CASWELL FILE

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

005541

OCT 20 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Assure (NC-302; ethyl 2-[4-(6-chloro-2-quioxalinyloxy)phenoxy]propionate): Evaluation of oncogenicity study in mice

Caswell No.: 215D
Project No.: 191/192
Action Code: 230
Record No. 151063; 152701

SUBMITTER: E. I. Du Pont De Nemours & Co.

TO: Robert J. Taylor
Product Manager (25)
Registration Division (TS-767C)

FROM: Whang Phang, Ph.D. Whang Phang 7/8/86
Pharmacologist
Toxicology Branch/HED (TS-769C)

THRU: Marcia van Gemert, Ph.D. M. van Gemert 9.29.86
Section Head
and
Theodore M. Farber, Ph.D.
Chief
Toxicology Branch/HED (TS-769C)

Action Requested:
Review oncogenicity study in mice to complete the data base for registration and tolerance for soybean and cotton.

Results:
The attached DER (EPA No. 68-02-4225; Dynamac No. 1-011-F; June 26, 1986) has been approved by Toxicology Branch. This oncogenicity study in mice (unpublished project No. 2076-109 prepared by Hazleton Laboratories America Inc. for Nissan Chemical Industries Ltd., Japan) is classified Core Guideline.

Assure was orally administered to groups of CD-1 mice (70/sex/dose) at dietary concentrations of 0, 2, 10, 80, and 320 ppm for 18 months. There was statistically significant increase in the incidence of ovarian tumors in high dose females. In males, significant decrease in survival rate at high dose and increased incidence of bilateral testicular atrophy were observed at 80 and 320 ppm. In both sexes of treated mice, hepatotoxicity which was characterized by enlarged and dark livers was reported, and histologically the livers contained diffuse hepatocyte enlargement, hepatocyte pigment, sinusoidal cell pigment, and focal pigmented macrophage. An increase in the incidence of liver tumors was also observed in high dose males. The NOEL for Assure is 10 ppm, and the LOEL is 80 ppm in CD-1 mice.

Comments:

The data of this study and other relevant data will be presented to the the Peer Review Committee to determine the carcinogenicity of Assure in mice at the end of October. Our final assessment will be forthcoming pending the final report from the Peer Review Committee.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

005541

EPA: 68-02-4225
DYNAMAC No. 1-011-F
June 26, 1986

DATA EVALUATION RECORD

ASSURE

Oncogenicity Study in Mice

STUDY IDENTIFICATION: Burdock, G. A., Alsaker, R. A., Kulwich, B. A., and Colpean, B. R. NC-302 oncogenicity (18-month feeding) study in mice. (Unpublished project No. 2076-109 prepared by Hazleton Laboratories America Inc. for Nissan Chemical Industries Ltd., Tokyo, Japan, and submitted by E.I. du Pont de Nemours and Co., Inc., Wilmington, DE; dated April 30, 1985.)

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

Date: 6-27-86

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1. CHEMICAL: NC-302 Assure.

2. TEST MATERIAL: NC-302 from batch No. 8002 was described as white powder containing 99% active ingredient.

3. STUDY/ACTION TYPE: 18 month feeding study in mice.

4. STUDY IDENTIFICATION: Burdock, G. A., Alsaker, R. A., Kulwich, B. A., and Colpean, B. R. NC-302 oncogenicity (18-month feeding) study in mice. (Unpublished project No. 2076-109 prepared by Hazleton Laboratories America Inc. for Nissan Chemical Industries Ltd., Tokyo, Japan, and submitted by E.I. du Pont de Nemours and Co., Inc., Wilmington, DE; dated April 30, 1985.)

5. REVIEWED BY:

Asit K. Lahiri, D.V.M., Ph.D.
Principal Reviewer
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Signature: Asit K Lahiri
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6. APPROVED BY:

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Whang Phang, Ph.D.
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Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: 7/1/86

7. CONCLUSIONS:

Under the conditions of the study, NC-302 caused an increase in the number of ovarian stromal cell tumors in female CD-1 mice. In addition, the test material caused a significant increase in mortality and bilateral testicular atrophy in the males and hepatotoxicity in both sexes of mice. An increase in the incidence of liver tumors was noted in the high-dose group of males. The maximum tolerated dose (MTD) was achieved in this study. The NOEL for NC-302 in this study is 10 ppm and the LOEL is 80 ppm in CD-1 mice.

