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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

CASWELL FILE

WASHINGTON, DC 20460

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OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: DEET: Review of a 90-day dermal toxicity study in castrated male rats

Caswell No. 346
MRID No. 411993-01
EPA ID No. TF-CSMA

HED Porj. No. 9-2036
EPA Record No. 250417

TO: D. Williams, PM (74)
Registration Division (H7505C)

FROM: Whang Phang, Ph.D. 1/26/90
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HFAS/Tox. Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head 1/26/90
and
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The registrant, Chemical Specialties Manufacturers Association, submitted a 90-day dermal toxicity in castrated male rats. This study has been reviewed, and the data evaluation report is attached. The conculsion is as follows:

The objective of this study was to determine the cause of the increase in the incidence of hyaline droplets in the renal tubules of DEET treated male rats seen in a 90-day dermal toxicity study. Prior to this study, the registrant thought that this increase might be strongly influenced by the male sex hormone, testosterone. The results of the current study showed that castration did not protect the DEET treated animals from hyaline droplet formation and other incidence of the kidney lesions. These results clearly demonstrated that the kidney lesions seen in the 90-day dermal toxicity study were compound-related effects.

Since this is a special study conducted to clarify a specific finding previous seen in another study, the classification system using the core guidelines does not apply to this study.

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Reviewer: Whang Phang, Ph.D. 1/26/90
HFAS/Tox. Branch II (H7509C)

Secondary Reviewer: K. Clark Swentzel, Section Head
HFAS/Tox. Branch II (H7509C) 1/26/90

DATA EVALUATION REPORT

Study Type: 90-Day dermal toxicity study in castrated male rats (special study)

Chemical: DEET (N, N-diethyl-m-toluamide)

Caswell No. 346
MRID No. 411993
EPA ID No. TF-CSMA
HED Porj. No. 9-2036
EPA Record No. 250417

Sponsor: DEET Joint Venture/Chemical Specialties Manufacturers Association

Testing Facility: International Research and Development Corp.
500 N. Main
Mattawan, Michigan 49071

Citation: Goldenthal, E.I. (1989) Evaluation of DEET in a 90-day toxicity study in castrated male rats. International Research and Development Corp.; Lab. Project No. 555-010. Aug. 9, 1989

Conclusion: The objective of this study was to determine the the cause of the increase in the incidence of hyaline droplets in the renal tubules of DEET treated male rats seen in a 90-day dermal toxicity study. Prior to this study, the registrant thought that this increase might be strongly influenced by the male sex hormone, testosterone. The results of the current study showed that castration did not protect the DEET treated animals from hyaline droplet formation and other incidence of the kidney lesions. These results clearly demonstrated that the kidney lesions seen in the 90-day dermal toxicity study were compound-related effects.

Since this is a special study conducted to clarify a specific finding previous seen in another study, the classification system using the core guidelines does not apply to this study.

Methods and Materials

Test Article: Technical DEET (98.3%) was "a mixture consisting of equal parts of four representative production runs" supplied by four manufacturers. The test article was assigned the ID No. IRDC 8812B at the testing laboratory. The test article was adminis-

tered as received.

Test Animals: The castrated and non-castrated male Charles River CD® rats were obtained from Charles River Lab., Portage, Michigan. The rats were observed for 20 days and were given a detailed physical examination before test.

Study Design

The objective of this study was to evaluate the effect of castration on renal lesions which have been observed in DEET treated male rats in a 90-day dermal toxicity study.

1. Animal assignment: Thirty castrated and 15 non-castrated males weighing from 251 to 315 gm were randomly assigned to the following dose groups:

Group Designation	Cast. Status	DEET Dose (mg/kg/day)	Number of male rats
Castrated Control	Castrated	0	15
Castrated Treated	Castrated	1,000	15
Positive Control	Non-castrated	1,000	15

2. Test article administration: The entire dorsal area of the trunk from the nape of the neck to the base of the tail of each test animal was shaved 24 hours (hrs) prior to the initiation of the test. This process was repeated on the last day of each study week or as needed. Based on a density of 1, the test article was applied dermally undiluted over the entire shaved area at 1000 mg/kg/day to animals in the positive control and castrated treated groups. Animals were treated 5 days per week. Castrated controls were treated with tap water at a volume 1 ml/kg/day in a similar manner to the DEET treated rats.

   The test animals received food and water ad. libitum.

