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

MAY 13 1981

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg.#677-231; Methane Arsonic Acid (MAA); 2-Year Chronic/Oncogenic Rat Feeding Study
CASWELL#549A; Accession#244061-067

FROM: William Dykstra, Toxicologist WJD
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TO: Richard Mountfort (23) WJD
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Recommendations:

1) In the 2-year chronic/oncogenic rat feeding study, adenomas and carcinomas of the thyroid of male rats receiving 200 ppm MAA diet occurred at a statistically significantly higher incidence ($p < 0.05$) than the corresponding control males. Although the number of animals with autolysis and the number of animals apparently not examined in low and mid-levels is considerable, sufficient evidence is available to conclude that MAA is oncogenic to the thyroid in male rats. This finding triggers an oncogenic RPAR criterion.

In addition, histopathological findings in the 200 ppm females (high dose level) which were present at increased incidences in comparison to the control female group were not evaluated at lower dosages to determine the NOEL for these chronic histological effects (spleen, kidney, trachea, ovary).

The study is considered as supplementary data. The registrant is required to upgrade the supplementary status of the study by further histopathological evaluation of the tissues of the mid and low female dosage levels which demonstrated statistically significant histological incidences at the high dose level and determine the NOEL for these effects.

Review:

1) Methane Arsonic Acid: Two-Year Chronic Feeding Study in Rats (Raltech Scientific Services; October 1, 1980 Report#T-526)

Test Material: MAA, 99.94% purity

After 9 weeks on MAA test diets F_0 generation Sprague-Dawley rats were mated to produce F_{1a} offspring which were continued on MAA through 2 years on test.

Feed (Purina Laboratory Chow) was provided ad libitum from specially capped clear glass jars that limit spillage and contamination and provide easy visability of amount and condition of feed. The MAA was administered as parts per million in the diet. The five test diets were mixed to contain the following levels of MAA: 0 ppm, 25 ppm, 50 ppm, 100 ppm and 200 ppm. Feed was mixed and offered fresh weekly.

Tap water was provided ad libitum and offered fresh at least three times weekly from 6-oz clear glass bottles fitted with rubber stoppers and stainless steel sipper tubes.

Animals randomly selected from F_{1a} litters were assigned to groups with the following experimental design.

Group	Sprague-Dawley Rats No. of Animals	Sex	Treatment ppm MAA
1	75	M	0
2	75	M	25
3	75	M	50
4	75	M	100
5	75	M	200
6	75	F	0
7	75	F	25
8	75	F	50
9	75	F	100
10	75	F	200

All animals in the study were observed at least twice daily, 7 days a week. While observing animals, the level and condition of feed and water, and environmental conditions were noted. Animals were carefully examined when body weights were taken and when being transferred to clean cages. Cages in which animals exhibited unusual behavior or appearance were tagged for inspection by the study leader or animal health veterinarian. Animals found in a moribund condition were sacrificed and all unusual findings were recorded.

Individual body weights (nearest gram) were recorded weekly through 13 weeks on test (post weaning) then monthly (every 4th week) through 104 weeks on test. Individual weekly feed consumptions (nearest gram) were recorded weekly for all animals through 13 weeks on test then monthly through 104 weeks on test. Shortly after study initiation (weaning of F_{1a} generation), 10 males and 10 females were randomly selected from groups on each test examination. Each animal was bled and had urine collected at 3, 6, 12, 18, and 24 months on test. If an animal that had been previously bled died or was sacrificed because of a moribund condition before the next collection, a new animal was selected in its place from the survivors in that group.

Urine was collected on a clean piece of wax paper placed under the cage. The paper was checked often to insure that a fresh, clean sample was examined. Blood samples were collected from the tail vein for hematology and blood chemistry.

The following data were collected from each animal.

Hematology: RBC, WBC, differential WBC, hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC).

Blood Chemistry: SGOT, BUN, SAP

Urinalysis: Specific gravity, pH, protein, glucose, ketones, blood, bilirubin.

In addition to terminal body weight, heart, liver, spleen, kidneys and gonads were weighed to three significant figures from each animal sacrificed at 104 weeks.

