OCT 2 1991

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OCT 2 - 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **IMAZAPYR**

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (H7509c)

TO: Addressees

Attached for your review is a package on **IMAZAPYR** prepared by **Dr. William Dykstra.**

A meeting to consider the carcinogenicity classification of **IMAZAPYR** is scheduled for **Wednesday Oct. 23, 1991,** at **10:00 am** in **Room 821, CM2.**

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
R. Beliles
K. Baetcke
L. Brennecke
M. Van Gemert
M. Copley
K. Dearfield
J. Parker
H. Pettigrew
W. Sette
G. Ghali
B. Fisher
J. Du
Y. Woo
G. Burin
J. Quest
E. Saito (for microfiche-with one-liner)
W. Dykstra
J. Parker

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Imazapyr

Tox. Chem. No.: 221G

FROM: William Dykstra, Ph.D.
Review Section I
Toxicology Branch
Health Effects Division (H7509C)

TO: Esther Rinde, Ph.D.
Manager, Peer Review for Oncogenicity
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)

C. Background Information

Imazapyr is 2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-pyridine carboxylic acid with 2-propanamine (1:1). It is sold under the trade names of Arsenal and Event. It is used for control of most annual and perennial grasses and broadleaf weeds in noncropland areas. There are no published tolerances.

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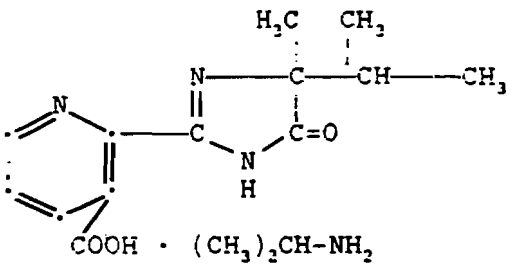
The structure of Imazapyr is shown below:



D. Evaluation of Carcinogenicity Data

1. Reference: Daly, Ira; April 6, 1988. A Chronic Dietary Toxicity and Oncogenicity Study with AC 243,977 in Rats. Unpublished Report No. 84-2862 prepared by BioDynamics, Inc. MRID No. 410395-03 (vol. 1-9).

a. Experimental Design

Randomized groups of 65 male and 65 female Sprague-Dawley rats were fed diets containing 0, 1000, 5000, and 10,000 ppm of technical imazapyr for 2 years. Criteria evaluated included toxic signs, mortality, body weight, food consumption, hematology, clinical chemistries, ophthalmological examinations, organ weights, and histopathology.

An interim sacrifice of 10/sex/dose was performed at 12 months.

In male rats, there was a slight dose-response in the time to death. Based on Kaplan-Meier estimated median survival times, the days on study for the control-, low-, mid-, and high-dose groups were 667, 689, 660, and 655 for males and 726, 734+, 708, and 740 for females. At 2 years, percent survival was 36, 40, 36, and 25 for males and 45, 37, 38, and 39 for females for the control-, low-, mid-, and high-dose groups, respectively.

There were no compound-related toxic effects in food consumption, body weight, clinical pathology, organ weights, and nonneoplastic lesions.

In female rats, at 10,000 ppm, there was an increased incidence of extramedullary hematopoiesis in the spleen and B-squamous cysts in the thyroid.

There were no compound-related effects in food consumption, body weight, clinical pathology, and organ weights.

b. Discussion of Tumor Data

Age-adjusted statistical analyses of the tumor data performed by SACB statisticians will be presented at the meeting.

1. Brain Tumors

There was an increased incidence of astrocytomas (a brain tumor) in high-dose male rats in comparison to controls.

Males Rats

Dose (ppm)	Animal Number	Week Death	Tumor
0	1051	106	B-astrocytoma
1000	2010	53	B-astrocytoma
5000	3059	106	B-granular cell tumor
5000	3046	55	B-oligodendroglioma
10,000	4005	106	M-astrocytoma
10,000	4028	106	B-astrocytoma
10,000	4037	106	B-astrocytoma
10,000	4051	106	B-astrocytoma

With respect to the incidence for the number examined by effective proportion, without survival disparity analyses, the summary of brain tumors for males is presented below:

	Male			
Groups:	1	2	3	4
No. Examined:	51	52	51	51
Brain:				
M-astrocytoma	0	0	0	1
B-astrocytoma	1	1	0	3
B-oligodendroglioma	0	0	1	0
B-granular cell tumor	0	0	1	0
Percentages	2.0	1.9	3.9	7.8

Since there was a survival disparity among the various groups of male rats, a complete statistical analysis of the data is necessary and is being performed by HED statisticians. Historical controls for brain tumors (all types) was provided by Ira Daly of Bio/dynamics.

In 14 studies submitted, the range of astrocytomas was 0 to 3.3 percent. The individual studies provided percentages of 1.7, 0.08, 0.86, 3.3, 0.08, 0, 0, 0, 0, 0.8, 0, 1.8, 0, and 1.7.

Other gliomas besides astrocytomas were recorded in rat brains in these 14 studies.

Additionally, it should be noted that the duration of the historical control studies generally exceeded 24 months (24-30 months, attached).

There was no compound-related effect in female rat brain tumors.

The Bio/dynamics historical control data are appended to the study report (attached).

2. Thyroid C-cell Tumors

The incidence of C-cell thyroid neoplasms showed an increase at the mid- and high-dose. More specifically, however, the C-cell carcinoma incidence was increased at the high-dose only.

The following data, without effective proportions, summarizes the findings.

	Male Rats			
Group	1	2	3	4
No. examined	65	65	63	65
C-cell carcinoma	1	1	1	5
C-cell adenoma	2	3	9	4

The high-dose male rats showed a higher incidence of C-cell carcinoma (5/65, 7.69%) when compared to the control (1/65, 1.53%), low-dose (1/65, 1.53%, and mid-dose (1/63, 1.58%) male rats. Additionally, the high-dose incidence (7.69%) was within the range of 0 to 13.7 percent from historical control data collected at Bio/dynamics. Also, the

high-dose was reported to be without statistical significance.

The fate of the individual male rats with C-cell carcinoma is presented below as tabulated in the report.

Male Rats with C-cell Carcinoma

Group	Sex	Animal No.	Death Code	Day of Study of Death	Week of Study of Death
I	M	1024	D	614	88
II	M	2027	D	691	99
III	M	3059	T	739	106
IV	M	4017	D	654	99
IV	M	4023	S	639	92
IV	M	4040	S	669	96
IV	M	4055	T	739	106
IV	M	4064	S	665	95

Key: T = Terminal Sacrifice; D = Spontaneous Death;
S = Sacrificed Moribund.

It can be seen from this table there is no apparent decrease in latency of the C-cell tumor.

Proliferative lesions of the male thyroid in this study are summarized below as presented in the report.

Table 1

Thyroid Gland

Summary - Incidence of Proliferative Lesions[a]

Sex	Males			
Group	I	II	III	IV
Thyroid gland No. Examined	65	65	63	65
C-cell hyperplasia	15	8	13	6
%	23.10	12.31	20.63	9.23
C-cell adenoma	2	3	9	4
%	3.10	4.62	14.29	6.15
C-cell carcinoma	1	1	1	5
%	1.54	1.54	1.59	7.69
C-cell adenoma and carcinoma	3	4	10	9
%	4.62	6.15	15.87	13.85
C-cell hyperplasia, adenoma, and carcinoma combined	17	12	21	15
%	26.15	18.46	33.33	23.08

[a]The totals for control and mid-dose do not add up to individual values since there was no double-counting.

The differences between the incidences of all groups are not statistically significant. The following tables, taken from the report, are of historical control data from Bio/dynamics. It should be noted that the duration of the historical control studies is generally longer than 24 months (24-30 months).

Table 2

Thyroid Gland - Selected Findings
Historical Control Data - Male Charles River Albino CD Rats
(Compiled from 14 Studies Conducted at Bio/dynamics, Inc.)

	Range of Incidence of Historical Data		Mean Incidence of Historical Data
	Low	High	
No. Examined	73	69	1413
C-cell hyperplasia	0	10	60
Percentage	0	14.59	4.25
No. Examined	73	69	1413
C-cell hyperplasia	0	10	60
Percentage	0	14.59	4.25
No. Examined	131	70	1413
C-cell adenoma	0	8	72
Percentage	0	11.43	5.10
No. Examined	129	131	1413
C-cell carcinoma	0	18	58
Percentage	0	13.74	4.10
No. Examined	54	70	1413
C-cell adenoma and carcinoma combined	0	12	129
Percentage	0	17.14	9.13
No. Examined	54	70	1413
C-cell hyperplasia, adenoma, and carcinoma combined	0	18	183
Percentage	0	25.71	12.95

For the purposes of a combined finding, those animals having more than one finding were counted only once.

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Table 3 shows the individual studies for the data from the Bio/dynamic files.

Table 3

Thyroid Gland - Selected Findings
Historical Control Data - Male Charles River Albino CD Rats

Study	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Date Initiated	1978	1977	1977	1978	1978	1977	1977	1978	1977	1977	1978	1978	1977	1979
Date Terminated	1980	1980	1980	1981	1980	1980	1980	1980	1980	1979	1981	1980	1980	1981
Duration (months)	25	27	29	30	26	30	29	24	27	24	29	28	29	26
# Examined	68	139	139	70	69	131	123	54	139	139	70	73	129	70
C-cell adenoma	0	14	6	8	4	0	0	0	5	8	8	2	13	4
%	0	10.1	4.3	11.4	5.8	0	0	0	3.6	5.8	11.4	2.7	10.1	5.7
C-cell carcinoma	2	4	4	4	2	18	12	0	3	1	0	0	0	8
%	2.9	2.9	2.9	5.7	2.9	13.7	9.8	0	2.2	0.7	0	0	0	11.4
C-cell adenoma and carcinoma	2	18	10	12	6	18	12	0	8	9	8	2	13	11
%	2.9	12.9	7.2	17.1	8.7	13.7	9.8	0	5.8	6.5	11.4	2.7	10.1	15.7
C-cell hyperplasia	4	3	9	6	10	9	2	0	11	2	2	0	1	1
%	5.9	2.2	6.6	8.6	14.5	6.9	1.6	0	7.9	1.4	2.9	0	0.8	1.4
C-cell adenoma, carcinoma, and hyperplasia	6	20	19	18	16	23	14	0	19	11	9	2	14	12
%	8.8	14.4	13.7	25.7	23.2	17.6	11.4	0	13.7	7.9	12.9	2.7	10.9	17.1

Evaluation of Tables 1, 2, and 3 shows that the incidences of C-cell proliferative lesions in the AC 243,997 study (C-cell hyperplasia, C-cell adenoma, C-cell carcinoma), individually or in combination, were within the range of Bio/dynamic historical control data.

As can be seen by comparing the incidences of proliferative lesions from Table 1 (the data from the current study) to the Bio/dynamic historical control data in Table 2, the incidences of the proliferative lesions at the high-dose are all within the range of the historical control data as shown below.

Table 4

Thyroid Findings

C-cell	High-Dose Incidence of Current Arsenal Study	Range of Hist. Controls from Bio/dynamics
Hyperplasia	9.23%	0 - 14.59%
Adenoma	6.15%	0 - 11.43%
Carcinoma	7.69%	0 - 13.74%
Adenoma and carcinoma	13.85%	0 - 17.14%
Hyperplasia, adenoma, and carcinoma	23.08%	0 - 25.71%

The report states that Suzuki et al. (1979) reported the incidence of medullary carcinoma in the thyroid gland of Sprague-Dawley rats to be 79 percent (33/42) in males and 49 percent (19/39) in females.

The registrant employed an outside consultant W. Roy Brown, D.V.M., Ph.D., to examine the thyroid gland of male rats and render an opinion.

Dr. Brown's analysis is presented below in Table 5.

Table 5

(Dr. Brown's Analysis)

Summary of Incidence of Proliferative Lesions of C-Cell Origin in the Thyroid Gland of Male Rats

Dose Group	I	II	III	IV
Number of Thyroid Glands Examined	65	65	65	65
C-cell hyperplasia (all degrees)				
Incidence	8	13	14	7
Percent	12.3	20.0	21.5	10.8
C-cell adenoma				
Incidence	2	1	8	6
Percent	3.1	1.5	10.8	3.1
C-cell carcinoma				
Incidence	1	1	1	4
Percent	1.5	1.5	1.5	6.2
C-cell adenoma and carcinoma				
Combined incidence	3	2	8	6
Percent	4.6	3.1	12.3	9.2
C-cell hyperplasia, adenoma, and carcinoma				
Combined incidence	11	15	22	13
Percent	16.9	23.1	33.8	20.0

Dr. Brown states:

> It is my opinion that the difference between the control and high dosage group male rats with respect to the C-cell carcinomas is of no biological significance. The incidence in the high-dose rats is consistent with that which can occur spontaneously and those that have been reported in control rats in studies of similar type at Bio/dynamics, the site of the study. An incidence of as high as 79% (33/42) of C-cell carcinoma have been reported in male Sprague-Dawley rats (Suzuki, et al.). Other studies indicate

an increase of 16-40% of C-cell carcinomas in other strains of rats, including Long-Evans, Sprague-Dawley, Wistar and wild rats (Rattus norvegicus). The highest group incidence of C-cell carcinomas in this study was 6.2%.

3. Adrenal Medullary Tumors

An additional tumor type of possible concern occurred in the adrenal gland.

In female rats, there was an increased incidence of adrenal medullary tumors at the high-dose. The incidence was as follows for the number of female rats examined by effective proportions:

Female Adrenal Medulla

Group	1	2	3	4
No. examined	27	34	22	31
Carcinoma	0	0	2	1
Adenoma	1	2	3	6
Carcinoma and adenoma (combined)	1	2	5	7
Percentages	4.0	5.8	23	22.5%

Animal Number	Dose	Week Death	Tumor
1540	0	106	Adenoma
2503	1000	104	Adenoma
2561	1000	106	Adenoma
4507	10,000	106 (unscheduled)	Adenoma
4521	10,000	106	Carcinoma
4524	10,000	106	Adenoma
4528	10,000	104	Adenoma
4534	10,000	107	Carcinoma
4537	10,000	106	Adenoma
4551	10,000	107	Adenoma

It can be seen from the week of death for the Arsenal female tumor-bearing animals that the earliest pheochromocytoma occurred at week 104. Therefore, when the number of animals examined is adjusted for effective proportions, the high-dose percentage is 22.5 percent.

The historical control data from Bio/dynamics for 14 studies showed pheochromocytomas ranging from 0 to 15.5 percent. The individual percentages were 6.7, 4.2, 0, 6.7, 1.9, 8.5, 11.0, 0, 4.5, 2.6, 8.7, 3.5, 5.3, and 15.5 percent.