CORE CLASSIFICATION: Core guideline.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS): (See Appendix a for detail)

A. Materials and Methods: The test material NC 302 (lot #8002) was a white powder and was 99% pure. Dietary levels of this compound for this study were adjusted to 100% of the active ingredient. Information regarding the stability and the chemical profile of the test material was not provided in this report. However, it was stated that this information was in the file of the registrant.

Four-and-half-week-old CD-1 mice were acclimatized for 2 weeks in the laboratory and distributed randomly in 10 groups of 70 animals/dose/sex. Two subgroups of 10 animals/dose/sex from each dose groups were designated as Satellite A and Satellite B. These animals were ear marked for interim sacrifices and for laboratory investigations (clinical pathology) at study weeks 26 and 52. All animals were housed individually (cages) in environmentally controlled rooms. Food (Purina Laboratory Chow®) and tapwater were provided ad libitum except for an overnight fast prior to the scheduled sacrifices and blood collections.

The dose levels used in the feed were 0, 2, 10, 80, and 320 ppm of the active ingredient of NC-302. Feed for each dose level was prepared in two steps: (a) premixing in quantities of 200 g in a Waring blender and (b) final mixing in a Patterson-Kelly Twin-Shell blender. The animals were treated with NC-302 for 78 weeks.

Chemical analyses of the dietary samples for each dose level were performed at various intervals (23 total) during the course of the study to determine the homogeneity and the level of the test material. In addition, stability of the test material in the feed (stored at room temperature) was routinely determined during the course of the study.

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[1] Only items appropriate to this DER have been included.

All mice were observed twice daily for mortality and once a week for clinical signs, including palpation for tissue masses.

Body weight and food consumption were recorded weekly for the first 14 weeks and biweekly thereafter.

Ophthalmoscopic examinations were performed on (a) 10 animals/sex prior to initiation, (b) all surviving Sattellite A animals at the end of the week 26, (c) all surviving Sattellite B animals at the end of the week 52, and (d) randomly selected 10 animals/dose/sex at the termination of the study.

Hematologic and clinical chemistry parameters were examined on blood obtained from: a) 10 animals per sex prior to initiation; b) all Satellite A animals at the end of the week 26; and c) all Satellite B animals at the end of week 52. Urinalyses were performed on the same groups of animals at the same intervals. At the end of week 78, only hematological examinations were performed for 10 animals/dose/sex.

Complete necropsies were performed on all surviving animals after phenobarbital anesthesia and exsanguination in the following schedule: a) Satelite A animals at the end of week 26, b) all the surviving animals in Satellite B at the end of week 52, and c) all the surviving animals at the end of week 78. Complete necropsy examination were performed on all animals found dead or sacrificed at moribund conditions.

Weights of the following organs from all animals sacrificed at scheduled intervals were recorded at necropsy: brain, heart, liver, spleen, kidney, testes, adrenal (fixed) and ovaries (fixed).

Representative portions of approximately 40 different organs and tissues in addition to gross lesions and masses from all animals were fixed in 10% neutral buffered formalin; they were subsequently processed and stained with hematoxylin and eosin for microscopic evaluation.

Appropriate statistical methods were used to analyze the study data (see Appendix A for detail). Significant levels for all statistical analyses were judged at $p \leq 0.05$ when compared to untreated control of the same sex unless otherwise indicated.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

Dietary Analysis: Analyses for homogeneity of the diet indicated that NC-302 was mixed uniformly in the feed.

Dietary analysis for the test compound at 23 intervals during the study indicated that the mean levels of test compound were 97.3, 96.8, 110.6, and 96.7% of the nominal values of 2, 10, 80, and 320 ppm, respectively.

Stability: Concentrations of the test material in the feed, following 7 days of storage under the condition of use, decreased by 5.0%, 5.8%, 0.2%, and 1.5% in diets with nominal concentrations of 2, 10, 80, and 320 ppm, respectively.

Mortality: The number of animals surviving at selected intervals is indicated in the weight body table (Table 1). Statistical analysis showed a significant trend towards decreased survival in the dosed males when compared to controls, and a significantly lower survival ($p < 0.05$) in males receiving 320 ppm when compared to controls. Female survival was comparable in the dosed and control groups (Table 1).

