6/25/1993

Reviewed by: William Dykstra Ph.D., Toxicologist William Dykstra 6/25/93
Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head, Toxicologist
Section I, Tox. Branch I Roger Gardner 6-25-93

DATA EVALUATION REPORT

STUDY TYPE: 82-1; 90 Day Rat Feeding Study with Imazapyr Technical TOX. CHEM NO: 221G

ACCESSION NUMBER: N/A MRID NO.: 42774401

TEST MATERIAL: AC 243,997 technical, 99.3% purity

SYNONYMS: Imazapyr, Arsenal

STUDY NUMBER: T-0486, Toxicology Report No. AX92-3

SPONSOR: American Cyanamid Company

TESTING FACILITY: Toxicology Department, American Cyanamid Company

TITLE OF REPORT: AC 243,997: A 13-Week Dietary Toxicity Study in the Albino Rat

AUTHOR(S): Joel E. Fischer

REPORT ISSUED: September 9, 1992

CONCLUSION: Randomized groups of 10/sex/dose of young adult Sprague-Dawley rats were fed dietary doses of 0, 15,000, and 20,000 ppm of imazapyr technical for 13-weeks. Parameters measured were food consumption, body weight, ophthalmological evaluations, hematology, clinical chemistries, urinalysis, gross necropsy, organ weights and complete histopathology.

The NOEL is 20,000 ppm (HDT). All animals survived the study and there were no overt signs of toxicity in any animals during the study period which could be attributed to the test material. Treated rats gained more weight than controls during the study period although the differences for the 13-week study period were not statistically significant. Increased body weight gains were 6.1% and 4.7% for males in the 15,000 and 20,000 ppm groups, respectively, and were 2.5% and 5.3% for females in those same groups, respectively. Food consumption was increased or equal to controls in both sexes at both dose levels during the 13-week study period. Compound-intake averaged 1248 and 1695 mg/kg/day

1

for males in the 15,000 and 20,000 ppm dose groups, respectively. For females, compound-intake averaged 1423 and 1784 for the 15,000 and 20,000 ppm groups,respectively. There were no compound-related ophthalmological findings at pre-test or at termination of the study. Results showed at termination, a diagnosis of unilateral focal retinopathy in 1 control female, 1 male and 1 female at 15,000 ppm, and 1 male at 20,000 ppm.

There were no statistically significant differences between means of hematological parameters of treated rats in comparison to controls for both sexes. Additionally, the mean values for hematological parameters for rats at both treated dose levels for both sexes were within the laboratory reference range of historical control values for those same parameters. Mean values for serum albumin were statistically significantly decreased in males at 20,000 ppm in comparison to controls. The mean albumin values were 4.9, 4.7, and 4.6 g/dL for the control, low-, and high-dose levels, respectively. The laboratory reference range for serum albumin is 2.9-6.0 g/dL. Since there was no increase in urinary protein at any level, no differences in serum albumin values in females, and the mean value of 4.6 g/dL in males was within the reference range of the laboratory, the statistically significant finding in males at 20,000 ppm was not considered compound-related. There were no other statistically significant differences between control and treated rats in any other clinical chemistry parameters. There were no statistically significant differences between control and treated urinalysis values for both sexes of rats. All findings were comparable between control and treated rats for all measured parameters.

Mean absolute and relative kidney weights were statistically significantly increased in female rats at 20,000 ppm ($p < 0.05$). The absolute kidney weights were increased by 14.5% and the relative kidney weights were increased by 12.3% in high-dose females in comparison to controls. There were no urinalysis changes, clinical chemistry findings, gross pathology results or histopathological effects to explain these small increases in kidney weights and, for these reasons, the increased absolute and relative kidney weights in 20,000 ppm females were not considered toxicologically significant. There were no other statistically

2

significant differences in absolute or relative organ weights between the controls and treated rats of both sexes. There were no compound-related gross necropsy findings observered in treated rats in comparison to controls. There were no compound-related microscopic findings in any of the examined tissues in treated rats of both sexes in comparison to controls.

