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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 128829. Imazapyr (Arsenal®) American Cyanamid's Response to Reviews of Chronic Rat and Mouse Studies and Requests of HED Peer Review Committee; ID #: 000241-00273

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ACTION REQUESTED: The Registrant, American Cyanamid Company, has submitted new information in response to the Toxicology Branch (TB-I) reviews of the chronic rat and mouse studies conducted on Imazapyr and the requests made by the HED Carcinogenicity Peer Review Committee. The new information consists of additional analyses of the non-neoplastic and neoplastic lesions in the chronic mouse and rat studies, as well as a new 13-week rat feeding study and a new pathology report of the brain tumor incidence in male rats.

TB-I has been requested to review the additional data and determine whether or not the two studies may be upgraded to an acceptable classification for regulatory purposes. In addition, TB-I is to summarize and present to the HED Carcinogenicity Peer Review Committee the Registrant's responses to their requests concerning the dose levels selected for the rat carcinogenicity study (the completion of the new rat subchronic feeding study) and the new microscopic examination of the brain tissue in males.

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CONCLUSIONS: TB-I has reviewed the additional data and has determined that both the mouse and rat oncogenicity studies are acceptable for regulatory purposes. They are both upgraded to Core Minimum. The following points summarize TB-I's responses to the Registrant's submitted additional data:

Mouse Study:

- The submission of historical control data for pulmonary edema in females has alleviated the concern over the incidences of these lesions. These are not considered to be related to treatment.

- A more detailed description of subscapular adrenal gland "cell reaction" was provided by the Registrant. No additional information is needed.

Rat Study:

- A complete statistical analysis for adrenal medullary neoplastic lesions in the female was provided. There was no statistical significance for this lesion in the treated groups when compared to the control group.

- The Registrant provided historical control data and additional arguments which demonstrated that the increased incidences of extramedullary hematopoiesis in the spleen and bilateral squamous cysts in the thyroid gland were not treatment-related. TB-I accepts their arguments and raises the NOEL for non-neoplastic lesions in females to 10,000 ppm (HDT).

- In response to the question concerning whether or not sufficiently high dose levels were used in the rat study (10,000 ppm), the Registrant provided an additional subchronic feeding study in the rat and a structure-activity argument based on other structurally related chemicals which were tested at the same dose levels and were accepted by the Agency as negative tests for carcinogenicity. TB-I has reviewed the subchronic feeding study and takes note of the structure-activity argument. Since this question was also asked by the HED Carcinogenicity Committee, this information will be presented to the Committee for their assessment.

- The Registrant re-cut and re-examined brains and brain slides from male rats in response to a request by the HED Carcinogenicity Peer Review Committee. TB-I has summarized the findings in a supplementary Data Evaluation Record (DER) attached to this memorandum. These findings will be presented to the Committee for their assessment. At that time, the Committee will determine a carcinogenicity classification for Imazapyr if it is possible to do so.

BACKGROUND:

HED'S Details of Deficiencies in Chronic Rat and Mouse Studies and the Results of the Peer Review of IMAZAPYR

1. In the Agency letter of August 19, 1991 from R. Taylor of Registration Division to B. Gingher of American Cyanamid, the following requests for additional data were made:

A. 18-Month Mouse Oncogenicity Study

1. Historical control data are needed to establish a NOEL for pulmonary edema, a non-neoplastic lesion in female mice.

2. Additionally, a more detailed description of subscapular adrenal gland "cell reaction" is needed.

3. This study can be upgraded to core-minimum after review of the needed data.

B. Combined Chronic Toxicity/Carcinogenicity Study - Rats

1. Complete statistical analysis for female adrenal medullary neoplastic lesions.

2. 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.

3. Information relative to the consideration of the MTD for the study needs to be submitted.

4. The study can be upgraded to core-minimum after review of the additional data.

2. Following the Carcinogenicity Peer Review of Imazapyr, the April 28, 1992 Agency letter from R. Taylor of Registration Division to M. Galley of American Cyanamid states:

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A. You must conduct a subchronic feeding study in the rat using a broad range of doses to facilitate a more reasoned evaluation of the adequacy of the dose levels used in the chronic rat study.

