10-21-86

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

005545

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

OCT 21 1986

MEMORANDUM

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

Caswell No.: 215D
Accession No.: 073531-5
Project No.: 736

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.
Pharmacologist
Toxicology Branch/HED (TS-769C)

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

Action Requested:
Review chronic/oncogenicity study in rats to complete data base for registration and tolerance for soybean and cotton.

Results:
The attached DER (EPA No. 68-02-4225; Dynamac I-01lE; June 2, 1986) has been approved by Toxicology Branch. This chronic/oncogenicity study in rats (unpublished study No. NSA 11-8575 by Huntingdon Research Centre, England; 3/27/85) is classified Core Guideline.

Dietary concentrations of 0, 25, 100, and 400 ppm of Assure were administered to Sprague-Dawley-derived CD rats. The data indicate an increase in incidence of combined benign and malignant liver cell tumors in both treated male and female rats. However, this increased incidence of liver tumors was within the range of historical control of the testing laboratory.

Male rats which were treated with 100 and 400 ppm of Assure showed red cell destruction early in the study whereas female rats which received 400

ppm also showed similar effects later in the study. However, histology findings relating to hemopoiesis did not show this effect.

An increased incioence of generalized hepatocytic enlargement was observed in both 400 ppm of Assure treated males and females. The same effect was also found in female rats treated with 100 ppm of Assure. Based upon these findings, LOEL is 100 ppm of Assure; NOEL, 25 ppm.

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EPA: 68-02-4225
DYNAMAC No. 1-011E
June 2, 1986

DATA EVALUATION RECORD

NC-302

Chronic/Oncogenic Toxicity Study in Rats

STUDY IDENTIFICATION: Pompey, C. A., Crome, S. J., Heywood, R., Street, A. E., Gopinath, C., Cherry, C. P., Anderson, A., and Barnard, A. V. Tumorigenicity/toxicity to rats by dietary administration. (Unpublished study No. NSA 11/8575 by Huntingdon Research Centre plc, Huntingdon, Cambridgeshire, England, for E. I. du Pont de Nemours and Co., Inc., Wilmington, DE; dated March 27, 1985.) Accession No. 073531-5.

APPROVED BY:

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

Signature: I Cecil Felkner
Date: 6-3-86

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1. CHEMICAL: NC-302; Assure; ethyl 2-[4-(6-chloro-2-quinoxalinyloxy) phenoxy]propionate.

2. TEST MATERIAL: Five samples of NC-302 were used in the study. The white powder was 99.1% pure. Samples were stored in daylight at ambient temperatures. The batch numbers for the five samples were 8002 HRC 2.7.82/2, 8002 HRC 3.11.82/4, 8002 HRC 25.2.83/5, 8002 HRC 24.6.83/4, and 8002 HRC 24.10.83.

3. STUDY/ACTION TYPE: Chronic/oncogenicity toxicity study in rats.

4. STUDY IDENTIFICATION: Pompey, C. A., Crome, S. J., Heywood, R., Street, A. E., Gopinath, C., Cherry, C. P., Anderson, A., and Barnard, A. V. Tumorigenicity/toxicity to rats by dietary administration. (Unpublished study No. NSA 11/8575 by Huntingdon Research Centre plc, Huntingdon, Cambridgeshire, England, for E. I. du Pont de Nemours and Co., Inc., Wilmington, DE; dated March 27, 1985.) Accession No. 073531-5.

5. REVIEWED BY:

Robert J. Weir, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Robert J. Weir
Date: June 3, 1986

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: June 3, 1986

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Chronic Toxicity/Oncogenicity
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Signature: I. Cecil Felkner
Date: 6-3-86

Whang Phang, Ph.D.
EPA Reviewer

Signature: W. Phang
Date: 6/26/86

Marcia Van Gemert, Ph.D.
EPA Section Chief

Signature: M. van Gemert
Date: 6.30.86

Page_____ is not included in this copy.

Pages 24 through 41 are not included.

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7. CONCLUSIONS:

A. Treatment of male and female rats with NC-302 for 104 weeks resulted in a LOEL of 100 ppm based on altered red cell parameters and slight/minimal centrilobular enlargement of the liver. The NOEL is 25 ppm.

B. This study is classified Core guideline.

Items 8, 9, and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Diets were prepared weekly by preparation of a premix in which the test material was mixed with Spratt's Laboratory Animal Diet No. 2. The required concentrations of 0, 25, 100, and 400 ppm were prepared by dilution and mixing in a double-cone blender for 7 minutes. Dietary homogeneity and stability were determined in a prior study. Dietary concentration was analyzed for agreement with nominal values in the first week and during months 3, 6, 9, 12, 15, 18, 21, and 24. Diet and tapwater were available ad libitum.