3. Observation: The test animals were observed twice daily for signs of toxicity and mortality throughout the period of the study. The application site was graded for skin irritation at the beginning of each treatment week (Monday) and again at each weekly physical examination (Thursday).

4. Body weight and food consumption: The test animals were weighed prior to the initiation of the test and weekly thereafter. Weekly food consumption of each animal was determined.

5. Pathology: At the end of 13-week treatment period, all test animals were weighed and sacrificed. At sacrifice, blood samples were collected. The kidneys of each test animal were removed and weighed. The fat and connective tissue were trimmed. Applying the tissue processing techniques, the analysis of alpha 2u globulin was carried out on the right

kidneys. The left kidney was processed for routine microscopic examination. Other tissues were not examined.

The details of the tissue preparations and microscopic examination were excerpted from the report and presented in Appendix A.

6. Statistics: Analysis of variance (one-way classification) and Bartlett's tests for homogeneity of variance were used to analyze the results of body weight, food consumption,and absolute and relative kidney weights. In comparing the results of different groups, appropriate t-statistic was used. Dunnett's multiple comparison tables were applied for determining the significance of differences.

7. A quality assurance statement was signed and included in the report.

Results

a. Clinical observations: Increased incidence of focal swelling, erythema, and anogenital staining were seen in both treated castrated and non-castrated groups (Table 1).

b. Mortality: No mortality was seen in any of the test groups.

c. Body weight and food consumption: In general, the mean body weights of the castrated animals were less than those of the non-castrated rats. While the mean body weights of treated castrated rats were statistically significantly ($p<0.01$)

lower than those of treated non-castrated rats, they did not show any significant difference from those of the castrated controls (Table 2).

The food consumption data showed a closely related pattern of differences as those of the body weight data (Table 3).

d. Kidney weights: The mean kidney weight of the castrated controls and that of the treated castrated rats were less than that of treated non-castrated rats (positive controls) whereas the mean kidney weights of the treated castrated and that of the castrated controls were not significantly different (Table 4). The difference in kidney weights appeared to be closely related to changes in the body weights.

e. Histopathology: In the kidneys, the increases in the incidence of renal tubular casts, hyaline droplets, chronic inflammation, the presence of regenerative tubules, and granular casts formation were found in treated non-castrated

and castrated male rats (Table 5). Essentially none of these findings were seen in the castrated control animals.

The report described the renal tubular casts as the presence of sloughed epithelial cells or hyaline (proteinaceous) materials within the tubular lumen. The presence of hyaline droplets were identified by hematoxylin and eosin, Mallory-Heidenhan or an immunochemical stain and were "characteristic of the droplets seen in the unique male rat phenomenon called 'chemical induced alpha 2u globulin nephrosis'". The presence of hyaline droplets as indicated by the positive stain for alpha 2u globulin was found in 15/15 non-castrated and 10/15 castrated males which were treated with DEET whereas the presence of hyaline droplets was not found in any of the castrated controls.

The severity of the kidney lesions shown in Table 5 was worse in treated non-castrated rat than that of the treated castrated rats.

Discussion

The objective of this study was to determine the cause of the increase in the incidence of hyaline droplets in the renal tubules of DEET treated male rats seen in a 90-day dermal toxicity study. Prior to this study, the registrant thought that this increase might be strongly influenced by the male sex hormone, testosterone. The results of the current study showed that castration did not protect the DEET treated animals from hyaline droplet formation and other incidence of the kidney lesions. These results clearly demonstrated that the kidney lesions seen in the 90-day dermal toxicity study were compound-related effects.

The renal changes seen in this study are similar to those described by Bomhard et al. (1988) in male F344 rats which received p-dichlorobenzene at the daily doses of 150-600 mg/kg by gavage for 13 weeks. Similar renal lesions were also described by other authors in male rats in response to the exposure to aliphatic and cyclic hydrocarbons (Alden, 1986). At the present, there are two hypotheses for the possible causes of this "chemical induced alpha 2u globulin nephrosis". This nephrosis develops as a result of increased absorption of the alpha 2u globulin by renal proximal tubular cells. This increased absorption may be the result of complexing of this protein with the hydrocarbons (Alden, 1986). Other researchers believe that the binding of hydrocarbons to the alpha 2u globulin may lead to a decrease in the ability of the lysosomes to degrade this protein and eventually leads to lysosomal dysfunction secondary to lysosomal protein overload (Short and Swenberg, 1988; Charbonneau and Swenberg, 1988).

