DATA EVALUATION RECORD

JAU 6476 (PROTHIOCONAZOLE)/113961
[OPPTS 870.6200a (§81-8)]

STUDY TYPE: ACUTE NEUROTOXICITY
MRID 46246417

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
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Task Order No. 87-2005

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DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats [OPPTS 870.6200a (§81-8)] OECD 424.

PC CODE: 113961 **DP BARCODE:** D303578
SUBMISSION NO.:

TEST MATERIAL (PURITY): JAU 6476 (Prothioconazole; 97.6–98.8% a.i.)

SYNONYMS: 2[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazol-3-thion

CITATION: Sheets LP (2000) An acute oral neurotoxicity screening study with technical grade JAU 6476 in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf Ave., Stilwell, KS 66085-9104. Study No. 98-412-RG, February 3, 2000. MRID 46246417. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 46246417), groups of fasted, 9-week-old Wistar rats (12/sex) were given a single oral (gavage) dose of JAU 6476 (97.6-98.8% a.i., batch# 898803005) in 0.5% methylcellulose/0.4% Tween 80 at doses of 0 (vehicle), 200, 750, or 2000 mg/kg bw and observed for 14 days. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed in 12 animals/sex/group on day 0 (four hours following treatment, the time of peak effect) and on days 7 and 14. Cholinesterase activity was not determined. At study termination, six animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no treatment-related effects on mortality, body weight, brain weight or gross and histologic pathology or neuropathology. The only treatment-related clinical sign was brown perianal stain (graded as slight) observed on 3/12, 7/12, 11/12, and 12/12 males and 0/12, 0/12, 8/12, and 11/12 females in the control through high-dose groups during days 1 through 5. This clinical sign was also observed, but at lower incidences, during the FOB on the day of treatment. The effect had resolved by FOB test day 7. Partially formed stools were also noted during the FOB. Although the incidences of perianal stain of 7/12 (males, 200 mg/kg) and 8/12 (females, 750 mg/kg) would appear similar, the incidence for males was within historical control values for this vehicle; whereas, the incidence in females showed a clear effect at the mid and high dose. No other parameters examined in the FOB were affected.

Motor activity (total beam breaks) was non-significantly reduced on the day of treatment (by 29 and 36% in males in the 750 and 2000 mg/kg groups, respectively, and by 45% in females in the 2000 mg/kg group). Subsession data (up to approximately 50 minutes) also showed correspondingly reduced motor activity. This effect on motor activity had resolved by the next test session on day 7. The effect on locomotor activity was similar. It is not clear whether the observed clinical sign and reduced motor activity were due to transient effects on the nervous system or were an effect on the gastrointestinal tract resulting from administration of a noxious substance. In the absence of other effects, the perianal stain observed on females in the 750 mg/kg group was considered a non-adverse effect.

The LOAELs for JAU 6476 in male and female rats are 750 and 2000 mg/kg, respectively, based on the transient effect of reduced motor activity. The NOAELs for male and female rats are 200 and 750 mg/kg, respectively.

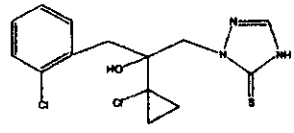
This neurotoxicity study is classified as **Acceptable/Guideline,** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424) provided the conducting laboratory provides positive control data demonstrating the ability to detect major neurotoxic endpoints, changes in motor activity, and nervous system pathology. Raw data on analysis for concentration, homogeneity, and stability of the test material should also be provided.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A flagging statement was not provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: JAU 6476

Description:	technical grade, beige powder, stable under conditions of study
Batch #:	*898803005*
Purity:	97.6% a.i. (March 1998); 98.8% a.i. (July 1999)
CAS # of TGAI:	178928-70-6
Structure:	

2. Vehicle and/or positive control: 0.5% methylcellulose/0.4% Tween 80 in deionized water.

3. Test animals:

Species:	Rat	
Strain:	Wistar (Crl:WI(HAN)BR	
Age/weight at dosing:	9 weeks; males: 204-205 g (mean); females 160-163 g (mean)	
Source:	Charles River Laboratories, Inc., Raleigh, NC	
Housing:	Individually, in suspended stainless steel wire-mesh cages	
Diet:	Purina Mills Rodent Lab Chow in "etts" form, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	Not given
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	Six days	