B. You must prepare and evaluate additional slides of brain tissue to further characterize the incidence and severity of brain tumors.

DETAILED REVIEW:

AMERICAN CYANAMID'S RESPONSE AND HED'S ASSESSMENT

A. In response to the deficiencies cited as needed to be addressed to upgrade the 18-month mouse oncogenicity study to core-minimum, American Cyanamid has provided the following data:

1. NOEL for Pulmonary Edema in Female Mice -

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. HED requested historical control data to address this concern. American Cyanamid has submitted historical control data from Bio/dynamics, Inc., the testing laboratory for the imazapyr mouse study. These 8 studies are from 18-month oncogenicity studies in CD-1 mice terminated between 1987-89 and are coded MO-4, MO-7, MO-8, MO-9, MO-11, MO-15, MO-16, and MO-19. For unscheduled deaths, the incidence of alveolar edema are 0/33, 6/25, 0/23, 0/18, 0/20, 3/39, 0/34, and 0/29. Therefore, the range of incidence is from 0 - 24% (6/25). The incidence in the imazapyr study are within the range of historical controls submitted by American Cyanamid.

The Registrant also notes that the incidence of two other pulmonary edematous lesions which are closely related to alveolar edema, "alveolar wall thickened: edema" and "perivascular edema", were not considered to be dose-related or compound-related by HED in the mouse study.

HED ASSESSMENT

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HED concludes that the NOEL for pulmonary edema is 10,000 ppm (HDT) in female mice and that the systemic NOEL for the mouse oncogenicity study is also 10,000 ppm.

2. Description of Subscapular Cell Reaction of the Adrenal Gland -

The Registrant provided a detailed description of subscapular cell reaction of the adrenal gland from the IARC publication entitled "Pathology of Tumors in Laboratory Animals, Vol.II, Tumors of the Mouse, page 475, Thelma B. Dunn (author), Tumors of the Adrenal Gland - Normal Structure, 1979".

HED ASSESSMENT

HED concludes that subscapular cell reaction of the adrenal gland has been sufficiently characterized by the Registrant and no additional information is needed.

3. Upgrading of the Mouse Oncogenicity Study -

HED ASSESSMENT

HED concludes that the 18-month mouse oncogenicity study can be upgraded from core-supplementary to core-minimum.

B. In response to the deficiencies cited as needed to be addressed to upgrade the 2-year chronic toxicity/carcinogenicity rat study to core-minimum, American Cyanamid has provided the following data:

1. Statistical Analyses of Adrenal Medullary Tumors in Female Rats -

Adrenal Medullary Tumors
in Female Rats

Dose (ppm)	0	1000	5000	10,000
No. Examined	65	65	65	65

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Adenoma	2	2	0	4
Carcinoma	0	1	0	2
Adenoma + Carcinoma	2	3	0	6

The Fisher Exact Test was used to compare each treatment group with the control. To use the Bonferroni significance levels with the Fisher Exact Test and a one tail statistic at a nominal $p < 0.05$, a p value less than 0.0170 has to be present in the comparison of the control to the treated group. By visual inspection, only the comparison of control vs. 10,000 ppm for adenomas and carcinomas combined may be statistically significant. The statistical analysis of this comparison yielded a one tail probability of 0.1367, which is greater than 0.0170. Therefore, there is no statistical significance in the occurrence of adrenal medullary tumors in treated groups in comparison to controls.

HED ASSESSMENT

There is no statistical significance in the comparison of control and treated groups for adrenal medullary tumors in female rats. Therefore, adrenal medullary tumors in treated female rats were not compound-related.

2. <u>Extramedullary Hematopoiesis in the Spleen and Bilateral Squamous Cysts in the Thyroid-</u>

The NOEL for extramedullary hematopoiesis of the spleen and bilateral (B)-squamous cysts in the thyroid gland in female rats was stated to be 5000 ppm by the Agency. In contrast, the Registrant has presented data, discussed below, which demonstrates that the NOEL for non-neoplastic lesions in female rats, specifically extramedullary hematopoiesis in the spleen and bilateral squamous cysts in the thyroid gland, is 10,000 ppm (HDT).