2. The test rats were from Charles River Breeding Laboratories (Portage, MI) and were of the Sprague-Dawley-derived CD strain. They were 28 days old on receipt. They were acclimated for 11 days and randomly distributed 50/sex/group to four dose groups. Twenty additional rats/sex were maintained for health checks. A satellite group of 35 animals of each sex was attached to each of the four main groups. Ten rats/sex/group were killed at 26 and 52 weeks for interim evaluation. All surviving satellite rats were killed at 78 weeks for histopathologic evaluation.

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

3. The rats were housed five/sex/cage. Room temperature and humidity were maintained at 21°C and 50%, respectively. Lighting was controlled to a 12-hour light/dark cycle.

4. All animals were observed twice daily for mortality, morbidity, and clinical or behavioral changes for the first 4 weeks and weekly thereafter. Body weights and food consumption were measured weekly. Water consumption was measured during weeks 11 and 24.

5. Hematologic and clinical chemistry evaluations were performed before dosing in 20/sex of the satellite animals and in 10 animals/sex at weeks 12, 26, 51, 78, and 101. The hematologic parameters measured were red blood cell, white blood cell, differential, reticulocyte, and platelet counts, hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), and mean corpuscular hemoglobin (MCH). Clinical chemistry parameters determined were glucose, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, lactate dehydrogenase, glutamate dehydrogenase, total bile acids, total bilirubin, urea nitrogen, total protein, albumin (A), globulin (G), A/G ratio, sodium, potassium, chlorine, calcium, inorganic phosphorus, creatinine, cholesterol, and plasma cholinesterase. Protein electrophoresis was used to determine $\alpha 1$, $\alpha 2$, β, and γ globulins beginning with week 25. During weeks 12, 25, 51, 77, and 103, urinalysis was performed to provide values for volume, pH, specific gravity, protein, reducing substance, glucose, ketones, bile pigments, urobilinogen, and hemoglobin; centrifuged residue was examined microscopically.

6. After 26 and 52 weeks, 10 males and 10 females from each satellite group were killed. After 78 weeks the surviving satellite animals were killed. Surviving animals of the main group were killed at 104 weeks. Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, thyroids, and uterus from all rats at scheduled sacrifices were weighed. Approximately 50 tissues per animal were preserved for histopathologic examination.

7. Statistical analysis for body weight gains and water and food consumption was performed using analysis of variance (ANOVA), and data from the dose groups were compared with control groups using Student's T-test.

   Clinical pathology data were analyzed for homogeneity of variance using Bartlett's test. Heterogeneous data were transformed using log transformations or square root transformations. Transformed and untransformed data that showed homogeneity of variance were analyzed using ANOVA, Student's T-test, and Williams' test. Heterogeneous data were analyzed using the Kruskal-Wallis analysis of ranks. Dose related trends were analyzed by the Mantel test.

Organ weight and terminal body weight data were subjected to Bartlett's test for homogeneity of variance and log transformed when necessary ($p < 0.01$). Further, the homogeneous data were analyzed by ANOVA. In cases where significant (10%) within-group differences between the organ and terminal body weights occurred, analysis of covariance was performed using the terminal body weight as the covariant. Williams' test for dose-related responses was used for intergroup comparisons.

Differences in tumor incidences were compared using chi-square or exact probability calculations. Log-rank methods were used to adjust for intergroup differences in mortality.

B. Protocol: A protocol was not provided in the report.

12. REPORTED RESULTS:

A. NC-302 was stable in the diet at ambient temperatures for 18 days. Data were presented to support homogeneity of the test material in the diets. Samples of diets were taken on a quarterly basis, and analysis showed them to be all within 13.2 percent of the nominal concentration.

B. Clinical signs were reported to be comparable between control and dosed groups. Individual animal observations were presented but there was no summary tabulation. Animals with ophthalmic lesions prior to study initiation were not included in the study. At week 100, there were no compound-related ophthalmic lesions in the groups receiving 400 ppm when compared to controls.

As evident in Table 1, mortality in the dosed animals was comparable to the controls.

C. Food consumption and body weights (Table 2) were comparable in the dosed and control animal values. Intake of NC-302 at nominal levels of 25, 100, and 400 ppm was, respectively, 0.9, 3.7, and 15.5 mg/kg/day for males and 1.1, 4.6, and 18.6 mg/kg/day for females. Water consumption, measured at weeks 11 and 24 only, did not differ between the dosed and control groups.

D. In males receiving 400 ppm, HCT was significantly decreased ($p \leq 0.01$) at weeks 12, 26, and 51 and RBC and Hb were significantly decreased ($p \leq 0.05$) at weeks 12 and 26. There were no changes in HCT, Hb, or RBC in males receiving 400 ppm at weeks 78 or 101, and only sporadic effects at doses of 25 and 100 ppm. In females receiving 400 ppm, RBC was decreased ($p \leq 0.05$) at weeks 26, 51, and 78, HCT was decreased at weeks 51 and 101, and Hb was decreased at week 101; only sporadic significant changes were seen at 25 and 100 ppm. The results of hematologic testing for weeks 12, 51, and 101, are presented in Tables 3, 4, and 5.

