UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 000959 Carcinogenicity 623A

DATE: January 7, 1981

SUBJECT: Oryzalin, TB/HED evaluation of 2-year feeding/oncogenicity study in the rat, submitted in support of requested tolerances at 0.05 ppm on certain fruits/nuts.

FROM: Dr. Mary L. Quaife, TB/HED MLQ 1/7/81
(TS-769)

TO: Mr. R. Taylor, PM
Registration Division (TS-767)

THRU: Mr. William Burnam, Acting Chief
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MLQ for RB Jaeger 1/29/81
WJ Burnam

PP No. 6F1859
EPA Registration Nos. 1471-96, 112

Eli Lilly and Company
Indianapolis, Indiana 46206

SUMMARY:

1. The two-year feeding study (Acc. Nos. 099517 and 099518) is judged acceptable to support tolerances (Core-minimum rating) with a "No-observed-effect level" of 300 ppm in the diet (= 15 mg/kg body weight/day) with respect to chronic (non-oncogenic) effects.

2. TB/HED requests the following information with respect to the two-year rat feeding study (Acc. Nos. 099517 and 099581) to enable TB/HED to evaluate the oncogenicity potential of oryzalin in the rat:

   a. Tables of incidence of each skin and each thyroid tumor for each of the following time-intervals of the study:
      15 months (test day 452),
      18 months (test day 543),
      20 months (test day 599, Study R-167; test day 598, Study R-177),
      and 22 months (test day 662) - as in Tables 30 and 32 (24 months).

      (Time intervals are chosen to correspond to those of Tables 3 and 4, which give survival data.)

   b. Resolution of apparent discrepancies in giving purity of test sample of oryzalin used:

      i. On p. 19 of this report, purity of lots X28607 and 9SY47 is given as 96.5 and 96.0%, respectively.

      ii. In Appendix C of the report, p. 761, purity of lots X28607 and 9SY47 is given as 97.4 and 98.9 mole %, respectively.

      iii. Report of rat reproduction study (Acc. No. 241742, Appendix C, p. 13), gives purity of lot X28607 (Sodyeco production lot 86343) as 99.0% (although a preliminary figure of 96.5% purity was used in protocols). P. 65 of the reproduction study report (Acc. No. 24172) states that the lot of test material used in the rat reproduction study was used in "chronic/oncogenic tests," also.

   c. Chemical identity of following impurities of test sample of oryzalin used in the 2-year rat study, noted on p. 759, Appendix C, Acc. No. 099518: [redacted]

NEW TOX STUDIES:

1. "The toxicological evaluation of oryzalin (compound 67019) given to Fischer 344 rats in the diet for two years," by James L. Carter et al., Toxicology Division, Eli Lilly & Co., Greenfield Indiana, March 1980, Acc.# 099517.

Test substance. Oryzalin* (compound 67019; EL-119; 3,5-dinitro-N^4,N^4-dipropyl-sulfanilamide, lots X28607 (96.5% pure, used until 6/21/78) and 9SY47 (96.0% pure, used until end of study). Assay for [redacted] of first sample, eleven times, and of second sample, six times (monthly), showed contents varying in random order from [redacted]

Procedure. Groups of 30 M and 30 F rats received in the diet either 0, 300, 900, or 2,700 ppm oryzalin (uncorrected for purity) for two years. Two identical experiments were conducted at the same time. Animals were 5 to 6 weeks old at start, and mean weights were approximately 140 (males) and 110 (females) g. Rats were kept individually in stainless steel, wire mesh cages. The basal diet was a natural food mash ration. Samples of control and test diets were assayed at start and after one or two weeks' aging. Every four months, new samples were assayed for content of test compound.

Animals were checked daily for dead or moribund ones and were observed carefully as to physical condition once a week. Rats were weighed and food consumption determined weekly. Two-year survivors were fasted overnight and blood samples collected for hematologic and clinical chemical testing. Packed cell volume, hemoglobin content, red cell count, total and differential white cell count, and erythrocyte morphology were determined on all rats; while the prothrombin time was measured on one-half of them. On all rats, the serum concentrations of glucose, urea nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase, and glutamic-pyruvic transaminase were each determined.