B. STUDY DESIGN:

1. **In life dates:** Start: June 8, 1998; End: June 25, 1998

2. **Animal assignment and treatment:** Following removal of animals that were ±20% of the mean weight for each sex, animals were randomly assigned to the test groups noted in Table 1 using INSTEM Computer Systems software. Body weight among groups was comparable. Following an overnight fast, rats were given a single dose by gavage in 0.5% methylcellulose/0.4% Tween 80 in deionized water. Rats were observed once daily for mortality or clinical signs and weighed weekly as a component of the Functional Observational Battery. Dose levels were chosen based on results of an acute oral range-finding study. In the range-finding study, 5 males/dose were administered 0 or 2000 mg/kg and observed for signs of toxicity until the following day. Males that received 2000 mg/kg had loose stools and perianal stain beginning at approximately three hours following treatment, with additional animals involved by 4 hours post-treatment. Perianal stain was the only observed sign the following day. Based on these results, doses of 0, 200, 750, and 2000 mg/kg (a limit dose) were chosen. The time of peak effect for neurotoxicity was 4 hours. Treatment was staggered over two days for each sex. Females were sacrificed 14 days after exposure and males were sacrificed 15 days after exposure. A necropsy was performed.

TABLE 1. Study design

Experimental Parameter	Dose group (mg/kg bw)			
	Control (0)	Low dose (200)	Mid dose (750)	High dose (2000)
Total number of Animals/sex/group	12/sex	12/sex	12/sex	12/sex
Behavioral Testing (FOB, Motor Activity)	12/sex	12/sex	12/sex	12/sex
Neuropathology	6/sex	—	—	6/sex

3. **Test substance preparation and analysis**: Details of the preparation of the dosing solutions and method and time of sample selection for concentration and homogeneity analyses were not provided. Samples with nominal concentrations of 20 and 200 mg/mL were analyzed by liquid chromatography. For stability analyses, samples were stored for 8 days prior to analysis.

Results:

Homogeneity analysis: Data were not provided. The report stated that the 20 mg/mL and 200 mg/mL solutions had coefficients of variation of 2.7 and 0.3%.

Stability Analysis: Data were not provided. The report stated that there was no appreciable decrease in concentration following eight days of storage.

Concentration analysis: Data were not provided. The report stated that doses of 0, 200, 750, and 2000 mg/kg ranged from 109-112% of nominal. Based on these results, the analytically-confirmed doses were 0, 218, 847, and 2240 mg/kg.

The summary analytical data (as reported by the study author) indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Continuous data were analyzed by an Analysis of Variance (ANOVA) followed by Dunnett's test if a significant F-value was determined in the ANOVA. Continuous FOB data and motor and locomotor activity were first analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction between dose group and test week. For weeks in which there was a significant treatment effect, Dunnett's test was used to determine which groups, if any, were significantly different from the control group. General Linear Modeling (GLM) and Categorical Modeling (CATMOD) procedures with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively, were used for categorical data in the FOB. Interval data for motor activity were subjected to a Repeated-Measures ANOVA to determine in which weeks there was a significant treatment by interval interaction. For those weeks, the data for each interval were subjected to a one-way ANOVA to determine at which intervals there was a significant treatment effect. For intervals with a significant treatment effect Dunnett's test was used to determine which groups were significantly different from the control group. The above analyses were performed using either INSTEM computer Systems or SAS. Significance was flagged at $p \leq 0.05$.

   Continuous pathology data were initially evaluated using Bartlett's Test to analyze for homogeneity of variances among groups. Homogeneous data were further analyzed using an ANOVA, followed by Dunnett's test for pair-wise comparisons. For non-homogeneous data, the non-parametric Kruskal-Wallis Test followed by a Mann-Whitney U Test was performed. Frequency data relevant to micropathology were analyzed using a Chi-Square Test followed by a one-tailed Fisher's Exact Test in cases of significant variation by the Chi-Square analysis. Significance was flagged at $p \leq 0.05$ except for Bartlett's Test in which a probability value of $p \leq 0.001$ was used.

   The Reviewer considers the statistical analyses appropriate.

C. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed once daily for mortality and morbidity. Detailed physical examinations were also carried out and recorded once daily.

2. **Body weight:** Animals were weighed weekly as a component of the FOB.

3. **Food consumption:** Food consumption was not recorded as part of the study.

4. **Cholinesterase determination:** Cholinesterase activity was not determined.

5. **Neurobehavioral assessment:**

   a. **Functional observational battery (FOB):** Twelve animals/sex/group were tested individually pretreatment and on the day of treatment, beginning 4 hours after treatment (the time of peak effect). Animals were tested again on days 7 and 14. Testing was staggered over two days for each sex, and groups were balanced across test times and test devices. Males and females were tested on separate days. Feeders were removed approximately 30 minutes prior to observation of home cage behavior. Observations were made in a specific order; scoring criteria were given for the appropriate parameters. Animals were observed for two minutes in the open field. The same technician made observations throughout the study. A second technician took measurements, and a third person recorded the results. The technicians were blind to the treatment status of the animals. The strain gauge used for grip strength was not described.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*		Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
X	Vocalizations	X	Respiratory rate+ (open field)	X	Urination / defecation*
X	Piloerection	X	Red/crusty deposits*		Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Approach response+		Eye prominence*	X	Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response*				Backing
X	Pain response*				Time to first step
X	Pupil response*			X	Piloerection
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
			OTHER OBSERVATIONS		Rotarod performance

*Required parameters; +Recommended parameters

b. **Motor/Locomotor activity:** Motor and locomotor activity were evaluated following the FOB (approximately seven hours following treatment). Total motor activity (total beam interruptions) was tested for 90 minutes in one of eight figure-eight mazes (Columbus Instruments Maze Monitoring System, Columbus, OH). Data were collected as 10-minute subsessions. Locomotor activity was calculated by eliminating consecutive counts for a given beam. Uniform background noise was provided by a white noise generator (Coulbourn Instruments).

6. **Sacrifice and pathology:** All animals were subjected to gross necropsy. The first six animals/sex/group were deeply anesthetized with an intraperitoneal injection of pentobarbital and then perfused via the left ventricle with phosphate buffered sodium nitrite followed by 10% formalin. The remaining animals were sacrificed by CO_2 asphyxiation. The brain was weighed. Brain (eight coronal sections) and spinal cord (four levels) sections were embedded in paraffin and stained with hematoxylin and eosin, luxol fast blue/cresyl violet, and Sevier Munger stains. Dorsal root ganglia, eyes, optic nerves, and gastrocnemius muscle were embedded in glycol methacrylate, sectioned at 2-3 µm and stained using a modified Lee's stain. Peripheral nerve fibers were embedded in epoxy resin, cross-sectioned at approximately 1 µm, and stained with toluidine blue. The sciatic nerve was also longitudinally sectioned at approximately 1 µm and stained with toluidine blue.

The CHECKED (X) tissues were evaluated.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Eight coronal sections [a]	X	Mid-thigh
			Sciatic Notch
			OTHER
		X	Sural Nerve
		X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
X	Thoracic swelling	X	Lumbar ventral root fibers
X	Cauda equina	X	Cervical dorsal root ganglion
	OTHER	X	Cervical dorsal root fibers
X	Gasserian Ganglion	X	Cervical ventral root fibers
	Trigeminal nerves		
X	Optic nerve		
X	Eyes		
X	Gastrocnemius muscle		

[a] Assumed to include the forebrain, cerebrum, midbrain, cerebellum, pons, and medulla oblongata.

7. **Positive controls:** Positive control data were not provided.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** Brown perianal stain was observed on males at all doses, including the control, and on females that received 750 and 2000 mg/kg (Table 2). Incidences on males appeared dose-related (3, 7, 11, and 12 of 12 animals in the control through high-dose groups). In females, only the 750 (8/12) and 2000 mg/kg dose groups (11/12) were affected. The study author noted that the incidences on males in the control and low-dose groups were within the range of historical control data for this vehicle. The perianal stain was resolved in all animals by day 5.

