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Reviewed by: Walter J. Kozumbo, Ph.D. Walter J. Kozumbo 4-29-92
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. J.M.I 4/29/92
Section I, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral/Rats (82-1)

TOX.CHEM.NUMBER: not assigned

MRID NUMBER: 421715-01 (PART A)

TEST MATERIAL: NC-319 Technical or MON 12000 Technical

STUDY NUMBER: 436605

TESTING FACILITY: Inveresk Research International
Musselburgh, EH21 7UB
Scotland

SPONSOR: Nissan Chemical Industries Limited
Kowa Hitotsubashi Building, 7-1, 3 chome
Kanda-Nishiki-cho, Chiyoda-ku
Tokyo 101, Japan

TITLE OF REPORT: NC-319: 13 Week Dietary Toxicity Study in Rats

AUTHORS: C. Atkinson; C.J. Perry; P. Hudson; J. Finch

REPORT ISSUED: June 14, 1988

CONCLUSIONS: NC-319 Technical was fed to Sprague Dawley rats (20/sex/dose) at 0, 100, 400, 1600 and 6400 ppm for 13 weeks. The dosages consumed in mg/kg/day for males and females, respectively, were 7.4 and 8.9 at 100 ppm, 28.8 and 37.3 at 400 ppm, 116 and 147 at 1600 ppm, and 497 and 640 at 6400 ppm. All significant effects considered to be treatment-related were observed in males and/or females at the highest dose (6400 ppm) only. Decreases were observed in body-weight gains and absolute organ weights (adrenals, liver, thymus, heart and kidneys), and in blood levels of cholesterol, bilirubin, protein, albumin and calcium. Increases were found in mean cell hemoglobin and its concentrations, in blood alanine aminotransferase activities and creatinine levels, and in vacuolated livers and pigmented kidney tubules. Underscored effects indicate dose-response relationships. The dose-dependent effect on kidney tubules was observed in both sexes. The NOEL for NC-319 Technical in rats was established at 1600 ppm and the LOEL at 6400 ppm for each sex. Based primarily on decreased body-weight gains, the MTD for each sex would appear to be slightly lower than 6400 ppm, the highest dose tested.

CLASSIFICATION:

Core Guideline --- This study satisfies guideline requirements for a subchronic feeding study in rats (82-1).

A signed "Quality Assurance Statement" was provided indicating that Standard Operating Procedures were followed and test facilities were inspected.

II. OBSERVATIONS AND RESULTS:

A. Mortality and Antemortem Observations:

No mortality was observed during the 13-week treatment except for one female in the 100 ppm group at week 10. No antemortem clinical observations were observed.

B. Body Weights:

All animals progressively gained weight throughout the 13-week study. (See attached p. 41.) During each week of the study, the highest-dosed groups (6400 ppm) of both males and females exhibited statistically significant reductions in their mean body weights relative to their respective control groups (0 ppm). Except for a few significant weight increases observed for the 400 ppm group of treated males during weeks 1, 2 and 5, none of the other treated groups were observed to be different from controls in their weekly mean absolute weights. The large differences that occurred only in body-weight gains between the 6400 ppm groups and appropriate controls (see text Table 1) reflect the significantly lower body weights of the highest treatment group at the termination of the study.

TABLE 1.

GRAM BODY-WEIGHT GAINS (BWG) AND MEAN FOOD EFFICIENCIES (MFE) FOR RATS TREATED WITH DIETARY NC-319 TECHNICAL FOR 13 WEEKS

Dose ppm	Males N=20		Females N=20	
	BWG (% Change)	MFE (% Change)	BWG (% Change)	MFE (% Change)
0	318* (0)	0.128** (0)	155 (0)	0.080 (0)
100	323 (+2)	0.123 (-4)	138 (-11)	0.076 (-5)
400	329 (+3)	0.128 (0)	150 (-3)	0.076 (-5)
1600	310 (-3)	0.124 (-3)	143 (-8)	0.075 (-7)
6400	264 (-17)	0.111 (-15)	100 (-35)	0.055 (-46)

* Data for BWG was taken from Table 1, p. 41 of the report.
** Values on MFE were calculated from weekly food efficiency data presented in Table 3, p. 43 of the report.