All animals dying on test, killed while moribund, or killed after two years on test were autopsied grossly "by qualified pathologists with experience in laboratory animal diseases and pathology." Following organs and tissues were collected and fixed in 10% formalin: Gross lesions, nodules, tumors, skin, mammary gland, salivary gland, lung, heart*, thyroid (parathyroid)*, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph node, liver*, skeletal muscle, thymus, pancreas, spleen*, kidney*, adrenal*, urinary bladder, prostate*, testis* ovary*, uterus*, cerebrum, cerebellum, brain stem, pituitary, eye, bone, and bone marrow. Organs starred were weighed. Fixed tissues were stained with hematoxylin and eosin. Evaluation was made by Pathologist ("with experience in diagnosis of lesions from rats on long-term toxicity studies") J. L. Carter, D.V.M., Ph.D. Other pathologists, including, Dr. W. Carlton of Purdue, were available for help. Criterion used in making diagnoses was "that of classical pathology." (Organs were weighed and relative organ weights calculated for two-year survivors, only.)

The following mean values were tested for statistical significance with the Dunnett's two-tailed "t" test: Weight gain; hematologic and blood chemical findings; terminal body weights; absolute and relative organ weights. Tumor and survival data were analyzed for linear trend using an arc sine transformation of proportions (Appendix I).

* Note: Appendix C lists the two as giving following assay results:
Lot X28607 96.5% pure and 97.4 Mole % (Assays of 2/26/80 and 1/22/80)
Lot 9SY47 96.0% pure and 98.9 Mole %

Results. Test diets, fresh and aged, gave assay results for oryzalin content that were very close to theoretical; one exception was a high assay, and new test diet was made when the high result was discovered.

Survival. Inspection of tables for survival at monthly intervals reveals no apparent difference between the sexes in either (replicate) study. No dose-related difference is shown for survival at 12 months, the rate being over 95% for combined sexes in all dose-groups of both studies. Same at 18 months exceeded 80%. However, survival at 24 months showed a "linear trend;" the "Z" statistic exceeded the allowable limits to show this (Appendix I) (in each replicate study). Respective survival rates were, control to highest dose, 76.7, 66.7, 60.0, and 55.0 (first study), and 73.3, 66.7, 61.7, and 51.7% (second study), according to table for "Z" statistic calculation (Appendix I).

We point out that these values differ, in 6/8 cases, from corresponding survival rates at term (Tables 3 and 4 of report) by at least one percent. However, the general trend is decreased survival at term - in Appendix or Tables.

According to Petitioner, there was no toxicologic or pathologic evidence to account for decreased survival rates of treated groups, which are dose-related.

Growth. During the first 12 months, males in either study showed no statistically significant growth retardation; while females in both studies gained one-fourth less than controls when ingesting 2,700 ppm oryzalin. However, both males (top-dose group in first study) and females (top-dose in both studies) had gained significantly less (females, one-third less) than corresponding controls by the end of the study. Body weight gain of mid-dose females was significantly decreased at intervals throughout both studies, also.

Food consumption. There was no evident effect of oryzalin ingestion on food consumption. Efficiency of food utilization was decreased, therefore, when growth was inhibited.

Clinical effects. No toxic effects were manifested overtly, according to the report. However, treated rats had yellow stained fur.

Hematologic values. High-dose males and high- and mid-dose females in both studies showed pronounced decreases in red blood cell values, and in most cases they differed statistically significantly from corresponding control values: Hematocrit, hemoglobin content, and red blood cell count. (Mean corpuscular volume was unaffected, but either mean corpuscular hemoglobin or mean corpuscular hemoglobin concentration was significantly decreased in high-dose males or females.) Prothrombin time was not affected by test compound.

There were statistically significantly increased mean leukocyte counts in some groups. Most groups had individual animals with greatly increased W.B.C.'s, including controls. According to Petitioner, "....alterations in leukocyte differential counts usually supported the chronic or neoplastic disease status in the respective individual animal."