Classification: **core-minimum**

Special Review Criteria (40 CFR 154.7) N/A

A. MATERIALS:

1. Test compound: AC 243,997 technical; Description - white powder, Batch # - AC 4866-62, Purity - 99.3%.

2. Test animals: Species: Albino Rat, Strain: Sprague-Dawley, Age: 4.5 Weeks Old, Weight: Males: 100-130 grams; Females: 102-120 grams, Source: Charles River Breeding Laboratories, Wilmington, MA.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 3 months		Interim Sac. None	
		male	female	male	female
1 Cont	0	10	10		
2 Low (LDT)	15,000	10	10		
3 Mid (MDT)	-	-	-		
4 High (HDT)	20,000	10	10		

2. Diet preparation

Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for homogeneity, stability and concentration at weekly intervals.

Results - With respect to homogeneity, duplicate 20 gram subsamples from three different site-specific feed samples were assayed at 15,000 and 20,000 ppm levels and averaged 99.7% and 103.2%, respectively. Stability results indicate that test material was stable in rodent diet at room temperature for 21 days. Overall averages for the 15,000 ppm diet stored in the animal room and in bulk storage were 99.0% and 98.6% of nominal concentrations, respectively. For the 20,000 ppm diets, corresponding results were 102.2% and 103.3% of nominal levels. Dietary analyses for concentration of prepared samples from weeks 0-13 at 15,000 ppm averaged 101.5% of nominal levels with a coefficient of

variation (CV) of 2.0%. At 20,000 ppm, prepared diets averaged 101.1% of nominal levels with a CV of 3.0%.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Standard one-way analysis of variance (ANOVA) was used to analyze the following data for each sex: body weight, body weight gain, food consumption, hematology, clinical chemistries, urinalysis, organ weights, and organ-body weight percentages. If ANOVA was significant, then Dunnett's t-test was used for pairwise comparisons between treated groups and control. All comparisons found to be statistically significant at the 5% level were indicated with an asterisk (*).

5. A quality assurance statement was signed by Kenneth A. Sund, and dated September 1, 1992.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected daily for signs of toxicity and mortality. Clinical evaluations were conducted weekly on each animal.

All animals survived the study and there were no overt signs of toxicity in any animals during the study period which could be attributed to the test material.

2. Body weight

Animals were weighed at study initiation (Day 0) and weekly for 13 weeks.

Body Weight (grams)

	Weeks			
	1	5	9	13
Males				
ppm				
0	173.9	372.5	481.6	534.9
15,000	175.7	377.5	499.1	558.7
20,000	175.8	382.6	499.2	553.4
Females				
ppm				
0	156.2	257.7	308.2	328.8
15,000	158.9	260.0	314.5	335.9
20,000	156.5	253.2	313.1	338.4

Treated rats gained more weight than controls during the study period although the differences for the 13-week study period were not statistically significant. Increased body weight gains were 6.1% and 4.7% for males in the 15,000 and 20,000 ppm groups, respectively, and were 2.5% and 5.3% for females in those same groups, respectively.

3. Food consumption and compound intake

Food consumption was determined weekly and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

Food consumption was increased or equal to controls in both sexes at both dose levels during the 13-week study period. Compound-intake averaged 1248 and 1695 mg/kg/day for males in the 15,000 and 20,000 ppm dose groups, respectively. For females, compound-intake averaged 1423 and 1784 for the 15,000 and 20,000 ppm groups,respectively.

4. Ophthalmological examination

Performed on all animals at pre-test and on all survivors at termination of the study.

There were no compound-related ophthalmological findings at pre-test or at termination of the study. Results showed at termination, a diagnosis of unilateral focal retinopathy in 1 control female, 1 male and 1 female at 15,000 ppm, and 1 male at 20,000 ppm.

5. Blood was collected at termination of the study for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Results - There were no statistically significant differences between means of hematological parameters of treated rats in comparison to controls for both sexes. Additionally, the mean values for hematological parameters for rats at both treated dose levels for both sexes were within the laboratory reference range of historical control values for those same parameters.

