid degeneration of mucosa (F2/5) Lung; hemorrhage of alveolus, edema of alveolus, inflammatory cell infiltration (F1/5) <u>survivals</u> Liver; hypertrophy of centrilobular hepatocyte (M2/4) Duodenum; hypertrophy of epithelial cell (M2/4, F6/7) Mesenteric lymph node; sinus histiocytosis (M1/4, F3/7)

Target organs	gastrointestinal tract, liver		
NOAEL	MF: 8 mg/kg/day		
Basis for NOAEL	MF 40: death, hypertrophy of epithelial cell of intestinal tract, hypertrophy of centrilobular hepatocyte, necrosis of centrilobular hepatocyte		
NOEL	MF: 8 mg/kg/day[1)]		
Basis for NOEL	MF 40: death, hypertrophy of epithelial cell of intestinal tract, hypertrophy of centrilobular hepatocyte, necrosis of centrilobular hepatocyte		
Reproductive and developmental toxicology			
Parent	NE	NE	NE
Offspring	NE	NE	NE
NOAEL	40 mg/kg/day		
Basis for NOAEL	Parent / offspring 40: no adverse effect		
NOEL	40 mg/kg/day		
Basis for NOEL	Parent / offspring 40: no effect		

NE; No effect ↑; increase ↓; decrease M; male F; female
A; absolute organ weight R; relative organ weight

Note

1) In the final report, NOEL for male was described as 1.6 mg/kg/day based on the retention of test article in the digestive tract, but since the retention was considered to be no biological significance, in the data sheet, NOEL was judged to be 8 mg/kg/day.
2) Findings noted in parentheses of rationale for dose selection were cited from the dose-range finding study report.