Additionally, it should be noted that the historical control data is unculled for mortality (although no pheochromocytomas were found prior to 12 months) and the duration of the studies was from 24 to 30 months.

The historical control data is appended to the report.

For males in the Arsenal study, the weeks at which pheochromocytomas were found were as follows:

Control		Low		Mid		High	
A.N.	Week	A.N.	Week	A.N.	Week	A.N.	Week
1001	106	2005	87	3003	91	4002	81
1008	106	2007	106	3012	106	4013	106
1020	93	2012	106	3018	105	4020	102
1023	92	2018	106	3039	98	4027	106
1031	106	2034	100	3040	96	4044	106
1043	106	2039	106	3059	106	4048	105
1052	97	2045	106			4051	106
1055	106	2052	106			4054	103
		2054	106			4059	104
		2055	88			4062	89
		2064	106				

The incidences, distributions, and time-to-tumor for the other benign and malignant neoplasms were considered unrelated to treatment.

c. Nonneoplastic

There were increased incidences of three nonneoplastic lesions in female rats. One lesion

was extramedullary hematopoiesis of the spleen. The second lesion was peliosis hepatis of the liver. The third lesion was B-squamous cysts of the thyroid.

1) Spleen

With respect to the spleen, the overall incidence of lesions was as follows:

Spleen Females

Group	1	2	3	4
No. examined	65	65	65	65
Extramedullary hematopoiesis	12	17	17	20
Grades of the lesions	2,3,2,4, 4,3,2,4, 2,2,2,2	3,2,2,2, 4,3,3,4, 2,4,4,5, 2,5,2,2, 2	3,2,2,2, 4,4,5,4, 2,2,2,2, 5,4,4,2, 2	2,2,3,3, 4,4,2,3, 2,2,4,4, 2,3,5,5, 3,5,2,2,

As can be seen from the incidence of grades of the lesion, the high-dose group is the LEL and the mid-dose group is the NOEL. The grade of 5 is associated with 0, 2, 2, and 3 lesions in the control, low-, mid-, and high-dose groups, respectively. Additionally, the grade of 4 is associated with 3/12 (25%), 4/17 (23%), 5/17 (29%). and 4/20 (20%) of the lesions in the control, low-, mid-, and high-dose groups, respectively.

Additionally, there did not appear to be any association of the increased incidence of this splenic lesion with earlier deaths based on analysis of the data. Also, associative anemia was not observed in high-dose females.

Although the full toxicological significance of this lesion is uncertain, it does not appear to be compensatory. In any case, due to the incidences and grades of the lesion, it appears to be a compound-related effect at the high-dose.

The distribution of the lesion in the spleen of female rats is shown below:

Interim Kill

Group	1	2	3	4
No. Examined	10	10	10	10
Lesion	0	0	0	0

Died on Study

Group	1	2	3	4
No. Examined	30	24	34	28
Lesion	8	7	10	11

Terminal Kill

Group	1	2	3	4
No. Examined	25	31	21	27
Lesion	4	10	5	9

2) Liver

In female rats, there was an increased incidence of peliosis hepatis of the liver in the mid- and high-dose groups. This lesion is the presence in hepatic lobules of multiple microscopic pools of blood which may become lined by endothelium. It is a rare condition that may result from the congestion of the liver with necrosis. However, there was no compound-related occurrence of hepatic necrosis (either by incidence or grade of lesion) in female rats in this study.

The incidence of the peliosis hepatis in the liver was as follows:

Female Liver

Group	1	2	3	4
No. Examined	65	65	65	65
Peliosis hepatis	4	3	6	8

The grades of the lesion were comparable between control and high-dose rats. Based

on these considerations, the slight increase in this lesion in the mid- and high-dose groups may not be compound-related.

3) Thyroid

The third lesion in female rats, which was ungraded (only P present in individual animal data) and which occurred at an increased incidence, was B-squamous cysts of the thyroid in female rats.

The occurrence was as follows:

	Female Thyroid			
Group	1	2	3	4
No. Examined	65	65	65	65
B-squamous cysts	5	7	5	12

The NOEL for this finding in the mid-dose of 5000 ppm and the LEL is the high-dose of 10,000 ppm.

Other nonneoplastic lesions occurred at similar frequency and grade between control and treated male and female rats.

4) Adequacy of Dosing for Assessment of Carcinogenic Potential

In males, there was a slight dose-response in the time to death. Based on Kaplan-Meier estimated median survival times, the days on study for the control-, low-, mid-, and high-dose groups were 667, 689, 660, and 655 for males and 726, 734+, 708, and 740 for females. At 2 years, percent survival was 36, 40, 36, and 25 for males and 45, 37, 38, and 39 for females for the control-, low-, mid-, and high-dose groups, respectively. These data indicate that an adequate dose was approached in males.

There were no life-threatening effects or body weight gain decreases to indicate that doses were adequate for females.

2. Reference: Auletta, Carol; dated November 3, 1988; unpublished report prepared by BioDynamics, Inc. A Chronic Dietary Toxicity and Oncogenicity Study with AC 243,997 in Mice.

Randomized groups of 65 male and 65 female CD-1 mice were fed dietary levels of 0, 1000, 5000, and 10,000 ppm of technical imazapyr for 18 months. An interim sacrifice of 10/sex/dose at 12 months was performed.

Criteria evaluated included toxic signs, mortality, body weight, food consumption, hematology, organ weights, and histopathology.

There were no compound-related effects in toxic signs, mortality, body weight, food consumption, hematology, organ weights, and tumors. Historical control data are required to establish the NOEL for pulmonary edema in female mice. Additionally, a more detailed description of subscapular adrenal gland cell reaction is required.

The carcinogenic potential was negative up to 10,000 ppm (HDT) which exceeds the limit dose of 7000 ppm for mice. Therefore, there were adequate doses for evaluating the carcinogenic potential.

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E. Additional Toxicology Data on Imazapyr

1. Metabolism of Imazapyr

One group of 15 male Sprague-Dawley rats was used in the study. Three rats were control animals and 12 rats were treated animals. Each treated rat received a single oral dose of C^{14}-label AC 243,997 equal to 1.1 mg (33 microcuries). Based on body weight of the rats (approximately 225 grams), this dose was 4.4 mg/kg.

Three treated rats were sacrificed at days 1, 2, 5, and 8. One control rat was sacrificed on day 5 and two were sacrificed on day 8.

Urine and feces were collected daily. At each sacrifice interval, blood was collected and liver, kidney, muscle, and fat were removed. All metabolism cages housing treated rats were rinsed with water and methanol and collected.

At day 1, 55.3 percent of the dose was excreted in the urine and 31.9 percent was excreted in the feces. Excretion was essentially complete by day 6 and was 95.1 percent of the total dose. Overall recovery of cage washes and excretion was 98.0 percent at day 8. Using TLC and mass spectrometry, the radiolabeled organic extracted material in feces and urine at day 1 was parent compound.

At day 1, kidney and liver contained 0.03 and 0.02 ppm, respectively, and less than 0.01 ppm on day 8.

Muscle, fat, and blood had less than 0.01 ppm at both days 1 and 8.

2. Mutagenicity

Imazapyr is negative in acceptable studies in the Ames assay, in vitro chromosomal aberration assay in Chinese hamster ovary cells up to a toxic dose (5000 mcg/mL), and the HGPRT mutation assay up to toxic doses (5000 mcg/mL).

Imazapyr was also tested in the dominant lethal assay in rats and UDS assay, but both studies were unacceptable, although reported as negative.

A data gap has been identified in the other genotoxic effects category.

3. Developmental Toxicity

In Sprague-Dawley rats, Imazapyr was negative for developmental toxicity at doses of 100, 300, and 1000 mg/kg/day. Maternal toxicity was observed at 1000 mg/kg/day as salivation.

On the basis of pilot study doses of 0, 250, 1000, and 2000 mg/kg/day in New Zealand white rabbits, imazapyr was negative for maternal and developmental toxicity in New Zealand white rabbits at doses up to 400 mg/kg/day (HDT). The doses were 0, 25, 100 and 400 mg/kg/day for the main study.

In a two-generation rat reproduction study, imazapyr had a NOEL of 10,000 ppm (HDT) in Sprague-Dawley rats.

4. Structure-Activity Relationships

There are no currently registered pesticides that are structurally related to imazapyr.

5. Acute, Subchronic, and Chronic

The acute oral LD_{50} is greater than 5000 mg/kg bw. The dermal LD_{50} in rabbits is greater than 2148 mg/kg bw. The inhalation LC_{50} is greater than 1.3 mg/L (gravimetric). Imazapyr is Toxicity Category III for eye and Category IV for skin irritation. Imazapyr is not a skin sensitizer.

The NOEL for chronic toxicity in a 1-year dog study was 10,000 ppm (HDT).

F. Weight-of-Evidence Considerations

The Committee should consider the following facts regarding the toxicology data on imazapyr in a weight-of-the-evidence determination on carcinogenic potential:

1. Imazapyr was associated with increased incidence of brain tumors in male rats, thyroid C-cell tumors in male rats, and pheochromocytomas in female rats.

2. Imazapyr was negative for carcinogenic potential in CD-1 mice up to 10,000 ppm (HDT)

3. Imazapyr was negative for mutagenic potential in the Ames assays, HGPRT assay, and in vitro chromosomal aberration assay in CHO cells.

4. Developmental toxicity potential was negative in rats and rabbits and the NOELs in the reproduction and chronic dog study were 10,000 ppm (HDT).

Attachments

PAGES 21 THROUGH 29 HAVE BEEN REMOVED FROM THIS DOCUMENT. THOSE PAGES CONSIST OF REGISTRANT-SUBMITTED DATA.

Reviewed By: William Dykstra 11/25/90
Section I, Toxicology Branch I IRS (H7509C)
Secondary Reviewer: Roger Gardner Acting Section Head
Section I, Toxicology Branch I IRS (H7509C)

(29)
06/14/
(for RG

DATA EVALUATION REPORT

Study Type: 83-5: Combined Chronic Toxicity/Oncogenicity Study
Study, Rats

TOX Chem. No. 2213
MRID No.: 410395-03
Vol. 1-9

Accession No.: N/A

Test Material: AC 243,997 Technical
99.5% a.i.

Synonyms: Arsenal (Imazapyr)

Study No.: 84-2862

Sponsor(s): American Cyanamid Company

Testing Facility: Bio/dynamics, Inc.

Title of Report: A Chronic Dietary Toxicity and Oncogenicity Study With AC 243,997 in Rats.

Author(s): Ira Daly

Report Issued: April 6, 1988

Conclusion:

Additional information is required. The registrant is required to submit complete statistical analyses for female adrenal medullary neoplasms and male brain neoplasms.

The issue of thyroid C-cell carcinoma is resolved.

There were no compound-related toxic effects in male food consumption, body weight, clinical pathology, organ weights, and non-neoplastic lesions. There was a slight dose-related decrease in survival of high-dose male rats, but not female rats.

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There were no compound-related toxic effects in female rats with respect to body weight and food consumption (although food efficiency in female rats showed a marginal toxic effect). Additionally there were no compound-related toxic effects in clinical pathology and organ weights. The NOEL for non-neoplastic lesions in female rats is the mid-dose of 5000 ppm. The LEL is the high-dose of 10,000 ppm and the effects are an increased incidence of extramedullary hematopoiesis in the spleen and B-squamous cysts in the thyroid. An MTD may not be established in the study.

Classification: Core-Supplementary

Special Review Criteria (40 CFR 154.7): N/A

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Review:

A. Materials:

1. Test Compound - AC 243,997; Description: Off-white chunky powder; Batch No.: AC 4866-062; Purity: 99.5%; Contaminants: List in CBI Appendix.

2. Test Animals - Species: Rat; Strain: Sprague-Dawley; Age: 29 days old; Weight (mean): Males 195 g, Females 148 g; Source: Charles River Breeding Labs, Kingston, NY.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study Total Nos. Male	Main Study Total Nos. Female	Interim Sac.[a/] 12 Months Male	Interim Sac.[a/] 12 Months Female	Months24[b/] Necropsy and Histopathology Male	Months24[b/] Necropsy and Histopathology Female
1 Control	0	65	65	13	14	52	51
2 Low (LDT)	1000	65	65	13	10	52	55
3 Mid (MDT)	5000	65	65	12	12	53	53
4 High (HDT)	10,000	65	65	13	10	52	55

a/Includes unscheduled deaths prior to month 12.
b/Includes unscheduled deaths between month 12 and study termination at month 24.

2. Diet Preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration.

Results - The dose levels of 1000 and 10,000 ppm were analyzed for homogeneity and found to have means of 100.9 and 106.2 percent, respectively, with coefficients of variation of 1.9 and 7.1 percent, respectively. The test material was stable in the diet for the 2-week period while it was exposed in the rat feeders. The low-dose batch lost an apparent 3.6 percent per week while the high-dose lost an apparent 4.0 percent per week. Additionally, diets prepared and dispensed to the rats weekly during the entire study were found to contain an average of 95.8 percent (1000 ppm), 96.0 percent (5000 ppm), and 96.8 percent (10,000 ppm) of nominal concentration. The coefficients of variation were 5.3 percent (low-dose), 4.5 percent (mid-dose), and 4.5 percent (high-dose).

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

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4. Statistics - The following procedures were utilized in analyzing the numerical data. parametric and nonparametric. The following is quoted from the study report:

"Body weight, food consumption, hematology and clinical chemistry parameters, organ weights, organ/body, organ/brain weight ratios and survivorship and tumor onset analyses were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables of appendices.

"Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

"A statistical test for trend in the dose levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used.

"The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level." [End of quotation.]

Life Table Analysis - The data on time to neoplastic lesion were analyzed for each sex separately by the series of programs included in the N.C.I. package for histopathologically proven tumors and time to tumor.

5. Quality assurance was performed and the report was signed.

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C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. Detailed physical examinations for signs of local or systemic toxicity, pharmacologic effects, and palpable masses were performed pretest and weekly thereafter.

Results:

a. Toxicity - There were no compound-related toxic signs. The most frequently observed in-life physical signs were chromodacryorrhea, opacity, lacrimation, teeth problems, and alopecia in males. In females excess lacrimation, chromodacryorrhea, ear problems, teeth problems, alopecia and stains were observed most frequently.

b. Survival - For males, there was a slight dose-response in the time to death. Based on Kaplan-Meier estimated median survival times, the days on study for the control, low-, mid-, and high-dose groups were 667, 689, 660, and 655 days, respectively. Using similar methods for the females, survival times were 726, 734+, 708, and 740 days for the control, low-, mid-, and high-dose groups, respectively.