TABLE 1. Mortality in Rats Fed NC-302 in the Diet for 2 Years

Dose Group (ppm)	Number of Deaths During Weeks (% Mortality)					% Survival
	1-26	27-52	53-78	79-106	Total	
Males						
Control	0/50 (0)	1/50 (2)	7/49 (14)	25/42 (60)	30/50 (60)	40
25	0/50 (0)	2/50 (4)	10/48 (21)	17/38 (45)	29/50 (58)	42
100	0/50 (0)	0/50 (0)	13/50 (26)	17/37 (46)	30/50 (60)	40
400	1/50 (2)	1/49 (2)	17/48 (35)	14/31 (45)	33/50 (66)	34
Females						
Control	0/50 (0)	3/50 (6)	7/47 (15)	17/40 (43)	27/50 (54)	46
25	0/50 (0)	2/50 (4)	11/48 (23)	16/37 (43)	29/50 (58)	42
100	0/50 (0)	1/50 (2)	2/49 (4)	19/47 (40)	22/50 (44)	56
400	0/50 (0)	0/50 (0)	5/50 (10)	16/45 (36)	21/50 (42)	58

TABLE 2. Mean Body Weights (g ± S.D.) at Selected Weekly Intervals for Male and Female Rats Fed NC-302 in the Diet for 2 Years

Week	Control	25 ppm	100 ppm	400 ppm
		MALES		
0	190± 10	187± 11	189± 10	186± 10
13	542± 55	536± 58	537± 57	513± 56
26	664± 83	658± 86	661± 86	638± 85
52	803±125	794±125	796±126	767±117
78	859±135	851±140	852±151	837±132
104	844±203	827±144	823±166	889±183
		FEMALES		
0	149± 8	146± 9	147± 9	145± 9
13	298± 30	299± 24	301± 29	295± 32
26	346± 46	350± 37	359± 45	354± 52
52	442± 81	457± 67	471± 87	454± 97
78	533±111	551± 86	567±117	539±125
104	566±134	573±114	591±120	552±114

*No values were statistically different from the control ($p < 0.05$).

TABLE 3. Mean Hematologic Values at Week 12 for Male and Female Rats Fed NC-302 for 2 Years

Parameter (Units)	Dose Level (ppm) 0	25	100	400
		MALES		
HCT[a] (%)	50.8± 2.2	50.8± 1.4*	49.9± 1.9	47.3± 1.4**
Hb (g/dL)	15.8± 0.7	15.2± 0.6*	14.6± 0.5**	14.2± 0.7**
RBC ($x10^6/mm^3$)	7.71± 0.51	7.56± 0.23	7.16± 0.30**	6.80± 0.35**
MCHC (%)	31.0± 1.2	29.8± 0.7**	29.2± 0.7**	29.6± 0.8**
MCV (fL)	66.0± 3.2	67.3± 1.9	69.8± 1.8**	70.6± 2.1**
MCH (pg)	20.5± 0.8	20.0± 0.7	20.4± 0.7	20.9± 0.5
Plat. ($x10^3/mm^3$)	780.0± 97.0	757.0± 49.5	693.0± 79.6**	634.0± 56.2**
		FEMALES		
HCT (%)	44.9± 1.4	46.8± 2.2*	47.0± 1.2*	47.9± 1.4**
Hb (g/dL)	14.7± 0.6	14.7± 0.6	14.8± 0.5	14.2± 0.7**
RBC ($x10^6/mm^3$)	6.70± 0.30	6.74± 0.40	6.75± 0.20	6.80± 0.35**
MCHC (%)	32.8± 1.5	31.5± 0.8*	31.7± 1.0*	29.6± 0.8**
MCV (fL)	67.0± 2.9	69.3± 3.1	68.4± 2.0	70.6± 2.1**
MCH (pg)	21.9± 0.4	21.8± 0.9	21.7± 0.5	20.9± 0.5
Plat. ($x10^3/mm^3$)	942.0± 74.7	876.2±118.7	1010.0±113.5	949.0± 80.2

*Statistically different from the control ($p < 0.05$).
**Statistically different from the control ($p < 0.01$).

[a]The abbreviations used are as follows: HCT (hematocrit), Hb (hemoglobin), RBC (red blood cells), MCHC (mean corpuscular hemoglobin concentration), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and Plat (platelet count).