TABLE 2. Clinical observations

Observation	Dose level (mg/kg bw)			
	Control (0)	Low dose (200)	Mid dose (750)	High dose (2000)
Males				
Perianal stain (brown)	3/12 (0-1)	7/12 (0-2)	11/12 (0-2)	12/12 (0-5)
Females				
Perianal stain (brown)	0/12	0/12	8/12 (0-1)	11/12 (0-2)

Data were extracted from Table 1, page 29, MRID 46246417.
Numbers represent the number of animals with the observed sign/total number of animals (range of days observed in parenthesis).
n=12.

2. **Mortality:** There were no deaths.

B. BODY WEIGHT AND BODY WEIGHT GAIN: There was no effect of treatment on final body weight or body weight gain in male or female rats (Table 3). On Day 0, the day of treatment (following the overnight fast), body weight of male rats showed a minimal increase and female rats had lost weight. Body weight and weight gain were unaffected thereafter (Days 7 and 14).

TABLE 3. Body weight and body weight gain (g±s.d.)				
Observation	**Dose level (mg/kg bw)**			
	Control (0)	**Low dose (200)**	**Mid dose (750)**	**High dose (2000)**
Body weight–Males				
Pretreatment	204±13	205±12	205±12	204±11
Day 0	213±13	214±10	214±12	215±10
Day 14	291±21	289±17 (99)	289±19 (99)	293±14 (101)
Body weight–Females				
Pretreatment	161±9	162±10	160±10	163±8
Day 0	155±9	154±10	155±10	156±8
Day 14	198±13	199±12 (101)	200±12 (101)	199±9 (101)
Body weight gain–Males				
Days 0-14	87	84 (97)	84 (97)	89 (102)
Body weight gain–Females				
Days 0-14	37	37 (100)	40 (108)	36 (97)

Data were extracted from Table 3, pp. 102, 103, 105, 106, 107, and 109, MRID 46246417.
Values represent mean ± s.d. (body weight gain calculated by Reviewer).
n=12.

C. **FOOD CONSUMPTION:** Food consumption was not measured.

D. **CHOLINESTERASE ACTIVITIES:** Cholinesterase activities were not measured.

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings:** During handling, brown perianal stain, graded as slight, was observed in male and female rats at the mid- and high dose following treatment on day 0. This sign had resolved by observation day 7. Although incidences were similar in the high-dose group of both sexes, statistical significance was attained only for females. The incidence in males in the mid-dose group, 6/12 animals, was not statistically significant. Although diarrhea was not observed for any animal during the open field observations, feces were characterized as partially formed on day 0 following treatment. Incidences in males in the control through high-dose groups were 0/12, 0/12, 4/12 ($p \leq 0.05$), and 3/12 (not statistically significant) (data not shown). Incidences in females on day 0 were 0/12, 2/12, 1/12, and 4/12 (not statistically significant), respectively. No other parameters were affected by treatment.

TABLE 4. Functional observation battery results				
Observation	Dose level (mg/kg bw)			
	Control (0)	Low dose (200)	Mid dose (750)	High dose (2000)
Males				
Perianal stain (brown)				
-Pretest	0/12	0/12	0/12	0/12
-Day 0	1/12	1/12	6/12	5/12
-Day 7	0/12	0/12	0/12	0/12
-Day 14	0/12	0/12	0/12	0/12
Females				
Perianal stain (brown)				
-Pretest	0/12	0/12	0/12	0/12
-Day 0	0/12	0/12	2/12	5/12*
-Day 7	0/12	0/12	0/12	0/12
-Day 14	0/12	0/12	0/12	0/12

Data were extracted from Table 2, pp. 30-101, MRID 46246417.
Values represent number of animals with sign.
n=12.
*=$p \leq 0.05$, compared with controls.

2. **Motor activity:** Motor activity (total beam breaks) is summarized in Table 5. Although not statistically significant compared with the respective control groups, motor activity was reduced following treatment on day 0 in males and females that received 2000 mg/kg (64 and 55% of the control values, respectively). The activity of males that received 750 mg/kg was 71% of the control level on day 0, and there appeared to be a dose-related trend in males, but not in females. In males in the 750 and 2000 mg/kg groups, the day 0 10-minute subsession values showed greatest reductions in the first two subsessions, although none of the subsession values were statistically significantly lower than the control values. At 2000 mg/kg, a similar trend was seen in subsession values for females through 50 minutes. The reduction in motor activity in males that received 200 mg/kg (79% of the control value) is close to the natural variability seen in this type of test (±20%), and the first 10-minute subsession data for both motor and locomotor activity did not show a clear effect of treatment (values were approximately 90% of the control value). Most of the inactivity occurred during subsession 5 (data not shown). Habituation of controls (and treated groups) was attained by the end of 60 minutes. There was no affect of treatment on motor activity on days 7 and 14 (the 22% increase in activity of females in the 750 mg/kg group on day 14 was considered incidental to treatment).