As reported in the summary data, animal survival (%) at selected intervals during the study was as follows:

Group	An.	Dose Level (ppm)	% Survivorship*			
Months	6 M/F	12 M/F	18 M/F	Term M/F
I	65	0	98/100	95/94	85/84	36/45
II	65	1000	100/100	98/100	85/89	40/37
III	65	5000	100/100	97/97	76/78	36/38
IV	65	10,000	98/100	97/100	75/85	25/49

*Excludes a 12-month interim sacrifice of 10 animals/sex/group and one accidental death.

The slight effect in survivorship in high-dose males, which is also slightly dose-related, may support a position that an MTD was approached for males in the study. This position is supported by the fact that the major effect in survivorship occurred during the last year of the study when, in addition to aging, cumulative toxicity is more apparent in males.

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2. Body Weight - Animals were weighed weekly for 14 weeks, then biweekly through 40 weeks, monthly thereafter, and terminally (after fasting).

Results - There were no compound-related toxic effects in body weight gain in treated male and female rats in comparison to controls. The following data shows the mean body weights of both sexes during the study. Slight increases in body weight gain were apparent for mid- and high-dose male rats in comparison to controls.

Mean Body Weight (grams)

	Males				Females			
	Dose				Dose			
	0	1000	5000	10,000	0	1000	5000	10,000
Month								
0	195	195	194	194	149	147	148	146
1	375	377	384	385	238	237	236	234
4	545	542	549	558	306	302	306	299
6	602	602	615	625	339	338	341	335
8	641	639	650	664	363	359	370	361
12	702	700	710	722	422	419	437	427
18	738	736	708	750	489	483	486	474
24	674	637	614	659	501	486	492	501

3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results - Mean food consumption values (g/kg/day) were, on occasion, statistically significantly increased in treated male groups, although the differences were not usually dose-related. Additionally the differences seldom exceeded 5 percent at the high-dose where the differences were most pronounced. This slight increase in food consumption can be correlated with the slight increase in body weight in these high-dose males.

In females, increases in food consumption occurred in all treated groups at about a 5 to 7 percent increased rate above controls during about the first 60 weeks of the study. These increased levels of food consumption are considered compound-related, although they were not

always dose-related and, in contrast to the males, were not reflected in higher body weight gains. This observation suggests that food efficiency was reduced in treated females. This slight reduction in food efficiency represents a marginal toxic effect.

Mean test substance intake calculated over the 2-year study period, as calculated in the study report, was as follows:

	AC 243,997 (mg/kg/day)		
Group	Dose Level (ppm)	Male	Female
II	1000	49.9	64.2
III	5000	252.6	317.6
IV	10,000	503.0	638.6

4. Ophthalmalogical examinations were performed at 12 and 24 months on all animals.

Results - There were no compound-related ophthalmalogical abnormalities noted by Dr. L.F. Rubin, the examining veterinary ophthalomalogist (Appendix D).

5. Blood was collected at 3, 6, 12, 18, and 24 months for hematology and clinical analysis from 10/sex/dose animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)
		X	Erythrocyte morphology

Results - The hematocrit of high-dose males was slightly, though significantly, increased (43% at high-dose vs. 40% in controls), at 3 months but not at later times. Inspection of individual values showed a generally higher percent hematocrit for most high-dose animals rather than the increase being the results of one or a few highly aberrant values. These findings in hematocrit in high-dose males are of no toxicological significance, since individual values ranged between 32 to 52 percent (Note: hematocrit values between 32-52% are 2 ± SD0 of mean

of 42%) which is within the historical control range for hematocrit values for young rats (strain unspecified) at IRDC. Other statistically significant hematological values observed during the 2-year period (and there were only a couple) did not occur at the high-dose and, therefore, were not dose-related. These statistically significant hematological findings were an increased percent hematocrit at the low-dose (42% vs. 40% control) at 3 months in females and an increased number of WBC (8.1 thousand/mL at mid-dose vs. 6.3 thousand/mL in control) in females at 12 months.

Since these aberrant values in hematology were not dose- or time-related, they were not considered compound-related.

b. Clinical Chemistry

X	Electrolytes:
X	Calcium*
X	Chloride*
	Magnesium*
	Phosphorous*
X	Potassium*
X	Sodium*

X	Other:
X	Albumin*
	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total Bilirubin*
X	Total Protein*
	Triglycerides
X	Direct Bilirubin

X	Enzymes
X	Alkaline phosphatase
	Cholinesterase
	Creatinine phosphokinase*
X	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*
X	Gamma glutamyl transpeptidase

Results:

3 Months - Slight, though significant, increase in BUN (mg/dl) in high-dose males (15.8 mg/dl vs. 13.3 mg/dl in controls). Significant increase in potassium in low-dose males (5.4 mEq/L vs. 4.9 mEq/L in control). There were no statistically significant differences between control and treated groups for any other parameters in males or for any parameters at all in females.

6 Months - No statistically significant differences between control and treated groups in males and females for any clinical chemistry parameter at all at 6 months.

12 Months - No statistically significant differences between control and treated groups in males and females for any clinical chemistry parameter at all at 12 months.

18 Months - A slight, though significant, decrease in total protein in mid-dose males (but not high-dose males). No statistically significant differences between control and treated groups in other clinical parameters in males or in any clinical parameter at all in females.

24 Months - No statistically significant differences between control and treated groups in males and females for any clinical chemistry parameter at all at 24 months.

Due to the absence of relationships to dose and time in the few abnormal clinical chemistry values, there were no compound-related effects in clinical chemistry.

6. Urinalysis - Urine was collected from fasted animals at 3, 6, 12, 18, and 24 months. The CHECKED (X) parameters were examined.

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

Results - There were no compound-related effects in urinalysis at 3, 6, 12, 18, or 24 months.

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		Cardiovas./Hemat.		Neurologic
X	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*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	XX	Thymus* (interim only)		Glandular
X	Ileum*		Urogenital	XX	Adrenals*

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X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*	X	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*	X	Uterus*	X	Skin
XX	Lung*			X	All gross lesions and masses

Results:

A. Organ Weights

12 Months - No statistically significant differences in means in organ weight or organ/body weight ratios or organ/brain weight ratios between control and treated male and female rats at 12 months in any weighed organs.

Terminal Sacrifice - No statistically significant differences in means in organ weights or organ/body weight ratios or organ/brain weight ratios between control and treated male and female rats at terminal sacrifice in any weighed organs.

B. Gross Pathology - There were no compound-related necropsy findings in male or female rats that died on study, or were sacrificed at 12 or 24 months.

C. Microscopic

1. Neoplastic

a. Brain Tumors

There was an increased incidence of astrocytomas (a brain tumor) in high-dose male rats in comparison to controls.

Male Rats

Dose (ppm)	Animal Number	Week Death	Tumor
0	1051	106	B-astrocytoma
1000	2010	53	B-astrocytoma
5000	3059	106	B-granular cell tumor
5000	3046	55	B-oligodendro-glioma

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Male Rats (cont'd)

Dose (ppm)	Animal Number	Week Death	Tumor
10,000	4005	106	M-astrocytoma
10,000	4028	106	B-astrocytoma
10,000	4037	106	B-astrocytoma
10,000	4051	106	B-astrocytoma

With respect to the incidence for the number examined by effective proportion, without survival disparity analyses, the summary of brain tumors for males, is presented below:

		Male			
	Groups:	1	2	3	4
Brain:	No. Examined:	51	52	51	51
M-astrocytoma		0	0	0	1
B-astrocytoma		1	1	0	3
B-oligodendroglioma		0	0	1	0
B-granular cell tumor		0	0	1	0
Percentages		2.0	1.9	3.9	7.8

Since there was a survival disparity among the various groups of male rats, a complete statistical analysis of the data is necessary and is being performed by HED statisticians.

Historical controls for brain tumors (all types) was provided by Ira Daly of Bio/dynamics.

In 14 studies submitted, the range of astrocytomas was 0 to 3.3 percent. The individual studies provided percentages of 1.7, 0.08, 0.86, 3.3, 0.08, 0, 0, 0, 0, 0.8, 0, 1.8, 0, and 1.7.

Other gliomas besides astrocytomas were recorded in rat brains in these 14 studies.

Additionally, it should be noted that the duration of the historical control studies generally exceeded 24 months.

The full significance of the oncogenic potential of Arsenal to male rat brains must await full statistical analysis and possibly Peer Review.

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There was no compound-related effect in female rat brain tumors.

The Bio/dynamics historical control data are appended to this report.

b. Thyroid C-cell Tumors

The incidence of C-cell thyroid neoplasms, showed an increase at the mid- and high-dose. More specifically, however, the C-cell carcinoma incidence was increased at the high-dose only.

The following data summarizes the findings.

Male Rats

Group	1	2	3	4
No. examined	65	65	65	65
C-cell carcinoma	1	1	1	5
C-cell adenoma	2	3	9	4

The high-dose male rats showed a higher incidence of C-cell carcinoma (5/65, 7.69%), when compared to the control (1/65, 1.53%), low-dose (1/65, 1.53%), and mid-dose (1/63, 1.58%) male rats. Additionally, the high-dose incidence (7.69%) was within the range of 0 to 13.7 percent from historical control data collected at Bio/dynamics. Also, the high-dose was reported to be without statistical significance.

The fate of the individual male rats with C-cell carcinoma is presented below as tabulated in the report.

Male Rats with C-cell Carcinoma

Group	Sex	Animal No.	Death Code	Day of Study of Death	Week of Study of Death
I	M	1024	D	614	88
II	M	2027	D	691	99
III	M	3059	T	739	106
IV	M	4017	D	654	99

Male Rats with C-cell Carcinoma (cont'd)

Group	Sex	Animal No.	Death Code	Day of Study of Death	Week of Study of Death
IV	M	4023	S	639	92
IV	M	4040	S	669	96
IV	M	4055	T	739	106
IV	M	4064	S	665	95

Key: T = Terminal Sacrifice; D = Spontaneous Death;
S = Sacrificed Moribund.

It can be seen from this table there is no apparent decrease in latency of the C-cell tumor.

Proliferative lesions of the male thyroid in this study are summarized below as presented the report:

Table 1

Thyroid Gland

Summary-Incidence of Proliferative Lesions

Sex	Males			
Group	I	II	III	IV
Thyroid gland				
# Examined	65	65	63	65
C-cell hyperplasia	15	8	13	6
%	23.10	12.31	20.63	9.23
C-cell adenoma	2	3	9	4
%	3.10	4.62	14.29	6.15
C-cell carcinoma	1	1	1	5
%	1.54	1.54	1.59	7.69
C-cell adenoma and carcinoma	3	4	10	9
%	4.62	6.15	15.87	13.85

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Summary-Incidence of Proliferative Lesions (cont'd)

Sex		Males			
Group		I	II	III	IV
C-cell hyperplasia, adenoma and carcinoma combined		17	12	21	15
	%	26.15	18.46	33.33	23.08

The differences between the incidences of all groups are not statistically significant. The following tables, taken from the report, are of historical control data from Bio/dynamics.

Table 2

Thyroid Gland - Selected Findings
Historical Control Data - Male Charles River Albino CD® Rats
(Compiled from 14 Studies Conducted at Bio/dynamics, Inc.)

	Range of Incidence of Historical Data		Mean Incidence of Historical Data
	Low	High	
# Examined	73	69	1413
C-cell hyperplasia	0	10	60
Percentage	0	14.59	4.25
# Examined	73	69	1413
C-cell hyperplasia	0	10	60
Percentage	0	14.59	4.25
# Examined	131	70	1413
C-cell ademoma	0	8	72
Percentage	0	11.43	5.10
# Examined	129	131	1413
C-cell carcinoma	0	18	58
Percentage	0	13.74	4.10
# Examined	54	70	1413
C-cell adenoma and carcinoma combined	0	12	129
Percentage	0	17.14	9.13

Thyroid Gland - Selected Findings (cont'd)

	Range of Incidence of Historical Data		Mean Incidence of Historical Data
	Low	High	
# Examined	54	70	1413
C-cell hyperplasia, adenoma and carcinoma combined	0	18	183
Percentage	0	25.71	12.95

For the purposes of a combined finding, those animals having more than one finding were counted only once.

Table 3 shows the individual studies for the data from the Bio/dynamic files.

Table 3

Thyroid Gland - Selected Findings
Historical Control Data - Male Charles River Albino CD® Rats

Study	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Date Initiated	1978	1977	1977	1978	1978	1977	1977	1978	1977	1977	1978	1978	1977	1979
Date Terminated	1980	1980	1980	1981	1980	1980	1980	1980	1980	1979	1981	1980	1980	1981
# Examined	68	139	139	70	69	131	123	54	139	139	70	73	129	70
C-cell adenoma	0	14	6	8	4	0	0	0	5	8	8	2	13	4
%	0	10.1	4.3	11.4	5.8	0	0	0	3.6	5.8	11.4	2.7	10.1	5.7
C-cell carcinoma	2	4	4	4	2	18	12	0	3	1	0	0	0	8
%	2.9	2.9	2.9	5.7	2.9	13.7	9.8	0	2.2	0.7	0	0	0	11.4
C-cell adenoma and carcinoma	2	18	10	12	6	18	12	0	8	9	8	2	13	11
%	2.9	12.9	7.2	17.1	8.7	13.7	9.8	0	5.8	6.5	11.4	2.7	10.1	15.7
C-cell hyperplasia	4	3	9	6	10	9	2	0	11	2	2	0	1	1
%	5.9	2.2	6.6	8.6	14.5	6.9	1.6	0	7.9	1.4	2.9	0	0.8	1.4
C-cell adenoma and carcinoma and hyperplasia	6	20	19	18	16	23	14	0	19	11	9	2	14	12
%	8.8	14.4	13.7	25.7	23.2	17.6	11.4	0	13.7	7.9	12.9	2.7	10.9	17.1

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Evaluation of Tables 1, 2, and 3 show that the incidences of C-cell proliferative lesions in the AC 243,997 study (C-cell hyperplasia, C-cell adenoma, C-cell carcinoma), individually or in combination, were within the range of Bio/dynamic historical control data. In addition, the incidences do not exhibit a stepwise dose-response progression from hyperplasia to adenoma to carcinoma (see Table 1).

As can be seen by comparing the incidences of proliferative lesions from Table 1 (the data from the current study) to the Bio/dynamic historical control data in Table 2, the incidences of the proliferative lesions at the high-dose are all within the range of the historical control data as shown below.