Animals that survived 104 weeks were denied food overnight, enthanized using CO_2, and exsanguinated. All animals, whether died or sacrificed on test or sacrificed at termination were subjected to complete necropsy and, unless severely autolyzed, the following tissues collected and preserved in alcohol-formalin-acetic acid (AFA) fixative:

Brain
Eye*
Thyroid/parathyroid
Lung
Pituitary
Pancreas
Uterus/prostate
Trachea
Bone/bone marrow*
Stomach
Jejunum
Cecum
Seminal vesicle
Urinary bladder
Adrenals
Skin*
Salivary glands
Mesenteric lymph node
Duodenum
Ileum
Spleen
Heart
Liver
Kidneys
Ovaries
Testes
*Sciatic nerve

*Collected but not examined histologically.

Tissues for histologic examination were embedded in paraffin, sectioned at 7 microns and stained with hematoxylin and eosin. Tissues examined included those from all animals in the control adn high level groups as required by the protocol. Also examined from the mid and low level groups were tissues from a majority of the animals that died on test, and major gross lesions noted at terminal necropsy.

Statistical methods used to analyze the data were noted in the results. Chi-square tests for significance were used to compare the incidence of lesions in the 200 ppm groups to that of the 0 ppm groups.

Results:

Observations during life revealed no significant differences in behavior or appearance of animals among any of the groups. Body weight and feed consumption data showed no significant effect on growth from the consumption of MAA. Growth curves were typical of Sprague-Dawley rats fed 2 years on test in the laboratory. Clinical data were generally normal for all animals throughout the studies. Some typical shifts in average values which accompany aging were seen in all groups, but there were no clinically significant differences among the groups during the test, with the exception of a dose-related decrease in SGOT levels seen at 3 months and 6 months in both males and females. However, no other indications of enzyme inhibition and no histopathology or other data which could be correlated with these depressed SGOT levels were found. There were no test related differences in survival over the 2-year period. At study termination, evaluation of organ weights showed no apparent toxicologically significant differences among the groups. A slightly increased mean heart weight, increased mean left kidney weight, and decreased mean spleen weight were noted in the 200 ppm female group when compared to the 0 ppm female group.

Terminal Sacrifice: The incidence of hemosiderin pigment deposition in the spleen was much higher in females in the 200 ppm group than in the control group (12/24, 50% vs. 7/39, 18%). This hemosiderin or hemosiderin-like pigment is the result of breakdown of red blood cells and subsequent hemoglobin degradation in the spleen. The difference in the incidence between the two groups was significant at the $p < 0.01$ level.

The lumens of the glands of the trachea were dilated in a larger number of the females in the high vs. the control level (7/24, 29% vs. 0/37, 0%), and the difference in the incidence levels between the two groups is significant at the $p < 0.01$ level.

No significant differences between the incidences of inflammatory, degenerative, or neoplastic changes in the uterus between the groups receiving 0 ppm and 200 ppm MAA were apparent. However, it should be noted that inflammatory changes occurred more frequently in Groups 7, 8, and 9 than in control Group 6 (Group 6, 4/39, 10%; Group 7, 13/19, 68%; Group 8, 11/18, 61%; Group 9, 14/18, 78%; Group 10, 6/24; 25%).

Ovarian follicular cysts occurred at a higher rate in the high dose females than in the control females (8/24, 33% vs 4/39; 10%) and the difference is significant at the $p < 0.05$ level.

Animals Examined Prior to Termination of the Study (DOT): Erythropoiesis (red blood cell production) was detected in 20% (10/50) of the spleens examined from females receiving 200 ppm MAA but in none (0/35) of the female control spleens. This difference is statistically significant at the $p < 0.01$ level.

The overall incidence of nephrosis and chronic nephrosis was high and occurred more frequently in females receiving 200 ppm than 0 ppm MAA (41/51, 80% vs 13/36, 36%). This difference is significant at the $p < 0.01$ level.

Parathyroid hyperplasia which frequently accompanies chronic nephrosis occurred in 22% (6/27) of the control and 27% (12/45) of the high dose females; this difference is not statistically significant.

Inflammatory uterine changes were significantly higher ($p < 0.01$) in the 200 ppm than in 0 ppm female group (21/49, 43% vs. 5/35, 14%).

There were no significant differences in the incidence of non-neoplastic lesions between the 200 ppm males and 0 ppm males.

