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

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FEB 28 1985

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

PC 121301

SUBJECT: PP 2F2707/FAP 2H5355. Cyromazine (Larvadex®). Final Review of the Mouse Oncogenicity Study on Larvadex® (IRDC Study No. 382-082).

Tox Chem. No. 167B

TO: Timothy A. Gardner, Product Manager #17
Registration Division (TS-767)

FROM: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

Budd 2/27/85

THRU: William L. Burnam, Deputy Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Theodore M. Farber, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Theodore M. Farber 2/27/85

Background:

In a previous re-evaluation of the mouse oncogenicity study on CGA-72662 submitted by Ciba-Geigy in support of the proposed registration and tolerances for Larvadex® (cyromazine), Toxicology Branch (TB) classified this study as Core Supplementary pending submission by the registrant of additional information consisting of (1) historical control data for malignant lymphomas in male and female mice, and (2) a re-reading of microscopic slides for male and female mice with "histiocytic" lymphoma. See memorandum from Edwin R. Budd to Herb Harrison, dated August 15, 1984.

Ciba-Geigy submitted the requested historical control data in a submission dated September 27, 1984 (EPA Accession No. 254878). A re-reading of the pertinent microscopic slides was not performed, however, by Ciba-Geigy. Instead, it was stated that EPAs questions concerning the pathological interpretation of these slides had apparently been satisfactorily answered in a discussion between Dr. Hardisty of EPL (who performed the original reading of the slides) and Dr. Kasza (TB pathologist). At that time, Dr. Kasza was not available

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to confirm this "mutual understanding." See memorandum from Edwin R. Budd to Timothy A. Gardner, dated December 20, 1984. Dr. Kasza, however, later indicated that written commentary would be required from Dr. Hardisty before TB could complete its review of this study. See memorandum from Edwin R. Budd to Timothy A. Gardner, dated January 11, 1985. This commentary was later received by Dr. Kasza. See letter from Jerry F. Hardisty, D.V.M. to Dr. Louis Kasza, dated January 8, 1985 (attached as reference 2).

Conclusions:

TB has now completed its re-evaluation of the mouse oncogenicity study on Larvadex®. All additional information requested from the registrant has been submitted and found to be satisfactory. This oncogenic study is classified as Core Guidelines and is acceptable in fulfillment of the requirement for one (of two) oncogenic studies. TB has concluded that there is insufficient evidence to establish a causal relationship between the occurrence of malignant lymphomas in the male or female mice and the test material in this study. No oncogenic potential was observed in male or female mice in this study at dosage levels up to and including 3000 ppm of Larvadex® in the diet for two years.

Detailed Considerations:

Basic to TBs assessment of malignant lymphomas in this study was the decision to consider not only separate incidences of malignant lymphoma subtypes (i.e., lymphocytic and histiocytic subtypes separately), but also that it was pathologically appropriate to consider the total incidences of combined malignant lymphomas (i.e., lymphocytic and histiocytic subtypes together). This decision was supported by Dr. L. Kasza (TB pathologist - see ref. 1), Dr. J. Hardisty (EPL pathologist - see ref. 2) and Dr. D. Goodman (Pathco pathologist - see ref. 3 and later in this memorandum).

Accordingly, TB determined the incidences of lymphocytic lymphoma, histiocytic lymphoma and total combined malignant lymphomas in this study to be as follows.

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Incidence (and Percent) of Malignant Lymphomas (1)

	Dosage Level (ppm)			
	0	50	1000	3000
Males				
lymphocytic lymphoma	3/68 (4.4)	5/68 (7.4)	6/68 (8.8)	7/68 (10.3)
histiocytic lymphoma	2/68 (2.9)	2/68 (2.9)	2/68 (2.9)	5/68 (7.4)
total combined lymphoma	5/68 (7.4)	7/68 (10.3)	8/68 (11.8)	12/68 (17.6)
Females				
lymphocytic lymphoma	3/68 (4.4)	13/68 (19.1)	7/67 (10.4)	10/61 (16.4)
histiocytic lymphoma	11/68 (16.2)	4/68 (5.9)	4/67 (6.0)	1/61 (1.6)
total combined lymphoma	14/68 (20.6)	17/68 (25.0)	11/67 (16.4)	11/61 (18.0)

(1) Incidence data taken (with adaptions) from memorandum from Edwin R. Budd to Herb Harrison, dated August 16, 1984. (Original incidence count by Carolyn Gregario, June 28, 1984.)