TABLE 4. Mean Hematologic Values at Week 51 for Male and Female Rats Fed NC-302 for 2 Years

Parameter (Units)	Dose Level (ppm)			
	0	25	100	400
		MALES		
HCT[a]	53.3± 5.4	48.9± 1.8**	47.7± 2.1**	46.9± 3.6**
Hb (g/dL)	16.1± 1.8	15.4± 0.7	15.3± 0.8	15.1± 1.1
RBC ($x10^6/mm^3$)	8.21± 0.67	8.14± 0.39	8.10± 0.46	7.81± 0.67
MCHC (%)	30.2± 1.4	31.4± 0.9*	32.0± 0.8**	32.3± 1.3**
MCV (fL)	65.1± 3.3	60.2± 2.1**	58.3± 1.9**	60.1± 2.8*
MCH (pg)	19.6± 0.7	18.9± 0.7	18.9± 0.6	19.4± 0.7
Plat. ($x10^3/mm^3$)	730.8± 65.5	722.0±103.1	755.0± 64.5	716.0± 77.1
		FEMALES		
HCT (%)	47.3± 2.7	46.9± 1.8	46.7± 1.5	45.0± 1.9*
Hb (g/dL)	15.5± 1.0	15.6± 0.8	15.8± 0.7	14.9± 0.6
RBC ($x10^6/mm^3$)	6.40± 0.45	6.38± 0.34	6.50± 0.37	5.70± 0.42**
MCHC (%)	32.8± 0.5	33.3± 0.6	33.9± 0.8	33.2± 0.8*
MCV (fL)	73.9± 2.4	73.6± 4.0	71.9± 2.9	78.8± 6.3*
MCH (pg)	24.2± 0.8	24.5± 1.4	24.5± 0.9	26.1± 2.2*
Plat. ($x10^3/mm^3$)	666.0± 77.5	652.2± 62.8	681.0± 94.0	611.0± 56.9

*Statistically different from the control ($p < 0.05$).
**Statistically different from the control ($p < 0.01$).

[a]The abbreviations used are as follows: HCT (hematocrit), Hb (hemoglobin), RBC (red blood cells), MCHC (mean corpuscular hemoglobin concentration), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and Plat (platelet count).

TABLE 5. Mean Hematologic Values at Week 101 for Male and Female Rats Fed NC-302 for 2 Years

Parameter (Units)	Dose Level (ppm) 0	25	100	400
		MALES		
HCT[a]	44.7± 7.7	45.8± 2.9	43.8± 5.5	44.3± 5.0
Hb (g/dL)	13.9± 2.5	14.1± 1.0	13.7± 1.7	13.7± 2.1
RBC ($x10^6/mm^3$)	7.11± 1.39	7.31± 0.52	7.24± 1.00	7.31± 1.23
MCHC (%)	31.1± 0.8	30.7± 0.6	31.2± 1.0	30.7± 1.8
MCV (fL)	63.5± 4.5	62.8± 2.0	60.7± 3.8	61.3± 5.1
MCH (pg)	19.7± 1.1	19.3± 0.6	18.9± 0.9	18.7± 0.9*
Plat. ($x10^3/mm^3$)	993.0±156.1	942.0±141.3	941.0±291.4	884.0± 97.3
		FEMALES		
HCT (%)	47.4± 2.5	45.3± 3.7	46.1± 2.7	42.5± 2.7**
Hb (g/dL)	15.5± 0.9	14.8± 1.4	15.1± 0.8	13.4± 0.9**
RBC ($x10^6/mm^3$)	7.30± 0.57	6.98± 0.76	7.19± 0.53	6.77± 0.61
MCHC (%)	32.7± 0.7	32.3± 0.8	32.5± 0.8	31.6± 0.9**
MCV (fL)	65.0± 2.7	64.6± 2.8	64.3± 2.1	63.4± 2.8
MCH (pg)	21.3± 0.9	20.2± 0.8	20.8± 0.7	19.9± 0.5**
Plat. ($x10^3/mm^3$)	53.0± 59.5	692.0±141.2	696.0± 69.8	766.0±131.5

*Statistically different from the control, $p < 0.05$.
**Statistically different from the control, $p < 0.01$.

[a]The abbreviations used are as follows: HCT (hematocrit), Hb (hemoglobin), RBC (red blood cells), MCHC (mean corpuscular hemoglobin concentration), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and Plat (platelet count).

Changes in MCHC, MCV, and MCH did not always correlate with Hb, HCT, and RBC. There was no consistency with time or dose when significant changes occurred and there were both increases and decreases.

Platelet counts were significantly ($p \leq 0.01$) decreased in the 100- and 400-ppm males at week 12 only. The platelet counts in the dosed females were comparable to the controls at all intervals.