7

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
x	Chloride*		Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorous*	x	Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
x	Creatinine phosphokinase*^		Serum protein electrophores
	Lactic acid dehydrogenase (LAD)		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

Results - Mean values for serum albumin were statistically significantly decreased in males at 20,000 ppm in comparison to controls. The mean albumin values were 4.9, 4.7, and 4.6* g/dL for the control, low-, and high-dose levels, respectively. The laboratory reference range for serum albumin is 2.9-6.0 g/dL. Since there was no increase in urinary protein at any level, no differences in serum albumin values in females, and the mean value of 4.6 g/dL in males was within the reference range of the laboratory, the statistically significant finding in males at 20,000 ppm was not considered compound-related. There were no other statistically significant differences between control and treated rats in any other clinical chemistry parameters.

6. Urinalysis^

Urine was collected from fasted animals at termination of the study. The CHECKED (X) parameters were examined.

X		X	
x	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*		Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*		Urobilinogen

^Not required for subchronic studies
* Required for chronic studies

Results - There were no statistically significant differences between control and treated urinalysis values for both sexes of rats. All findings were comparable between control and treated rats for all measured parameters.

7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	x	Aorta*	xx	Brain*$_{+}$
x	Salivary glands*	xx	Heart*	x	Periph. nerve*#
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*#
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen	x	Eyes (optic n.)*#
x	Jejunum*	x	Thymus*	Glandular	
x	Ileum*	Urogenital		xx	Adrenal gland*
x	Cecum*	xx	Kidneys*+		Lacrimal gland#
x	Colon*	x	Urinary bladder*	x	Mammary gland*#
	Rectum*	xx	Testes*$^{+}$	xx	Parathyroids*$^{++}$
xx	Liver *$^{+}$	x	Epididymides	xx	Thyroids*$^{++}$
	Gall bladder*	x	Prostate	Other	
x	Pancreas*	x	Seminal vesicle	x	Bone*#
Respiratory		xx	Ovaries*$^{+}$	x	Skeletal muscle*#
x	Trachea*	xx	Uterus*	x	Skin*#
x	Lung*			x	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
$^{+}$ Organ weight required in subchronic and chronic studies.
$^{++}$ Organ weight required for non-rodent studies.

a. Organ weight - Mean absolute and relative kidney weights were statistically significantly increased in female rats at 20,000 ppm ($p < 0.05$). The absolute kidney weights were increased by 14.5% and the relative kidney weights were increased by 12.3% in high-dose females in comparison to controls. There were no urinalysis changes, clinical chemistry findings, gross pathology results or histopathological effects to explain these small increases in kidney weights and, for these reasons, the increased absolute and relative kidney weights in 20,000 ppm females were not considered toxicologically significant. There were no other statistically significant differences in absolute or relative organ

weights between the controls and treated rats of both sexes.

b. Gross pathology - There were no compound-related gross necropsy findings observered in treated rats in comparison to controls. The following gross findings are tabulated below.

11

MALES

Dose (ppm)	Organ	Finding
0	lung	mottled,pink/red,w.f.
0	lung	mottled,pink/dark red
0	adrenals	Rt. enlarged, lf.sm.
15,000	kidney	Rt. mod. hydronephro.
15,000	lungs	pale, small blk. foci
15,000	lungs	mottled,pink/red,r.f.
20,000	lungs	mottled,pink/red,r.f.
20,000	lungs	mottled,pink/red,r.f.

FEMALES

0	uterus	Distended w. clear fl.
0	lungs	mottled,pink/red,r.f.
15,000	lungs	mottled,pink/red,r.f.
15,000	lungs	mottled,pink/red,r.f.
15,000	lungs	mottled,pink/red,r.f.
15,000	lungs	small dark spots, min.
20,000	lungs	mottled,red/dark, r.f.
20,000	pituitary	enlarged
20,000	skin,fore-legs	alopecia
20,000	urinary bladder	discolored mucosa

c. Microscopic pathology - There were no compound-related microscopic findings in any of the examined tissues in treated rats of both sexes in comparison to controls.