Historical control data for malignant lymphomas in male and female mice, submitted by Ciba-Geigy, covered sixteen long-term studies conducted by IRDC on Charles River CD-1 mice from October, 1979 to December, 1981. A total of 1423 male mice and 1424 female mice were examined. The incidences of total combined malignant lymphomas in the male control animals in these studies ranged from 2.9 to 17.5% (mean = 9.4%) and in the female control animals from 8.3 to 40.0% (mean = 19.2%). In as much as different pathological criteria were used to classify the various subtypes of malignant lymphomas observed in historical control animals and in the Larvadex® mouse study, it was necessary to rely upon the expert judgement of a pathologist to determine the incidences of lymphocytic lymphoma and histiocytic lymphoma in the historical control data. This determination was made by Dr. D. Goodman (see ref. 3). Accordingly, the historical control data for malignant lymphomas in male and female mice is as follows.

Range and Mean of Malignant Lymphomas in Historical Control Mice

	Range (in %)	Mean (in %)
Males		
lymphocytic lymphoma	0.0 - 12.5	7.0
histiocytic lymphoma	0.0 - 6.1	2.5
total combined lymphoma	2.9 - 17.5	9.4
Females		
lymphocytic lymphoma	5.0 - 27.5	14.0
histiocytic lymphoma	0.0 - 24.0	5.2
total combined lymphoma	8.3 - 40.0	19.2

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Regarding the occurrence of lymphocytic lymphomas in the male mice, neither a statistically significant dose-response trend according to Cochran-Armitage and Peto statistics ($p = 0.13$) nor a statistically significant pair-wise exact test of control vs high dosage level ($p = 0.16$) was observed (see ref. 4). Furthermore, since the incidences observed at all test dosage levels were similar to the historical mean, it appeared that the control incidence may have been low. In addition, all observed incidences fell within the historical control range. TB concluded that the lymphocytic lymphomas observed in the male mice in this study were not likely to be related to the test material. Incidences of histiocytic lymphomas observed in control, low and mid dosage level male mice were all nearly identical to the historical control mean, but the 7.4% incidence in the high dosage level group exceeded the historical control upper limit of 6.1%. A dose-response trend test was of borderline significance ($p = 0.07$). A pair-wise test of control vs high dosage level ($p = 0.22$) was not statistically significant. In as much as no clear dose response relationship was observed across the treatment levels, the pair-wise test was not statistically significant, and the incremental increase in percent above the historical control upper limit was quite small (only 1.3%), TB concluded that there was insufficient evidence to support a causal relationship between histiocytic lymphomas in the male mice in this study and the test material. Incidences of total combined malignant lymphomas for the male mice in this study all fell within the historical control range or, in one case, was almost identical to the upper limit. Although a statistically significant dose-response trend ($p = 0.03$) was observed, the pair-wise test was only of borderline significance ($p = 0.06$). In view of the foregoing discussion and conclusions for lymphocytic and histiocytic lymphomas (considered separately) and the historical control data for combined lymphomas, the observed postive trend was not considered to be biologically meaningful.

With respect to lymphocytic lymphomas in female mice, incidences in all Larvadex® treated groups were greater than that of the concurrent control group. Incidences in all treated female groups, however, were similar to the historical control mean and well within the historical control range. It is likely that the 4.4% incidence in the concurrent control group was, in fact, low since it actually fell below the historical control lower limit of 5.0%. TB concluded that lymphocytic lymphomas observed in the Larvadex® treated female mice in this study were not likely to be related to the test material. Incidences of histiocytic lymphomas in all Larvadex® treated female groups were considerably lower than that in the concurrent control group. Incidences of total combined malignant lymphomas were similar in the concurrent control and all Larvadex® treated groups and in all instances were also similar to the historical control mean.

Considering the totality of all available experimental data and other pertinent information, TB concluded that the malignant lymphomas observed in the Larvadex® treated male and female mice in this study are not likely to be related to the administration of test material.