Individual animals in all groups had marked changes in "erythocytic and/or leukocytic parameters."

Some conditions which caused the "erythrocytic and/or leukocytic" changes in individual animals are identified as malignant lymphoma, purulent endometritis, purulent prostatitis, and extramedullary hematopoiesis.

Blood chemistry values. Blood urea nitrogen (BUN) and serum glutamic-pyruvic transaminase (S-GPT) mean values were significantly increased in high-dose females of first study (R-167). BUN mean value was significantly increased in mid-dose females of second study (R-177). Statistically significantly lower mean value for creatinine was found in high-dose males of first study.

Some individual rat values for AP, SGPT, and BUN were increased, according to report, due to chronic diseases, such as progressive glomerulonephritis, bile duct hyperplasia in the liver, and malignant lymphoma. Total bilirubin was also increased, according to report, in some cases. Inspection of tables of individual values did not reveal serious deviations.

Organ weights. There were statistically significantly increased mean liver weights in mid-dose and high-dose males of first study, along with a significantly increased mean heart weight in high-dose males of the study. Adrenal mean weight of high-dose females was significantly increased. Mean weights of livers and of thyroids of high-dose males were significantly greater than those of corresponding controls in second study. In both studies, high-dose females showed approximately twice as high mean ovarian weights as those of corresponding controls; although variance around the mean was so great in each case as to preclude statistical significance of the differences occurring.

Relative liver and kidney weights in mid-dose and high-dose males and females of first study and of mid- and high-dose females of second study were increased significantly in dose-related fashion.

Following additional increased organ-weight/body-weight ratios, compared to controls, occurred: For high-dose males of first study, heart; for high-dose females of first study, heart, spleen, adrenals; for high-dose males of second study, liver, thyroids, testes; and for high-dose females of second study, heart and adrenals.

According to the report, a cause for increased liver weights in mid- and high-dose groups is "not evident," but it is not due to any condition evident on microscopic examination. Increased thyroid weights in high-dose males in second study (R-177) are ascribed to "antithyroid action" of oryzalin. Other weight changes are not discussed.

Nor does this reviewer find noteworthy explanation of the organ weight changes, enumerated above, which can be related to ingestion of test compound.

Pathologic findings. No explanation, toxicologic or pathologic, is available for decreased survival rates in male and female rats of all test groups, according to report.

A myeloproliferative disease was diagnosed as malignant lymphoma due to lymphoid differentiation of many of the neoplastic cells; it has been described as a mononuclear cell leukemia (Coleman, G. L. et al.: Pathological Changes during Aging in Barrier-reared Fischer 344 Male Rats. J. Gerontol. 32, 258-78 (1977)) and occurs in aged Fischer 344 rats, report says.

The pathologist's summary describes an increase in the "total number of benign skin and adnexal tummors....in all dose groups," However, our Pathologist, Dr. L. Kasza, rejects this grouping as not being a sound one (told to M. Quaife, In discussion, 12/4/80).

The total number of thyroid follicular neoplasms for both studies shows dose-related increases (control, low-, mid-, and high-dose groups, respectively): Female, 1/60, 1/60, 3/60, and 9/60; male, 1/60, 6/60, 5/60, and 7/60. When the total is combined over sexes, and this is tested by "Z-statistic," for a "linear trend in tumorigenesis," a "Z-statistic" value (= 3.493) which exceeds a range of from -2.48 to +2.48 is calculated; this value is significant. Thus, there is an increase in total thyroid follicular cell tumors in all dose-groups. (Cf. Appendix I.) (We note that only 6 tumors for high-dose males are listed in Appendix I, Table 5; however, the tables in the text (p. 159 and p. 170, Tables 30 and 32) list 7 for them.)