Clinical Observation: Numerical, but not statistically significant, increases in the incidences of swollen abdomen were noted among the high-dose male and female mice. Ophthalmoscopic examinations did not reveal any compound-related effect for any dose group of either sex.

Body Weight: Group mean body weights at selected intervals are summarized in Table 1. During the course of the first 52 weeks of the study the growth rates were in general significantly increased in males receiving 320 ppm and females receiving 80 and 320 ppm (at the analyzed intervals). This was with the exception of females receiving 80 ppm at week 8, 16, and 20.

Food Consumption: In general, mean weekly food consumption was similar in dosed and control groups. However, sporadic changes in food consumption were noted in both sexes at 4 different weekly intervals.

Hematology: There were no changes in mean hematologic values that correlate with dose level in either sex. Statistically significant ($p < 0.05$) increases in the group mean values for hemoglobin, hematocrit, and mean corpuscular volume were noted for females receiving 2 ppm at week 27, and mean corpuscular volume for females receiving 320 ppm was significantly increased at week 53.

Clinical Chemistry: Statistically significant changes in the group mean values for total protein, albumin, A/G ratio, and alkaline phosphatase were noted in males receiving 320 ppm more often than those of the females in the same dose group (Table 2). See also Reviewers' Discussion.

Urinalysis data for the dosed animals were essentially unremarkable when compared to the respective controls.

Organ Weight: An overview of the significant changes in organ weights as presented by the authors is given in Table 3. Absolute

TABLE 1. Selected Mean Body Weight Values and Survival in Mice Fed NC-302 for 78 Weeks

Dietary Level (ppm)	Mean Body Weight (g ± S.D.) at Week				
	0	13	26	52[a]	78
			Males		
0	27.1±1.40 (70)[b]	35.5±2.21 (70)	37.0±2.65 (70)	37.7±2.96 (56)	39.2±3.21 (41)
2	27.2±1.69 (70)	35.0±2.64 (69)	36.5±3.06 (69)	37.0±2.81 (57)	37.5±2.90 (39)
10	26.5±1.60 (70)	35.1±2.24 (70)	36.4±2.82 (69)	36.8±2.87 (57)	37.9±3.19 (40)
80	26.4±1.90 (70)	35.3±2.58 (69)	36.4±2.79 (67)	37.6±3.08 (55)	38.6±3.99 (34)
320	26.2±1.69[S-] (70)	36.3±2.83 (69)	37.2±3.22 (67)	38.8±2.89[S+] (50)	38.0±3.5 (27)*
			Females		
0	22.7±1.18 (70)	28.9±2.18 (70)	31.2±2.37 (69)	33.4±2.91 (58)	35.2±3.38 (37)
2	22.7±1.26 (70)	28.8±2.05 (70)	30.9±2.28 (70)	33.7±3.38 (59)	35.9±3.64 (39)
10	22.1±1.35[S-] (70)	28.8±2.29 (69)	31.1±2.37 (67)	33.4±2.72 (53)	34.6±2.76 (33)
80	22.4±1.35 (70)	29.5±2.09 (70)	31.4±2.08[S+] (68)	34.4±2.94[S+] (51)	36.1±2.78 (34)
320	22.2±1.10[S-] (70)	30.9±2.01 (70)	32.4±2.18[S+,s+] (70)	35.1±2.52[S+,s+] (58)	34.9±4.41 (31)

[a]Ten/sex/group were sacrificed at 26th and 52nd week of treatment.

[b]The value in parentheses is the number of animals.

[S+]Statistically significantly higher mean body weight compared to control ($p \leq 0.05$).

[S-]Statistically significantly lower mean body weight compared to control ($p \leq 0.05$).

[s+]Statistically significantly increased growth rate compared to control ($p \leq 0.05$).

*Statistically significant decreased survival ($p \leq 0.05$).