The results of clinical chemistry determinations at weeks 12, 51, and 101 are presented in Table 6. Albumin was significantly increased in the males ($p \leq 0.01$) receiving 400 ppm at weeks 12, 26, 27, and 78 but not at weeks 51 or 104. Albumin was significantly ($p < 0.01$) increased in the 100- and 400-ppm female groups at weeks 12 and 51 and in the 400-ppm group only at week 101. There were no appreciable changes in the A/G ratio at termination but the ratio was increased in high-dose males at 12, 26, and 78 weeks and in high-dose females at weeks 12, 26, 51, and 78. Plasma cholinesterase was significantly ($p < 0.05$ or $p < 0.01$) increased in the high-dose males at weeks 12, 26, 51, and 78, in all female groups at weeks 12 and 26, and in high-dose females at week 78. Alkaline phosphatase activity was significantly increased ($p < 0.05$) in the high-dose males at all intervals and in the high-dose females at 101. Electrophoresis indicated a reduction in $\alpha 2$ and β globulins in the high-dose males at weeks 27 and 78. It was reported that intergroup differences for other parameters, although in some instances attained levels of significance, were without consistency with regard to dose and time and were of no toxicological importance.

E. The results of selected organ weights taken at interim sacrifices at weeks 27, 53, and 79 and those measured at termination (weeks 105-107) are presented in Table 7. Liver weights were significantly increased ($p < 0.05$ to $p < 0.01$) in the high-dose group of both sexes at weeks 27, 53, and 78; however, at weeks 105-107 the liver weights were only significantly increased in the high-dose female group. There appeared to be a general reduction in spleen weights; although the reduction was dose related, no morphologic correlation was seen in histopathologic examination.

F. Gross lesions considered to be related to NC-302 dosing were found at necropsy and were limited to the liver and lungs. The increased incidence of liver masses (Table 8) in mid- and high-dose females from the main group indicated the presence of neoplasia. In addition, there was an increased incidence of dark areas of the liver in the high-dose males and in mid- and high-dose females. There was also liver enlargement in the satellite group observed in the high-dose males at weeks 26, 52, and 78 and in the high-dose females at week 52. Mid- and high-dose males showed pale foci in the lungs; however, the study authors did not consider this to be toxicologically important.

TABLE 6a. Selected Mean Chemistry Values for Male Rats Fed NC-302 for 2 Years

Parameter (units)	Dose Level (ppm) 0	25	100	400
			Week 12	
ALB[a] (g/dL)	3.3 ± 0.1	3.4 ±0.12	3.4 ±0.07**	3.7 ±0.11**
A/G ratio	0.98± 0.061	0.98±0.068	1.02±0.075	1.32±0.137**
ALP (mU/mL)	21.0 ± 2.6	26.2 ±7.7	25.4 ±5.3	43.3 ±8.8*
CHOL (mg/dL)	37.7 ± 4.9	38.9 ±9.0	47.7 ±6.0*	44.1 ±0.16**
P-ChE (µmol/ nL/min)	0.48 ± 0.07	0.45 ±0.07	0.57 ±0.18	0.65±0.16**
			Week 51	
ALB (g/dL)	3.5 ± 0.3	3.2± 0.3	3.4± 0.1	3.5± 0.4
A/G ratio	0.91± 0.144	0.83±0.116	0.88±0.075	1.00±0.269
ALP (mµ/mL)	109.9 ±44.5	146.0± 67.3	132.7± 42.8	238.4± 75.9**
CHOL (mg/dL)	96.3 ±22.8	81.9± 18.7	97.1± 29.7	79.7± 22.6
P-ChE (µmol/ nL/min)	0.68± 0.13	0.69± 0.13	0.62± 0.16	0.88± 0.27*
			Week 101	
ALB[a] (g/dL)	3.2 ± 0.5	3.0 ± 0.6	3.3 ± 0.7	3.2 ± 0.8
A/G ratio	0.87± 0.260	0.80± 0.210	0.86± 0.234	0.83± 0.330
ALP (mµ/mL)	86.8 ±25.3	85.1 ±18.6	101.3 ±33.9	187.2 ±62.5**
CHOL (mg/dL)	129.9 ±48.0	133.0 ±41.1	150.5 ±80.0	80.5 ±21.8*
P-ChE (µmol/ nL/min)	0.86± 0.17	0.86 ± 0.26	0.92± 0.47	1.08± 0.29

*Significantly different from control ($p < 0.05$).
**Significantly different from control ($p < 0.01$).

[a]Abbreviations used are as follows: ALB (albumin), A/G (albumin/globulin) ALP (alkaline phosphatase), CHOL (cholesterol), and P-ChE (plasma cholinesterase).