Since this is a study conducted to clarify a specific finding previous seen in another study, the core guideline classification system does not apply to this study.

References:

Alden, C. L. (1986). A review of unique male rat hydrocarbon nephropathy. Toxicol Pathol 14: 109-111.

Bomhard, E. et al. (1988). Induction of light hydrocarbon nephropathy by p-dichlorobenzene. Arch Toxicol 61: 433-439.

Charbonneau, M. and Swenberg, J.A. (1988). Studies on the biochemical mechanism of alpha 2u- globulin nephropathy in rats. CIIT Activities 8: No. 6 (June), PP. 1, 3-5.

Short, B. and Swenberg, J. A. (1988). Pathologic investigations of the mechanism of unleaded gasoline-induced renal tumors in rats. CIIT Activities 8: No.7 (July), pp. 1-6.

TABLE 1*

Summary of Clinical Findings
MALES

Observation	Interval: 1 - 13 Week CASTRATED CONTROL (15)	NON-CASTRATED 1,000 MG/KG/DAY (15)	CASTRATED 1,000 MG/KG/DAY (15)
APPEARANCE AND CONDITION			
No visible abnormalities for entire interval	9 [60.0]	0	0
Subcutaneous mass	3 [20.0]	0	3 [20.0]
EXCRETION			
Decreased defecation	0	1 [6.7]	0
BODY SURFACE			
Abrasion	0	0	1 [6.7]
Focal swelling	0	1 [6.7]	4 [26.7]
Edema	1 [6.7]	0	0
Erythema	1 [6.7]	15 [100.0]	15 [100.0]
Hair loss	1 [6.7]	0	0
Scabbed area	2 [13.3]	0	4 [26.7]
RESPIRATION			
Rales	0	1 [6.7]	0
ANOGENITAL			
Anogenital staining	0	4 [26.7]	9 [60.0]

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( ) = Number of animals observed at start of interval
[ ] = Percent of animals with observation during interval

* TABLE EXCERPTED FROM THE REPORT (IRDC No. 555-010)

TABLE 2*

Males: Summary of Body Weight Values

Parameters Measured	WEEK OF STUDY	CASTRATED CONTROL MEAN	S.D.	N	NON-CASTRATED 1000 MG/KG/DAY MEAN	S.D.	N	CASTRATED 1000 MG/KG/DAY MEAN	S.D.	N
Body Weight	ALLOC	287	17.3	15	294	14.2	15	285	17.8	15
grams	-1	295	17.2	15	303	16.5	15	294	17.8	15
	1	331	19.1	15	341	19.5	15	328	21.2	15
	2	356	20.2	15	362	20.9	15	350	20.9	15
	3	374	19.8	15	396	28.1	15	368[4]	26.5	15
	4	392	22.0	15	420	32.2	15	383[4]	27.0	15
	5	405	23.9	15	439	36.2	15	393[4]	30.6	15
	6	416	25.8	15	455	36.8	15	401[4]	31.2	15
	7	425	24.0	15	468	39.8	15	408[4]	31.3	15
	8	434	26.9	15	482	40.0	15	414[4]	36.8	15
	9	438	28.6	15	493	43.0	15	415[4]	36.1	15
	10	446	28.7	15	503	49.6	15	420[4]	38.5	15
	11	450	30.2	15	513	45.9	15	422[4]	36.9	15
	12	455	32.2	15	523	48.5	15	428[4]	36.7	15
	13	456	34.5	15	527	47.2	15	424[4]	36.8	15

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S.D. - Standard Deviation
N - Number of Animals

[4]Significantly different from the non-castrated treated group; $p<0.01$

* TABLE EXCERPTED FROM THE REPORT (IRDC NO. 555-010)