Extramedullary Hematopoiesis

In eight 24-month chronic rat historical control studies from Bio/dynamics terminated between 1985-88 and identified as AA, BB, CC, DD, EE, II, JJ, KK, the incidence of hematopoiesis of the spleen in female rats was 62/70, 15/76, 9/60, 66/69, 75/80, 11/50, 68/70, and 56/59, respectively. Thus the incidence ranged from 9/60 (15.0%) to 68/70 (97.1%). The incidence in the imazapyr 2-year rat study were 18.4, 26.1, 26.1, and 30.7% for the

control, low, mid, and high-dose groups, respectively. Therefore, the highest incidence of 30.7% in the high-dose group in the imazapyr study is within the range of historical controls for this lesion. Additional information provided by the Registrant to further support their position was that (1) there was no dose-related increase in the incidence of rats with high grades (4 and 5) for this microscopic change; (2) associative anemia was not observed hematologically in high-dose females at 3, 6, 12, 18, and 24 months; and (3) associative increased splenic weights (absolute and relative) were not seen in high-dose females in comparison to controls at 12-month interim sacrifice and terminal kill.

Bilateral (B) Squamous Cysts in the Thyroid Gland

The incidence of bilateral squamous cysts in female rats at the high-dose (12/65) was increased in comparison to controls (5/65), but the increase was not statistically significant. The Registrant states that both unilateral incidence as well as bilateral incidence should be combined in the evaluation of the incidence of squamous cysts. This combination was verified by the HED consulting pathologist, Dr. Brennecke, during the Imazapyr Peer Review. When combined Bilateral and Unilateral Squamous Cysts are examined in both sexes the total incidence are 35/65, 32/65, 22/65, and 26/65 in males for the control, low, mid, and high-dose groups, respectively and, in females, the total incidence are 30/65, 31/65, 32/65, and 36/65 for the control, low, mid, and high-dose groups, respectively.

HED ASSESSMENT

HED concludes that the NOEL for extramedullary hematopoiesis of the spleen and bilateral squamous cysts in female rats is 10,000 ppm (HDT).

3. <u>Information relative to the consideration of the MTD of the study needs to be considered.</u>

The Registrant has provided several supporting rationale to support the position that an MTD dose was used in the chronic rat study and that the chronic rat study should be acceptable.

a. A new 13-week rat feeding study at doses of 0, 15,000 and 20,000 ppm in 10/sex/dose Sprague-Dawley rats did not demonstrate any compound-related effects in the parameters evaluated. These parameters were clinical signs, mortality, body weight, food consumption,

ophthalmologic evaluations, hematology, clinical chemistries, urinalysis, gross pathology, organ weights, and histopathology. The doses tested in this new rat study were approximately 1.34 and 1.74 g/kg B.W./day, which were above the 500 - 640 mg/kg B.W./day in the chronic rat study. These findings indicate that the MTD for Imazapyr exceeds the limit dose of 20,000 ppm for testing nontoxic pesticides in chronic toxicity/carcinogenicity rat studies. The 500 - 640 mg/kg B.W./day level used in the 2-year imazapyr rat study is approximately 50% of the limit dose of 1.0 mg/kg B.W./day for oncogenicity studies.

b. The imazapyr 2-year rat study was initiated in 1984, three years before the Agency established the 1.0 mg/kg B.W./day limit dose (20,000 ppm in rats and 7,000 ppm in mice) for non-toxic pesticides in the Federal Register in December, 1987. The imidazolinone series which includes Imazapyr, Assert, Pursuit, and Scepter all had mouse and rat chronic studies conducted at 10,000 ppm.

c. Imazapyr (Arsenal) is structurally related to three other herbicides, imazethapyr (Pursuit), imazaquin (Scepter), and imazamethabenz-methyl (Assert). All three structurally related herbicides have been tested in rats and mice at 10,000 ppm and have been found to have negative carcinogenic potential by the Agency. These herbicides are also negative for mutagenic potential in a variety of genotoxic studies. Therefore, the data base for these related chemicals supports the lack of carcinogenic potential in imazapyr. The doses used to test for the carcinogenic potential in mice, i.e., 10,000 ppm, for all of the imidazolinone series (Arsenal, Pursuit, Scepter, and Assert) exceeds the dietary limit dose of 7,000 ppm for mouse oncogenicity studies.