Second (Independent) Pathological Assessment:

With respect to the potential relationship between malignant lymphomas observed in the male and female mice in this study and the test material, TB considered it prudent to also seek a second and totally independent pathological assessment of the results of this study. Accordingly, TB authorized Dawn G. Goodman, V.M.D. (Pathco Inc. pathologist and Diplomate of the American College of Veterinary Pathologists) to perform such an assessment. In a review dated February 18, 1985 (see ref. 3), she presented the results of her findings. She stated that the pathologic terminology and groupings used in the Larvadex® study were consistent with current concepts and that, in her opinion, a re-evaluation of the microscopic slides (originally requested by TB) was not necessary. Her "SUMMARY AND CONCLUSIONS" are quoted below.

> "The EPA Larvadex study report and selected data from the 2-year oncogenicity study of Larvadex were reviewed to assess the significance of the incidence of lymphomas in both male and female CD-1 mice. The incidence of lymphomas in both male and female mice does not appear to be related to the administration of Larvadex."

Dr. Kasza's comments on Dr. Goodman's review and his own interpretation of the results of this study, with respect to malignant lymphomas in the male and female mice, are also attached (see ref. 1).

Attachments:

cc: Anne Barton (HED)
Herb Harrison (RD)

OPP:HED:TOX:E.BUDD:sb 2/26/85 X77395 #14-D1

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REFERENCES

1. Memorandum from Louis Kasza, D.V.M., Ph.D. to Edwin Budd, dated February 26, 1985.

2. Letter from Jerry F. Hardisty, D.V.M. to Dr. Louis Kasza, dated January 8, 1985.

3. "Review of the Incidence of Lymphomas in the Mouse Oncogenicity Study on Larvadex," by Dawn G. Goodman, V.M.D., Pathco Inc., dated February 18, 1985.

4. Memorandum from Bertram Litt to Edwin Budd, dated February 27, 1985.

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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Statistical Evaluation of Lymphoma Data from Mouse Oncogenicity Study of Larvadex®.

FROM: Bertram Litt, Leader Biostatistics Team
Mission Support Staff, Toxicology Branch
Hazard Evaluation Division (TS-769)

2/27/85

TO: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

The data for analysis are taken from the report of Dr. L. Kasza on this subject dated February 26, 1985. Dr. Kasza's table is reproduced below, modified by showing Larvadex dosage in ppm:

INCIDENCE OF LYMPHOMA IN THE LARVADEX 2-YEAR MOUSE ONCOGENICITY STUDY

	Group I (0 ppm)	Group II (50 ppm)	Group III (1,000 ppm)	Group IV (3,000 ppm)	Historical control mean	Historical control range
MALES						
Lymphocytic Lymphoma	3/68(4.4%)	5/68(7.3%)	6/68(8.8%)	7/68(10.3%)	7.0%	0-12.5%
Histiocytic Lymphoma	2/68(2.9%)	2/68(2.9%)	2/68(2.9%)	5/68(7.3%)	2.5%	0-6.1%
TOTAL	5/68(7.3%)	7/68(10.3%)	8/68(11.8%)	12/68(17.6%)	9.4%	2.9-17.5%
FEMALES						
Lymphocytic Lymphoma	3/68(4.4%)	12/68(17.6%)	7/67(10.4%)	10/61(16.4%)	14.0%	5.0-27.5%
Histiocytic Lymphoma	11/68(16.2%)	4/68(5.9%)	5/67(7.5%)	1/61(1.6%)	5.2%	0-24.0%
TOTAL	14/68(20.6%)	16/68(23.5%)	12/67(17.9%)	11/61(18.0%)	19.2%	8.3-40.0%

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The basic issues concern the probability that lymphocytic lymphoma and, more appropriately, the total incidence of lymphocytic and/or histiocytic lymphoma were observed in this study at unexpectedly high incidence rates. The Table indicates that the rates observed in female mice closely approximates the mean of the historical rate and is well within the range of historical controls. This is not the case for the males, we observed that the control rates for lymphocytic lymphoma and for the combined incidence is at the low end of the expected or historical control range and the high dose rates exceeded those reported for historical controls. As the only dose level which indicates a possibly meaningful increase in the high (3000 ppm) dose, we performed Fisher's exact test comparing control and high doses:

Lymphocytic lymphoma	$p \simeq 0.15$, N.S.
Histocytic lymphoma	$p \simeq 0.22$, N.S.
Histocytic and/or lymphocytic lymphoma	$p \simeq 0.06$, Borderline

Dose-response trend tests which utilize the dose and the response rate at each dose level are more sensitive indicators of the toxicological effect of the chemical. The Cochran-Armitage and Peto statistics indicates $p \simeq 0.13$, a possibly borderline trend for lymphocytic lymphoma; $p \simeq 0.07$, borderline finding for the histiocytic lymphoma; and $p \simeq .03$ statistically significant linear-trend for lymphocytic and/or histiocytic lymphoma.