This increased incidence of thyroid follicular neoplasms in the treated groups is ascribed by the study report (p. 25) to the "antithyroid activity of a sulfonamide with a para-substituted aminobenzene grouping, with or without aliphatic substitution of the amino nitrogen." This is believed due to increased pituitary thyrotropin release secondary to a deficiency of circulating thyroid hormone. Report claims sulfonamides have antithyroid actions in rats, but (they) "are not clinically goitrogenic in man."*

In further statistical comparison (in Appendix I), female rats with benign tumors were significantly increased in all treated groups (Z = 3.407). However, female rats with malignant tumors and male rats with either benign or malignant tumors did not vary significantly with treatment (testicular tumors were not counted; evidently, they are very common in aged male rats of this strain (IARC monograph, Supplement 2, "Long-term and short-term screen assays for carcinogens: A critical appraisal," IARC, Lyon, 1980, p. 73)).

Twenty-four-month (but not 12-month) survival was found to be significantly decreased in males and females (Appendix I). For males, control to high-dose, in order, 24-month survival was 46, 40, 36, and 33 rats, each, out of 60, and for females, correspondingly, it was 44, 40, 37, and 31, each out of 60. Corresponding "Z-statistics" are -2.642 and -2.530; both are significant.

In further analysis (Appendix I), omission of skin and mammary tumors, results in decrease in benign tumor-bearing males, with no effect of treatment on females. The incidence of benign mammary tumors is increased (10, 21, 37, and 29, each out of 60) in female rats, controls on up, "Z statistic" being 4.554, which is significant.

Top-dose females (Appendix I) were classified as to mammary tumor positive or negative and follicular (thyroid) cell positive (hyperplasia, adenoma, carcinoma) or negative. However follicular cell problems were as prevalent in non- (9 positive, 21 negative) as in positive-mammary-tumor-bearing animals (10 positive, 20 negative.

* Refs. include: McLaren, E. H. and Alexander, W. D.: Goitrogens. Clin. End. Metabolism. 8, 129-44 (1979); MacKenzie, C. G. and McKenzie, J. B.: Effect of Sulfonamides and Thioureas on the Thyroid Gland and Basal Metabolism. Endocrinology 32, 185-209,(1943); (contd. on next page)

In males, there is a significant (Z = -2.436) decrease in pituitary tumors (actual numbers, control to high-dose males, in order, being 16, 17, 5, and 9, each out of 60).

Discussion. Petitioner's own analysis of the data shows that oryzalin is oncogenic to the Fischer 344 rat, having caused increased, dose-related incidence of thyroid follicular cell tumors in males and females and increased incidence of benign mammary tumors in females. The occurrence of the benign mammary tumors in females is apparently independent of the occurrence of the follicular thyroid tumors. In addition, two-year survival was decreased, significantly, in dose-related fashion, in both males and females.

Although Petitioner speaks of increased numbers of skin tumors, he does not provide statistical analysis of their occurrence for degree of significance. Because differences in occurrence of such tumors at two years could be masked by differences in survival among controls and treated rats, we ask Petitioner to provide further information on this point.

Specifically, Petitioner is asked to provide tables of incidence of all skin and all thyroid tumors at each of the following time-intervals of the study: 15 months (test day 452)
18 months (test day 543)
20 months (test day 599, Study R-167; test day 598, Study R-177)
and 22 months (test day 662)
The time intervals are chosen to correspond to those of Tables 3 and 4 which give survival data; so that we can calculate incidence in actual survivors, if we wish to do so. (The request for these data is based on discussions of the reviewer with Dr. L. Kasza, TB/HED Pathologist.) The tables will give incidence figures for all individual skin and thyroid tumors - at requested time-intervals - exactly like the tables on neoplastic lesions (Table 30, pp. 156 and 159, and Table 32, pp. pp. 168 and 170) do.

Petitioner's figures for tumor incidence in various parts of the report do not agree with each other. An example is given in second paragraph of the preceding page. For another example: Summing total incidence of benign mammary tumors from Table 30, p. 159, and Table 32, p. 170, gives, in order of control to high-dose groups, in females: 10, 21, 38, and 31, each out of 60; these comprise total incidence of mammary gland adenomas and fibroadenomas. Yet in Appendix I, Table 4, incidence for two top dose-groups is given as 37 and 29, respectively.

It appears, by inspection, that these slight differences in tumor incidences - in various parts of the report - do not affect the conclusions as to statistical significance of their occurrence in treated rats to an extent which will affect conclusions as to the biological effect of oryzalin ingestion on tumor production in these rats.