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C. Food Consumption:

Throughout the study, food consumption by both sexes at all treatment levels appeared to be nearly equal, with no large changes relative to negative controls. After 13 weeks, total food consumption by treated males ranged between +6 and -4% of negative controls, and treated females between +2 and -6% of controls (see attached p. 42, note the arithmetic mistake made in calculating the total food consumption by males treated at 6400 ppm). When food efficiencies were computed by comparing the steady food intakes to the body-weight gains observed in the various treatment groups of each sex, an effect level is clearly indicated for each sex at 6400 ppm (see text Table 1).

D. Diet Analysis and Actual Dosages:

Identification and quantitation of test article in the diet was accomplished using reversed phase HPLC with UV detector at 240 λ following extraction of the diet with acetonitrile. During the course of the study, the diet was analyzed 3 times and found to range no more than 5.6% higher and 7.2% lower than the expected (theoretical) concentrations, indicating that the actual and theoretical concentrations of test article in the diet were approximately equal.

The actual dosages in mg/kg/day that each group of treated animals received weekly was then derived using weekly food consumption data and the theoretical concentrations of the test article in the diet. Table 2 shows the actual mean dosages achieved during the 13-week feeding study in rats. Since all treatment groups for either sex consumed approximately equivalent levels of food (see attached p. 42) and since the test article in the diet approached theoretical concentrations, it is not surprising to find that the theoretical dose ratios of 1:4:16:64 (parenthetical numbers in Table 2) were

TABLE 2.

ACTUAL MEAN DOSAGES ACHIEVED IN mg/kg/day FOR RATS FED NC-319 TECHNICAL FOR 13 WEEKS		
Dose ppm	Males N=20	Females N=20
100 (1)*	7.4 (1.0)	8.9 (1.0)
400 (4)	28.8 (3.9)	37.3 (4.2)
1600 (16)	116 (15.6)	147 (16.5)
6400 (64)	497 (67.2)	640 (71.9)

* Numbers in parentheses represent the ratios of the designated doses to the lowest level dose of 100 ppm, 7.4 or 8.9 mg/kg/day.

closely approximated for the actual dosages of both males and females. It is also apparent that male dosages were somewhat lower than female dosages, in fact they were equal to 79.2 ± 2.7% of what females received. Not readily apparent from Table 2 is the fact that the dosaging of every group was highest in the first week of the study and progressively decreased to a point at week 13 where the dosages were 44.3 ± 2.3% and 52.4 ± 0.8% of the initial week's dosage. The dosaging decrease seemed to plateau out by week 9 or 10 of the study as the weights of all animals began to level out (close to 500 g for males and to 300 g for females).

TABLE 3.

HEMATOLOGY PARAMETERS MEASURED	
x	hematocrit (HCT) or packed cell volume (PCV)*
X	hemoglobin (Hb)*
X	leukocyte count (WBC)*
X	erythrocyte count (RBC)*
	platelet count*
X	total plasma protein (TP)
X	leukocyte differential count
X	mean corpuscular hemoglobin (MCH)
X	mean corpuscular hemoglobin concentration (MCHC)
X	mean corpuscular volume (MCV)

* 1982 Subdivision F guideline requirements for a chronic study.

TABLE 4.