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

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February 26, 1985

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

TO: Edwin Budd, Section Head
Review Section #II
Toxicology Branch/HED (TS-769)

FROM: Louis Kasza, D.V.M., Ph.D. LK
Branch Pathologist
Toxicology Branch/HED (TS-769)

SUBJECT: Evaluation of Lymphomas in Two Pathology Reports.
Mouse Oncogenicity Study on Larvadex.

In the Experimental Pathology Laboratories, Inc., pathology report, and in the Pathco Inc. pathology report, the distribution of malignant lymphomas in male and female mice was illustrated in the following table:

INCIDENCE OF LYMPHOMA IN THE LARVADEX 2-YEAR MOUSE ONCOGENICITY STUDY

	Group I	Group II	Group III	Group IV	Historical Control Mean	Historical Control Range
MALES	C	L	M	H		
Lymphocytic Lymphoma	3/68(4.4%)	5/68(7.3%)	6/68(8.8%)	7/68(10.3%)	7.0%	0-12.5%
Histiocytic Lymphoma	2/68(2.9%)	2/68(2.9%)	2/68(2.9%)	5/68(7.3%)	2.5%	0-6.1%
TOTAL	5/68(7.3%)	7/68(10.3%)	8/68(11.8%)	12/68(17.6%)	9.4%	2.9-17.5%
FEMALES						
Lymphocytic Lymphoma	3/68(4.4%)	12/68(17.6%)	7/67(10.4%)	10/61(16.4%)	14.0%	5.0-27.5%
Histiocytic Lymphoma	11/68(16.2%)	4/68(5.9%)	5/67(7.5%)	1/61(1.6%)	5.2%	0-24.0%
TOTAL	14/68(20.6%)	16/68(23.5%)	12/67(17.9%)	11/61(18.0%)	19.2%	8.3-40.0%

At the high dose level, there was an increased incidence both in lymphocytic and histiocytic lymphomas in male mice. Also, there were increased incidences in test groups of lymphocytic lymphomas, and there were decreased incidences in histiocytic lymphomas in test groups in female mice.

Two questions arose related to these differences. The first was: whether the lymphocytic and histiocytic lymphomas should be counted together or separately; the second was: what is the biological significance of the increased incidences of lymphomas in test groups, both in male and in female mice?

Two independent pathology laboratories were asked to study these questions. Their opinions are summarized as follows:

Experimental Pathology Laboratories, Inc.: "For statistical purposes, it is only appropriate to compare the incidence of each type (Malignant Lymphoma, Lymphocytic Type or Malignant Lymphoma, Histiocytic Type) and of all Malignant Lymphomas combined. It is inappropriate to further subclassify either of these at the light microscopic level without the use of immunohistochemistry to demonstrate antigenic differences."

"The incidences of lymphoma observed in this study are variable among treatment groups but are all within the normal range of variation expected for untreated female mice of this strain and age. Although the incidence of lymphocytic lymphoma is lower in the control female mice than the treated groups, it is not considered to be a treatment-related effect. There is an absence of a dose-related increase and all incidences are within the expected range."

Pathco, Inc.: "For the purposes, of evaluating a potential carcinogenic effect with tumors of the hematopoietic system as the endpoint, it is appropriate to combine all types of malignant lymphomas in mice (Board of Scientific Counselors, National Toxicology Program, 1984). Thus, since the combined incidence seen in the high dose males is within the historical control range (albeit at the upper end), this does not represent a positive carcinogenic response)."

SUMMARY AND CONCLUSION from Pathco, Inc., report: "The EPA Larvadex study report and selected data from the 2-year oncogenicity study of Larvadex were reviewed to assess the significance of the incidence of lymphomas in both male and female CD-1 mice. The incidence of lymphomas in both male and female mice does not appear to be related to the administration of Larvadex."