TABLE 2a. Selected Mean Clinical Chemistry Values for Male Mice Fed NC-302

Dietary Level (ppm)	Total Protein at Week 27	Total Protein at Week 53	Albumin at Week 27	Albumin at Week 53
		Males		
0	5.4±0.27 (10)	5.8±0.39 (10)	3.1±0.16 (10)	3.3±0.18 (10)
2	5.6±0.31 (10)	5.7±0.20 (10)	3.3±0.19 (10)	3.2±0.22 (10)
10	5.5±0.18 (9)	5.8±0.28 (9)	3.2±0.28 (9)	3.3±0.23 (9)
80	5.4±0.32 (9)	5.7±0.29 (8)	3.3±0.26 (9)	3.4±0.21 (8)
320	5.9±0.23^{S+} (10)	6.0±0.36 (9)	3.8±0.20^{S+} (10)	3.6±0.23^{S+} (9)

	A/G[a] Ratio at Week 27	A/G[a] Ratio at Week 53	Alkaline Phosphatase at Week 27	Alkaline Phosphatase at Week 53
		Males		
0	1.38±0.110 (10)	1.34±0.143 (10)	34±11.3 (10)	37±9.2 (10)
2	1.46±0.198 (10)	1.32±0.192 (10)	41± 8.2 (10)	41±11.9 (10)
10	1.47±0.269 (9)	1.33±0.125 (9)	38±14.6 (9)	36±0.23 (9)
80	1.54±0.134 (9)	1.45±0.201 (8)	46±16.5 (9)	63±39.2 (7)
320	1.85±0.202^{S+} (10)	1.53±0.170 (9)	105±23.9^{S+} (10)	81±28.6^{S+} (9)

$^{S+}$Statistically significantly higher than control value ($p \leq 0.05$).

[a] A/G, Albumin/Globulin.

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TABLE 2b. Selected Mean Clinical Chemistry Values for Female Mice Fed NC-302

Dietary Level (ppm)	Total Protein at Week 27	Total Protein at Week 53	Albumin at Week 27	Albumin at Week 53
			Females	
0	5.6±0.30 (9)	5.7±0.29 (10)	3.6±0.14 (9)	2.0±0.21 (9)
2	5.5±0.17 (10)	5.5±0.32 (9)	3.5±0.17 (10)	2.1±0.12 (10)
10	5.4±0.26 (9)	5.4±0.24 (10)	3.5±0.22 (9)	1.9±0.09 (9)
80	5.4±0.39 (10)	5.7±0.47 (7)	3.5±0.25 (10)	1.9±0.17 (10)
320	5.5±0.19 (9)	5.6±0.58 (10)	3.7±0.15 (9)	1.9±0.17 (9)

	A/G[a] Ratio at Week 27	A/G[a] Ratio at Week 53	Alkaline Phosphatase at Week 27	Alkaline Phosphatase at Week 53
			Females	
0	1.79±0.157 (9)	1.60±0.173 (10)	45± 7.6 (9)	53±14.8 (10)
2	1.69±0.147 (10)	1.46±0.133 (9)	49± 9.4 (10)	50±17.2 (9)
10	1.84±0.132 (9)	1.61±0.123 (10)	48±11.2 (9)	48±12.6 (10)
80	1.84±0.112 (10)	1.66±0.231 (7)	51±16.9 (10)	53±11.5 (7)
320	2.00±0.216[S+] (9)	1.72±0.186 (10)	70± 5.8[S+] (9)	76± 8.9[S+] (10)

[S+] Statistically significant increase ($p \leq 0.05$).

[a] A/G, Albumin/Globulin.

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TABLE 3. Summary Table of Statistically Significant Organ Weights at All Sacrifice Intervals in Mice Fed NC-302 for 78 Weeks

Organ	Males/Dose (ppm)				Females/Dose (ppm)			
	2	10	80	320	2	10	80	320
Week 26 Interim Kill								
Absolute kidney weight								S+
Kidney/body weight						S+	S+	S+
Absolute liver weight			S+	S+				S+
Liver/body weight			S+	S+				S+
Week 52 Interim Kill								
Absolute kidney weight				S+				
Kidney/body weight		S+						
Absolute liver weight			S+	S+				S+
Liver/body weight			S+	S+			S+	S+
Terminal Kill								
Absolute kidney weight							S+	S+
Kidney/body weight							S+	S+
Absolute liver weight				S+			S+	S+
Liver/body weight				S+			S+	S+
Absolute testes weight				S-				
Testes/body weight				S-				
Absolute ovary weight					S+	S+	S+	S+
Ovary/body weight					S+	S+	S+	S+
Absolute adrenal weight		S+	S+	S+				
Adrenal/body weight		S+	S+	S+				

Source: CBI p. 24.