TABLE 3*

Males: Summary of Food Consumption Values

Parameters Measured	WEEK OF STUDY	CASTRATED CONTROL MEAN	S.D.	N	NON-CASTRATED 1000 MG/KG/DAY MEAN	S.D.	N	CASTRATED 1000 MG/KG/DAY MEAN	S.D.	N
Food Consumption g/animal/day	1	26.0	1.68	15	27.2	1.77	15	25.3[3]	2.09	15
	2	26.5	1.93	15	26.8	2.15	15	26.5	2.31	15
	3	26.5	1.69	15	29.3	2.88	15	26.4[4]	2.92	15
	4	27.0	1.94	14	30.0	2.36	15	26.7[4]	2.54	15
	5	26.5	2.03	15	30.2	2.34	15	26.5[4]	2.49	15
	6	25.7	2.26	15	29.3	2.11	13	25.4[4]	2.63	15
	7	24.6	1.75	15	28.0	4.35	14	24.8[3]	2.71	15
	8	24.1	2.63	15	28.2	2.63	14	23.4[4]	2.79	15
	9	22.4	2.39	15	27.9	2.72	15	22.8[4]	2.47	15
	10	22.8	1.89	15	27.0	4.09	15	22.5[4]	2.55	15
	11	23.9	1.47	15	29.0	2.43	15	23.7[4]	2.41	15
	12	23.0	1.91	15	28.0	1.94	15	23.0[4]	2.71	15
	13	22.4	1.63	15	26.9	2.43	14	22.8[4]	2.41	15

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S.D. - Standard Deviation
N - Number of Animals

[3]Significantly different from the non-castrated treated group; $p<0.05$
[4]Significantly different from the non-castrated treated group; $p<0.01$

* TABLE EXCERPTED FROM THE REPORT (IRDC No. 555-010)

TABLE 4*

Males: Summary of Organ Weight Values - Terminal Sacrifice

Parameters Measured	Castrated Control			Non-castrated 1,000 mg/kg/day			Castrated 1,000 mg/kg/day		
	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N
Body Weight g	450	33.7	15	508	63.2	15	425[4]	36.4	15
Kidney g	3.13	0.381	15	5.20	0.763	15	3.46[4]	0.546	15
Kidney/Body Weight %x10	6.99	1.125	15	10.27	1.324	15	8.14[1,4]	1.011	15

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S.D. - Standard Deviation
N - Number of Animals

[1]Significantly different from the castrated control group; $p<0.05$
[4]Significantly different from the non-castrated treated group; $p<0.01$

* TABLE EXCERPTED FROM THE REPORT (IRDC NO. 555-010)

TABLE 5*

Microscopic Findings in the Kidneys of DEET Treated Rats

Tissue Observation	Castrated Control	Non-Castrated 1,000 mg/kg/day	Castrated 1,000 mg/kg/day
Cast, hyaline	0/15	11/15	1/15
Hyaline droplet	0/15	15/15	5/15
Inflammation	1/15	14/15	8/15
Regeneration	0/15	15/15	7/15
Granular cast	0/15	8/15	2/15
Mallory-Heidenhein ⊕	0/15	15/15	10/15
Immunocytochemistry ⊕	0/15	15/15	10/15

* TABLE EXERPTED FROM THE REPORT (ERDC 555-010) Page 22.

Appendix A*

After weighing, the right kidney was processed for alpha 2u globulin analysis. It was first placed on a 2" x 2" square parafilm and sliced into 2 to 3 mm thick cross sections. Tissue samples were then dropped into 15 ml glass vials containing B-5 fixative. A solution of 90 ml of B-5 fixative was diluted with 10 ml of 40% formaldehyde and used for tissue fixation. The vials were placed on a rocking platform at room temperature and shook gently for 4 hours. The B-5 fixative was removed by washing the tissue samples six times (5 minutes each) in 70% ethanol and then placed in neutral buffered formalin for approximately 20 hours.

The left kidney was processed for routine light microscopy. After weighing it was placed in neutral buffered formalin. All other tissues and carcasses of all animals were discarded without examination.

Microscopic Examination

Tissue from the right kidney was embedded in paraffin and 5 micrometer cross sections made and placed on albuminized slides. Six slides were prepared for each animal. These slides were shipped to the Sponsor's representative, Dr. Arun K. Roy. Processed tissue from the left kidney was placed on slides and stained with hematoxylin and eosin and also with Mallory-Heidenhain stain. Slides from both the left and right kidneys were examined by Dr. Donald N. Kitchen, a resident consulting pathologist of IRDC.

A four-step grading system of trace, mild, moderate and severe was used to define gradable lesions for comparison between dosage groups. Slides with Mallory-Heidenhain stain or by immunocytochemical techniques were designated as either negative or positive for the presence of hyaline droplets or alpha 2u globulin, respectively. A complete listing of tissue accountability can be found in the Microscopic Tissue Inventory (Appendix H).

* EXCERPTED FROM THE REPORT (IRDC # 555-010)