Table 4

Thyroid Findings

C-cell	High-Dose Incidence of Current Arsenal Study	Range of Hist. Controls from Bio/dynamics
Hyperplasia	9.23%	0 - 14.59%
Adenoma	6.15%	0 - 11.43%
Carcinoma	7.69%	0 - 13.74%
Adenoma and Carcinoma	13.85%	0 - 17.14%
Hyperplasia and Adenoma and Carcinoma	23.08%	0 - 25.71%

The report states that Suzuki et al. (1979) reported the incidence of medullary carcinoma in the thyroid gland of Sprague-Dawley rats to be 79 percent (33/42) in males and 49 percent (19/39) in females.

The registrant employed an outside consultant, W. Roy Brown, D.V.M., Ph.D., to examine the thyroid gland of male rats and render an opinion.

Dr. Brown's analysis is presented below in Table 5.

Table 5
Dr. Brown's Analysis

Summary Incidence of Proliferative Lesions of C-cell Origin in the Thyroid Gland of Male Rats

Dose Group	I	II	III	IV
Number of Thyroid Glands Examined	65	65	65	65
C-cell hyperplasia (all degrees)				
Incidence	8	13	14	7
Percent	12.3	20.0	21.5	10.8
C-cell adenoma				
Incidence	2	1	7	2
Percent	3.1	1.5	10.8	3.1
C-cell carcinoma				
Incidence	1	1	1	4
Percent	1.5	1.5	1.5	6.2
C-cell adenoma and carcinoma				
Combined Incidence	3	2	8	6
Percent	4.6	3.1	12.3	9.2
C-cell hyperplasia adenoma and carcinoma				
Combined Incidence	11	15	22	13
Percent	16.9	23.1	33.8	20.0

Dr. Brown states "It is my opinion that the difference between the control and high dosage group male rats with respect to the C-cell carcinomas is of no biological significance. The incidence in the high-dose rats is consistent with that which can occur spontaneously and those that have been reported in control rats in studies of similar type at Bio/dynamics, the site of the study. An incidence of as high as 79% (33/42) of C-cell carcinoma have been reported in male Sprague-Dawley rats (Suzuki, et al.). Other studies indicate an increase of 16-40% of C-cell carcinomas in other strains of rats, including Long-Evans, Sprague-Dawley, Wistar and wild rats (Rattus norvegicus). The highest group incidence of C-cell carcinomas in this study was 6.2%." [End of quotation].

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c. Adrenal Medullary Tumors

An additional tumor type of possible concern occurred in the adrenal gland.

In female rats, there was an increased incidence of adrenal medullary tumors at the high-dose. The incidence was as follows for the number of female rats examined by effective proportions:

Female Adrenal Medulla

Group	1	2	3	4
No. examined	27	34	22	31
Carcinoma	0	0	0	1
Adenoma	1	2	0	6
Carcinoma and Adenoma (Combined)	1	2	0	7
Percentanges	4.0	5.8	0	22.5%

Animal Number	Dose	Week Death	Tumor
1540	0	106	Adenoma
2503	1000	104	Adenoma
2561	1000	106	Adenoma
4507	10,000	106 (unscheduled)	Adenoma
4521	10,000	106	Carcinoma
4524	10,000	106	Adenoma
4528	10,000	104	Adenoma
4534	10,000	107	Adenoma
4537	10,000	106	Adenoma
4551	10,000	107	Adenoma

It can be seen from the week of death for the Arsenal female tumor-bearing animals that the earliest pheochromocytoma occurred at week 104. Therefore, when the number of animals examined is adjusted for effective proportions, the high-dose percentage is 22.5 percent.

The historical control data from Bio/dynamics for 14 studies showed pheochromocytomas ranging from 0 to 15.5 percent. The individual percentages were 6.7, 4.2, 0, 6.7, 1.9, 8.5, 11.0, 0, 4.5, 2.6, 8.7, 3.5, 5.3, and 15.5 percent.

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Additionally, it should be noted that the historical control data is unculled for mortality (although no pheochromocytomas were found prior to 12 months).

The historical control data is appended to the report.

Reviewer's Conclusion - The incidences of thyroid proliferative lesions and neoplasms in this study are considered unrelated to the administration of AC 243,997.

Additional statistical analyses of the incidences of astrocytomas in male rats need to be performed. The increase in these tumors at the high-dose may be compound-related.

With respect to the adrenal medullary gland in females, it appears that the increased incidence at the high-dose may be compound-related depending on interpretation by statistics. However, survival disparity analysis and statistical analysis are needed.

If the number of female adrenal medullary gland tumors is not adjusted for effective proportions and the essentially unculled proportions are examined, the following incidences are observed.

Female Adrenal Medullary Tumors

Group	1	2	3	4
No. examined	55	55	55	55
Adenomas and carcinomas	1	2	0	7
Percentages	1.8%	3.6%		12.7%

In this situation, the unculled data are within the range of Bio/dynamics historical controls from 0 to 15.5 percent which are also unculled.

Additionally, in male rats in the Arsenal study, pheochromocytomas were more frequent and occurred at earlier periods than in females.

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What may be being observed in females is 008729
a compound-related geriatric increase of pheochromocytoma.

For males in the Arsenal study, the weeks at which pheochromocytomas were found were as follows:

Control		Low		Mid		High	
A.N.	Week	A.N.	Week	A.N.	Week	A.N.	Week
1001	106	2005	87	3003	91	4002	81
1008	106	2007	106	3012	106	4013	106
1020	93	2012	106	3018	105	4020	102
1023	92	2018	106	3039	98	4027	106
1031	106	2034	100	3040	96	4044	106
1043	106	2039	106	3059	106	4048	106
1052	97	2045	106			4051	106
1055	106	2052	106			4054	103
		2054	106			4059	104
		2055	88			4062	89
		2064	106				

The incidences, distributions, and time-to-tumor for other benign and malignant neoplasms were considered unrelated to treatment.

2. Non-Neoplastic

There were increased incidences of three non-neoplastic lesions in female rats. One lesion was extramedullary hematopoiesis of the spleen. The second lesion was peliosis hepatis of the liver. The third lesion was B-squamous cysts of the thyroid.

With respect to the spleen, the overall incidence of the lesion was as follows:

	Spleen Females			
Group	1	2	3	4
No. examined	65	65	65	65
Extramedullary hematopoiesis	12	17	17	20
Grades of the lesion	2,3,2,4, 4,3,2,4, 2,2,2,2	3,2,2,2, 4,3,3,4, 2,4,4,5, 2,5,2,2, 2	3,2,2,2, 4,4,5,4, 2,2,2,2, 5,4,4,2, 2	2,2,3,3, 4,4,2,3, 2,2,4,4; 2,3,5,5, 3,5,2,2

As can be seen from the incidence and grades of the lesion, the high-dose group is the LEL and the mid-dose group is the NOEL. The grade of 5 is associated with 0, 2, 2, and 3 lesions in the control, low-, mid-, and high-dose groups, respectively. Additionally, the grade of 4 is associated with 3/12 (25%), 4/17 (23%), 5/17 (29%), and 4/20 (20%) of the lesions in the control, low-, mid-, and high-dose groups, respectively.

Additionally, there did not appear to be any association of the the increased incidence of this splenic lesion with earlier deaths based on analysis of the data. Also, associative anemia was not observed in high-dose females.

Although the full toxicological significance of this lesion is uncertain, it does not appear to be compensatory. In any case, due to the incidences and grades of the lesion, it appears to be a compound-related effect.

The distribution of the lesion in the spleen of female rats is shown below:

Interim Kill

Group	1	2	3	4
No. Examined	10	10	10	10
Lesion	0	0	0	0

Died on Study

Group	1	2	3	4
No. Examined	30	24	34	28
Lesion	8	7	10	11

Terminal Kill

Group	1	2	3	4
No. Examined	25	31	21	27
Lesion	4	10	6	9

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In female rats, there was an increased incidence of peliosis hepatis of the liver in the mid- and high-dose groups. This lesion is the presence in hepatic lobules of multiple microscopic pools of blood which may become lined by endothelium. It is a rare condition that may result from the congestion of the liver with necrosis. However, there was no compound-related occurrence of hepatic necrosis (either by incidence or grade of lesion) in female rats in this study.

The incidence of the peliosis hepatis in the liver was as follows:

Female Liver

Group	1	2	3	4
No. Examined	65	65	65	65
Peliosis hepatis	4	3	6	8

The grades of the lesion were comparable between control and high-dose rats and there was no indication of a decrease in latency of the lesion at the high-dose. Based on these considerations, the slight increase in this lesion in the mid- and high-dose groups is not considered compound-related.

The third lesion in female rats, which was ungraded (only P present in individual animal data), which occurred at an increased incidence was B-squamous cysts of the thyroid in female rats.

The occurrence was as follows:

Female Thyroid

Group	1	2	3	4
No. Examined	65	65	65	65
B-squamous cysts	5	7	5	12

The NOEL for this finding is the mid-dose of 5000 ppm and the LEL is the high-dose of 10,000 ppm.

Other non-neoplastic lesions occurred at similar frequency and grade between control and treated male and female rats.

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Reviewer's Conclusion - The NOEL for non-neoplastic lesions is considered to be the mid-dose of 5000 ppm. The LEL is the high-dose of 10,000 ppm and the effects are increased incidences of extramedullary hematopoiesis of the spleen and B-squamous cysts of the thyroid in female rats. These compound-related lesions do not appear to be life-threatening and may not be used to establish an MTD for female rats.

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R:55662:Dykstra:C.Disk:KENCO:01/22/90:CT:VO:SW:CT:ka
R:55665:Dykstra:C.Disk:KENCO:01/25/90:CT:VO:CT

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Review By: William Dykstra William Dykstra 7/13/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian
Section I, Toxicology Branch I - IRS (H7509C) 7/13/89

DATA EVALUATION REPORT [Amended 06/14/91 (for RG)]

Study Type: 83-2 - Oncogenicity, Mouse TOX Chem No.: 821C

Accession No.: N/A MRID No.: 410395-04; Vol. 1-6

Test Material: AC 243,997 technical

Synonyms: Imazapyr; Arsenal 008426

Study No.: 86-3074

Sponsor: American Cyanamid

Testing Facility: Bio/dynamics

Title of Report: A Chronic Dietary Toxicity and Oncogenicity Study with AC 243,997 in Mice.

Author: Carol Auletta

Report Issued: November 3, 1988

Conclusions:

The oncogenic potential is negative up to 10,000 ppm (HDT). The HDT exceeds the 7000 parts per million (ppm) limit dose for mouse oncogenicity studies and is therefore the maximum tolerated dose (MTD).

There were no compound-related effects in toxic signs, mortality, body weight, food consumption, hematology, organ weights, and tumors. Historical control data are required to establish the NOEL for pulmonary edema in female mice. Additionally, a more detailed description of subscapular adrenal gland cell reaction is required.

Following the submission of the historical control data and descriptive material, a NOEL for the study will be determined.

Classification:

Core-Supplementary, which can be upgraded after review of historical control data.

Special Review Criteria (40 CFR 154.7): N/A

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A. Materials:

1. Test Compound - AC 243,997, Description: Off white powder, Batch No. AC 4866-062, Purity 99.5%.

2. Test Animals - Species: mice, Strain: CD-1, Age: 42 days, Average Weight: males, 27 g, females, 21 g, Source: Charles River, Kingston, NY.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 18 Months Male	Main Study 18 Months Female	Interim Sac. 12 Months Male	Interim Sac. 12 Months Female
1. Control	0	65	65	10	10
2. Low (LDT)	1000	65	65	10	10
3. Mid (MDT)	5000	65	65	10	10
4. High (HDT)	10,000	65	65	10	10

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

Results - The test material was stable in the diet for the required 2-week interval based on analyses of the 1000 and 10,000 ppm batches.

The concentration of the test material in the diet for 80 weeks ranged from 888 to 1026 ppm for the low dose, 4758 to 5479 ppm for mid dose, and 9178 to 11,273 ppm for the high dose.

At the low dose, assays averaged 97.6 percent with a coefficient of variation (CV) of 4.8 percent of nominal. At the mid dose, assays averaged 100.4 percent with a CV of 4.6 percent of nominal. At the high dose, assays averaged 99.6 percent with a CV of 4.9 percent of nominal. Technical material was stable for the duration of the study.

3. Animals received food (Purina Certified Rodent Chow No. 5002) and water ad libitum.

4. Statistics - The following level was utilized in analyzing the numerical data: $p < 0.05$, $p < 0.01$.

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5. Quality Assurance was performed and the report was signed by the Study Director.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. Detailed examinations were performed weekly.

Results:

a. Toxicity Signs - There were no compound-related toxic signs during the study. The most frequently observed toxic signs were, in males, ear problems, yellow stains on fur and genital area, scabs and alopecia. The frequency of these findings was comparable between control and treated male groups.

The most frequently observed toxic signs in females were ear problems, scabs, and alopecia. The incidence of these toxic signs were comparable between control and treated female mice.

b. Mortality - There were no compound-related effects in mortality between control and treated male and female groups.

The mortality at 18 months for males and females, excluding the 10 mice/sex/group sacrificed at 12 months, was as shown below:

	Mortality (%)			
Group	I	II	III	IV
Dose (ppm)	0	1000	5000	10,000
Males Percent	27/55 (49%)	19/54[a] (35%)	20/54[b] (37%)	22/55 (40%)
Females Percent	19/55 (35%)	18/55 (33%)	27/55 (49%)	24/54[b] (44%)

a/Excludes one animal which escaped and was missing for more than 24 hours, found and killed.
b/Excludes one animal which died accidently.

As can be noted from the data, total survival among male treated groups was better than the control

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group. In females, total survival in the mid- and high-dose groups was slightly decreased in comparison to controls. None of the differences in survival between control and treated male and female groups were statistically significant and in females, the decreased survival was not dose-related.

There was no compound-related effect in the time course of cumulative mortality in treated males and the slight increase in mortality in high-dose females is not considered compound-related.

Total Cumulative Mortality

Group (ppm)	No. of Mice	Month	1	4	8	12[a/]	15	18
		Males						
I 0	65		0	0	2	4	5	27
II 1000	65		0	0	2	2	3	19
III 5000	65		0	0	1	2	8	20
IV 10,000	65		0	0	3	4	8	22
		Females						
I 0	65		0	0	0	2	7	19
II 1000	65		0	0	1	3	6	18
III 5000	65		0	0	2	4	8	27
IV 10,000	65		0	2	4	5	12	24

[a/]Ten mice/sex/group were sacrificed at 12 months.

These mortality data indicate that the greatest number of mice dying in each group occurred between month 15 and at termination in month 18.

