DATA EVALUATION RECORD

JAU 6476 (PROTHIOCONAZOLE)/113961
[OPPTS 870.6200b (§82-7)]

STUDY TYPE: SUBCHRONIC ORAL NEUROTOXICITY
MRID 46246416

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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Oak Ridge, TN 37831
Task Order No. 87-2005

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity [OPPTS 870.6200b (§82-7)] oral (gavage) - rat; (No OECD guideline).

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

TEST MATERIAL (PURITY): JAU 6476, technical (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, Lake SG (2001) A subchronic 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. Laboratory study number 98-412-RY, April 12, 2001. MRID 46246416. Unpublished.

SPONSOR: Bayer Corporation

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 46246416) JAU 6476 (97.6-98.8% a.i., batch#s 898803005 and 6233/0031) was administered to 12 Wistar (Crl:WI(HAN)BR) rats/sex/group at nominal dose levels of 0, 100, 500, or 1000 mg/kg bw/day, five days/week, for 13 weeks (analytically determined doses of 0, 98, 505, and 1030 mg/kg/day). The dose was administered by gavage in 0.5% methylcellulose/0.4% Tween 80 in deionized water. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 12 animals/sex/group pretreatment and during weeks 4, 8, and 13. 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 deaths. Treatment-related clinical signs included urine stain on 8/12 males and 9/10 females in the 500 mg/kg/day group and 12/12 males and 11/ 11 females in the 1000 mg/kg/day group. Urine stain was first observed on day 18 on males treated with 1000 mg/kg/day. Urine stain increased in frequency with duration of exposure. Urine stain was considered an effect of treatment, but, in the absence of other clinical signs or histological correlates, not an adverse effect. Oral stain was observed on 3/12 males (beginning on day 25) and 1/11 females in the 1000 mg/kg/day group. Males in the mid- and high-dose groups lost weight during the first week of the study (3-4%); weight loss was not accompanied by a decrease

in food consumption during the first week. Final body weight was slightly (non-statistically) reduced in males treated with 500 (6%) and 1000 mg/kg/day (8%). Body weight gain was reduced by 16 and 24% in males in the 500 and 1000 mg/kg/day groups, respectively. Body weight and body weight gain of females were unaffected by treatment. Food consumption was unaffected in both sexes. Urine stain was the only compound-related parameter affected during the FOB.

Compared with the respective control groups, slight reductions were observed in motor and locomotor activity (up to 26%, non-statistically significant) in both sexes in the high-dose groups. These reductions occurred in males and females during week 4 and in females during weeks 4 and 13. However, the values for females were not dose-related, and differences in values between the control and high-dose females for all weeks (8-15%) were less than or similar to the difference between the pretest control and high-dose group value (14%). Therefore, the effect on motor activity for females cannot be considered a clear effect of treatment. The effect on motor activity of males is questionable for the same reasons. There were no compound-related ophthalmic findings or microscopic lesions of the central or peripheral nervous system. Brain weight was unaffected by treatment.

Based on the non-toxicologically significant effects seen in this study, the LOAEL for JAU 6476 in male and female rats was not attained; the NOAEL for male and female rats was ≥1000 mg/kg/day.

This neurotoxicity study is classified as **Acceptable/Guideline**, and satisfies the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b; no OECD) provided the conducting laboratory provides positive control data demonstrating their 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.

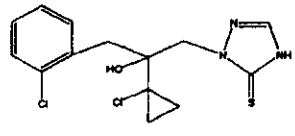
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: JAU 6476

Description:	technical grade, beige powder, stable under conditions of study
Batch #s:	898803005, 6233/0031
Purity:	898803005: 97.6% (March 1998), 98.8% (July 1999); 6233/0031: 98.8% (June 1998), 98.1% (November 1998)
CAS # of TGAI:	178928-70-6
Structure:	



2. **Vehicle and/or positive control:** 0.5% methylcellulose/0.4% Tween 80; Lot/Batch # not provided.