HEMATOLOGY EFFECTS IN RATS FED NC-319 TECHNICAL FOR 13 WEEEKS (% CHANGE)						
Effects	Sex N=10	Doses, ppm				
		0	100	400	1600	6400
MCHC g/dl	♂	36.9 ± 1.0	37.0 ± 0.6	36.9 ± 0.4	36.5 ± 0.4	37.4* ± 0.6 (+1)
MCHC g/dl	♀	36.0 ± 0.5	36.1 ± 0.7	36.0 ± 0.4	36.0 ± 0.5	36.9*** ± 0.5 (+3)
MCH pg	♀	21.2 ± 0.5	20.9 ± 0.2	21.1 ± 0.5	21.1 ± 0.4	21.8** ± 0.4 (+3)

* Statistically significant from controls p<0.05
** Statistically significant from controls p<0.01
*** Statistically significant from controls p<0.001

D. Hematology:

Various hematologic parameters were measured for males and females at 13 weeks of the study. See Table 3 for an indication of measured parameters. Although platelet counts were not assessed, an Hepato Quick test was performed to measure various clotting factors in whole blood. Total protein measurements are included under clinical chemistry (see below).

In males, there was a small but statistically significant increase in MCHC, but only at the highest dose (see Table 4). In females, there were similar small and significant increases in both MCHC and MCH.

E. Clinical Chemistry:

Blood chemistry parameters were also analyzed in males and females at the end of the 13-week study. See Table 5 for an indication of measured parameters. Magnesium and creatinine phosphokinase were not measured.

TABLE 5.

CLINICAL CHEMISTRY PARAMETERS			
Electrolytes		**Other**	
X	calcium*	X	albumin*
X	chloride*	X	blood creatinine*
	magnesium*	X	blood urea nitrogen*
X	phosphorous*	X	cholesterol*
X	potassium*	X	albumin/globulin ratio
X	sodium*	X	glucose*
Enzymes		X	total bilirubin*
X	alkaline phosphatase	X	total protein*
	cholinesterase		triglycerides
	creatinine phosphokinase*		
X	lactic acid dehydrogenase		
X	serum alanine aminotransferase (ALT or SGPT)*		
X	serum aspartate aminotransferase (SGOT)*		

*1982 Subdivision F guideline requirements for a chronic study.

Several blood chemistry parameters were significantly altered during the study. These occurred in either sex, at the highest dose only, and without evidence of a clear dose-dependent activity (see text Table 6). Levels of cholesterol and total bilirubin were found to be reduced in both sexes. Relative to control values, cholesterol decreased by 37 and 29% and total bilirubin by 46 and 26%

in males and females, respectively. In males, alanine aminotransferase (ALT) increased by 25% and creatinine by 12%. In females, total protein, albumin and calcium decreased by 7, 8 and 5%, respectively.

TABLE 6.

BLOOD CHEMISTRY EFFECTS IN RATS FED NC-319 TECHNICAL FOR 13 WEEEKS (% CHANGE)

Effects	Sex N=9&10	Doses, ppm 0	100	400	1600	6400
Cholesterol mmol/l	♂	2.7 ± 0.5	2.6 ± 0.6	2.7 ± 0.4	2.4 ± 0.4	1.7*** ± 0.2 (-37)
Cholesterol mmol/l	♀	3.1 ± 0.7	2.8 ± 0.4	2.7 ± 0.4	2.7 ± 0.6	2.2*** ± 0.3 (-29)
Bilirubin umol/l	♂	2.8 ± 0.8	2.6 ± 0.4	2.7 ± 0.3	2.5 ± 0.3	1.5*** ± 0.3 (-46)
Bilirubin umol/l	♀	3.1 ± 0.6	3.2 ± 0.4	3.3 ± 0.5	3.3 ± 0.9	2.3** ± 0.5 (-26)
ALT IU/l	♂	57 ± 11	56 ± 6	49 ± 6	52 ± 8	71** ± 11 (+25)
Creatinine umol/l	♂	50 ± 3	51 ± 3	48 ± 2	49 ± 2	56*** ± 5 (+12)
Protein g/l	♀	73 ± 5	75 ± 5	73 ± 4	73 ± 5	68** ± 3 (-7)
Albumin g/l	♀	38 ± 3	39 ± 3	37 ± 2	37 ± 3	35* ± 2 (-8)
Calcium mmol/l	♀	2.72 ± 0.10	2.68 ± 0.14	2.70 ± 0.04	2.69 ± 0.10	2.58** ± 0.10 (-5)

* Statistically significant from controls p<0.05
** Statistically significant from controls p<0.01
*** Statistically significant from controls p<0.001

Although not statistically significant (due to large sample variations), lactate dehydrogenase activities exhibited a dose-dependent increase in males ranging from 397 in control males to 794 IU/l in high-dosed males (see attached pp. 49 and 50). Females were unaffected.