S+ Statistically significant increase, $p \leq 0.05$.

S- Statistically significant decrease, $p \leq 0.05$.

and relative liver weights and gonad weight (and statistical significance) are presented in Tables 4 and 5. The authors considered the changes in liver weight and testes weights to be of toxicologic significance since they were correlated with histologic changes; however, they stated "the biologic importance of the increased ovary weights in all dosed females at 78 weeks was unknown". Mean liver weights and liver-to-body weight ratios were significantly increased in both males and females receiving 320 ppm at weeks 26, 52, and 79. In males receiving 80 ppm, mean liver weights were increased at weeks 26 and 52 and liver-to-body weight ratios were increased at all intervals. In females receiving 80 ppm, both absolute and relative liver weights were significantly increased at week 79; there were increases in both parameters at weeks 26 and 52 but only the mean relative liver weight at week 52 was significantly ($p \leq 0.05$) different from the control value. Increases in absolute and relative kidney weight ($p \leq 0.05$) in females receiving 80 and 320 ppm were noted at 78 weeks; other changes in kidney weights were sporadic.

Gross Pathology: An increase in the incidence of dark and enlarged livers was noted in 320-ppm males as early as the 27-week sacrifice and in females as early as the 52-week sacrifice, and in 320 ppm animals that died or were sacrificed moribund. There was an apparent dose-related trend in these gross findings at the terminal sacrifice (Table 4).

Histopathology: Compound-related microscopic changes were noted in the livers of the high dose groups of animals of both sexes sacrificed at all intervals including those died naturally and were sacrificed moribund during the course of the study (Table 4). These changes included a) diffuse enlargement of hepatocytes, b) hepatocellular pigmentation, c) sinusoidal cellular pigmentation, and d) occurrences of focal pigmented macrophages. The hepatic alterations of the the first three categories were noted in animals sacrificed in week 27, all four types of changes occurred often in high-dose males and females sacrificed at weeks 53 and 79 including those died naturally and were sacrificed moribund. Hepatic changes noted above also appeared in some 80-ppm animals of both sexes at the 52 and 79 week sacrifices.

Testicular atrophy noted in the dosed males was considered to be compound related because of the statistically significant ($p = 0.01142$) increase in bilateral testicular atrophy among the combined high dose group of spontaneously dead, moribund sacrifice and terminally sacrificed animals when compared to untreated males (Table 5).

Amyloidosis was more frequently observed in males and females receiving 320 ppm at week 26 and at terminal sacrifice than in controls.

Dosed female mice that died (or sacrificed moribund) in the first 26 weeks had an increased incidence of malignant lymphoma. However,

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TABLE 4. Correlation of Parameters Indicating an Effect on the Liver in Mice Fed NC-302 for 78 Weeks

	Males/Dose (ppm)					Females/Dose (ppm)				
	0	2	10	80	320	0	2	10	80	320
				Week 27						
Gross Findings										
Enlarged liver	0/10	0/10	0/9	0/9	2/10	0/9	0/10	0/9	0/10	0/9
Dark liver	0/10	0/10	0/9	0/9	5/10	0/9	0/10	0/9	0/10	0/9
Histologic Findings										
Diffuse hepatocyte enlargement	0/10	0/10	0/9	0/9	10/10	0/9	0/10	0/9	0/10	9/9
Hepatocyte pigment	0/10	0/10	0/9	0/9	10/10	0/9	0/10	0/9	1/10	1/9
Sinusoidal cell pigment	0/10	0/10	0/9	0/9	9/10	0/9	0/10	0/9	0/10	1/9
Hepatic tumor (adenoma and carcinoma)	0/10	0/10	1/9	0/9	0/10	0/9	0/10	0/9	0/10	0/9
Liver Weight										
Gram	1.34	1.39	1.34	1.54*	2.48*	1.22	1.16	1.13	1.25	2.01*
% of body weight	4.273	4.415	4.334	4.983*	8.186*	4.608	4.453	4.449	4.844	7.165*
Clinical Chemistry										
Albumin (g/dL)	3.1	3.3	3.2	3.3	3.8*	3.6	3.5	3.5	3.5	3.7
A/G ratio	1.38	1.46	1.47	1.54	1.85*	1.79	1.69	1.84	1.84	2.00*
Total protein (g/dL)	5.4	5.6	5.5	5.4	5.9*	5.6	5.5	5.4	5.4	5.5
Alkaline phosphatase	34	41	38	46	105*	45	49	48	51	70*

(Continued)

*Significantly different from control value ($p \leq 0.05$).