My conclusion is in agreement with both pathologists' opinions at Experimental Pathology Laboratories, Inc., and at Pathco, Inc., that the oncogenic potency of Larvadex in the submitted mouse experiment can not be established. Also, I would like to mention the high frequency of leukemia (lymphoma) in different strains of mice. In nine different laboratory strains of mice the spontaneous leukemia incidences vary from 19-100% (Pathology of

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Laboratory Animals, Volume II, page 1054). The high incidences of leukemia are probably due to the frequent contamination of mice with many different leukemia viruses, and very often, it is difficult to determine whether the test material is an inducer or a promoter. These are some of the reasons why the mouse is not the best laboratory animal for the evaluation of the effects of test material related to leukemia.

cc: T. Farber, Branch Chief
W. Burnam, Deputy Branch Chief
C. Gregorio

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EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
P. O. BOX 12766, RESEARCH TRIANGLE PARK, NC 27709 (919)544-8061

January 8, 1985

Dr. Louis Kasza
Environmental Protection Agency
Toxicology Branch
TS769C
Washington, D.C. 20460

Dear Dr. Kasza:

I am writing to clarify my interpretation of the incidence of Malignant Lymphoma in female mice in the Oncogenicity Study with CGA-72662 Technical in Albino Mice (IRDC Study No. 382-082) submitted to CIBA-GEIGY Corporation on May 31, 1982. As you stated during our phone conversation on January 8, 1985, the incidence of lymphoma in female mice were as follows:

	Group 1	Group 2	Group 3	Group 4
Malignant Lymphoma, Lymphocytic Type	3/68	13/68	7/67	10/67
Malignant Lymphoma, Histiocytic Type	11/68	4/68	4/67	1/67

I have enclosed a letter dated July 6, 1984, sent to Dr. Phelps at the CIBA-GEIGY Corporation. In this letter, I discussed my criteria and terminology used to classify malignant lymphoma in mice. In this letter, I stated that for statistical purposes it might be appropriate to compare the incidence of each type and all of the malignant lymphomas combined. I do not feel that it is appropriate to further subclassify either malignant lymphoma, lymphocytic type or malignant lymphoma, histiocytic type without the use of immunochemistry to demonstrate antigen differences.

The incidences of lymphoma observed in this study are variable among treatment groups but are all within the normal range of variation expected for untreated female mice of this strain and age. Although the incidence of lymphocytic lymphoma is lower in the control female mice than the treated groups, it is not considered to be a treatment-related effect. There is an absence of a dose-related increase and all incidences are within the expected range.

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I hope that this answers your concern regarding the incidence of malignant lymphoma in this study. If I may be of an other assistance, please do not hesitate to contact me.

Sincerely,

Jerry F. Hardisty DVM

JERRY F. HARDISTY, D.V.M.
Pathologist

JFH:amw

c: Campbell

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EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
P. O. BOX 12766, RESEARCH TRIANGLE PARK, NC 27709 (919) 598-1495

July 6, 1984

Dr. Warner Phelps
CIBA-GEIGY Corporation
P.O. Box 18300
Greensboro, NC 27419

Dear Dr. Phelps:

I am writing in reference to the classification system that I used for diagnosis of Malignant Lymphoma in the Oncogenicity Study with CGA-72662 Technical in Albino Mice (IRDC Study No. 382-082). I submitted my final report to CIBA-GEIGY on May 31, 1982.

The criteria and terminology that I used to classify Malignant Lymphoma in mice were those established by the Tumor Pathology Section of the National Cancer Institute for use in the NCI Carcinogenesis Bioassay Testing Program. The criteria described by Dunn[1] in which neoplasms of the reticular system were classified as stem cell leukemia, lymphosarcoma, granulocytic leukemia, reticulum-cell sarcoma type A, reticulum-cell sarcoma type B, plasmacytoma and mastocytoma were not being used by the NCI Bioassay Program at that time. The classification used by the NCI Bioassay Program separated malignant lymphoma into three categories. These were Malignant Lymphoma, Lymphocytic Type; Malignant Lymphoma, Mixed Cell Type and Malignant Lymphoma, Histiocytic Type which correspond to Dunn's classifications of lymphosarcoma, reticulum-cell sarcoma type B and reticulum-cell sarcoma type A respectively.