TABLE 6b. Selected Mean Chemistry Values for Female Rats Fed NC-302 for 2 Years

Parameter (units)	Dose Level (ppm) 0	25	100	400
		Week 12		
ALB (g/dL)	3.5 ± 3.3	3.6 ±0.2	3.9 ±0.1**	4.0 ±0.2**
A/G ratio	0.97± 0.121	0.98±0.076	1.19 ±0.080*	1.30±0.079**
ALP (mU/mL)	15.1 ± 4.4	14.2 ±3.0	14.4 ±4.1	16.9 ±5.7
CHOL (mg/dL)	1.41± 0.32	49.4 ±7.1	40.3 ±8.1	38.3 ±4.6
P-ChE (µmol/ nL/min)	1.41 ± 0.32	1.94 ±0.49*	1.94± 0.62*	1.96±0.55*
		Week 51		
ALB (g/dL)	4.1 ± 0.3	4.2 ± 0.2	4.6 ± 0.3**	4.8 ± 0.3**
A/G ratio	1.27± 0.189	1.33± 0.999	1.34± 0.202	1.59± 0.205**
ALP (mµ/mL)	42.8 ±12.2	40.5 ±10.5	56.0 ±20.6	54.2 ±16.6
CHOL (mg/dL)	103.2 ±36.3	81.7 ±17.4	81.1 ±18.6	87.7 ±17.9
P-ChE (µmol/ nL/min)	1.89± 0.49	1.96± 0.48	1.98± 0.40	2.21± 0.46
		Week 101		
ALB (g/dL)	4.2 ± 0.3	3.9 ± 0.2	4.0 ± 0.3	4.0 ± 0.5
A/G ratio	1.13± 0.154	1.02± 0.133	1.07± 0.174	1.02 ± 0.206
ALP (mµ/mL)	52.6 ±24.5	60.5 ±17.5	66.9 ±20.4	83.3 ± 43.5*
CHOL (mg/dL)	112.5 ±21.3	137.4 ±81.2	99.2 ±23.5	117.2 ± 63.5
P-ChE (µmol/ nL/min)	1.37± 0.26	1.52± 0.40	1.65± 0.63	1.57± 0.39

*Significantly different from control ($p < 0.05$).
**Significantly different from control ($p < 0.01$).

[a]Abbreviations used are as follows: ALB (albumin), A/G (albumin/globulin) ALP (alkaline phosphatase), CHOL (cholesterol), and P-ChE (plasma cholinesterase).

TABLE 7. Selected Organ Weights[a] for Male and Female Rats Fed NC-302 in the Diet for 2 Years

Dose (ppm)	27 Weeks		53 Weeks		79 Weeks		105-107 Weeks	
	Liver (g)	Spleen (g)	Liver (g)	Spleen (g)	Liver (g)	Spleen (g)	Liver (g)	Spleen (g)
Male								
0	23.6 (23.8)	0.90 (0.90)	28.6 (28.3)	1.04 (1.03)	30.7 (30.3)	1.40 (1.40)	26.7 (27.4)	1.38 (1.38)
25	24.0 (23.5)	0.85 (0.84)	28.4 28.4	1.05 (1.05)	29.6 (30.8)	1.13 (1.19)	24.9 (25.0)	1.19 1.17
100	24.2 (24.3)	0.90 (0.90)	31.7 (33.2)	0.94 (0.97)	28.0 (27.2)	1.28 (1.25)	28.7 (29.6)	1.27 (1.21)
400	33.1* (33.3)	0.79 (0.79)	35.8** (34.7)	0.96 (0.94)	35.0* (34.9)	1.05** (1.05)	29.4 (31.2)	1.09 (1.13)
Female								
0	11.5 (11.1)	0.58 (0.56)	16.7 (15.7)	0.58 (0.56)	17.6 (18.0)	0.74 (0.73)	20.1 (20.0)	0.85
25	11.3 (11.0)	0.53 (0.52)	16.3 (16.9)	0.55 (0.56)	17.1 (17.2)	0.77 (0.76)	20.0 (20.1)	0.75
100	12.1 (2.3)	0.49* (0.50)	15.9 (16.5)	0.58 (0.59)	19.4 (20.7)	0.68 (0.70)	21.6 (22.2)	1.01
400	13.1** (13.7)	0.49* (0.52)	20.6** (20.6)	0.61 (0.61)	20.9** (20.9)	0.67 (0.66)	24.2** (23.7)	0.71

[a]Mean values adjusted for body weight as the covariant (unadjusted means are in parentheses).

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

**Significantly different from the control value ($p \leq 0.01$).

TABLE 8. Incidence of Grossly Evident Liver Masses in Rats Fed NC-302 for 2 Years

Group	Males/Dose Level (ppm)				Females/Dose Level (ppm)			
	0	25	100	400	0	25	100	400
Main Group	(50)[a]	(50)	(50)	(50)	(52)	(50)	(50)	(50)
Liver masses	1	3	3	2	0	0	4	4
Satellite Group	(33)	(34)	(32)	(31)	(34)	(33)	(33)	(33)
Liver masses	0	0	2	1	0	0	0	0
Total	(83)	(84)	(82)	(81)	(86)	(83)	(83)	(83)
	1	3	5	3	0	0	4	4

[a]Number of rats examined/dose and sex.