TABLE 4. Correlation of Parameters Indicating an Effect on the Liver in Mice Fed NC-302 for 78 Weeks (Continued)

	Males/Dose (ppm)					Females/Dose (ppm)				
	0	2	10	80	320	0	2	10	80	320
Week 52 Interim Sacrifice										
Gross Finding										
Enlarged liver	0/10	0/10	0/9	0/8	9/9	1/10	0/9	2/10	1/7	6/10
Dark liver	0/10	0/10	0/9	2/8	9/9	0/10	0/9	0/10	2/7	6/10
Histologic Findings										
Diffuse hepatocyte enlargement	0/10	0/10	0/9	0/8	9/9	0/10	0/9	0/10	0/7	9/10
Hepatocyte pigment	0/10	0/10	0/9	3/8	9/9	0/10	0/9	0/10	3/7	8/10
Sinusoidal cell pigment	1/10	0/10	0/9	3/8	9/9	1/10	0/9	3/10	5/7	10/10
Focal pigmented macrophages	0/10	2/10	4/9	3/8	9/9	2/10	5/9	5/10	4/7	10/10
Hepatic tumor (adenoma and carcinoma)	0/10	2/10	0/9	1/8	0/9	0/10	0/9	0/10	0/7	0/10
Liver Weight										
Gram	1.27	1.43	1.33	1.56*	2.47*	1.23	1.33	1.35	1.51	2.17*
% of body weight	3.964	4.406	4.199	5.211*	7.588*	4.397	4.657	4.679	5.255*	7.419*
Clinical Chemistry										
Albumin (g/dL)	3.3	3.2	3.3	3.4	3.6*	3.5	3.3	3.3	3.5	3.5
A/G ratio	1.34	1.32	1.33	1.45	1.53	1.60	1.46	1.61	1.66	1.72
Total protein (g/dL)	5.8	5.7	5.8	5.7	6.0	5.7	5.5	5.4	5.7	5.6
Alkaline phosphatase	37	41	36	63	81*	53	50	48	53	76*

(Continued)

*Significantly different from control value ($p \leq 0.05$).

TABLE 4. Correlation of Parameters Indicating an Effect on the Liver in Mice Fed NC-302 for 78 Weeks (Continued)

	Males/Dose (ppm)					Females/Dose (ppm)				
	0	2	10	80	320	0	2	10	80	320
Week 79 Terminal Sacrifice										
Gross Findings										
Enlarged liver	0/41	0/38	1/40	0/33	17/27	0/35	2/39	0/33	2/34	11/28
Dark liver	1/41	0/38	2/40	14/33	26/27	0/35	1/39	2/33	5/34	26/28
Histologic Findings										
Diffuse hepatocyte enlargement	0/41	0/38	0/40	1/33	27/27	0/35	0/39	0/33	0/34	17/28
Hepatocyte pigment	0/41	0/38	1/40	18/33	26/27	2/35	0/39	0/33	6/34	20/28
Sinusoidal cell pigment	4/41	4/38	2/40	13/33	27/27	10/35	10/39	10/33	21/34	20/28
Focal pigmented macrophage	6/41	12/38	8/40	13/33	24/27	14/35	21/39	19/33	25/34	24/28
Hepatic tumor (adenoma and carcinoma)	6/41	8/38	6 (w.f.) 1/40	6/33	9 (w.f.) ~~12~~/27	1/35	0/39	0/33	0/34	4/28
Liver Weight										
Gram	1.65	1.48	1.56	1.70	2.71*	1.47	1.54	1.40	1.75*	2.45*
% of body weight	5.035	4.658	4.811	5.230*	8.489*	4.969	5.077	4.880	5.714*	8.078*
Deaths and Moribund Sacrifices										
Gross Findings										
Enlarged liver	0/9	0/12	0/12	2/20	3/24	2/16	2/12	3/18	4/19	6/23
Dark liver	0/9	0/12	1/12	2/20	8/24	0/12	0/12	1/18	0/19	8/23
Histologic Findings										
Diffuse hepatocyte enlargement	0/9	0/11	0/11	1/19	13/24	0/16	0/12	0/17	0/19	8/22
Hepatocyte pigment	0/9	0/11	0/11	8/19	16/24	0/16	0/12	0/17	5/19	19/22
Sinusoidal cell pigment	0/9	1/11	1/11	8/19	18/24	0/16	0/12	1/17	6/19	18/22
Focal pigmented macrophage	0/4	2/11	1/11	2/19	17/24	5/16	1/12	3/17	6/19	16/22
Hepatic tumor (adenoma and carcinoma)	1/9	0/11	0/11	1/19	6/24	0/16	0/12	0/17	0/19	0/22