F. Urine Analysis:

See Table 7 below for urinalysis measurements. Centrifuged urine sediments (erythrocytes, leukocytes, epithelial cells, casts, crystals, organisms and abnormal constituents) were semi-quantitatively analyzed by microscopic examination.

TABLE 7.

URINALYSIS: PARAMETERS MEASURED			
X	appearance*	X	glucose*
X	volume*	X	ketones*
X	specific gravity*	X	bilirubin*
X	pH	X	blood*
X	sediment (microscopic)*		nitrate
X	protein*	X	urobilinogen

* 1982 Subdivision F guideline requirements for a subchronic study.

Virtually no changes were observed in urine parameters except for a non-statistically significant decrease in volumes and an indicated presence of blood pigments. Volumes in males decreased from 2.6 ± 1.4 in controls to 1.4 ± 0.8 in the highest-dosed animals (see attached pp. 53 and 54). At 6400 ppm, trace amounts of blood pigments were detected in urines obtained from 7 of 10 males and 5 of 10 females as compared to untreated controls with incidences of 0 and 2, respectively. At 400 and 1600 ppm, no traces of blood pigments were found in urines from animals of either sex.

G. Organ Weights:

For a list of organs weighed see Table 10 (although not noted, pituitaries were also weighed).

Except for a decrease in thymus weight at 100 ppm in females (see Tables 8 & 9), all statistically significant differences in absolute organ weights were found at the highest dose (6400 ppm). These included a reduction in weights of adrenals, livers and thymuses of both sexes relative to controls. In females, the hearts and kidneys also weighed significantly less than controls, with kidneys demonstrating the only apparent dose-dependent reduction in organ weights. No changes in organ weights were specific to males alone.

When covariance analyses were performed on the absolute organ-weight data, none of the organ weights were found to be significantly changed relative to the appropriate control values. However, if relative weights (absolute organ weight/absolute body weight X 100) were calculated using individual absolute organ and body weights provided

TABLE 8.

STATISTICALLY SIGNIFICANT CHANGES IN ABSOLUTE (% CHANGE) AND RELATIVE ORGAN WEIGHTS OF MALES FED NC-319 AT 6400 PPM FOR 13 WEEKS				
Organ	Absolute Wgts (g)		Relative Wgts (%)	
	Control	6400 ppm	Control	6400 ppm
Adrenal	0.050 § ± 0.009	0.045* ± 0.006 (-10)	0.00934 ± 0.00247	0.00974 ± 0.00101
Liver	18.99 ± 2.70	16.27*** ± 2.31 (-14)	3.71 ± 0.35	3.44* ± 0.40
Thymus	0.34 ± 0.06	0.28** ± 0.07 (-18)	0.0676 ± 0.0141	0.0604 ± 0.0132

§ Mean ± standard deviations.
* Statistically significant from controls $p<0.05$
** Statistically significant from controls $p<0.01$
*** Statistically significant from controls $p<0.001$

TABLE 9.