G. Histopathologic findings were limited to the liver, although there was a higher incidence of mineralization of the pelvic/papillary region of the kidneys in the female rats from all dose groups (Table 9). The latter is a common spontaneous lesion in rats of this age and strain. There was a slight, but generalized, hepatocytic enlargement of the liver in the high-dose male rats at termination. Centrilobular enlargement was observed in rats of both sexes fed the high dose and in a few animals fed the mid dose. Cytoplasmic eosinophilia of the centrilobular hepatocytes occurred in 400-ppm rats of both sexes.

Nonneoplastic liver damage was also apparent at interim sacrifices. Hepatocyte enlargement in the high-dose males occurred in 6/10, 9/10, and 6/11 rats at 6, 12, and 18 months, respectively. Occurrences in the high-dose females were 0/10, 7/10, and 4/11 rats for the same intervals; at the 100-ppm dose, hepatocyte enlargement was seen in 1/10 and 1/12 rats at 12 and 18 months, respectively. The controls of either sex showed no incidence. Cytoplasmic eosinophilia was observed in the high-dose males and females at an incidence of 2/10 and 3/10 at 12 months and 6/11 and 8/13 at 18 months. The controls showed no effect.

Liver tumor incidence is presented in Table 10. At 100 ppm, two malignant cell tumors in livers were found at 12 months in males and one at 18 months in females. In the 400-ppm male dose group, one malignant tumor was found at the 18-month interim sacrifice. Statistical analysis, using the time-to-tumor technique accepted by the International Agency for Research on Cancer, revealed that there was no significant treatment-related effect on the incidence of benign liver cell tumors in male or female rats.

There was, however, a statistically significant trend with dose ($p < 0.01$) in which the incidence of malignant liver cell was higher in the the high-dose females; however, this parameter showed no effect on the males and there was no effect in either sex when the benign and malignant tumors were analyzed together.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. "Treatment with NC-302 for 104 weeks at a dietary level of 400 ppm did not reveal any definite carcinogenic effect. The effect seen on the incidence of liver cell tumours in female rats receiving 400 ppm was not clear-cut as there was no significant increase when compared with controls, although a positive trend for malignant liver cell tumours was observed in female rats when all groups were analysed. However, the incidence of combined benign and malignant liver cell tumours in males and females of the low dose group (25 ppm) was comparable with the controls, and no malignant liver cell tumours were observed in females receiving 25 ppm."

TABLE 9. Incidences of Selected Nonneoplastic Histopathologic Findings in Rats Fed NC-302 for 2 Years[a]

Organ and Finding	Males/Dose Level (ppm)				Females/Dose Level (ppm)			
	0	25	100	400	0	25	100	400
Heart	(50)[b]	(50)	(50)	(50)	(50)[a]	(50)	(50)	(50)
myocarditis	36	27	31	39	27	25	31	28
myocardial fibrosis	42	46	38	43	30	29	33	32
Lungs	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
alveolar macrophages	22	28	25	28	21	25	30	24
arterial median calcification	41	38	41	40	30	35	36	35
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
hepatocyte enlargement	0	0	1	16	0	0	1	15
cytoplasmic eosinophilia	0	0	0	15	0	0	0	34
dilated/congested sinusoid	12	11	13	22	27	24	46	36
Spleen	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
hemosiderosis	34	28	25	37	41	41	44	43
extramedullary hemopoiesis	0	6	4	3	10	5	7	7
Kidneys	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
tubular basophilia/dilation	2	10	12	10	6	9	11	9
pelvic/papillary mineralization	2	0	1	3	17	31	30	31
Adrenals	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
cortical hyperplasia	2	1	3	1	9	5	5	4
enlarged/eosinophilia cortical cells	11	6	5	7	1	3	1	3
enlarged zona glomerulosa cells	25	25	22	25	24	27	30	38
cortical degeneration/hemorrhage	4	7	9	8	38	32	39	29

[a] Includes animals at terminal sacrifice and those dying or killed during the study; does not include animals sacrificed by design at 26, 52, and 78 weeks.

[b] The number of tissues examined microscopically are in parentheses.

TABLE 10. Incidence of Liver Cell Tumors Found Histopathologically in Rats Fed NC-302 for 2 Years

Type of tumor	Males/Dose Level (ppm)				Females/Dose Level (ppm)			
	0	25	100	400	0	25	100	400
(No. of rats examined)	(83)	(84)	(82)	(81)	(86)	(83)	(83)	(83)
Benign liver cell tumor	0	1	1	2	3	1	1	1
Malignant liver cell tumor	1	1	5	2	0	0	2	4[a]
Combined benign or malignant liver cell tumors	1	2	6	4	3	1	3	5

[a]One rat with multiple tumors.

