UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: January 10, 1980

SUBJECT: Review of an 18-month study of the carcinogenicity and chronic toxicity of Avadex in hamsters. EPA Reg. No. 524-119, 524-151 Acc. No.241087, 241088, 241089 CAS No. ~~299~~ 299

FROM: Roger Gardner
Toxicologist R. Gardner WSW
Toxicology Branch/HED (TS-769)

TO: Robert J. Taylor
Product Manager (25)
RD (TS-767)

M. Adrian Gross, Chief William S. Butler for M. Adrian Gross
Toxicology Branch/HED (TS-769)

Action Requested

Review of an 18-month carcinogenicity and chronic toxicity study of Avadex in hamsters.

Recommendations

Attached is my review of the 18-month hamster study with Avadex.

The study provides suggestive evidence that Avadex is carcinogenic in male and female hamsters receiving diets containing 600 or 2000 ppm Avadex. No effects were observed in hamsters fed diets containing 200 ppm Avadex.

The high mortality observed in all control and treatment groups makes clear interpretation of results difficult, but the results appear to be consistent with those of three other studies (see references in the attached review) in other rodent species.

Since Avadex is under RPAR review, there is no further action to be recommended.

cc: Richard Troast, SPRD (TS-791)

EPA Form 1320-6 (Rev. 3-76)

Data Evaluation Record

(1) Compound: Avadex (diallate)

(2) Formulation: CP-15336 (Avadex), purity unspecified. Provided by Monsanto Co. St. Louis, Mo.

(3) Citation:

An eighteen month oral toxicity/carcinogenicity study of CP-15336 in hamsters. Monsanto September 14, 1979. R.D. Number 253. Special Report MSL-0871. Volumes 1, 2, and 3.

(4) Reviewed by: Roger Gardner Signature: Roger Gardner
Toxicologist Date: 1-10-1980
Toxicologist Branch/HED (TS-769) 557-1511

(5) Approved by:

(6) Test type: Chronic toxicity/carcinogenicity

(7) Conclusion: The study provides suggestive evidence that Avdex is carcinogenic in Syrian Golden hamsters. The high mortality makes a clear interpretation of the results difficult, but the results suggest the increased incidences of skin, liver, and thyroid tumors in male and femal hamsters is associated with Avadex treatment.

(8) Materials and methods:
(a) Test substance: see item (2) above.

(b) Animals: Seven to eight week old Syrian Golden hamsters (Charles River, Lakeview, Newfield, N.J.) of both sexes were dividied into two control groups (IA and IB), and three treatment groups (III, IV and V). Each group contained 50 of each sex. Males averaged 86.9 g (69-104) in weight while females averaged 88.1 g (69-105) per individual. Animals were housed one per cage.

(c) Dosing: Groups IA and IB received diets containing no Avadex while groups II, III, and IV received diets containing 200, 600, and 2000 ppm. Food was available to the animals ad libidum, and a fresh supply was provided weekly. Male hamsters received test diets for 548 days while female hamsters got test diets for 507 days.

(d) Parameters: Animals were examined daily for mortality and gross signs of toxicological effects for the first 9 months of the study and twice each day during the last half of the study. Detailed physical examinations were conducted weekly for signs of local or systemic toxicological or pharmacological effects. Tissue masses were palpated weekly.

Ophthalmoscopic examinations were done pretest and at 6 and 12 months. This examination was also done near termination of the test.

Body weights and food consumption wee measured weekly from one week prior to beginning through the 14th week of treatment, biweekly from the 14th to the 16th weeks, and monthly thereafter. Compound consumption was calculated from food consumption data.

Laboratory studies were done at 45 days, 3, 6, and 12 months on 10 hamsters of each sex in groups IA, IB, and IV (controls and highest dose groups). At 17 months all survivng female hamsters were tested while 10 male hamsters (if available) from each group were tested. Blood was collected from the retro orbital sinus. The tests done are as follows:

Hematology
- hemoglobin
- hematocrit
- erythrocyte count
- clotting time
- total and differential leukocytes
- erythrocyte morphology

Clinical Chemistry
- serum glutamic pyruvic transaminase
- alkaline phosphatase
- blood urea nitrogen
- fasting blood glucose

Urinalysis
- appearance
- pH
- protein
- glucose
- ketone
- biirubin
- occult blood
- specific gravity

Gross necropsy was done on all animals including those dying spontaneously, killed in extremis, or sacrificed at the end of the test. Organs were weighed, organ/dody weight ratios and organ/brain weight ratios were calculated. These organs are as follows:

adrenal	ovary
brain	pituitary
kidney	spleen
liver	testis

These parameters were measured for all surviving females (at 17 month) and 10 males from each group (it availabel) at 18 month.

Histological examinations of tissues (see list below) from 10 hamsters of each sex in each group (if possible) and all tissues from those animals with black dermal lesions were done. Black dermal lesions, tissue masses, and other lesions were also examined histologically.

Tissues preserved

- adrenal (2)
- bone marrow (sternal)
- brain (2 sections)
- eye (2)
- gall bladder
- heart (with coronary vessels)
- Intestine:
  - colon
  - duodenum
  - ileum
- kidney (2)
- liver (2 sections)
- lung (2 sections)
- lymph node (mesenteric)
- mammary gland (right inguinal)
- ovary (2)
- pancreas
- pituitary
- prostate
- salivary gland (submaxillary)
- skeletal muscle (right femoral)
- skin (right inguinal)
- spinal cord (cervical)
- spleen
- stomach
- testis (2)
- thyroid
- urinary bladder
- uterus
- all gross lesions and tissue masses.