(Concluded)

*Significantly different from control value ($p \leq 0.05$).

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TABLE 5. Effects of Dosing Mice for 78 Weeks on Gonad Weights and Histologic Findings

	Males/Dose (ppm)					Females/Dose (ppm)				
	0	2	10	80	320	0	2	10	80	320
Week 27										
Ovaries										
Weight (g)	--	--	--	--	--	0.028	0.031	0.029	0.038	0.030
Tumor (luteoma and granuloma cell)	-	--	--	-	--	0/9	0/10	0/9	0/10	0/9
Cyst	--	--	--	--	--	4/9	4/10	5/9	7/10	2/9
Testes										
Weight (g)	0.35	0.39	0.37	0.40	0.36	--	-	--	-	--
Atrophy (unilateral)	0/10	0/10	1/9	0/9	0/10	-	--	--	-	--
Week 53 Interim Sacrifice										
Ovaries										
Weight (g)	--	--	--	--	--	0.031	0.035	0.036	0.039	0.048
Tumor (luteoma and granuloma cell)	--	--	--	--	--	0/10	0/9	0/10	0/7	0/10
Cyst	--	--	--	--	--	4/10	3/9	4/10	3/7	8/10
Testes										
Weight (g)	0.39	0.36	0.36	0.37	0.39	--	--	--	-	--
Atrophy	0/10	0/10	1/9	0/8	1/9	--	--	--	-	--
Week 79 Terminal Sacrifice										
Ovaries										
Weight (g)	--	--	--	--	--	0.028	0.058*	0.073*	0.061*	0.056*
Tumor (luteoma and granuloma cell)	-	-	-	-	--	0/35	0/39	1/33	0/34	3/28
Cyst	--	--	--	--	--	16/35	25/39	20/33	21/34	16/28
Testes										
Weight (g)	0.40	0.40	0.39	0.39	0.34	--	--	--	-	--
Atrophy (bilateral	4/41	3/38	5/40	8/33	9/27	-	-	--	-	--
Deaths and Moribund Sacrifices										
Ovaries										
Weight (g)	--	--	--	--	--					
Tumor (luteoma and granuloma cell)	--	--	--	--	--	0/16	0/12	0/16	0/19	1/22
Cyst	--	-	-	-	--	10/16	5/12	8/16	8/19	10/22
Testes										
Weight (g)						--	--	--	-	--
Atrophy, bilateral	1/9	2/12	2/12	4/20	6/23	--	-	-	-	--

*Statistically significantly higher than control value ($p \leq 0.05$).

this pattern did not persist in the animals that died or were sacrificed subsequently. The occurrence of lymphoma was not considered compound related.

The incidence of hepatic carcinoma and adenoma was increased among the high-dose male animals, but it was not significantly different (Fisher exact test, $p < 0.05$) from that of the untreated control (Table 6).

TABLE 6. Incidence of Hepatocellular Neoplasms in Male Mice Fed NC-302 for 79 Weeks

	Dietary Level (ppm)				
	0	2	10	80	320
Hepatocellular adenoma	3/70	6/69	6/69	7/69	8/70
Hepatocellular carcinoma	4/70	4/69	2/69	1/69	10/70
Adenoma/carcinoma[a]	7/70	10/69	7/69	8/69	15/70

[a]Number of animals with either or both types of neoplasms, not the number of neoplasms.