"Treatment with NC-302 for 104 weeks induced non-neoplastic changes in the liver consisting of slight generalised hepatocyte enlargement in male rats receiving 400 ppm; slight/minimal centrilobular enlargement in rats of both sexes receiving 400 or 100 ppm; and cytoplasmic eosinophilia of centrilobular hepatocytes in rats of both sexes receiving 400 ppm. For rats receiving 400 ppm, increased liver weight and increased plasma alkaline phosphatase activity were also noted."

"Minimal changes in red blood cell parameters were essentially confined to the high dose level of 400 ppm. Minor increases in plasma cholinesterase activity which became less evident as the study progressed are of dubious toxicological significance. It should be noted that in a 13-week study in rats (see HRC Report No. NSA 3/8 1669) a similar effect upon plasma cholinesterase activity at 1280 ppm was shown to be completely reversible following a 6-week withdrawal period."

"It is concluded that the no effect level in this study was 25 ppm; 100 ppm represented a minimal toxic effect level and 400 ppm a definite toxic effect level."

B. A quality assurance statement was signed and dated March 27, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Data in the report demonstrated that the stability and homogeneity of the test substance in the diet was acceptable. The nominal and actual diet concentrations were found to be comparable within 13.2%.

B. The hematologic values indicate that there is a trend to very mild anemia or red cell destruction in the mid- and high-dose males early in the study and in high-dose females late in the study. Since the histopathologic findings related to hemosiderosis or hemopoiesis do not reflect a change, this is not considered a biologically meaningful effect.

C. Of the clinical chemistry determinations found to be altered, only the alkaline phosphatase values had biological significance. The high-dose males at all intervals and high-dose females at week 101 showed elevated values. These clinical chemistry effects were correlated with the histopathologic alterations seen in the liver. Except for cholinesterase activity, the remaining clinical chemistry determinations, although found to be different from the control values, varied too much with doses and time to be biologically meaningful. There is no toxicological meaning associated with the elevated cholinesterase values (higher than normal).

D. There were no dose-related alterations observed in urinalysis data that could be related to the test material at any interval.

E. No ophthalmoscopic alteration could be related to the administration of NC-302 in the diet at any dose level when compared to control animals.

F. Terminal studies indicated that the number of liver masses seen at necropsy were increased in male and female rats fed the mid and high doses. Histopathologically, there was an increased incidence of benign and malignant liver cell tumors in both sexes. Nevertheless, statistical examination indicated that the incidences were comparable to those in the control. There was, however, a significant trend$^{\updownarrow}$ ($p < 0.01$) in the female rats. The incidence of tumors of the liver in dosed groups was within the range found spontaneously in Sprague-Dawley rats.[2] It is of interest to note that there were no preneoplastic or hyperplastic lesions in the liver, an observation that is unusual for this strain. Nonneoplastic lesions consisted of generalized hepatocytic enlargement in the 400-ppm males. Slight centrilobular enlargement and cytoplasmic eosinophilia of the centrilobular hepatocytes occurred in some of the male and female rats receiving 400 ppm. In a few rats of both sexes receiving 100 ppm, minimal centrilobular enlargement was observed. The nonneoplastic changes in the liver are fragmented primarily because of a lack of collection of similar pathologic terminology, e.g., vacuoles, vacuolated, and eosinophilic/vacuoles are used as separate diagnostic entities.

The higher incidence of pelvic/papillary mineralization observed in high-dose female rats was considered of no toxicologic significance because of its common spontaneous occurrence.

The statistically increased liver weights (generally $p \geq 0.01$) in the high-dose males and females correlated with the histopathological findings in the liver of both sexes at 400 ppm at 27, 53, and 78 weeks and at termination (females only).

All other alterations in organ weights were incidental on the basis of lack of correlation with histopathologic findings and clear dose/effect relationships.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-14, and Appendix B, Historical Data in Sprague-Dawley Rats--Liver Tumors.

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[2] Hazelton Laboratories America Inc. (1984); see Appendix B.

TABLE 11. Selected Nonneoplastic Liver Lesions Observed in Male and Female Rats Killed at 6, 12, and 18 Months of a 2-Year Feeding Study with NC-302

No. of rats showing:	Time (Mos.)	Males: No. rats Examined	Males: ppm in diet 0	Males: ppm in diet 100	Males: ppm in diet 400	Females: No. rats Examined	Females: ppm in diet 0	Females: ppm in diet 100	Females: ppm in diet 400
Hepatic enlargement	6	(10)	0	0	6	(10)	0	0	0
	12	(10)	0	1	9	(10)	0	0	7
	18	(11)	0	1	6	(13)	0	0	4
Total			0/31	2/31	21/31		0/33	0/33	11/33
Cytoplasmic eosinophilia	6	(10)	0	0	0	(10)	0	0	0
	12	(10)	0	0	2	(10)	0	0	3
	18	(11)	0	0	6	(13)	0	0	8
Total			0/31	0/31	8/31		0/33	0/33	11/33
Dilated/congested sinosoids	18	(11)	3	1	5	(11)	3	4	8

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