STATISTICALLY SIGNIFICANT CHANGES IN ABSOLUTE (% CHANGE) AND RELATIVE ORGAN WEIGHTS OF FEMALES FED NC-319 AT 6400 PPM FOR 13 WEEKS				
Organ	Absolute Wgts (g)		Relative Wgts (%)	
	Control	6400 ppm	Control	6400 ppm
Adrenal	0.062 § ± 0.009	0.052*** ± 0.008 (-16)	0.0214 ± 0.0023	0.0219 ± 0.0030
Heart	0.98 ± 0.10	0.89** ± 0.08 (-9)	0.337 ± 0.024	0.374*** ± 0.032
Kidney	1.97 ± 0.20	1.75*** ± 0.16 (-11)	0.681 ± 0.062	0.737** ± 0.050
Liver	9.72 ± 1.49	8.24** ± 1.05 (-15)	3.34 ± 0.28	3.45 ± 0.27
Thymus	0.29 ± 0.07	0.22*** ± 0.04 (-24)	0.1007 ± 0.0219	0.0906 ± 0.0140

§ Mean ± standard deviations.
* Statistically significant from controls $p<0.05$
** Statistically significant from controls $p<0.01$
*** Statistically significant from controls $p<0.001$

in appendix 13 for the control and highest-dosed groups (6400 ppm), and if T-test analyses were performed to compare relative organ weights of treated to control groups, then livers of males and hearts and kidneys of females were found to be significantly different from controls (see Tables 8 & 9).

H. Pathology:

All animals were necropsied at the end of study. Tissues that were examined <u>in situ</u> for gross pathology and fixed for histological examination are marked X below in Table 10. Organs marked with XX were also weighed.

TABLE 10.

TISSUES EXAMINED HISTOLOGICALLY					
Digestive		**Hemato/Immuno-logic**		**Neurologic**	
X	tongue	X	aorta*	XX	brain*
X	salivary gland*	XX	heart*	X	periph. nerve*
X	esophagus*	X	bone marrow*	X	spinal cord
X	stomach*	X	lymph nodes*	X	pituitary*
X	duodenum*	XX	spleen*	X	eyes(optic n.)*
X	jejunum*	XX	thymus*	**Glandular**	
X	ileum*	**Urogenital**		XX	adrenals*
X	caecum*	XX	kidneys*		lacrimal gland
X	colon*	X	urin. bladder*	X	mammary gland
X	rectum*	XX	testes*	X	parathyroids*
XX	liver*	X	epididymis	X	thyroids*
	gall bladder*	XX	prostate	**Other**	
X	pancreas*	X	seminal vesicle	X	bone*
Respiratory		XX	ovaries	X	skel. muscle*
X	trachea*	XX	uterus*	X	skin
XX	lung*			X	gross lesions

*1982 Subdivision F guideline requirements for a subcrhonic study.

No gross pathological lesions were found that were related to administration of test article. Observations of pathological irregularities appeared to be distributed randomly across treatment groups and were thus considered to be independent of dose.

Histopathology data showed an absence of abnormalities occurring at the microscopic level in <u>nearly</u> all analyzed tissues. In males, apparent increases in liver vacuolations were observed at the highest dose (see attached p. 69). There appeared to be a dose-dependent

increase in total abnormalites in the female kidneys and only a high-dose (6400 ppm) effect in males. Marginal to moderate tubular pigmentation (containing hemosiderin) of the kidneys was the most obvious irregularity observed in either sex (see attached p. 70 & 71). The increase in tubular pigmentation was dose-dependent in both sexes and observed to be greater than control levels in each sex at the 2 highest concentrations (1600 and 6400 ppm).

I. Ophthalmoscopy:

Ophthalmoscopic examination revealed no effects related to treatment.

III. DISCUSSION

Technical grade NC-319 or MON 12000 was mixed into the rat diet at concentrations of 0, 100, 400, 1600 and 6400 ppm and fed ad libitum to the animals for 13 weeks. Chemical analytical data were provided indicating that the test article in the diet was homogeneously mixed and relatively stable under experimental conditions (for at least 2 weeks). As verified by chemical analysis, the technical grade of the test article was employed at an acceptable level of purity (98.6%).