   Data were presented separately for locomotor activity. Locomotor activity followed the same trends as motor activity (data not shown in Table 5). Compared with the respective control values, no statistically significance difference was seen in total locomotor activity or subsession data on days 0, 7, or 14.

TABLE 5. Motor activity (total activity counts for 90-minute sessions and subsessions on day 0)

Test day	Dose level (mg/kg bw)			
	Control (0)	Low dose (200)	Mid dose (750)	High dose (2000)
Males				
Pre-test	527±178	580±146	499±194	554±204
Day 0 *(total)*	312±148	247±111 (79)	220±135 (71)	199±96 (64)
Interval 1	114±29	106±45	99±41	91±37
Interval 2	90±37	68±34	62±40	66±42
Interval 3	51±28	43±37	29±33	17±23
Interval 4	24±36	17±24	6±11	3±5
Interval 5	18±32	4±10	1±1	0±1
Interval 6	4±8	1±3	4±7	4±10
Interval 7	6±16	1±3	10±21	2±4
Interval 8	4±6	2±6	8±12	12±23
Interval 9	2±3	5±9	3±6	4±12
Day 7	556±213	554±149 (100)	587±152 (106)	526±132 (95)
Day 14	537±214	550±160 (102)	489±172 (91)	489±185 (91)
Females				
Pre-test	775±288	764±196	859±232	705±294
Day 0 (total)	462±93	415±193 (90)	454±156 (98)	254±120 (55)
Interval 1	151±44	136±48	149±52	97±49
Interval 2	116±28	105±46	112±39	77±44
Interval 3	74±22	63±39	70±34	31±25
Interval 4	48±29	32±32	42±31	11±19
Interval 5	29±22	19±24	21±27	7±15
Interval 6	11±14	18±23	18±24	2±3
Interval 7	9±15	19±33	22±31	7±13
Interval 8	7±14	7±12	11±21	6±10
Interval 9	18±25	16±33	11±16	16±16
Day 7	665±155	645±125 (97)	733±212 (110)	553±230 (83)
Day 14	661±155	730±184 (110)	809±199 (122)	651±258 (98)

Data were extracted from p. 12, Table 4 (pp. 110 and 111), and Table 6 (pp. 115 and 119), MRID 46246417.
Values represent mean number of beam breaks ± s.d.
Numbers in parenthesis represent percent of control value, calculated by Reviewer.
n=12.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** There were no treatment-related grossly observable pathological changes.

2. **Brain weight:** There were no effects of treatment on absolute brain weight or brain to body weight ratio (Table 6). All values were within 98-101% of the control values.

TABLE 6: Absolute and relative brain weights (n=6/sex)				
Weights (mg)	Dose level (mg/kg bw)			
	Control (0)	Low dose (200)	Mid dose (750)	High dose (2000)
Males				
Body wt	293.5±9.5	286.4±20.7	293.6±23.0	290.8±14.2
Brain wt	1.851±0.096	1.780±0.094	1.816±0.071	1.815±0.051
Brain/body wt	0.631±0.046	0.622±0.022	0.621±0.038	0.625±0.034
Female				
Body wt	202.8±12.8	194.7±5.9	200.9±8.8	197.9±7.9
Brain wt	1.754±0.075	1.749±0.070	1.704±0.036	1.741±0.059
Brain/body wt	0.868±0.068	0.898±0.034	0.849±0.034	0.881±0.045

Data were extracted from Table OW1K-SUM, pp. 391 and 392, MRID 46446417.