1) Non-neoplastic - The incidence of non-neoplastic microscopic lesions in control and treated rats of both sexes are presented below.

12

MALES

Organ	0 ppm	15,000 ppm	20,000 ppm
Heart			
No. Examined	10	0	10
Vent. Acu.Inf.	1	0	0
Vent. Chron.Inf.	1	0	0
Vent. Mononuc.C.I.	1	0	3
Kidneys	10	10	10
(U) Med.Cyst	1	2	3
(U) Cort. Scar	0	1	1
(U) Hydronephrosis	0	1	0
Liver	10	10	10
H.C. Vacuolation	1	1	0
H.C. Mic. Necrosis	4	2	3
H.C. Necrosis	0	1	0
Lungs/Bronchi	10	10	10
Vascular Congestion	3	3	4
Neutro.Cell.Infilt.	1	2	3

FEMALES

Heart	10	0	10
Vent. Mononuc. C.I.	3	0	1
Atrial Endocard.Inf.	1	0	0
Vent. Epicard. A.I.	0	0	1
Vent. Epicard. C.I.	0	0	1
Kidneys	10	10	10
(U) Med. Cyst	0	1	0
(U) Cort. Scar	0	1	0
(U) Tub. Casts	2	2	2
(U) Mineral. Cort-M	1	0	0
(B) Mineral. Cort-M	4	4	4
(U) Hydropelvis	0	1	0
Liver	10	10	10
H.C. Mic. Necrosis	1	3	1
Lungs/Bronchi	10	10	10
Vas. Congestion	4	6	2
Lympho. C.I.	1	1	0
Neutro.Cell Infilt.	3	1	0

2) Neoplastic - There were no tumors detected in this 13-week feeding study.

D. DISCUSSION: This 13-week rat feeding study was conducted to determine if any significant toxicological results could be attributed to technical imazapyr at dietary levels up to 20,000 ppm. Since the NOEL for this 13-week study is 20,000 ppm, it is concluded that no significant toxic

effects were produced in this study. This study was requested by the Peer Review Committee (PRC) as a supplement to the 2-year rat study which was conducted at a HDT of 10,000 ppm without any significant toxicity. Therefore, the question arose as to whether or not an MTD or "life-threatening" toxic effects could be observed at the limit dose of 20,000 ppm in a 13-week study.

The NOEL in this 13-week study is 20,000 ppm (HDT) and no toxic effects were produced by technical imazapyr in either sex of young adult Sprague-Dawley rats. The study was well conducted and all significant clinical pathology parameters were evaluated, as well as a thorough gross necropsy and complete histopathology. Statistical analysis of the numerical data was conducted.

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 6/15/93
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I
Roger Gardner 6-25-93

DATA EVALUATION REPORT

STUDY TYPE: 83-5; Combined Chronic Toxicity/Carcinogenicity Study in Rats-Additional Histopathological Evaluation of Male Rat Brains as Requested by the PRC

TOX. CHEM NO: 221G

MRID NO.: 42774401

TEST MATERIAL: AC 243,997 Technical, imazapyr, Arsenal

SYNONYMS: Imazapyr, Arsenal

STUDY NUMBER: Bio-Research Project No. 99055; Bio/dynamics Project No. 84-2862; American Cyanamid Protocol No. 981-84-140

SPONSOR: American Cyanamid Company

TESTING FACILITY: Bio-Research Laboratories, Quebec, Canada

TITLE OF REPORT: Histopathology of Brain of Male Rats-" A Chronic dietary Toxicity and Oncogenicity Study with AC 243,997 in Rats"

AUTHOR(S): Brian Broxup, B.V.Sc., D.V.Sc., M.R.C.V.S.