Although many statistically significant effects were observed in male and female animals, they all occurred (with the exception of female thymus weight that occurred at the lowest dose as well as highest) at the highest dose (6400 ppm) (see text Table 11 for a qualitative summary of the statistically significant effects of this study). There were, however, only a few effects that could be classified as demonstrating significance at the highest dose in addition to some indication of a dose-dependent relationship at a lower dose or doses. These effects were essentially limited to reductions in blood cholesterol levels, increases in the incidence of kidney tubular pigmentation and decreases in absolute kidney weights. The former effects were observed in both sexes and the latter effect in females only. In the females, the kidneys were doubly affected in that they exhibited not only reductions in absolute weights but increases in tubular pigmentations by iron-containing hemosiderin. It is also noteworthy to mention that both of these kidney effects appeared to be dose-dependent. Although increased levels of tubular pigmentation relative to controls were evident at both 1600 and 6400 ppm, the 1600 ppm level failed to achieve statistical significance. In addition, the intensity of this response at 1600 ppm was scored at the lowest grade (+/-).

Based on this study, the maximum tolerated dose (MTD) would appear to be between 1600 and 6400 ppm (1600 < MTD < 6400). The highest dose (6400 ppm) may be too high for an MTD due to relatively large losses in the body-weight gains of 17 and 35 % that were observed at this dose for males and females, respectively. Also, at 6400 ppm the appreciable number of effects that were observed in relatively young rats during the suchronic treatment period (13 weeks) may result in excessivemortality when the test article is chronically adminsistered

TABLE 11.

QUALITATIVE SUMMARY OF THE STATISTICALLY SIGNIFICANT EFFECTS OBSERVED IN RATS FED NC-319 TECHNICAL FOR 13 WEEKS

Effects	Males					Females				
	* DR	Doses **				DR	Doses			
		L	LI	HI	H		L	LI	HI	H
MCHC					↑					↑
MCH										↑
ALT					↑					
Creatinine					↑					
Bilirubin					↓↓					↓
Calcium										↓
Albumin										↓
Protein										↓
Choles-terol	✓				↓↓	✓				↓↓
Body wgt. gain					↓					↓↓
Adrenal wgt.					↓					↓
Liver wgt.					↓					↓
Thymus wgt.					↓		↓			↓
Heart wgt.										↓
Kidney wgt.						✓				↓
Tubular Pigmenta. (kidney)	✓				↑↑	✓				↑↑
Vacuoles (liver)					↑					

* Indication of dose-response (DR) relationship.
** L=100, LI=400, HI=1600 and H=6400 ppm.

for nearly the entire lifespan of the test animal (104 weeks). At 1600 ppm, on the other hand, the reductions in body-weight gains for males and females were only 3 and 8 %, respectively, and less than the preferred 10 to 15% decrement thought to be essential for predicting an MTD in a chronic study. In addition, the absence of significant effects observed at this penultimate dose would serve to support the notion that a chronic MTD of 1600 ppm would be inadequate.

IV. CONCLUSIONS

NC-319 Technical was fed to Sprague Dawley rats (20/sex/dose) at 0, 100, 400, 1600 and 6400 ppm for 13 weeks. The dosages consumed in mg/kg/day for males and females, respectively, were 7.4 and 8.9 at 100 ppm, 28.8 and 37.3 at 400 ppm, 116 and 147 at 1600 ppm, and 497 and 640 at 6400 ppm. All significant effects considered to be treatment-related were observed in males and/or females at the highest dose (6400 ppm) only. Decreases were observed in body-weight gains and absolute organ weights (adrenals, liver, thymus, heart and kidneys), and in blood levels of cholesterol, bilirubin, protein, albumin and calcium. Increases were found in mean cell hemoglobin and its concentrations, in blood alanine aminotransferase activities and creatinine levels, and in vacuolated livers and pigmented kidney tubules. Underscored effects indicate dose-response relationships. The dose-dependent effect on kidney tubules was observed in both sexes. The NOEL for NC-319 Technical in rats was established at 1600 ppm and the LOEL at 6400 ppm for each sex. Based primarily on decreased body-weight gains, the MTD for each sex would appear to be slightly lower than 6400 ppm, the highest dose tested.