3. **Test animals:**

Species:	Rat	
Strain:	Wistar (Crl:WI(HAN)BR)	
Age/weight at study initiation:	Nine weeks of age; males: 236.8±17.9-241.7±17.9 g; females: 157.7±10.8-162.5±7.3 g	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually, in suspended stainless steel wire-mesh cages	
Diet:	Purina mills Rodent Lab Chow, "etts" form, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	Not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	6 days	

B. STUDY DESIGN:

1. **In life dates:** Start: August 22, 1998; End: November 19, 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. Rats were administered the gavage dose in 0.5% methylcellulose/0.4% Tween 80 in deionized water. Doses were administered once daily, in the morning, five days/week, for 13 weeks. On days on which the FOB was administered, doses were administered in the afternoon. All animals were subjected to a gross necropsy.

   Dose levels were chosen based on results of a 4-week study and a developmental toxicity study in young adult Wistar rats. In both studies, the limit dose of 1000 mg/kg/day resulted in effects on body weight gain, food consumption, and clinical chemistry, with undefined effects on the liver and kidney. Based on these studies, doses of 0, 100, 500, and 1000 mg/kg/day were selected for the subchronic study.

TABLE 1. Study design[a]

Experimental parameter	Dose group (mg/kg bw/day)[a]			
	Control (0)	Low dose (100)	Mid dose (500)	High dose (1000)
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

[a] Based on analytical results, actual doses were 0, 98, 505, and 1030 mg/kg/day.

3. **Test substance preparation and analysis**: Preparation of the dosing suspensions was not described. The test substance was prepared/dissolved in 0.5% methylcellulose/0.4% Tween

80 in deionized water. Samples with nominal concentrations of 2 and 100 ppm were analyzed for homogeneity and concentration by liquid chromatography. For concentration analysis, samples were taken during weeks 1, 5, and 9 of the study. For stability analyses, samples were stored for 35 days in the refrigerator prior to analysis.

Results:

Homogeneity analysis: Data were not provided. The report stated that the 2 and 100 ppm suspensions had coefficients of variation of 1.1 and 1.7%, respectively.

Concentration analysis: Data were not provided. The report stated that concentrations of doses of 0, 100, 500, and 1000 mg/kg ranged from 98-103% of nominal. Based on these results, the analytically-confirmed doses were 0, 98, 505, and 1030 mg/kg.

Stability analysis: Data were not provided. The report stated that there was no appreciable decrease in concentration following 35 days of storage under refrigeration.

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:** Statistical evaluations were performed using software from either INSTEM Computer Systems or SAS. 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 (session data) 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 from the FOB. Interval data for motor activity were subjected to a two-way Repeated-Measures ANOVA, using both test interval and test occasion as the repeated measures. This was followed by a Repeated Measures ANOVA to determine on 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. 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 twice daily (once daily on holidays and weekends) for mortality and moribundity. The authors noted two exceptions to the twice daily observations. These exceptions did not invalidate the study. Detailed clinical observations were recorded once each week. Ophthalmic examinations were conducted pre-exposure and during week 12.

2. **Body weight:** Animals were weighed weekly.

3. **Food consumption:** Food consumption was determined weekly (method was not described).

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

5. **Neurobehavioral assessment:**

   a. **Functional observational battery (FOB):** All 12 animals/sex/group were tested individually (in groups of 8) pretreatment and during weeks 4, 8, and 13, prior to dosing. 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, with the cages cleaned between intervals to reduce the residual scent from the other sex. Feeders were removed approximately 30 minutes prior to observations of home cage behavior. Observations were made in a specific order; scoring criteria were given for the appropriate parameters. Animals were observed for 2 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 approximately 1 to 2.5 hours following the FOB. 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 (three levels and the cauda equina) 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:** Clinical signs are summarized in Table 2. Urine stain was observed on 8/12 and 12/12 males that received 500 and 1000 mg/kg/day, respectively. In females, incidences in the respective groups were 9/10 and 11/11. This sign first appeared on males on day 18 and on females on day 25, and in most cases continued throughout the remainder of the treatment period. Urine stain increased in frequency with duration of exposure. Oral stain was observed only in the high-dose groups. Perianal stain was not clearly treatment related. Alopecia was observed in low numbers in all female dose groups and was not dose-related (data not shown). The eyes were not affected by treatment.