There was no apparent increase in the 200 ppm group over the 0 ppm group for most tumors, and the numbers were low and generally sporadic, with only one or two of the animals examined having any one particular type of tumor. There were no high incidences of tumors in the liver, kidneys or other major organs. Only the mammary gland, pituitary, thyroid, and uterus had more than a minimal incidence of associated neoplasms.

The majority of the mammary gland tumors were diagnosed as fibroadenomas. There was no significant difference in the incidence of these tumors between the 0 ppm or 200 ppm groups; seventeen tumors of 37 tissues examined in 0 ppm animals and 22 tumors of 46 tissues in the 200 ppm animals.

Pituitary chromophobe adenomas occurred with a high incidence in female groups and with a low incidence in male groups. A total of 40 adenomas was found of 66 pituitaries examined in the 200 ppm female groups, and 40 adenomas of 67 pituitaries examined in the 0 ppm female group. These adenomas are considered to be spontaneous "old age" lesions in rats, and, although occurring to some degree in males, they usually occur in greater numbers in females.

Tumors of the uterus were essentially of three types: adenocarcinomas, adenoacanthomas, and squamous cell carcinomas.

There was no increase in the total number of these types of tumors in the 200 ppm group, 8 of 73 tissues examined (11%), when compared to the 0 ppm group, 8 of 74 tissues examined (11%).

Follicular thyroid adenomas occurred at a statistically higher incidence ($p < 0.01$) in males receiving 200 ppm than 0 ppm MAA (12/70, 17% vs. 2/73, 3%). This neoplasm was also responsible for parallel increase ($p < 0.05$) in the total number of thyroid neoplasms occurring in that group (14/70, 20% vs 5/73, 7%).

Thyroid Neoplasms

Sex:	M	M	M	M	M	F	F	F	F	F
Group No:	1	2	3	4	5	6	7	8	9	10
No. Examined DOT:	53	51	56	54	53	29	39	33	35	49
No. Examined TS:	20	2	5	0	17	37	1	3	2	24
Total No. Examined:	73	53	61	54	70	66	40	36	37	73
Cell Type and Neoplasm										
Follicular Cell										
Adenomas										
DOT†	0	0	0	1	3	2	0	0	0	2
TS	2	0	0	0	9	3	0	0	0	0
Total	2	0	0	1	12*	5	0	0	0	2
Carcinoma										
DOT	0	0	1	1	1	0	0	1	1	0
TS	1	0	1	0	0	0	0	0	0	0
Total	1	0	2	1	1	0	0	1	1	0
C-cell										
Adenoma										
DOT	0	1	0	0	0	0	1	0	0	1
TS	1	0	1	0	0	3	0	0	0	2
Total	1	1	1	0	0	3	1	0	0	3
Carcinoma										
DOT	0	0	0	0	0	0	0	0	0	0
TS	0	0	0	0	0	0	1	3	2	0
Total	0	0	0	0	0	0	1	3	2	0
DOT animals only										
Adenoma	0	0	0	0	1	0	0	1	0	0
Adenocarcinoma	1	0	0	0	0	0	0	0	0	0
Total	1	0	0	0	1	0	0	1	0	0
Total Neoplasms	5	1	3	2	14**	8	2	5	3	5

†DOT = Died on Test or Moribund
TS = Terminal Sacrifice
*Significantly difference from control males at the $p \leq 0.01$ level
**Significantly difference from control males at the $p \leq 0.05$ level

Conclusions:

Adenomas and carcinomas of the thyroid of male rats receiving 200 ppm MAA diet occurred at a statistically significantly higher incidence ($p < 0.05$) than the corresponding control males. Although the number of animals with autolysis and the number of tissues apparently not examined in low and mid-levels is considerable, sufficient evidence is available to conclude that MAA is oncogenic to the thyroid in male rats. This finding triggers an oncogenic RPAR criterion.

In addition, histopathological findings in the 200 ppm females (high-dose level) which were present at increased incidences in comparisons to the control female group were not evaluated at lower dosages to determine the NOEL for these chronic histological effects (spleen, kidney, trachea, ovary). The study is supplementary data. The registrant is required to upgrade the supplementary status of the study by further histopathological evaluation of the affected issues of the mid and low female dosage levels and determine the NOEL for these effects.

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