2. Body Weight - They were weighed weekly for 14 weeks, then biweekly for 10 weeks, and monthly, thereafter.

Results - There were no compound-related adverse effects in body weight gain between control and treated male and female groups. Occasionally, there were statistically significant differences in body weight between the control and treated groups but the treated mice had gained more weight than the controls. An increase in body weight gain by the treated mice is not considered a toxic effect. The following table shows the mean body weights during the study:

Males (Body Weight in Grams)

Dose (ppm)	0	1000	5000	10,000
Week				
0	27.4	27.2	27.3	27.7
4	31.3	31.6	31.3	31.7
8	33.8	34.1	33.6	34.1
16	37.4	37.7	37.0	37.3
30	38.4	38.5	38.1	39.4
64	40.0	40.4	39.3	39.6
77	41.3	40.7	41.0	41.4

Females (Body Weight in Grams)

Dose (ppm)	0	1000	5000	10,000
Week				
0	20.7	21.5*	20.7	21.9**
4	25.0	25.3	24.8	25.5
8	27.1	27.0	26.9	28.3**
16	29.3	29.0	29.4	29.1
30	31.4	32.4	33.2	32.1
64	31.4	34.6	34.0	33.1
77	33.9	35.7	33.5	35.6

* $p < 0.05$
** $p < 0.01$

3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

a. Food Consumption - There were no compound-related adverse effects in food consumption between control

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and treated male and female mice. The occasionally statistically significant increases and decreases observed between control and treated groups were, for the most part, not consistent in a time or dose-related fashion and, in frequent instances, the treated mice consumed more food than the controls. The following table shows the mean food consumption during the study:

Males (Food Consumption: mg/kg/day)

Dose (ppm)	0	1000	5000	10,000
Week				
0	229.0	241.8	223.8	212.9
4	214.8	220.1	218.0	205.4
8	225.0	220.9	221.0	216.6
16	190.6	195.4	150.6**	155.6**
30	160.5	158.8	158.0	152.1
64	123.8	125.7	137.1**	135.3*
77	138.5	149.5	163.4**	148.4

Females (Food Consumption: mg/kg/day)

Dose (ppm)	0	1000	5000	10,000
Week				
0	306.1	296.5	315.8	285.4
4	269.5	269.9	279.7	285.1
8	293.0	294.7	300.1	282.2
16	287.8	264.6**	203.0*	210.8**
30	192.1	175.5*	163.3**	184.7
64	155.5	159.1	155.3	180.6**
77	197.4	182.7	185.0	171.3**

* $p < 0.05$
** $p < 0.01$

b. Compound-Intake - The range of test material intake in mg/kg/day as presented in the report is shown below:

Dose (ppm)	1000	5000	10,000
Males	126-254	674-1194	1301-2409
Females	151-303	776-1501	1639-3149

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4. Ophthalmological Examinations - The mice were not examined in life by an ophthalmologist for ocular lesions.

5. Blood was collected at 12 and 18 months for hematology analysis from 10/sex/group animals. The CHECKED (X) parameters were examined.

- Hematology

X		X	
X	Hematocrit (HCT)*		
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)

Results - There were no compound-related effects in mean hematological values at 12 and 18 months in treated male and female mice in comparison to controls. Additionally, there were no statistically significant differences.

The following tables show the results of the 12-month and 18-month hematological findings:

12 Month Analysis

	Males					Females			
Dose ppm Mean values	0	1000	5000	10,000	Dose ppm Mean values	0	1000	5000	10,000
HgB (g/dL)	13.8	13.6	13.9	14.3	HgB (g/dL)	14.2	14.7	14.9	14.5
HCT (%)	37	36	37	38	HCT (%)	37	38	37	37
RBC (mil/uL)	6.95	7.06	7.16	7.31	RBC (mil/uL)	7.35	7.63	7.54	7.42
Plat (100T/uL)	15.65	16.58	15.02	16.32	Plat (100T/uL)	12.67	14.69	12.78	13.66
WBC (thous/uL)	4.9	4.2	4.0	7.9*	WBC (thous/uL)	3.9	4.6	3.9	4.6

*Male mouse No. 4031 of the high-dose group had a WBC count of 34.1 (thous/uL) due to an increased segmented neutrophil count. This isolated finding was not considered treatment-related.

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18 Month Analysis

Dose ppm Mean values	Males 0	1000	5000	10,000	Dose ppm Mean values	Females 0	1000	5000	10,000
HgB (g/dL)	14.3	13.5	14.0	14.7	HgB (g/dL)	14.3	13.4	15.0	13.2
HcT (%)	41	39	41	43	HcT (%)	42	40	44	38
RBC (mil/uL)	7.85	7.44	7.72	7.95	RBC (mil/uL)	7.82	7.42	8.15	7.21
Plat (100T/uL)	25.83	20.23	28.25	25.55	Plat (100T/uL)	20.74	20.24	16.62	21.05
WBC (thous/uL)	4.7	3.8	4.5	3.6	WBC (thous/uL)	4.0	4.6	5.6	3.0

6. 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
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (2 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenals*
X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
	Rectum*	XX	Testes*	XX	Parathyroids*
XX	Liver*	XX	Epididymides	XX	Thyroids*
X	Gallbladder*	X	Prostate		Other
X	Pancreas*	X	Seminal vesicle	X	Bone*
	Respiratory	XX	Ovaries	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin (mammary area)
XX	Lung*			X	All gross lesions and masses

Results:

a. Organ Weight at 12 Months - There were no compound-related effects in organ weight, organ-to-body weight, and organ-to-brain weight ratios for male and female mice sacrificed at 12 months. Additionally, there were no statistically significant differences between control and treated groups of male and female mice.

b. Organ Weight at 18 Months - At the terminal sacrifice, there were no compound-related effects in organ weight, organ-to-body weight, and

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organ-to-brain weight ratios for male and female mice sacrificed terminally at 18 months.

The statistically significant differences observed between control and treated mean values in absolute and relative organ weight observed occasionally were not dose-related, and therefore, were not considered compound-related.

These statistically significant differences in means included for males: increased absolute liver weight at mid dose.

For females, the following data were observed: decreased brain-to-body weight ratio at low dose, increased heart-to-brain weight ratio at low dose, increased kidney-to-brain weight ratio at low dose, and increased liver-to-brain weight ratio at low dose.

c. Gross Pathology

1) Twelve Months - There were no compound-related or toxicologically significant effects in gross necropsy findings at 12 months. Uterine cysts occurred at an increased incidence in treated female mice (2/10, 3/10, 7/10, and 6/10 for the control, low-, mid-, and high-dose groups, respectively). This lesion was not considered toxicologically significant since the incidence of this gross lesion in female mice dying on study and mice terminally sacrificed was randomly distributed without any relationship to dose.

2) All Unscheduled Deaths - There were no compound-related or toxicologically significant effects or dose-trends in groups or pathological findings in mice which were unscheduled deaths.

3) Terminal Sacrifice - There was one possible compound-related effect in mice terminally sacrificed.

The incidence of enlarged seminal vesicles in male mice was 3/28 (11%), 6/35 (17%), 9/34 (27%), and 10/33 (30%) for the control, low-, mid-, and high-dose groups, respectively. Histologically, there was no increase in microscopic lesions in terminally sacrificed male mice which correlated with the gross findings. The report states that the incidence of the seminal vesicle gross

findings were not statistically significant, but a probability value was not presented.

Toxicology Branch (TB) does not consider the gross findings incidence in the seminal vesicles to be toxicologically significant.

Additionally, there was an increased incidence of kidney cysts in high dose male mice in comparison to controls. The incidence was 2/28 (7%), 0 (0%), 3/34 (9%), and 5/33 (15%) in control, low-, mid-, and high-dose groups, respectively. Histologically, there were no microscopic increases in kidney lesions which correlated with the gross findings.

Although the finding is dose related, it is not considered toxicologically significant.

d. Microscopic Pathology

1. Non-neoplastic

a) 12 Month Sacrifice - The most frequently observed microscopic lesion observed at 12 months was amyloidosis and included the kidney, heart, mesenteric lymph nodes, stomach and intestines, tongue, ovaries, liver, thyroid gland, and adrenal gland. Although there were occasional increased incidences in treated mice in comparison to controls, the increases were usually less than twice the control level at the high dose. Additionally, the incidences of amyloidosis in these and other organs in mice dying on study or terminally sacrificed were distributed in a similar random pattern. Also, the grades of the lesion were comparable among control and treated groups.

Therefore, the occurrence of amyloidosis is not considered compound-related in mice.

Another 12-month microscopic finding that occurred at slightly higher incidences in treated mice in comparison to controls was unilateral (but not bilateral) subscapular cell reaction of the adrenal gland in male and female mice. The incidence of the unilateral lesion was 0/10, 1/10, 2/10, and 5/10

in male mice of the control, low-, mid-, and high-dose groups, respectively. Additionally, in female mice the incidence was 4/10, 3/10, 1/10, and 7/10 in the control, low-, mid-, and high-dose groups, respectively.

The occurrence of the unilateral (and bilateral) subscapular cell reaction of the adrenal gland in treated mice that died on study or were terminally sacrificed, did not show any treatment-related distribution. Also, the grades of the lesion were comparable among control and treated groups. A more detailed description of this lesson is required.

In light of these findings, the occurrence of unilateral subscapular cell reactions of the adrenal is not considered compound-related.

b) Unscheduled Deaths - There was an increased incidence at the high-dose group in females of congestion of the brain. The incidences were 3/19 (16%), 2/17 (12%), 3/27 (11%), and 7/25 (28%) in the control, low-, mid-, and high-dose groups, respectively.

There were no occurrences of congestion in the brain in the control and treated groups of both sexes at 12 months and terminal sacrifice. The grades of the lesion were comparable between control and treated females in the unscheduled deaths.

Additionally, the increased occurrence of this lesion in high dose females is not greater than 2X of the controls.

The incidence, but not the grade, of edema in the alveoli of the lungs in female mice occurred in an increased manner. The incidence was 2/19 (11%), 4/18 (22%), 5/27 (19%), and 6/25 (24%) for the control, low-, mid-, and high-dose groups, respectively, in the unscheduled deaths. There was no alveoli edema in mice sacrificed at 12 months and no compound-related increase in female mice sacrificed terminally. The

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incidence of this lesion in all female mice on study was 3/65, 5/65, 5/65, and 7/65 in the control, low-, mid-, and high-dose groups, respectively.

The increased incidence at the high dose may be compound-related.

The registrant is required to provide historical control data to resolve this issue.

The incidence and grades of other lesions in the tissues and organs of both sexes of mice were comparable between control and treated groups.

c) Terminal Sacrifice - There was an increased incidence, but not increased grade, of erythrocytes in the sinus of mediastinal lymph nodes in treated female mice in comparison to controls. The incidences were 3/29 (10%), 5/33 (15%), 2/22 (9%), and 10/28 (36%) in the control, low-, mid-, and high-dose groups, respectively. For all female animals on study, the incidence was 5/48 (10%), 9/48 (18%), 10/46 (21%), and 12/52 (23%) for the control, low-, mid-, and high-dose groups, respectively.

The increased incidence of this lesion at the high dose in terminally sacrificed female mice is not considered compound-related.

There was an increased incidence, but not grade, of brown pigment in the Harderian gland of female mice sacrificed terminally. The incidence was 7/14 (50%) in the controls compared to 18/21 (85%) in the high-dose group. For all female mice on study, the incidence of this lesion was 8/15 in controls compared to 20/25 at this high dose.

Since the incidence of this lesion at the high dose is not 2X greater than controls, the lesion is not considered compound-related.

The incidences and grades of lesions in other tissues and organs of both sexes of mice were comparable between control and treated groups.

2) Neoplastic Lesions - There were no compound-related increases in benign or malignant neoplasms in the various tissues and organs of both sexes of mice and no decrease in latency for any tumors.

The most frequently observed neoplasms were in the lungs. The overall incidences for all mice on study are shown below:

	Lung							
	Males				Females			
Dose (ppm)	0	1000	5000	10,000	0	1000	5000	10,000
No. examined	65	65	65	65	65	65	65	65
Adenoma	12	9	12	9	9	5	14	6
Percent (%)	18	14	18	14	14	8	22	9
Carcinoma	3	1	1	0	1	0	0	0
Percent (%)	5	2	2	0	2	0	0	0

The distribution of the adenomas and carcinomas between mice of the 12-month sacrifice, the unscheduled deaths, and terminal kill are shown below:

	Lung							
	Males				Females			
Dose (ppm)	0	1000	5000	10,000	0	1000	5000	10,000
Adenomas								
12 Months	2	1	2	0	1	1	1	1
Unsch. Deaths	4	4	2	4	0	1	2	1
Term. Kill	6	4	8	5	8	3	11	4
Carcinomas								
12 Months	1	0	0	0	0	0	0	0
Unsch. Deaths	1	1	1	0	1	0	0	0
Term. Kill	1	0	0	0	0	0	0	0

It can be concluded from the data that there is no compound-related decrease in latency for lung adenoma and carcinoma.

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R:55897:Dykstra:C.Disk:KENCO:7/26/99:CL:MO:EK:AS

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D. Discussion:

The oncogenic potential is negative up to 10,000 ppm (HDT). The HDT exceeds the 7000 ppm limit dose for mouse oncogenicity studies. A NOEL for various non-neoplastic gross and microscopic lesions could not be established. Historical control data for these lesions are required to be submitted to resolve these issues. The historical control data required are the following: 1) incidence of gross pathological findings of enlarged seminal vesicles in 18-month old male mice and a statistical analysis of this lesion in male mice in the study; 2) incidence of congestion of brain in female mice; 3) incidence of edema of alveoli in female mice; and 4) incidence of erythrocytes in the sinus of mediastinal lymph nodes in female mice.

From: J. J. Slaughter 7-26-89

To: E.R. Dykstra

- I dont think any request for historical control data is warranted at this time. The incidence of all neoplastic + non-neoplastic lesion spontaneously occur in CD, mice. However I would suggest that you get or give a more detailed description of the unicapsular adrenal gland "cell reaction. Also; it would (maybe) important to know what other lesion (s) were associated with the pulmonary edema.

REVIEWER 67

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TOX Chem No.: 352H
TB Project No.: 9-1736
RD Record Nos.: 247,679

MEMORANDUM

SUBJECT: Imazapyr (ARSENAL) - Mutagenicity Data Submitted under Accession No. 260,000
EPA ID No. 241-273

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

11/03/89

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Karl P. Baetcke 11/07/89

Registrant: American Cyanamid (AC), Princeton, NJ

Request

Review and evaluate the following mutagenicity studies:

Study 1a. Cytotoxicity Pilot Study in Male Albino Rats with AC 243,997, performed by ToxiGenics Inc., Decatur, IL, Project No. 450-1283, Final Report dated August 30, 1983.