TABLE 2. Clinical observations				
Observation	**Dose level (mg/kg bw/day)**			
	Control (0)	**Low dose (100)**	**Mid dose (500)**	**High dose (1000)**
Males				
urine stain	0	0	8 (days 46-88)	12 (days 18-88)
perianal stain	0	0	3 (days 46-60)	0
oral stain	0	0	0	3 (days 25-74)
Females				
urine stain	0	0	9 (days 32-88)	11 (days 25-88)
perianal stain	0	0	0	0
oral stain	0	0	0	1 (74)

Data were extracted from Table 1, p. 32, MRID 46246416.
Numbers represent number of animals with at least one instance of the observation; numbers in parenthesis are days the sign was observed.
n=10-12 (numbers <12 due to deaths in female groups).

2. **Mortality:** Three females died prior to study termination. One mid-dose female was found dead on day 55; the cause of death could not be determined. Two females, one each in the 500 and 1000 mg/kg dose groups succumbed to gavage error.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** There was no statistically significant effect of treatment on body weight or body weight gain in either sex (Table 3). In males during the first week of treatment, body weight increased only slightly in the control and low-dose groups, whereas, the mid- and high-dose groups lost weight (3-4%). All male groups gained weight thereafter, with final body weight in the 100, 500, and 1000 mg/kg dose groups of 98, 94, and 92% of the control value, respectively. For males total weight gain in the 100, 500, and 1000 mg/kg groups were 95, 84, and 76% of the control value.

Except for the last week of the study, where weight gain was minimal in all groups, females in all dose groups gained weight throughout the study. During the first week, weight gain in the treated groups was lower than the control value in a dose-related manner. Final body weight in the 100, 500, and 1000 mg/kg dose groups was 96, 93, and 96% of the control weight, respectively. Respective weight gain values for females were 93, 83, and 93% of the control value.

TABLE 3. Body weight and body weight gain (g ± s.d.)

Observation	Dose level (mg/kg bw/day)			
	Control (0)	Low dose (100)	Mid dose (500)	High dose (1000)
Body weight–Males				
Day 0	237.8±20.8	236.8±17.9	240.5±20.8	241.7±17.9
Day 7	243.2±16.2	238.4±12.8	233.0±13.0	231.5±11.6
Day 42	351.7±38.3	345.0±27.9	340.2±32.9	330.2±32.5
Day 91	396.1±45.8	386.9±35.1 (98)	373.4±31.9 (94)	362.7±38.4 (92)
Body weight–Females				
Day 0	162.5±7.3	157.7±10.8	158.7±8.5	159.1±11.7
Day 7	174.7±10.5	167.8±8.6	165.9±13.5	165.5±12.0
Day 42	220.4±14.8	209.8±9.9	209.0±12.5	214.5±20.0
Day 91	239.8±15.2	229.3±8.8 (96)	223.0±11.4 (93)	231.2±24.8 (96)
Body weight gain–Males				
Days 0-7	5.4	1.6	-7.5	-10.2
Days 0-91	158.3	150.1 (95)	132.9 (84)	121.0 (76)
Body weight gain–Females				
Days 0-7	12.2	10.1	7.2	6.4
Days 0-91	77.3	71.6 (93)	64.3 (83)	72.1 (93)

Data were extracted from Table 2, pp. 34-35, MRID 46246416.
Values represent mean ± s.d.
Numbers in parenthesis represent percent of control value, calculated by Reviewer.
n= 12 except for female deaths on days 55 and 85 (mid-dose) and 51 (high-dose).