The study authors noted an increase in leuteoma, and reported two cases of luteal hyperplasia in ovaries of females receiving 320 ppm. The incidence of luteoma in high-dose females was not statistically significant when compared to the concurrent control. However, the study authors did not rule out the possibility of these lesions being compound related in view of its rare occurrence in their historical control population (see Section 14, Reviewers' Discussion of Study Results).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that a) the MTD was approached or exceeded at the high-dose level administered to both sexes of mice; b) the NOEL was 10 ppm for mice of both sexes (the statistically significant increase in ovarian weight in the females of this dose group was considered to be due to ovarian cysts and trimming difficulty); c) the toxic effects of the test material were limited to testes and liver; d) the incidence of hepatic neoplasms was within the range of historical data of the laboratory (adenoma: 0-16%; carcinoma: 6-28%); and e) the incidence of luteoma in females receiving 320 ppm was low, but it was above that usually seen in the historical data of the laboratory.

B. A quality assurance statement was signed and dated April 8, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study authors noted the absence of statistical significance in the occurrence of luteoma in the high-dose group when compared to the concurrent control; however, they expressed the possibility that a compound-related effect could not be ruled out solely on the basis of its low incidence in the historical control population.

We observed that in addition to two cases of luteal hyperplasia (2/50), one case of granulosa cell tumor also occurred in the high dose group females in this study. Since both luteoma and granulosa cell tumor belong to the ovarian stromal cell type, we assess that it is appropriate to combine them in evaluating the possibility of chemical induction. Therefore, our reviewers have statistically analyzed the data in this study, and compared them to the combined historical data provided in this report (Table 7).

TABLE 7. Incidence of Ovarian Tumors in High Dose Female Mice Fed NC-302 for 78 Weeks

	Dietary Level (ppm)					
Neoplasm/Hyperplasia	0	2	10	80	320	Historic Control
Luteoma	0/51	0/51	1/46	0/53	3/50*	0/196
Granulosa cell tumor	0/51	0.51	0.46	0/53	1/50	1/196
Combined stromal tumors	0/51	0/51	1/46	0/53	4/50*	1/196
Luteal hyperplasia	0/51	0/51	0/46	0/53	2/50	[a]

* Statistically significant when compared to the historical control by Fisher's exact test ($p < 0.05$).

[a]Data not available.

The incidence of luteoma in the high-dose group is statistically significant when compared to the historical data (provided by the laboratory) both by itself and also in combination with the incidence of granulosa cell tumor. In view of the above observation, test material NC-302 appears to be carcinogenic in inducing ovarian stromal cell tumors in CD-1 mice under the conditions of the study.

A substantial increase in the incidence of hepatic tumors noted in the high-dose male animals at terminal sacrifice (Table 4) also points to the possibility of tumorigenic potential of NC-302, specifically in view of the gamut of toxic effects it induces to liver.

Table 4 summarized the important compound-related changes noted in the dosed animals during the study. The test material was hepatotoxic for CD-1 mice of both sexes at 80 and 320 ppm dose levels, and caused simultaneous correlated changes in term of a) organ weight, b) gross appearance, c) clinical chemistry parameters (albumin, total protein, A/G ratio and alkaline phosphatase), and d) histomorphology.

A statistically significant decrease in testicular weight was noted in males receiving 320 ppm at the terminal sacrifice. This was correlated with the histomorphological observation of bilateral atrophy of the organ, and was considered to be compound related. We also consider that changes in ovarian weight in dosed females occurred because of the presence of ovarian cysts and consequent trimming difficulties as stated by the study authors. We assess that the significant changes in kidney weights in females receiving 80 or 320 ppm and adrenal weights in dosed males at termination were not of toxicologic importance because there were no histologic correlations or any consistent effects observed at the time of interim sacrifices.

We concur with the study authors in concluding that a) the MTD was achieved in the study; b) the LOEL is 80 ppm; and c) the NOEL is 10 ppm of NC-302 for CD-1 mice of both sexes under the conditions of this study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-17.

APPENDIX A

Materials and Methods

ASSURE TOXR 005541

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