Study 1b. (DLT) Dominant Lethal Assay in Male Albino Rats with AC 243,997, performed by ToxiGenics, Inc., Decatur, IL, Project No. 450-1284, Final Report dated January 30, 1984.

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Study 2. (UDS) Unscheduled DNA Synthesis Rat Hepatocyte Assay with AC 243,997, performed by Hazleton Laboratories America, Inc., Vienna, VA, Study No. 362-170, Final Report dated January 21, 1984.

Study 3. (CA) Vitro Chromosomal Aberrations in Chinese Hamster Ovary Cells with AC 243,997, performed by Hazleton Laboratories America, Inc., Vienna, VA, Study No. 362-169, Final Report dated February 3, 1984.

Study 4. (HGPRT) Mutagenicity Testing of AC 243,997 in the in vitro CHO/HGPRT Mutation Assay, performed at American Cyanamid, GTOX Volume 4, Number 1, Final Report dated February 17, 1984.

TB Conclusions

Study	Reported Results	TB Evaluation
1. DLT	Although reported as negative, major procedural and reporting deficiences exist	UNACCEPTABLE
2. UDS	Although reported as negative, major procedural and reporting deficiencies compromise the study	UNACCEPTABLE
3. CA	Negative up to a toxic dose, 5000 mcg/mL)	ACCEPTABLE
4. HGPRT	Negative up to toxic doses (5000 mcg/mL +)	ACCEPTABLE

Detailed reviews are appended to this memorandum.

Attachments (DERs)

Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

008729

11/07/89

DATA EVALUATION RECORD

008729

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128,821
Caswell No.: 352H
Project No.: 9-1736

Study Type: Mutagenicity - Foreward gene mutation in mammalian cells (Hypoxanthine-guanine phosphoribosyl transferase in Chinese hamster cells, HGPRT/CHO)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: American Cyanamid, Princeton, NJ

Title of Report: Mutagenicity Testing of AC 243,997 in the in vitro CHO/HGPRT Mutation Assay.

Authors: E. Johnson and J.S. Allen

Study Number: 0493

Date of Issue: February 17, 1984

TB Conclusions:

Test substance was demonstrated to be negative for inducing forward mutations at the HGPRT locus in CHO cells, when these cells were exposed to concentrations into the toxic range (5000 mcg/mL and higher).

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - AC 243,997 (Imazapyr)

Description: White powder
Batch (Lot): AC 4391-97
Purity (%): 93
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Established cell line

Species: Chinese hamster ovary (CHO)
Strain: K_1-BH_4
Source: J.P. O'Neill, University of Vermont

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of AC 243,997 when administered in vitro to CHO cells with/without metabolic activation. The procedures employed were those established in the literature by expert practitioners.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was provided.

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing, triplicate cultures of cells were exposed for 5 hours to test substance at each of five concentrations (up to the limit 5000 mcg/mL), in the absence or presence of a mammalian metabolic activation system prepared from Aroclor 1254-induced rat liver (S9 mix). In addition to a solvent control (DMSO), positive controls were run concurrently, respectively the direct-acting mutagen ethylmethane sulfonate (EMS, 200 mcg/mL) for the nonactivated series, and 7,12-dimethylbenz(a)anthracene (DMBA, 7 mcg/mL) in the presence of S9.

One day after transfer to fresh culture medium without test substance, treated cells were subcultured for phenotypic expression (every 2 days until day 9 post-dose), then plated onto selection medium containing 6-thioguanine (TG) for mutant colony enumerations. Mutation frequency (MF) was calculated by dividing the total number of mutant colonies by the number of cells tested (corrected for cloning efficiency) and expressed as mutants per 10^6 surviving cells.

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Concurrent determinations of cytotoxicity and cloning efficiency were made from aliquots of the same treated cultures.

The entire experiment was repeated once at doses up to 12,000 mcg/mL test substance.

E. Results - In the preliminary toxicity test, only the HDT, 5000 mcg/mL, showed evidence of toxicity (Report Tables 1 and 2), moderate in the presence of S9 (56.8% relative survival) but severe in nonactivated cultures (100% lethal). Hence, the initial mutation experiment was performed at five doses of 250, 500, 1000, 2500, and 5000 mcg/mL with S9, but only up to 2500 mcg/mL in the absence of S9.

In this first trial, cloning efficiencies were unaffected by AC 243,997 treatment at any dose, being equivalent to solvent controls (Report Tables 3 to 6), and mutation frequencies were likewise similar to solvent controls (Report Tables 7 and 8, attached to this DER).

In the repeat assay, dose levels up to 12,000 mcg/mL +S9, and up to 10,000 mcg/mL -S9, were used in preliminary cytotoxicity testing, but the highest concentrations proved too severely toxic: In S9-activated cultures 100 percent lethality at 12,000 mcg/mL, 46 percent survival at 9,000 mcg/mL (Table 9); without S9, no cells survived 7500 or 10,000 mcg/mL, but survival was at control levels below that (Table 10). As in the first trial, all dose levels of test substance produced mutation frequencies comparable to solvent control values (Report Tables 13 and 14, attached here).

By contrast, both positive controls performed as expected, inducing MFs from 12 to > 20 times DMSO values.

The authors concluded that AC 243,997 was not mutagenic at the HGPRT locus in CHO cells when assayed in repeat experiments up to toxic concentrations.

F. TB Evaluation - ACCEPTABLE. The test compound was assayed adequately with appropriate controls such that the negative results obtained may be judged valid.

Attachments

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PAGES 74 THROUGH 78 HAVE BEEN REMOVED FROM THIS DOCUMENT. THOSE PAGES CONSIST OF REGISTRANT-SUBMITTED DATA.

Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

11/07/89

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DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128821
Caswell No.: 352H
Project No.: 9-1736

Study Type: Mutagenicity = Chromosome damage in vitro (in Chinese hamster ovary cells, CHO)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: Hazleton Labs America (HLA), Vienna, VA

Title of Report: In Vitro Chromosomal Aberrations in Chinese Hamster Ovary Cells with AC 243,997.

Authors: M.G. Farrow and T. Cortina

Study Number: 362-169

Date of Issue: February 3, 1984

TB Conclusions:

The test compound was demonstrated to have no clastogenic activity in CHO cells exposed to concentrations up to 5000 ug/mL, a dose producing some signs of toxicity.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - AC 243,997

Description: Off-white powder
Batch (Lot): AC 4361-97
Purity (%): 93
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Established cell line

Species: Chinese hamster (ovary)
Strain: K-1
Source: American Type Culture Collection (ATCC), Rockville, MD (CCL61)

C. Study Design (Protocol) - This study was designed to assess the clastogenic (chromosome-breaking) potential of imazapyr when administered in vitro to CHO cells exposed to limit doses. Standardized procedures were used for this assay.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was provided.

D. Procedures/Methods of Analysis - Following preliminary dose-selection testing, monolayer cultures of CHO cells were exposed for 2 hours in triplicate to five concentrations of test substance (50, 170, 500, 1700 or 5000 ug/mL) in the absence or presence of metabolic activation (referred to as "S-9 mix," and prepared from Aroclor 1254-induced rat liver). Control cultures were run concurrently: solvent controls exposed only to DMSO; nonactivated positive controls treated with mitomycin-C (MC, 1.0 ug/mL), and activated cultures with cyclophosphamide (CP, 140 ug/mL).

Cultures were harvested 3, 8 and 12 hours after treatment, following exposure to the metaphase-arresting agent, colcemid, and five microscope slides prepared for each treatment group according to conventional cytological procedures.

One hundred metaphase cells from each group's coded slides (50 per duplicate flask) were scored for number and type of chromosome aberration (according to established convention) and modal number. Mitotic indices were determined from the number of cells in metaphase per 1000 cells counted.

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Data on aberrations were analyzed by Chi-Square (percent aberrant metaphases), and ANOVA with Students t-test (for mean aberrations per cell; and mean modal number). The level of significance was chosen as $p \leq 0.01$.

E. Results - The test compound was apparently nontoxic at dose levels up to 4000 ug/mL, which generated a mitotic index of 5.8 (compared to a solvent control value of 6.4) and relative growth of 91 percent (Report Table 2).

In the main assay the test material did not induce any statistically significant increases in percent aberrant cells or mean aberrations/cell at any dose level up to 5000 ug/mL, or at any sampling time, with or without metabolic activation (Report Table 4, attached to this DER). Both positive controls performed appropriately at the 8- and/or 12-hour sampling.

Modal number analysis revealed statistically significant ($p < 0.01$) differences from the strain mean of 20 chromosomes for nonactivated test groups at 12 hours; however, mean values ranged between 19 and 21 chromosomes (Report Table 6). Since the CHO line can vary in karyotype and chromosome number, the investigators did not consider these differences compound-related.

The test substance produced some decrease in mitotic index, as reflected in reductions in relative growth percentage, in nonactivated cultures. No such trend was evident under activated conditions (Table 7).

The authors concluded that AC 243,997 did not appear to be clastogenic under the conditions of this assay.

F. TB Evaluation - ACCEPTABLE. The test substance appeared to have been assayed in an appropriate manner and with proper controls. Hence the negative result obtained is considered a valid conclusion from the procedures employed.

Attachment

PAGES 82 THROUGH 87 HAVE BEEN REMOVED FROM THIS DOCUMENT. THOSE PAGES CONSIST OF REGISTRANT-SUBMITTED DATA.

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Karl Baetcke 11/07/89

DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128,821
Caswell No.: 352H
Project No.: 9-1736

Study Type: Mutagenicity = DNA damage/repair in vitro (Unscheduled DNA Synthesis in rat hepatocytes, HPC/UDS)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: Hazleton Labs America (HLA), Vienna, VA

Title of Report: Unscheduled DNA Synthesis Rat Hepatocyte Assay with AC 243,997.

Authors: M.G. Farrow and R.C. Sernau

Study Number: 362-170

Date of Issue: January 26, 1984

TB Conclusions:

The test substance was reported as negative for inducing unscheduled DNA synthesis in hepatocytes from a male Sprague-Dawley rat treated in vitro up to 5000 ug/mL, as measured by nuclear grain counts.

Classification (Core-Grade):

UNACCEPTABLE, due to a number of major procedural and reporting deficiencies (see TB Evaluation at F).

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II. DETAILED REVIEW

A. Test Material - AC 243,997

Description: Off-white powder
Batch (Lot): AC 4361-97
Purity (%): 93
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Rodent hepatocytes

Species: Rat
Strain: Sprague-Dawley
Age: "Adult"
Weights - Males: (not given)
Females: (not used)
Source: Charles River, Kingston, NY

C. Study Design (Protocol) - This study was designed to assess the genotoxic potential of imazapyr when administered in vitro to primary rat hepatocyte cultures.

A copy of the procedures employed is appended to this DER (from the investigator's FINAL REPORT).

A statement affirming compliance with Agency GLPs was provided as well as a Statement of Quality Assurance measures (inspections/audits).

D. Procedures/Methods of Analysis - From preliminary cytotoxicity testing, 5000 ug/mL was selected as the highest dose to be assayed, based on minimal toxicity and solubility considerations. Accordingly, primary monolayer cultures of fresh hepatocytes from an SD male were established on coverslips, and exposed (in triplicate) to solvent control (DMSO) or to six concentrations of test material (10, 50, 100, 500, 1000 and 5000 ug/mL), together with 10 uCi/mL tritiated thymidine (^{3}H-TdR). A series of positive control cultures were treated with 2-acetylaminofluorene (2AAF) at levels of 0.05, 0.10, or 0.5 ug/mL. After 24 hours incubation, cytotoxicity was determined by trypan blue exclusion, and the remaining treated cultures fixed, mounted on microscope slides and treated with photographic emulsion for the development of silver grains. Following 4 days storage in the dark at refrigerator temperatures, the slides were treated with D-19 developer, fixed, then stained with 1:25 (v/v) Giemsa:Dulbecco's saline.

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Nuclei of 50 morphologically normal cells on coded slides from each treatment were scored for silver grains under oil immersion by an automated colony counter, and net nuclear grain count (NNGC) calculated for each cell by subtracting the mean background cytoplasmic count.

For statistical purposes, "zero" was adopted for any calculated NNGC less than zero. The criterion for a significant (positive) test result was considered to be a mean NNGC greater than three standard deviations of the solvent control value, preferably at two or more consecutive doses.

E. Results - Compared to the definitively positive response of the 2AAF control cultures (over 38 times the vehicle control), at no concentration up to 5000 ug/mL did the test substance increase grain counts above the stated criterion for a positive, namely, three times the SD plus control value = 1.62 x 3 + 0.65 = 5.51 (Report Table 1, appended to this DER as ATTACHMENT B).

Hence, the authors concluded that AC 243,997 was negative for UDS in this rat HPC assay.

F. TB Evaluation - This purported negative study was not conducted according to currently recognized procedures of expert practitioners. The following deficiencies compromise the acceptability of the study results:

1. Storage time of the photographic emulsion-covered slides (to allow the appearance of sufficient silver grains to discriminate a positive response) was only 4 days, insufficient compared to the usual 7 to 10 days employed by some experts, and up to 2 weeks by others.

2. The tabulation provided (Report Table 1 see Appendix B to this DER) does not reflect the method of displaying grain count data as stated in the Methods section. "Mean net nuclear grain counts" were to be obtained, but Table 1 shows "mean nuclear grain count," as though background cytoplasmic counts were not taken into account.

3. Further absent are tabulations of:

   % of cells in repair
   % of cells with > 20 grains
   % cells in replicative (scheduled) DNA synthesis (SDS)

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4. Finally, the assay was not repeated (preferably with heptatocytes isolated from a female rat), to confirm the presumptive negative.

Attachments

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Reviewed By: Irving Mauer, Ph.D., Geneticist 11/07/89
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 11/07/89
Toxicology Branch I - IRS (H7509C)

009729

DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128821
Caswell No.: 352H
Project No.: 9-1726

Study Type: Mutagenicity - Chromosome damage in vivo (Rat dominant lethal test, DLT)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: Toxigenics, Decatur, IL

Title of Report: Dominant Lethal Assay in Male Albino Rats with AC 243,997.

Authors: Dale A. Mayhew, Clare M. Salamon and Peter V. Enloe

Study Number: 450-1284

Date of Issue: January 30, 1984

TB Conclusions:

The test substance appeared to be nontoxic on oral administration, as well as apparently without effect on reproductive (fertility) or mutagenic (induction of dominant lethals) indices at doses up to 1000 mg/kg/day for 5 days.