C. **FOOD CONSUMPTION:** Food consumption was unaffected by treatment.

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

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings:** Urine stain was the only FOB parameter associated with treatment (Table 4). Urine stains were graded as (1) slight or (2) moderate to severe. Urine stain was observed on 4/12 males in the 1000 mg/kg/day group during week 4 (all slight) and on 3/12 males in the 1000 mg/kg group during week 8 (2 slight and 1 moderate to severe) and on 4/12 males of both the 500 and 1000 mg/kg groups during week 13 (all slight). During week 8, urine stain was observed on 7/12 and 8/11 females in the 500 and 10000 mg/kg dose groups. Respective incidences were 4/10 and 9/11 during week 13. All stains were graded as slight with the exception of one female in each dose group during each week (graded moderate to severe). None of the incidences, reported separately as slight and moderate to severe by the study author, attained statistical significance.

TABLE 4. Functional observational battery results

Observation	Dose level (mg/kg bw/day)			
	Control (0)	Low dose (100)	Mid dose (500)	High dose (1000)
Males				
Urine stain				
Pretest	0/12	0/12	0/12	0/12
Week 4	0/12	0/12	0/12	4/12
Week 8	0/12	0/12	0/12	3/12
Week 13	0/12	0/12	4/12	4/12
Females				
Urine stain				
Pretest	0/12	0/12	0/12	0/12
Week 4	0/12	0/12	0/12	1/12
Week 8	0/12	0/12	7/12	8/11
Week 13	0/12	0/12	4/10	9/11

Data were extracted from Table 4, pp. 39-111, MRID 46246416.
Values represent number of animals affected/total number of animals.

2. **Motor activity:** Total motor activity is summarized in Table 5. Total motor activity was unaffected by treatment with JAU 6476. With the exception of males in the high-dose group during week 4 (a decrease of 22%), all values were within 20% of the respective control values (the normal range of variation among groups). Subsession data for both sexes in the high-dose group followed the same pattern: occasional, slight, non-statistically significant reductions in motor activity during the first 5-6 subsessions of week 4. Total and subsession locomotor activity followed the same above pattern for both sexes. The largest decreases in locomotor activity occurred for males in the high-dose group during week 4 (26%) and for females in the high-dose group during week 13 (24%) (data not shown). Habituation was attained for controls and all treated groups. It should be noted that pre-test motor activity data were lower for both high-dose groups compared with the respective controls values (males, 87%; females 86%).

TABLE 5. Motor activity (total activity counts for session)				
Test day	Dose level (mg/kg bw/day)			
	Control (0)	Low dose (100)	Mid dose (500)	High dose (1000)
Males				
Pre-test	749±328	646±240	660±359	649±393 (87%)
Week 4	537±167	473±182 (88)	557±205 (104)	420±219 (78)
Week 8	579±208	588±209 (102)	559±241 (97)	476±243 (82)
Week 13	420±274	397±226 (95)	402±258 (96)	354±209 (84)
Females				
Pre-test	981±351	837±360	1009±396	841±279 (86%)
Week 4	797±229	730±288 (92)	854±305 (107)	701±255 (88)
Week 8	745±217	651±218 (87)	755±339 (101)	682±192 (92)
Week 13	630±180	571±146 (91)	734±286 (117)	538±115 (85)

Data were extracted from Table 6, pp. 121-123, MRID 46246416.
Values represent mean ± s.d.
n=10-12.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** Other than wet/stained ventrum areas, discussed in clinical signs above, there were no grossly observed effects related to treatment. Red discoloration of the lungs of two females that died early, one each in the 500 and 1000 mg/kg groups, confirmed the diagnosis of gavage error.

2. **Brain weight:** Absolute brain weight and brain weight relative to body weight data are summarized in Table 6. There were no statistically or biologically significant effects of treatment on absolute or relative brain weight. The slightly increased relative brain weight of high-dose males reflects the decreased body weight with no effect on absolute brain weight.