At this time, investigations[2,3] into the appropriate classification of these neoplasms had demonstrated that neoplasms which had previously been classified as reticulum-cell sarcoma type B or mixed cell lymphoma were actually B-cell lymphoid neoplasms derived from follicular center cells. The only type of neoplasm which was distinctly different and could be accurately separated at the light microscopic level was the histiocytic lymphoma or reticulum-cell sarcoma type A. For these reasons, during my evaluation of this study I diagnosed all lymphocytic neoplasms as Malignant Lymphoma, Lymphocytic Type (includes lymphosarcoma and reticulum-cell sarcoma type B) and all histiocytic neoplasms as Malignant Lymphoma, Histiocytic Type (corresponds to reticulum cell sarcoma type A).

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For statistical purposes, it is only appropriate to compare the incidence of each type (Malignant Lymphoma, Lymphocytic Type or Malignant Lymphoma, Histiocytic Type) and of all Malignant Lymphomas combined. It is inappropriate to further subclassify either of these at the light microscopic level without the use of immunohistochemistry to demonstrate antigenic differences.

If you have any further questions concerning this study, please do not hesitate to call me.

Sincerely,

Jerry F. Hardisty DVM

JERRY F. HARDISTY, D.V.M.
Pathologist

JFH:amw

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[1]Thelma B. Dunn, "Normal and Pathologic Anatomy of the Reticular Tissue in Laboratory Mice," JOURNAL OF THE NATIONAL CANCER INSTITUTE (Vol. 14, No. 6, June 1954).

[2]G. Della Porta, L. Chieco-Bianchi and N. Pennelli, "Tumours of the Haematopoietic System," PATHOLOGY OF TUMOURS IN LABORATORY ANIMALS, (Vol. II, 1979) p. 527-575.

[3]Paul K. Pattengale, M.D. and Charles H. Frith, D.V.M., Ph.D., "Immunomorphologic Classification of Spontaneous Lymphoid Cell Neoplasms Occurring in Female BALB/c Mice," JNCI, Vol. 70 (January 1983) p. 169-179.

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E. BUDD.

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PATHCO INC.

9200 Pavonia Court, Potomac, MD 20854
(301) 340-8714

P. O. Box 12796, Research Triangle Park, NC 27709
(919) 541-1607

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REVIEW OF THE INCIDENCE OF LYMPHOMAS IN THE MOUSE ONCOGENICITY STUDY ON LARVADEX

SUMMARY AND CONCLUSIONS

The EPA Larvadex study report and selected data from the 2-year oncogenicity study of Larvadex were reviewed to assess the significance of the incidence of lymphomas in both male and female CD-1 mice. The incidence of lymphomas in both male and female mice does not appear to be related to the administration of Larvadex.

INTRODUCTION

EPA reviewed the mouse oncogenicity study on Larvadex entitled "Oncogenicity Study with CGA-72662 in Albino Mice" International Research and Development Corporation (IRDC) Study No. 382-082, June 30, 1982 submitted by Ciba-Geigy. In its review, EPA determined there was a question regarding the significance of the incidence of lymphomas in both male and female CD-1 mice. PATHCO, Inc. was asked to provide an independent assessment and was supplied with the EPA Larvadex study report prepared by EPA reviewers along with the supporting documentation from the sponsor's final report cited above.

RESULTS AND DISCUSSION

In the sponsor's report, malignant lymphoma, lymphocytic and malignant lymphoma, histiocytic were reported in both male and female CD-1 mice. No other types of lymphoma were reported. In the summary tables, the incidences of these neoplasms were reported on a per tissue basis rather than on a per animal basis. In their review, EPA compiled an incidence table of the number of animals per group with each of these neoplasms. PATHCO, independently, also extracted this information from the individual animal histopathology reports (Table I). There were some minor disagreements in the incidences tabulated in Groups II and III in female mice; however, these have no impact on interpretation of the data.

In male mice, there was a slight increase in the incidence of lymphocytic lymphomas in treated mice compared to controls and in the incidence of histiocytic lymphomas. The combined incidence of histiocytic and lymphocytic lymphomas in male mice was increased in treated animals, with a dose related trend, compared to controls. In female mice, there was an increased incidence of lymphocytic lymphomas in treated animals when compared to controls, albeit not in a dose-related fashion. The incidence of histiocytic lymphomas was greater in controls than in any treatment group. The combined incidences of histiocytic and lymphocytic lymphomas in female mice were comparable between control and treated groups.