Classification (Core-Grade)

UNACCEPTABLE, because 1) there was insufficient documentation that the test material was absorbed and transported to target; and 2) the full spermatogenic cycle was not sampled.

II. DETAILED REVIEW

A. Test Material - AC 243,997 [nicotinic acid, 2-(4-isopropyl-4-methyl)-5-oxo-2-imidazolin-2-yl].

Description: Light tan powder
Batch (Lot): AC 4391-97
Purity (%): 94 (nitrosamines < 1 ppm)
Solvent/Carrier/Diluent: 0.1% Tween 80 in deionized water (DW)

B. Test Organism - Rodent

Species: Rat
Strain: CD Albino
Age: 68 days
Weights - Males: 390 g
Females: (not provided)
Source: Charles River, Portage, MI

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of imazapyr when administered by gavage to male rats for 5 days. The procedures employed nominally followed standard techniques as reported in published literature.

Statements affirming compliance with GLPs as well as Quality Assurance measures (inspections/audits) were provided in the Final Report.

D. Procedures/Methods of Analysis - Dose selection for this study was determined by a preliminary toxicity assay in males of the same strain of rat,* which received test compound at oral doses of 125, 250, 300, 1000 or 2000 mg/kg/day for 5 days. Animals were observed for 6 days, weighed on postdose days 1, 2, and 5, then injected with colchicine (for cytogenetic examination of the bone marrow cells) and sacrificed 2 to 4 hours later. Femoral bone marrow was harvested,** and thoracic and abdominal organs examined for gross changes.

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*(Unpublished) Cytotoxicity Pilot Study in Male Albino Rats with AC 243,997, performed by ToxiGenics, Inc., Decatur, IL, ToxiGenics Study No. 450-1283, August 30, 1983.

**Cytogenetic analysis of bone marrow preparations was conducted by Microbiological Associates, Bethesda, MD (see below).

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For the dominant lethal study, groups of 10 males each were gavaged with AC 243,997 at levels of 0 (DW), 250, 500 or 1000 mg/kg/day for 5 days, while a fifth group of 10 animals served as positive control, and received the mutagen, triethylenemelamine (TEM, 0.5 mg/kg), as a single ip injection on test day 5 of the study. Two days after the final treatment, males were caged with untreated females (1 male:2 females) for 1 week, then caged sequentially with fresh females weekly for an additional 7 weeks of matings.

Females of each week's matings were sacrificed 10 days following the final day of cohabitation, and uteri examined for implantation sites, viable fetuses, early fetal deaths (deciduomata), and late fetal deaths. Males were killed after completion of mating, and testes (with epididymides) fixed for later histological examination.

The following reproduction parameters were calculated for each treatment group for each mating week:

1. Male fertility index = $\frac{\text{Number of Males Siring at Least 1 Litter}}{\text{Total Number of Males Paired}} \times 100$

2. Female fertility index = $\frac{\text{Number of Pregnant Females}}{\text{Total Number of Females Paired}} \times 100$

3. Preimplantation loss = $\frac{\text{Number of Corpora Lutea} - \text{Number of Implantations}}{\text{Number of Corpora Lutea}} \times 100$

4. Mutation rate = $\frac{\text{Number of Deciduomata}}{\text{Number of Implantation Sites}} \times 100$

These four indices were statistically analyzed by Chi-Square; all other enumeration data (numbers of corpora lutea, implants, resorptions, and live fetuses) were analyzed by ANOVA, with any resulting differences further tested (if needed) by Tukey's or Scheffe's analysis for multiple comparisons.

E. Results - In the preliminary toxicity test, salivation was the only recurring clinical observation, especially in 1000 and 2000 mg/kg-dosed animals. Gross necropsy revealed no compound-related alterations, and evaluation of bone-marrow slides for mitotic index (MI) was also negative for significant differences ($p > 0.05$, one-way ANOVA) between test groups (MI ranging from 1.4% at 125 mg/kg to 2.3% at 2000 mg/kg) and the vehicle control (MI = 1.6%) [Report APPENDIX A].

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All males treated in the dominant lethal (main) assay survived the entire study period, without any apparent adverse clinical effects. Final body weights of treated animals were comparable to controls (548, 546 and 549 g for test groups 1, 2 and 3, compared to 544 g for vehicle control) and, except for a small left testis recorded in two animals (one control and one high-dose), no other gross pathologic alterations were found.

In contrast to definitively positive reproductive and mutagenic findings in positive control females mated to males treated with TEM (decreased implants in weeks 1 through 4; increased deciduomata, with concomitant decrease in viable fetuses in weeks 1-5) random significant fluctuations from control values were recorded for the three test groups (Report Tables 2 and 3, data extracts from which are summarized on the page following). These changes were considered by the authors as reflecting strain variance rather than compound-related, since they were sporadic, without consistent direction and unrelated to dose.

No reproductive effects different from control were found in test groups, and fertility was unaffected by imazapyr treatment.

Hence the authors concluded the test substance was not mutagenic in this assay under the conditions designed.

F. TB Evaluation - UNACCEPTABLE because of the following:

1. The authors have not demonstrated absorption from the gi tract, and transport of the test material to target tissue in sufficient concentrations to be effective. The highest dose tested in the main assay, 1000 mg/kg/day, was nontoxic, and did not affect fertility or any reproductive parameters. That the material was apparently not absorbed by the oral route to any great extent was confirmed in the preliminary toxicity trial, where mitotic indices in bone marrow cells were reportedly not impacted by the administration of double the high dose of the main assay, namely 2000 mg/kg/day. The positive control was effective, perhaps because it was given ip, assuring greater availability systemically.

2. Since the spermatogenic cycle in rats ranges up to 10 weeks, the investigators did not sample that portion of the cycle whose elements are probably the most important for risk assessment, namely, spermatogonia.

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Effect of Imazapyr on Reproductive
And Mutagenic Indices in Rats[1/]

Mating Week	Index	Dose Groups (mg/kg) 0	250	500	1000	TEM
1	PI[2/]	4.6	3.7	3.5	6.1	24.2**
	MR[3/]	8.0	2.4**	4.9	4.3	61.7**
2	PI	9.7	12.7	9.7	10.4	46.7**
	MR	5.6	4.4	6.1	4.7	60.5**
3	PI	14.1	8.6	7.6*	10.4	70.9**
	MR	9.1	6.4	6.6	2.4**	100.00**
4	PI	16.2	10.8	12.6	13.5	70.4**
	MR	8.3	2.6**	4.7	4.6	89.6**
5	PI	8.9	10.5	9.0	19.8**	9.0
	MR	10.5	3.7**	5.4*	8.4	46.9**
6	PI	6.6	16.8**	12.3*	15.5**	20.7**
	MR	10.4	4.6*	3.0**	4.7*	9.7
7	PI	14.9	22.7	14.9	14.0	15.5
	MR	8.0	6.4	5.8	2.4**	7.9
8	PI	11.6	12.4	12.5	13.3	14.0
	MR	11.5	6.0*	7.0	7.8	4.8**

*Statistically significant difference, $p < 0.05$.
**Statistically significant difference, $p < 0.01$.
1/Extracted from Tables 2 and 3 of the Final Report.
2/PI, preimplantation loss.
3/MR, mutation rate.

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TB recommends repeating the same assay (subacute treatment followed by 10 weeks of mating) but employing ip administration (following proper dose selection for this route), with the full cycle of mating weeks for rats, in order to a) assure distribution of test material in effective amounts to the target, male germinal epithelium, and b) to sample all stages of the cycle. Alternatively, the investigators may treat males for the full 10 weeks of the spermatogenic cycle, and sample but twice thereafter. This saves animals, while still satisfying the data requirement for this type of mutagenic assay.

Attachments

PAGES 107 THROUGH 122 HAVE BEEN REMOVED FROM THIS DOCUMENT. THOSE PAGES CONSIST OF REGISTRANT-SUBMITTED DATA.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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008729

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imazapyr - Tox Data Originally submitted under Accession Nos. 00151640 and 260000.
EPA Reg. No. 241-317.

Chemical: 352H
RD Record: 263,386
HED Project: 0-1151

FROM: Irving Mauer, Geneticist
Toxicology Branch-I (IRS)
Health Effects Division (H7509C)

6-28-90

TO: Robert J. Taylor/A.E. Barnes, PM 25,
Herbicide-Fungicide Branch
Registration Division (H7505C)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I (IRS)
Health Effects Division (H7509C)

7/9/90

Registrant: American Cyanamid, Princeton NJ

Request: Review and evaluate the following mutagenicity studies, originally submitted under

A) Accession No. 00151640:

(1) In Vitro Chromosomal Aberrations in Chinese Hamster Ovary Cells With AC-243,997, performed by Hazleton Labs. America, Vienna VA, Project No. 363-169, report dated February 3, 1984.

B) Under Accession No. 00260000:

(2) Cytotoxicity Pilot Study in Male Albino Rats With AC-243,997, performed by Toxigenics Inc., Decatur IL, Study No. 450-1283, report dated August 30, 1983.

(3) Dominant Lethal Assay in Male Albino Rats With AC-243,997, performed by Toxigenics Inc., Decatur IL, Project No. 450-1284, dated January 30, 1984.

008729 (122)

Taylor Barnes June 28, 1991

- Unscheduled DNA Synthesis Rat Hepatocyte Assay With AC-243,997, performed by Hazleton Labs America, Vienna VA, Project No. 362-[illegible], dated January 2[illegible]

E In Vitro Chromosomal Aberrations In Chinese Hamster Ovar Cells With AC-243,997, performed by Hazleton Labs., Project No. 362-[illegible], dated February 3, 1984.

N.B.: Duplicate of No. (1). /

'6 Mutagenicity Testing of AC-243,997 in the In Vitro CHO/HGPRT Mutation Assay, performed by American Cyanamid, GTOX, Vol. 4, No. [illegible], dated February 17, 1984.

IB Conclusions:

All of these studies have been previously reviewed and evaluated by the Agency (see attached memo: MAUER TO TAYLOR, date-stamped NOV. 22, 1989 --- HED/TB Doc. No. 007626 --- with accompanying DERs /

ATTACHMENT

REVIEWER 123

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

007626
008729

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TOX Chem No.: 352H
TB Project No.: 9-1736
RD Record Nos.: 247,679

MEMORANDUM

SUBJECT: Imazapyr (AC [illegible]) - Mutagenicity Data Submitted under Accession No. 260,000
EPA ID No. 241-273

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

11/03/89

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Karl P. Baetcke 11/07/89

Registrant: American Cyanamid (AC), Princeton, NJ

Request

Review and evaluate the following mutagenicity studies:

Study 1a. Cytotoxicity Pilot Study in Male Albino Rats with AC 243,997, performed by ToxiGenics Inc., Decatur, IL, Project No. 450-1283, Final Report dated August 30, 1983.

Study 1b. (DLT) Dominant Lethal Assay in Male Albino Rats with AC 243,997, performed by ToxiGenics, Inc., Decatur, IL, Project No. 450-1284, Final Report dated January 30, 1984.

Study 2. (UDS) Unscheduled DNA Synthesis Rat Hepatocyte Assay with AC 243,997, performed by Hazleton Laboratories America, Inc., Vienna, VA, Study No. 362-170, Final Report dated January 21, 1984.

Study 3. (CA) Vitro Chromosomal Aberrations in Chinese Hamster Ovary Cells with AC 243,997, performed by Hazleton Laboratories America, Inc., Vienna, VA, Study No. 362-169, Final Report dated February 3, 1984.

Study 4. (HGPRT) Mutagenicity Testing of AC 243,997 in the in vitro CHO/HGPRT Mutation Assay, performed at American Cyanamid, GTOX Volume 4, Number 1, Final Report dated February 17, 1984.

TB Conclusions

Study	Reported Results	TB Evaluation
1. DLT	Although reported as negative, major procedural and reporting deficiences exist	UNACCEPTABLE
2. UDS	Although reported as negative, major procedural and reporting deficiencies compromise the study	UNACCEPTABLE
3. CA	Negative up to a toxic dose, 5000 mcg/mL)	ACCEPTABLE
4. HGPRT	Negative up to toxic doses (5000 mcg/mL +)	ACCEPTABLE

Detailed reviews are appended to this memorandum.

Attachments (DERs)

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Classification: Core Minimum Data.

7. Teratology pilot study in albino rabbits with AC 243,977 (Toxigenic's study 450-1223; 8/2/83)

Test Material: AC 243,997; Lot #: AC 4361-97; 93% purity; light tan/beige powdery solid.

Groups of five bred female NZW rabbits were orally gavaged with 0, 250, 500, 1000 or 2000 mg/kg/day of test material during gestation days 6-18. Surviving animals were sacrificed at gestation day 28. Reproductive status of female rabbits was determined.

Results:

Two of the five 250 mg/kg/day group rabbits, 4 of the five 1000 mg/kg/day group rabbits, and all 5 of the 2000 mg/kg/day rabbits died before final sacrifice. Necropsy examination revealed stomach ulcers, and gastrointestinal lesions which can be considered compound-related.

Toxic signs and body weight data of surviving (day 28 of gestation) does were comparable between control and treated animals.

Corpora lutea, implantation sites, resorption sites and viable fetuses were comparable between control and treated surviving animals. Necropsy of surviving does revealed no compound-related lesions.

Conclusions:

Dosages used in this pilot study showed that at 1000 and 2000 mg/kg/day, maternal death resulted from exposure. The findings at 250 and 500 mg/kg/day showed that these doses are appropriate for the main teratology study.

Classification: Supplementary Data.

8. Bacterial/Microsome Reverse Mutation (Ames) Test on AC 243,997 (Cyanamid project # 0493; 6/17/83).

Caswell # [redacted] 003F

Test Material: AC 243,997; Lot #: AC4361-97; 93% purity positive controls: 2-nitrofluorene (2-NF), 9-aminoanthracene (2-AA), N-methyl-N-nitro-N-nitrosoguaridine (MNNG).

The assay employed both the plate and disc methods. S. typhimurium strains TA-98, TA-100, TA-1535, TA-1538 and E. coli WP-2-UVRA were used.

Dosages of test material were 0, 50, 158, 500, 1581 and 5000 (HDT) micrograms/plate both with and without S-9 metabolic activattion and 1000 micrograms/disc both with and without activation. The plate assay was conducted in triplicate twice to confirm initial results and the disc test was repeated to confirm initial results. Positive controls were used for each tester strain in the appropriate metabolic system at every assay.