V. CLASSIFICATION

Core Guideline --- This study satisfies guideline requirements for a subchronic feeding study in rats (82-1).

SYNOPSIS:

28-DAY REPEATED ORAL TOXICITY STUDY IN RATS WITH NC-319 (a non-guideline study). MRID No. 421715-01 (PART B)

This preliminary dose range-finding study (PART B) was performed in a different laboratory (Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia, 22180) than was the guideline study (PART A) reviewed above. Inspite of study completion dates which indicate that the preliminary study was completed (August 12, 1988) after the guideline study (June 14, 1988), it should be noted that the necropsies of the preliminary study were actually completed (December 16, 1987) nearly 3 months prior to initiating animal treatment in the guideline study (March 8, 1988).

The preliminary study was performed on Sprague-Dawley rats (10/sex/dose) to determine dose levels of NC-319 Technical (98.5% pure) to be used in a prospective subchronic dietary study (i.e., the subchronic study of PART A). Test article was mixed into diet at the following concentrations: 0, 300, 1000, 3000 and 10,000 ppm, and fed to rats for 28 days ad libitum. Unforeseen mortalities occurred in two animals on the day prior to sacrifice (1 male at 3000 ppm and 1 female at 10,000 ppm). Moribundity and clinical signs of toxicity were not apparent during the study; gross necropsies, urinalyses and ophthalmoscopic examinations performed at or near the end of the study revealed no abnormalities relative to control animals. Positive effects were observed primarily at the 2 highest concentrations (3000 and 10,000 ppm) in males and females. At 10,000 ppm, males exhibited decreases in body weights, body-weight gains, food consumption and blood glucose levels; increases in relative weights of liver, testes and epididymis; and cell degeneration/necrosis of the pancreas. Females exhibited decreases in body weights, body-weight gains, relative kidney weights and blood levels of protein, albumin, globulin and glucose; increases in hemoglobin, hematocrit and blood chloride levels; and cell degeneration/necrosis of the pancreas. At 3000 ppm, males demonstrated increases in body-weight gains and degeneration/necrosis of the pancreas. Females demonstrated decreases in body-weight gains and food consumption; increases in blood chloride levels; and cell degeneration/necrosis of the pancreas. At the lower concentrations of 300 and 1000 ppm, the only observed effects were decreases in the female blood levels of protein, albumin and globulin. From these results, the authors estimated an apparent NOEL for dietary NC-319 Technical of between 1000 and 3000 ppm.

Based on this short preliminary study, certain observations would have been predicted to occur in a longer subchronic study. These predictions of subchronic observations include: that the reduction in female blood protein levels would be observed down to the 400 ppm dose; that blood bilirubin levels would be unaffected; that the kidneys would be free of microscopic lesions; and that pancreatic lesions would be found at the 6400 ppm dose. Instead, blood protein reduction was found to occur only at 6400 ppm in females, blood

bilirubin levels were decreased in both sexes at 6400 ppm, and kidney -- but not pancreatic -- lesions were observed. (Because blood cholesterol was not measured in the preliminary study, the dose-dependent cholesterol reductions observed in the subchronic study have no basis for comparison.) On the other hand, the increases in relative kidney weights observed in females at 10,000 ppm in the preliminary study agree with findings from the subchronic study showing increases in relative kidney weights of females at 6400 ppm.

The reasons for not observing the occurrence of kidney lesions or decreases in blood bilirubin in the preliminary study may possibly relate to the short exposure time of the preliminary study relative to the subchronic. However, the reasons for not subchronically observing either decreases in blood protein levels at lower doses or microscopic lesions of the pancreas at 6400 ppm, are not readily apparent. Close attention should be paid in the future to chronic studies on NC-319 and the possibility that it may produce effects on blood protein levels and/or the pancreas.