* Refs contd. from previous page: Astwood, E. B.: In: Pharmacological Basis of Therapeutics. Goodman, L. S. and Gilman, A. eds., 1483-7, Macmillan, New York, 1970; Swarm, R. L., Roberts, G. K. S., Levy, A. C., Hines, L. R.: Observations on the Thyroid Gland in Rats Following the Administration of Sulfamethoxazole and Trimethoprim. Toxicol. Applied Pharmacol. 24, 351-63 (1973); Astwood, E. B., Sullivan, J., Bissel, A., and Tyslowitz, R.: Action of Certain Sulfonamides and of Thiourea upon the Function of the Thyroid Gland of the Rat. Endocrinology 32, 210-25 (1943); Furth, J.: Pituitary Cybernetics and Neoplasia. Harvey Lect.: 63, 47-71 (1969).

There are discrepancies in the chemical composition of the test compound given in varying places.

On p. 19 of this report, purity of lots X28607 and 9SY47 is given as 96.5 and 96.0%, respectively.

In Appendix C of this report, p. 761, however, the respective contents of oryzalin are listed as 97.4 and 98.9 "mole percent" for lot X28607 (Soyleco Corp.) and lot 9SY47 (Eli Lilly & Co.).

Appendix C, p. 759, lists [redacted] in these two preparations, as follows:

[redacted]

The impurities are not identified, and they should be. Petitioner is asked to do so.

Petitioner should also clear up the discrepancies between reported values for content of oryzalin and is asked to do so.

We note that it is very important that the test compound in this chronic/oncogenicity study be entirely representative of the grades produced commercially; else the toxicity of the latter is not truly being evaluated.

In addition, we note that the report of the reproduction study in rats, carried out on technical oryzalin (Acc. No. 241742), states (on p. 65) that the lot of material used in the reproduction study was also that used in chronic/oncogenic tests. (Whether that means the rat chronic/oncogenic test is not specified there.)

Acc. No. 241742, Appendix C, gives 99.0% purity for "lot X-28607," Soyleco production lot 86343, while stating (on p. 134, Appendix C) that a preliminary figure used in the protocols was 96.5% - for degree of purity.

Again, we ask Petitioner to tell whether that batch (lot X-28607, Soyleco production lot 86343) was used in this rat chronic/oncogenic study (Accession No. 099518), and, if so, to resolve the discrepancy between the 99.0% pure value given in the reproduction study report and the two values given in the chronic/oncogenic rat study report (cf. second and third paragraphs, this page.

Summary, oncogenicity study. Pending answering questions respecting occurrence of all individual skin tumors and thyroid tumors seen in the rats in this study at the specificied time-intervals, the study cannot be evaluated, finally, as to oncogenic potential of oryzalin in the rat, nor can a CORE rating be assigned.

Summary, chronic study. The study is judged to provide, with respect to chronic effects, a "No-observed-effect-level," though there was statistically significant, dose-related decrease in survival in the test rats (24-month survival) - cf. Appendix I, p. 769-70. Hoever, according to Mr. B. Litt, EPA statistician, effect is not signif. at 300 ppm in the diet (= 15 mg/kg body weight/day). There was pronounced growth inhibition in rats on the top dose and some in mid-level rats, as well.

Also, such parameters as red blood cell count, hemoglobin concentration, and hematocrit were significantly decreased in high- and mid-dose groups. Also, relative and absolute liver weights were increased in high- and mid-dose rats (mean values of); although adverse effects were not seen on microscopic examination of livers from these rats.

Thus for rat chronic feeding (but not for oncogenicity - not yet evaluated), the NOEL is 300 ppm in diet or 15 mg/kg body weight/day, and the lowest effect level is 900 ppm (45 mg/kg BW/day), effects including decreased red cell count, hemoglobin content, and hematocrat and increased absolute and relative liver weight; some growth inhibition

Rat chronic (2-year) feeding study is rated Core-minimum.

1. Only terminal hematologic and blood chemical examinations (or assays) were made.