Results:

The test material did not produce a mean number of revertants which was twice the number found on solvent control plates and no plate containing a disc impregnated with test material showed a ring of revertants around the disc. Positive controls showed the expected results which demonstrated the mutagenic assay was functional.

Conclusion: Caswell # 003F

AC 243,997 was not mutagenic in the Ames assay.

Classification: Acceptable. Caswell # 003F

9. Herbicide AC 243,997; the absorption, excretion, tissue residues and metabolism C^{14}-labeled AC 243,997 in the rat (AC Project # 0493; 6/6/83)

One group of 15 male Sprague-Dawley rats were used in the study. Three rats were control animals and 12 rats were treated animals. Each treated rat received a single oral dose of C^{14}-label AC 243,997 equal to 1.1 mg (33 microcuries). Based on body weight of the rats (approximately 225 grams), this dose was 4.4 mg/kg.

Three treated rats were sacrificed at days 1, 2, 5 and 8. One control rat was sacrificed on day 5 and two were sacrificed on day 8.

Urine and feces were collected daily. At each sacrifice interval, blood was collected and liver, kidney, muscle and fat were removed. All metabolism cages housing treated rats were rinsed with water and methanol and collected.

Results:

At day 1, 55.3% of the dose was excreted in the urine and 31.9% was excreted in the feces. Excretion was essentially complete by day 6 and was 95.1% of the total dose. Overall recovery of cage washes and excretion was 98.0% at day 8.

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Using TLC and mass spectrometry, the radiolabeled organic extracted material in feces and urine at day 1 was parent compound.

At day 1, kidney and liver cotnained 0.03 and 0.02 ppm, respectively and less than 0.01 ppm on day 8.

Muscle, fat and blood had less than 0.01 ppm at both days 1 and 8.

Conclusion:

The half-life of AC 243,997 was less than one day. No significant radiolabelled compound in the rat was present from tissue residues.

Classification: Core Minimum Data.

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TOXCHEM NO. 003F - AC 243,997 FILE LAST PRINTED: 08/13/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-1(a) and 83-2(a) Feeding/oncogenic-2 year Species: rat Bio/dynamics Inc. 84-2862; 4/6/88	AC 243,997 99.5% pure batch AC4866-862	410395-03	Oncogenic potential is inconclusive. Peer review is required for male brain tumors & female adrenal medullary tumors. NOEL = 5000 ppm (M&F) LEL = 10,000 ppm; effects in males are decreased survival & in females are increased incidences of thyroid cysts & extramedullary hematopoiesis of the spleen. Doses: 0, 1000, 5000 & 10,000 in Sprague-Dawley rats.		Supplementary 008426
83-1(a) and 83-2(b) Oncogenic-18 month Species: mice Bio/dynamics Inc. 86-3074; 11/3/88	AC 243,997 99.5% pure lot # Ac 4866-62	410395-04	Oncogenic potential is negative up to 10,000 ppm (HDT) (MTD). Historical control data are needed to establish NOEL for pulmonary edema in female mice. Doses: 0, 1000, 5000 & 10,000 ppm in diet of CD-1 mice at 65/sex/dose.		Supplementary 008426
83-1(b) Feeding-1 year Species: dog Tegeris Labs 86002; 5/29/87	AC 243,997 99.5% pure lot # AC 4866-62	410395-02	NOEL = 10,000 ppm (HDT). Doses: 0, 1000, 5000 & 10,000 ppm in diet in 6/sex/dose pure bred beagle dogs for one year.		Guideline 008426
83-3(a) Developmental Toxicity Study Species: rat Toxigenics Inc. 450-1222; 9/9/83	AC243997 Lot#4361-97 93% pure	251502	Levels tested by gavage in Sprague - Dawley strain - 0, 100, 300, and 1000 mg/kg (HDT). Maternal NOEL = 300 mg/kg/day Maternal LEL = 1000 mg/kg/day (salivation) Developmental NOEL > 1000 mg/kg (HDT)		Minimum 006997
83-3(a) Developmental Toxicity Study Species: rat Toxigenics Inc.. 450-1221; 8/2/83	AC4361-97 Lot#4361-97 93% pure	251502	Pilot Study - Levels tested by gavage in Sprague - Dawley strain: 0, 250, 500, 1000, and 2000 mg/kg/day on gestation days 6 - 15. Maternal NOEL < 250 mg/kg/day (1/5 salivation)		Supplementary 006997
83-3(b) Developmental Toxicity Study Species: rabbit Toxigenics Inc. 450-1224; 9/21/83	AC243997 Lot#4361-97	251502	Levels tested by gavage to NZW strain during 6 - 18 day of gestation - 0, 25, 100, and 400 mg/kg. Developmental NOEL > 400 mg/kg ; Maternal NOEL > 400 mg/kg		Minimum 006997
83-3(b) Developmental Toxicity Study Species: rabbit Toxigenics Inc. 450-1223	AC243997 Lot#4361-97 93% pure	251502	Pilot Study -- Levels tested by gavage: 0, 250, 500, 1000, and 2000 mg/kg/day on 6 - 18 day of gestation. Maternal NOEL = 500 mg/kg Maternal LEL = 1000 mg/kg (death)		Supplementary 006997

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-4 Reproduction-2 generation Species: rat Biosearch Inc. 42408; 5/6/87	AC 243,997 99.5% pure Lot # AC 4866-062	410395-05	NOEL = 10,000 ppm (HDT). Doses = 0, 1000, 5000 & 10,000 ppm in diet of Sprague-Dawley rats (25 M & 25 F) Fo.		Guideline 008426
82-2 Dermal-3 week Species: rabbit 11/11/85 AC4361-97	AL243997 Lot#AC4361-97 93% pure	251502	Levels tested in NZW strain for 6 hrs/day/5 days/wk for 3 weeks - 0, 100, 200 and 400 mg/kg. Systemic NOEL > 400 mg/kg (HDT) Dermal NOEL > 400 mg/kg (HDT)		Minimum 006997
82-2 Dermal-3 week Species: rabbit 9/15/83 187830123083	AC252925 Lot# AC4396-77		Levels tested in NZW strain for 6 hrs/day/5days/3weeks - 2ml/kg of sterile saline (0), 25%, 50% and 100% of test material (maybe equal to 250, 500, and 2000 mg/kg). Dermal NOEL < 25% ai (250 mg/kg). Systemic NOEL = 25% ai (250 mg/kg)		Minimum 006997
84-2(a) Mutagenic-Ames Species: bacteria 5/17/83	AC243997 Lot#AC4361-97 93% pure	251502	Levels tested: 0, 50, 158, 500, 1581, and 5000 ug/plate with and without activation. Negative		Acceptable 006997
84-2(b) Mut- Chrom aberr. in vivo Species: rat Toxigenics Inc. 450-1284; 1/30/84	Imazapyr Tech. 94%	260000	Although reported as negative for inducing dominant lethals at oral doses up to 1000 mg/kg/day for 5 days, insufficient absorption for any systemic effects, and the entire sperm cycle was not sampled.		Unacceptable 007626
84-2(b) Mutagenic-DNA repair test Species: mamnal cell(HPC/UDS) Hazleton 462-170; 1/26/84	Imazapyr Tech. 93%	260000	Although reported as negative for inducing unscheduled DNA synthesis (repair), major procedural and reporting deficiencies.		Unacceptable 007626
84-2(b) Mut- Chrom. aberr. in vitro Species: CHO cells Hazleton 462-164; 2/3/84	Imazapyr Tech. (93%)	260000	Negative for clastogenicity in chinese hamster ovary cells, tested up to toxic concentrations (5000 mcg/ml), with/without activation.		Acceptable 007626

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-4 Mutagenic-(HGPRT) Species: CHO cells American Cyanamid Co. 1493; 2/17/84	Imazapyr tech (93%)	260000	Negative for inducing forward mutation at the HGPRT locus of CHO cells, treated up to toxic concentrations (5000 mg/ml +), with/without metabolic activation.		Acceptable 007626
85-1 Metabolism Species: rat	AC243997	251502	Half - life is less than 24 hrs. No significant residue in rat tissue.		Minimum 006997
81-1 Acute oral LD50 Species: rat American Cyanamid Co. 484-175; 10/8/84	Arsenal (4 lb./gal. aq. conc.) (CL 243,997)	255338	No deaths. LD50 > 5000 mg/kg.	4	Minimum 004218
81-1 Acute oral LD50 Species: rat American Cyanamid Co. 483-24; 7/19/83	AC243997 #83-62 93% pure tech	252004	LD50 > 5000 mg/kg	3	Minimum 006998
81-1 Acute oral LD50 Species: rat American Cyanamid Co. 486-57; 1/22/87	Imazapyr 0.5%	400703-01	Dose: 5000 mg/kg. LD50 > 5000 mg/kg.	4	Guideline 005993
81-1 Acute oral LD50 Species: rat American Cyanamid Co. 487-3; 1/16/87	Imazethapyr 16.1%, Imazapyr 0.61%, Event Grass Growth Regulator	407634-02	LD50 > 5000 mg/kg. No signs of toxicity -- either males or females or both combined.	4	Guideline 007121
81-2 Acute Dermal LD50 Species: rabbit American Cyanamid Co. 484-175; 10/8/84	Arsenal (4 lb./gal. aq. conc.) (CL 243,997)	255338	One female died. LD50 > 2000 mg/kg.	3	Minimum 004218

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-2 Acute Dermal LD50 Species: rabbit American Cyanamid Co. 83-24; 7/19/83	AC243997 #83-62 93% pure tech	252004	LD50 > 2148 mg/kg	3	006998
81-2 Acute Dermal LD50 Species: rabbit American Cyanamid Co. 83-24; 7/19/83	AC243997 #83-62 93% pure tech	252004	LD50 > 2000 mg/kg	3	Minimum 006998
81-2 Acute Dermal LD50 Species: rabbit American Cyanamid Co. 86-57; 1/22/87	Imazapyr 0.5%	400703-01	Dose: - 2000 mg/kg. LD50 > 2000 mg/kg.	3	Guideline 005993
81-2 Acute Dermal LD50 Species: rabbit American Cyanamid Co. 87-3; 1/16/87	Imazethapyr 16.1%, Imazapyr 0.61%, Event Grass Growth Regulator	407634-02	LD50 > 2000 mg/kg for either males or females -- or both. No clinical signs of toxicity.	3	Guideline 007121
81-3 Acute Inhalation LC50 Species: rat Food and Drug Research Lab 7624; 9/1/83	AC243997 #83-62 93% pure tech	252004	LC50 > 1.3 mg/l (gravimetric); LC50 > 5.1 mg/l (nominal)	3	Minimum 006998
81-3 Acute Inhalation LC50 Species: rat Food and Drug Research Lab 7607; 9/1/83	AC252925 Arsenal formulation	252004	LC50 > 0.2 mg/l (gravimetric), LC50 > 5.0 mg/l (nominal)	3	Minimum 006998
81-3 Acute Inhalation LC50 Species: rat Bioresearch Inc. 87-5952; 2/2/88	Imazethapyr 16.1%, Imazapyr 0.61%, Event Grass Growth Regulator	408657-02	LC50 for 4 hrs > 3.24 mg/l air (imazapyr) and 3.07 mg/l air (imazethapyr). Maxium analytical concentration which could be attained.	3	Guideline 007121

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-4 Primary eye irritation Species: rabbit American Cyanamid Co. A84-175; 10/8/84	Arsenal (4 lb./gal. sq. conc.) (CL 243,997)	255338	No corneal opacity and no iritis; conjunctivitis at 1 hr. cleared at 24 hrs.	3	Minimum 004218
81-4 Primary eye irritation Species: rabbit American Cyanamid Co. A83-24; 7/19/83	AC243997 #83-62 93% pure tech	252004	Corneal opacity in 2/6 at 24 hrs conjunctivitis in 6/6 at 24 hrs and 0/6 at day 7.	4	Minimum 006998
81-4 Primary eye irritation Species: rabbit American Cyanamid Co. A83-24; 7/19/83	AC243997 #83-62 93% pure tech	252004	No corneal opacity in unwashed eyes. Corneal opacity in 4/6 in washed eyes which reversed at 72 hrs. Conjunctivitis in 6/6 at 72 hrs. which reversed by day 7.	3	Minimum 006998
81-4 Primary eye irritation Species: rabbit American Cyanamid Co. A86/57; 1/22/87	Imazapyr 0.5%	400703-01	48 hrs.: all irritation cleared.	3	Guideline 005993
81-4 Primary eye irritation Species: rabbit American Cyanamid Co. A87-3; 1/16/87	Imazethapyr 16.1%, Imazapyr 0.61%, Event Grass Growth Regulator	407634-02	Non-irritating. PIS = 0	4	Guideline 007121
81-5 Primary dermal irritation Species: rabbit American Cyanamid Co. A84-175; 10/8/84	Arsenal (4 lb./gal sq. conc.) (CL 243,997)	255338	Slight erythema and edema. PIS = 0.63.	4	Minimum 004218
81-5 Primary dermal irritation Species: rabbit American Cyanamid Co. A83-24; 7/19/83	AC243997 #83-62 93% pure tech	252004	PIS = 1.29	4	Minimum 006998

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORE GRADE/ DOCUMENT#
81-5 Primary dermal irritation Species: rabbit American Cyanamid Co. A83-24; 7/19/83	AC243997 #83-62 93% pure tech		PIS = 0.083	4	Minimum 006998
81-5 Primary dermal irritation Species: rabbit American Cyanamid Co. A86/57; 1/22/87	Imazapyr 0.5%	400703-01	No irritation	4	Guideline 005993
81-5 Primary dermal irritation Species: rabbit American Cyanamid Co. A87-3; 1/16/87	Imazethapyr 16.1%, Imazapyr 0.61%, Event Grass Growth Regulator	407634-02	Non-irritating. PIS = 0	4	Guideline 007121
81-6 Dermal sensitization Species: guinea pig	AC243997 Lot#AC4361-97 93% pure tech	252004	Negative. Tested at level of 0.3 g/pig once a week for 3 weeks for 6 hrs per application. Challenged at 14 days post treatment with a level of 0.3 g/pig.		Minimum 006998
81-6 Dermal sensitization Species: guinea pig 7/29/83 187A20123183	AC252925 Arsenal formulation Lot# AC4396-77	252004	Negative. Tested at 0.3 g/pig once a week for 3 weeks for 6 hrs/application. Challenged at 14 days post treatment with 0.3 g/pig.		Minimum 006998
81-6 Dermal sensitization Species: guinea pig Bioresearch Inc. 87-5951A; 1/18/88	Imazethapyr 16.1%, Imazapyr 0.61%, Event Grass Growth Regulator	408657-03			Guideline 007121

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