When evaluating the incidences of neoplasms in a study where there is an increase in treated animals compared to concurrent controls, it is useful to look at historical control data, including the range of variation in incidence between different control groups. If an observed tumor incidence in treated animals is within the historical control range, it can be considered to be within the biologic range of variation for that tumor. The National Toxicology Program uses this approach when evaluating carcinogenicity studies (Haseman, et al, 1984).

The historical control data on CD-1 mice for lymphoma in 2-year studies conducted at IRDC was part of the final report (Attachment 14) and was also included as part of "Ciba-Geigy's Response Document to EPA's August 8, 1984 Letter Requesting Additional Information on Cryomazine" (Larvadex). A summary of these data is included in Table I. For summary purposes, lymphoma, malignant (not otherwise specified); lymphoma, malignant, mixed; lymphoma, malignant, lymphocytic; lymphoma, malignant, thymic; and lymphoma, malignant, unclassified were grouped as lymphoma, lymphocytic. As can be seen from Table I, the incidences of histiocytic and lymphocytic lymphomas, either singly or combined fall within the range of variation for incidences observed in control CD-1 mice in 2-year studies except for histiocytic lymphomas in high dose males. In this group, however, the combined incidence of histiocytic and lymphocytic lymphoma is within the range of variation, albeit at the upper limit. For the purposes of evaluating a potential carcinogenic effect with tumors of the hematopoietic system as the endpoint, it is appropriate to combine all types of malignant lymphomas in mice (Board of Scientific Counselors, National Toxicology Program, 1984). Thus, since the combined

incidence seen in the high dose males is within the historical control range (albeit at the upper end), this does not represent a positive carcinogenic response.

Differences in survival rates between control and treated groups should also be considered when evaluating the significance of the observed incidences of a neoplasm. Comparable incidences between control and treatment groups may become significant if there is poor survival in the treated animals compared to controls, thus shortening the time available for tumors to develop in the treated animals. In this study, survival appears comparable amongst control and treatment groups in male mice. In females, survival was slightly decreased in the high dose compared to controls, but probably not enough to have any impact on interpretation of the study.

In summary, the incidences of lymphomas observed in this study do not appear to be related to the administration of Larvadex.

EPA, in their Larvadex study report, suggested that a re-evaluation of microscopic slides for mice with histiocytic lymphoma, to differentiate type A from type B, might be appropriate. Dr. J. Hardisty in his letters to Dr. L. Kasza dated January 8, 1985 and to Dr. W. Phelps dated July 6, 1984 described the criteria used when making the original diagnoses in the Larvadex study. Based on these criteria, the terminology and groupings used in the Larvadex study are consistent with current concepts and a re-evaluation of the microslides is not necessary.

Dawn G. Goodman, V.M.D.
Diplomate, American College
of Veterinary Pathologists
February 18, 1985

REFERENCES

Board of Scientific Counselors, National Toxicology Program. (1984). Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation. U.S. Department of Health and Human Services, Public Health Service, U.S. Government Printing Office: 1984-421-132:4726.

Haseman, J.K., Huff, J. and Boorman, G.A. (1984). Use of Historical Control Data in Carcinogenicity Studies in Rodents. Tox. Pathol. 12: 126-135.

TABLE I

INCIDENCE OF LYMPHOMA IN THE LARVADEX 2-YEAR MOUSE ONCOGENICITY STUDY

	Group I	Group II	Group III	Group IV	Historical control mean	Historical control range
MALES						
Lymphocytic Lymphoma	3/68(4.4%)	5/68(7.3%)	6/68(8.8%)	7/68(10.3%)	7.0%	0-12.5%
Histiocytic Lymphoma	2/68(2.9%)	2/68(2.9%)	2/68(2.9%)	5/68(7.3%)	2.5%	0-6.1%
TOTAL	5/68(7.3%)	7/68(10.3%)	8/68(11.8%)	12/68(17.6%)	9.4%	2.9-17.5%
FEMALES						
Lymphocytic Lymphoma	3/68(4.4%)	12/68(17.6%)	7/67(10.4%)	10/61(16.4%)	14.0%	5.0-27.5%
Histiocytic Lymphoma	11/68(16.2%)	4/68(5.9%)	5/67(7.5%)	1/61(1.6%)	5.2%	0-24.0%
TOTAL	14/68(20.6%)	16/68(23.5%)	12/67(17.9%)	11/61(18.0%)	19.2%	8.3-40.0%