HED ASSESSMENT

The final assessment of the acceptability of the doses of the imazapyr chronic rat study will be addressed by the HED Peer Review Committee

4. <u>Upgrading of the 2-Year Rat Chronic Toxicity/Carcinogenicity Study -</u>

HED ASSESSMENT

HED concludes that the 2-year rat chronic toxicity/carcinogenicity study can be upgraded from core-supplementary to core-minimum.

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C. American Cyanamid's Response to Agency's Carcinogenicity Peer Review of Imazapyr -

1. The Agency's request for the conduct of a subchronic feeding study in the rat using a broad range of doses to facilitate a more reasoned evaluation of the adequacy of the dose levels used in the chronic rat study.

The Registrant conducted a 13-week feeding study in Sprague-Dawley rats to address the concerns of the HED Peer Review Committee. Randomized groups of 10/sex/dose of young adult Sprague-Dawley rats were fed dietary doses of 0, 15,000, and 20,000 ppm of imazapyr technical for 13-weeks. Parameters measured were food consumption, body weight, ophthalmological evaluations, hematology, clinical chemistries, urinalysis, gross necropsy, organ weights and complete histopathology.

The NOEL is 20,000 ppm (HDT). All animals survived the study and there were no overt signs of toxicity in any animals during the study period which could be attributed to the test material. Treated rats gained more weight than controls during the study period although the differences for the 13-week study period were not statistically significant. Increased body weight gains were 6.1% and 4.7% for males in the 15,000 and 20,000 ppm groups, respectively, and were 2.5% and 5.3% for females in those same groups, respectively. Food consumption was increased or equal to controls in both sexes at both dose levels during the 13-week study period. Compound-intake averaged 1248 and 1695 mg/kg/day for males in the 15,000 and 20,000 ppm dose groups, respectively. For females, compound-intake averaged 1423 and 1784 for the 15,000 and 20,000 ppm groups,respectively. There were no compound-related ophthalmological findings at pre-test or at termination of the study. Results showed at termination, a diagnosis of unilateral focal retinopathy in 1 control female, 1 male and 1 female at 15,000 ppm, and 1 male at 20,000 ppm.

There were no statistically significant differences between means of hematological parameters of treated rats in comparison to controls for both sexes. Additionally, the mean values for hematological parameters for rats at both treated dose levels for both sexes were within the laboratory reference range of historical control values for those same parameters. Mean values for serum albumin were statistically significantly decreased in males at 20,000 ppm in comparison to controls. The mean albumin values were 4.9, 4.7, and 4.6* g/dL for the control, low-, and high-dose levels, respectively. The laboratory

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reference range for serum albumin is 2.9-6.0 g/dL. Since there was no increase in urinary protein at any level, no differences in serum albumin values in females, and the mean value of 4.6 g/dL in males was within the reference range of the laboratory, the statistically significant finding in males at 20,000 ppm was not considered compound-related. There were no other statistically significant differences between control and treated rats in any other clinical chemistry parameters. There were no statistically significant differences between control and treated urinalysis values for both sexes of rats. All findings were comparable between control and treated rats for all measured parameters.

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

2. The Agency's request to prepare and evaluate additional slides of brain tissue to further characterize the incidence and severity of brain tumors.

The Registrant has recut and re-examined brains and brain slides from male rats in the 2-year chronic rat study. This new pathology report involved a re-examination of the original 3 brain sections per animal as well as a new examination of an additional 6 brain sections per animal per dose group from the original paraffin blocks and a new examination of 5 sections of forebrain per animal from new paraffin blocks from control and high-dose male animals. Each of the 5 sections represented two separate pieces of tissues from right and left forebrain. Therefore, for this new histopathological evaluation, approximately 14 brain sections per animal were examined microscopically in the high-dose and control groups and 9 sections per animal were examined in the low- and mid-

dose groups.

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

HED ASSESSMENT

The HED Peer Review Committee will address the carcinogenic potential of imazapyr with respect to the brain tumors in male rats. Additionally, the Committee will determine the adequacy of the new 13-week feeding study in rats as a measure of the MTD of the 2-year chronic study.