The eyes and testes were fixed in Bouin's solution while all other organs were fixed in 10% neutral buffered formalin. Hematoxylin-eosin stain was used.

(e) Statistics: Hematology and clinical chemistry values from control groups and treatment groups were compared by the F-test and student's t test. When the F-test showed significantly different variances a modified t test was done using Cochran's approximation (t'). Comparison of body weight, organ weights, and organ/body weight ratios as well as food consumption data from control and treatment groups were analysed by Dunnet's procedure (references are cited in Appendix A of the report).

(9) Reported Results: Table 1 shows the number of animals that died by the end of the study. When a group had only 5 hamsters of one sex left, all the animals of that sex in all groups were sacrificed. As a result female hamsters were sacrificed at 17 months. There were two non-treatment related deaths during the first week of the study, and these animals were replaced.

No effects on body weight or behavior were observed. Mean hemoglobin and hematocrit values were depressed in the 600 and 2000 ppm group males (See Table 2). The erythrocyte count was depressed in males getting the 2000 ppm diet. None of the observed difference were found to be statistically significant. A significant elevation in blood urea nitrogen was observed in 2000 ppm males (61.3 mg/dl $\pm$ 47.1 compared with 24.8 mg/dl $\pm$ 8.9, $p<0.05$). The investigators did not associate this effect with Avadex treatment since they saw no histopathology in the kidneys.

No ophthalmic effects were observed.

The only treatment related gross lesions found were cutaneous pigmented masses which occurred in the mid and high-dose groups of both sexes (7 of 99 and 24 of 100, respectively, none were seen in 199 untreated hamsters).

No effects were observed on organ weights or weight ratios.

The investigators concluded that there were dose related incidences of benign and malignant melanomas, papillary adenocarcinomas of the thyroid, and neoplastic nodules in the liver of male and female hamsters receiving diets of 600 or 2000 ppm. No significant increase in neoplasia in animals receiving the 200 ppm diet was seen.

Table 3 summarizes the incidence of lesions of the skin and Table 4 shows the incidence data for appropriate hepatic lesions. The incidence of papillary adenocarcinoma in the thyroids of animals from groups IA, IB, II, III, and IV were 0/13, 0/15, 0/7, 1/11, 2/27, respectively. These results are for both sexes combined. Because of the uncommon occurrence of such thyroid lesions in hamsters, and since the tumors were found at the two higher dose levels, the investigators consider the results suggestive of a comopound related effect. No summary of total tumors was presented nor were statistical analyses of tumor incidence data perfromed.

The highest dietary concentration without chronic or carcinogenic effects is stated as 200 ppm. These kinds of effects were observed in hamsters receiving diets containing 600 and 2000 ppm Avadex.

(10) Discussion: The value of this study is limited by high mortality (75% in males and 86% in females) during the study. The number of observations of many of the tissues was so small (less than 10 per group) that statistical comparisons cannot be made. The authors of the report characterize evidence for compound related effects as suggestive. The results of this study are apparently consistant with the three other studies of Avadex carcinogenicity (see references listed below).

(11) References: Innes, J.R.M., B.M. Ulland, M.O. Valerio, L. Petrucelli, L. Fishbein, E.R. Hart, A.J. Pollotta, R.R. Bates, H.L. Falk, J.J. Gart, M. Klein, I. Mitchell, and J. Peters. 1969. Bioassay of pesticides and industrial chemicals for tumorigencity in mice. A preliminary note. J. Nat. Cancer Instit. 42:1101.

Ulland, B., E.K. Weisburger, and J.H. Weisburger. 1973. Chronic toxicity and carcinogenicity of industrial chemicals and pesticides. Toxicol. Appl. Pharmacol. 25:416.

Keplinger, M.L. 1976. Two-year chronic oral toxicity study with Avadex technical in albino rats. ETL-74-12. Industrial Bio-Test Laboratories, Inc. Dec. 17, 1976, submitted to EPA by Monsanto Company, February 16, 1977. EPA Acc. No. 228094.

Table I

Number of animals dying during the experiment per number initially. (Includes animals that were replaced).

Group	Diet (ppm)	Males		Females
IA	0	38/51(a)	45/50	
IB	0	37/50		40/50
II	200	44/51(a)	45/50	
III	600	35/50		42/50
IV	2000	35/50		43/50

(a) Only one animal died during the first week and was replaced in the group.

Table 2

Hematocrit values - % (at 18 months)
and hemoglobin values - g% (at 18 months

Group	Hematocrit	Hemoglobin
IA	47±4	15.6±1.4
IB	45±5	15.1±1.5
II	45±6	14.9±1.7
III	43±7	14.1±2.3
IV	41±7	13.4±2.3

Table 3

Incidence of skin lesions in males and females combined from each group. (Number with lesion/number observed).

	Dermal melanomas			Focal dermal
Group	benign	malignant	total	melanosis
IA	0/23	0/23	0/23	2/23
IB	1/26	0/26	1/26	7/26
II	0/15	0/15	0/15	5/15
III	1/16	7/16	8/16	7/16
IV	9/46	19/46	28/46	15/46

Table 4
Liver lesions found (Number with lesion/number observed)

Group	Hepatocytic pigmentation	Focal cellular alteration[a]	Hepatocytic Hypertrophy	Hepatoctic hyperplasia	Benign neoplastic nodules
IA	9/74	5/74	1/74	0/74	0/74
IB	3/69	8/69	4/69	0/69	0/69
II	0/69	8/69	1/69	0/69	0/69
III	4/70	5/70	7/70	0/70	0/70
IV	33/79	13/79	10/79	2/79	2/79

(a) Cytoplasmic vacuolation and alterations in nuclear or hepatocyte size.