3. **Neuropathology:** No neuropathological lesions attributed to treatment were observed. The most common lesions were hyperplasia of the brain (several levels) and degeneration of nerve fibers of the spinal cord. These lesions were observed at nearly equal incidences in the control and high-dose groups. Due to the absence of treatment-related lesions, the lower dose groups were not examined.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: The investigator concluded that compound-related clinical signs were evident in males and females treated with 750 and 2000 mg/kg. The clinical sign of brown perianal stain was observed on the day of treatment and resolved by day 5. During the FOB, the same compound-related sign of brown perianal stain (accompanied by partially formed stools) was observed on males and females treated with 750 and 2000 mg/kg. Perianal stain was observed on day 0 and had resolved by the next observation time (day 7). Motor and locomotor activity were reduced on day 0 in males that received 750 and 2000 mg/kg and in females that received 2000 mg/kg. There was no effect of treatment on body weight or brain weight. No gross or microscopic lesions attributed to treatment were observed. The NOEL was 200 mg/kg in both males and females.

B. REVIEWER COMMENTS: The Reviewer agrees with the Investigator's descriptions of effects, but would change the LOAEL and NOAEL. There were no deaths, and JAU 6476 had no effect on body weight or body weight gain. A single clinical sign - brown perianal stain (graded as slight) - was observed on the day of treatment on several males and females, primarily in the 750 and 2000 mg/kg groups, both during the clinical examination and during the FOB. Partially formed stools were also observed during the FOB. The perianal stain had resolved by day 5. The brown perianal stain may be partially attributed to the effect of the vehicle (methylcellulose/Tween 80) on the digestive tract. But, as pointed out by the Investigator, the incidences in the 750 and 2000 mg/kg groups (both sexes) were higher than historical control rates with the use of this vehicle, and may be caused by the test substance.

The differences in incidence of perianal stain between the daily clinical examinations (higher incidence) and the FOB on Day 0 is explained by the fact that this sign was not present in some animals until Day 1, a day after the FOB was conducted. The Reviewer considers the perianal stain an effect of treatment, but, taken alone, not an adverse effect.

Total motor activity was reduced in all groups (including controls) on the day of treatment. The Reviewer concurs with the Investigator's statement that motor activity is generally reduced following the overnight fast prior to treatment. In addition, total motor activity in males in the 750 and 2000 mg/kg groups and in females in the 2000 mg/kg group was non-significantly reduced compared with the respective control values on the day of treatment. These reductions, 64-71% and 55% of control levels in males and females, respectively, are considered an effect of treatment. Subsession data, primarily 10-minute intervals 1-5, indicated that motor activity was consistently, non-significantly reduced as noted above for total motor activity. This effect on motor activity was resolved by day 7, when the next test session was conducted. The effect on locomotor activity was the same. Motor and locomotor activity of males and females in the lower dose groups was unaffected by treatment. The value for males in the 200 mg/kg/day group (79% of the control value) was considered close to the range of natural variation for this type of test (±20%), and the first 10-minute subsession data for both motor and locomotor activity did not show a reduction.

There was no effect of treatment on absolute or relative brain weight. No gross organ/tissue lesions or microscopic lesions of the nervous system attributed to treatment were observed. While the observed clinical signs are mild and may be attributed to a noxious effect on the gastrointestinal tract, the effect on motor activity is biologically significant.

The LOAELs for JAU 6476 in male and female rats are 750 and 2000 mg/kg, respectively, based on the transient effect of reduced motor activity. The NOAELs for male and female rats are 200 and 750 mg/kg, respectively.

C. **STUDY DEFICIENCIES:** Raw data for the homogeneity, concentration, and stability analysis were not provided. The study is not acceptable unless positive control data demonstrating the ability of the conducting laboratory to detect major neurotoxic endpoints, changes in motor activity, and nervous system pathology are provided.

DATA FOR ENTRY INTO ISIS

Acute Neurotoxicity Study - rats (870.6200a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
113961	46246417	acute neurotoxicity	rats	1 dose	oral	gavage	200-2000	0, 200, 750, 2000	200 (males); 750 (females)	750 (males); 2000 (females)	reduced motor activity, gastro-intestinal tract	

13544

R154821

Chemical:

PC Code:

HED File Code: **258 Contractor DER TOX Warning: This Document (review) was completed by a contractor and has not undergone secondary review. This document may not reflect Agency policies**

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File ID: ***00000000***

Accession #: **000-00-0123**

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