TABLE 6. Absolute and relative brain weight (n=6/sex)				
Weight	Dose level (mg/kg bw/day)			
	Control (0)	Low dose (100)	Mid dose (500)	High dose (1000)
Males				
Body wt (g)	407.6±60.5	396.1±39.5	365.9±38.8	353.4±31.4
Brain wt (g)	1.979±0.079	1.981±0.088	2.008±0.049	1.936±0.099
Brain/body wt (%)	0.493±0.059	0.503±0.033	0.554±0.062	0.550±0.033
Female				
Body wt (g)	234.5±13.1	230.9±8.4	222.7±12.2	241.2±25.1
Brain wt (g)	1.871±0.118	1.886±0.041	1.829±0.090	1.845±0.085
Brain/body wt (%)	0.800±0.066	0.817±0.029	0.823±0.053	0.771±0.070

Data were extracted from Table OW1K-SUM, pp. 420-422, MRID 46246416.

3. **Neuropathology:** The microscopic lesions of the nervous system (occasional incidences of inflammation of the spinal cord and axonal degeneration), were not dose-related. All lesions were graded (1) minimal or (2) mild or slight on a scale of 1 to 5. Only control and high-dose animals were examined.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: There were no compound-related deaths. Compound-related clinical signs included urine stain in both sexes treated with 500 and 1000 mg/kg/day and oral stain in the high-dose groups. Body weight was non-statistically reduced in males treated with 500 or 1000 mg/kg/day; body weight of females was unaffected by treatment. Food consumption was unaffected in both sexes. Urine stain and reduced body weight (males) were the only compound-related effects observed during the FOB. The non-statistically reduced motor and locomotor activity in both sexes in the high-dose group was considered treatment-related. These reductions occurred in males and females during week 4 and in females during week 13. There were no compound-related ophthalmic findings or microscopic lesions of the central or peripheral nervous system. The NOEL was 100 mg/kg/day based on reduced body weight (males only) and urine stain (both sexes). The NOEL for microscopic lesions was 1000 mg/kg/day.

B. REVIEWER COMMENTS: The Review agrees with the observations of the study authors, but the conclusions are too conservative. The Reviewer would change the NOAELs and LOAELs based on the following reasons. Final body weight of males and females in the high-dose group were reduced by <10% of the respective control values. Lower final body weight is an effect of treatment, but, at <10%, is not considered an adverse effect. Males in the mid- and high-dose groups lost weight during the first week of the study (3-4%). This weight loss was not accompanied by a decrease is food consumption during the first week. This apparent paradox may be explained if the males consumed little food during the first few days of treatment (in reaction to gavage with a noxious substance) and then consumed more

food during the latter part of the week. The observed effect in males is mild (weight loss during the first week of treatment as a possible reaction to administration of a noxious substance) and is a possible indication of gastrointestinal tract disturbance. As noted, final body weight was not significantly affected in either sex. Urine stains are also an effect of treatment, but, in the absence of other signs or histological correlates, are not considered adverse. Motor and locomotor activity in the high-dose group of both sexes was reduced up to 26% compared with respective control values (males during week 4 and females during weeks 4 and 13). However, the values for females were not dose-related, and differences in values between the control and high-dose females for all weeks (8-15%) were less than or similar to the difference between the pretest control and high-dose group value (14%). Therefore, the effect on motor activity for females cannot be considered a clear effect of treatment. The effect on motor activity of males is questionable for the same reason. 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.

Treatment-related effects seen in this study were mild (<10% lower final body weight, 3-4% weight loss in males during the first week, and urine stains) and not toxicologically significant. The Reviewer considers the NOAEL for both sexes to be ≥ 1000 mg/kg/day, the limit dose. A LOAEL was not achieved.

Based on the non-toxicologically significant effects seen in this study, the LOAEL for JAU 6476 in male and female rats was not attained; the NOAEL for male and female rats was ≥ 1000 mg/kg/day.

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

Subchronic Neurotoxicity Study - rats (870.6200b)

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
113691	46246416	subchronic neurotoxicity	rats	90 days	oral	gavage	100-1000	0, 100, 500, 1000	≥1000 (males and females)	not attained (males and females)	none	

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R154820

Chemical:

PC Code:

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