REPORT ISSUED: June 4, 1992

CONCLUSION: The results of the new examination showed only two additional diagnoses of astrocytomas, one in the control group and one in the high-dose group. The new incidence of astrocyomas in high-dose males was 7.7% (5/65) and the new incidence in controls was 3.1% (2/65). According to the pathology report, there was no statistical significance ($p > 0.05$) in the Fisher's Exact Test between the control group and the high-dose group. Additionally, there was no decreased time in appearence of tumors in high-dose males in comparison to controls, no evidence of preneoplastic lesions, and all high-dose brain tumors appeared well-differentiated and non-expansive beyound the outer contours of the brain. Based on these considerations, the observed

increase in astrocytomas in the high-dose males **may** have occurred by chance rather than as a result of treatment with imazapyr. These data, together with the future statistical analysis by SAB, will be presented to the PRC for assessment and classification.

Core Classification: **Supplementary**

17

A **GLP Statement of Compliance** was present and signed by the Study Pathologist, Brian Broxup, the Sponsor's Representative, Frederick Hess, and the Submitter, Mark W. Galley and dated June 4, 1992 and a **Quality Assurance Statement** was present, signed by A. Gagne and P. Sidney and dated June 4, 1992.

METHODS

This new pathology report involved a re-examination of the original 3 brain sections per animal as well as a new examination of an additional 6 brain sections per animal per dose group from the original paraffin blocks and a new examination of 5 sections of forebrain per animal from new paraffin blocks from control and high-dose male animals. Each of the 5 sections represented two separate pieces of tissues from right and left forebrain. Therefore, for this new histopathological evaluation, approximately 14 brain sections per animal were examined microscopically in the high-dose and control groups and 9 sections per animal were examined in the low- and mid-dose groups.

RESULTS

DOSE ppm	0	1000	5000	10,000
No. Examined	65	65	65	65
BRAIN				
Primary Neoplasms				
Astrocytoma	2	0	1	5
Granular Cell Tumor	0	0	1	0
Malignant Reticulosis	0	1	0	0
Secondary Neoplasms				
Pituitary Adenoma	1	1	2	1
Granulocytic Leukemia	0	1	0	0
Nonneoplastic				
Vacuolation of neutropil	5	0	0	2
Dilated ventricles	5	10	10	10
Depression in ventral Diencephalon	5	6	5	12
Hemorrhage	2	2	2	4
Congestion	0	0	1	0
Cyst	0	0	0	1
Mineralization	10	0	1	12
Gliosis	2	1	0	0
Neutrophil infiltation	1	0	0	0
Mononuclear cell infiltation	0	0	0	1

BRAIN TUMOR-BEARING RATS

DOSE (ppm)	Animal No.	Tumor Type	Death
0	1042	Pituitary Adenoma	UD
0	1051	Astrocytoma	TS
0	1057	Astrocytoma	UD
1000	2010	Malignant Reticulosis	UD
1000	2021	Granulocytic Leukemia	UD
1000	2030	Pituitary Adenoma	UD
5000	3032	Pituitary Adenoma	UD
5000	3040	Pituitary Adenoma	UD
5000	3046	Astrocytoma	UD
5000	3059	Granular Cell Tumor	TS
10,000	4004	Astrocytoma	UD
10,000	4005	Astrocytoma	TS
10,000	4028	Astrocytoma	TS
10,000	4037	Astrocytoma	TS
10,000	4039	Pituitary Adenoma	UD
10,000	4051	Astrocytoma	TS

UD = Unscheduled Death

TS = Terminal Sacrifice

CONCLUSION

The results of the new examination showed only two additional diagnoses of astrocytomas, one in the control group and one in the high-dose group. The new incidence of astrocyomas in high-dose males was 7.7% (5/65) and the new incidence in controls was 3.1% (2/65). According to the pathology report, there was no statistical significance ($p > 0.05$) in the Fisher's Exact Test between the control group and the high-dose group. Additionally, there was no decreased time in appearence of tumors in high-dose males in comparison to controls, no evidence of preneoplastic lesions, and all high-dose brain tumors appeared well-differentiated and non-expansive beyound the outer contours of the brain. Based on these considerations, the observed increase in astrocytomas in the high-dose males **may** have occurred by chance rather than as a result of treatment with imazapyr. These data, together with the future statistical analysis by SAB, will be presented to the PRC for assessment and classification.

20