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DATA EVALUATION RECORD

ASSURE

Chronic Feeding Study in Dogs

STUDY IDENTIFICATION: Varney, P. NC-302: 52-week oral (dietary administration) toxicity study in the beagle dog. (Unpublished study No. 4297-306/1 prepared by Hazleton Laboratories Europe Ltd., England, for Nissan Chemical Industries Ltd., Japan; dated April 1985.) Accession No. 073536.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 4-4-86

1. CHEMICAL: Assure; NC-302.

2. TEST MATERIAL: NC-302 from batch No. 8002 was described as a white powder containing 99.1% active ingredient.

3. STUDY/ACTION TYPE: Chronic feeding study in dogs.

4. STUDY IDENTIFICATION: Varney, P. NC-302: 52-week oral (dietary administration) toxicity study in the beagle dog. (Unpublished study No. 4297-306/1 prepared by Hazleton Laboratories Europe Ltd., England, for Nissan Chemical Industries Ltd., Japan; dated April 1985.) Accession No. 073536.

5. REVIEWED BY:

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7. CONCLUSIONS:

NC-302 was administered to beagle dogs for 52 weeks at dietary levels of 25, 100, or 400 ppm. There were no effects of dosing on survival, food consumption, and hematologic or urinalysis parameters. One male control and one female receiving 100 ppm NC-302 died during the study, but the deaths were not related to the test material. Body weight data were erratic. There were no compound-related effects of toxicological importance on clinical laboratory findings; dose-related creatine phosphokinase (CPK) changes were reported in analyses performed prior to study initiation as well as during the study. Statistically significant changes were found in brain weight data for males and females. Nonsignificant dose-response effects were reported for other organ weight parameters. The histopathologic data did not support changes in organ weights of treated males or females. The basis to establish a LOEL was not provided. The NOEL for NC-302 in dogs is 400 ppm, the highest dose tested.

Core Classification: Core Supplementary.

8. RECOMMENDATIONS: This study may be upgraded from Core Supplementary to Core Minimum with the submittal of experimental ophthalmologic data for substantiation of the conclusion that there were no treatment-related ocular changes. In addition, results of the 26-week feeding study used for dose selection should be submitted.

9. BACKGROUND: The doses for this study were based on a 26-week feeding study in which 0, 25, 100, and 400 ppm NC-302 were tested. The test animal used in this 26-week study and results of this study were not provided in this report.

Item 10-see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, NC-302, 99.1% pure, was administered to dogs orally in a measured quantity of basic powdered diet (SQC Laboratory Diet A, Special Diet Services, Ltd.) at concentrations of 25, 100, or 400 ppm. Control animals were fed the basic powdered diet alone. Diets were prepared weekly and samples were collected at 13 intervals for analysis of test compound at each dietary level. At study initiation, samples were taken from the 25- and 400-ppm diet to check homogeneity and stability of test compound in the diet.

2. Twenty-four male and 24 female purebred beagle dogs (Hacking and Churchill Ltd., Abbots Ripton Road, Wyton, Huntington)

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

between 4 and 6 months old were used for this study. Prior to delivery, the animals received treatment for parasites and were vaccinated against distemper, hepatitis, Leptospira canicola, and L. icterohaemorrhagiae. Upon delivery, the animals were housed by sex in two experimental rooms that were maintained at a temperature of 16 to 22°C. The animals were acclimatized in the rooms for a period of 7 weeks, during which time they were vaccinated against Parvovirus. Twelve animals (six males and six females) were assigned to each of four groups (control animals and animals receiving 25, 100, or 400 ppm) using a stratified body weight procedure.

A measured quantity of the test diet (400 g), containing the specified concentrations of test material, was offered to the animals daily for a period of 52 weeks. Animals were fasted prior to blood collection. Water was available ad libitum.

3. Animals were observed several times daily for clinical signs and body weights were recorded weekly before each feeding. Food consumption was estimated daily by the amount (weight) of food left from the measured amount that was offered, and mean water consumption values were calculated from data collected over 3 consecutive days each week.

4. The eyes of all animals were examined ophthalmologically prior to the start of treatment and during weeks 25 and 52.

5. Hematologic and clinical chemistry determinations and urinalysis were performed prior to the start of treatment and during weeks 13, 26, and 52.

   Urinary parameters included specific gravity, pH, blood, reducing substances, ketone bodies, microscopy of spun deposits, protein, bilirubin, urobilinogen, and glucose.

   Hematologic parameters included hemoglobin concentration, mean cell volume, red blood cell count, mean cell hemoglobin, packed cell volume, mean cell hemoglobin concentration, total and differential white cell count, platelet count, and prothrombin time.

   Clinical chemistry parameters included glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, blood urea nitrogen, glucose, total bilirubin, sodium, potassium, calcium, chloride, phosphorus, cholesterol, creatinine, CPK, total protein, albumin, albumin/globulin ratio, plasma cholinesterase, and protein electrophoresis.

   At each sacrifice, organ weights were determined for adrenals, kidneys, pituitary, thyroids, brain, liver spleen, heart, ovaries, and testes. Each animal received a complete gross examination, and 36 tissues and all unusual lesions were preserved for histologic examination.

The data were evaluated by determining group mean values and standard deviations, where appropriate. Statistical analysis of liver-to-body weight ratios was performed using Kruskal-Wallis and Wilcoxon's rank sum tests.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Dietary Analysis: The mean concentration of test material in the diet (weeks 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49) were 23.85±1.0, 96.4±3.5, and 386.0±8.4 for nominal levels of 25, 100, and 400 ppm, respectively. All dietary concentrations were within ±20 percent of the nominal levels. Test material was stable in the diets when stored under cool to ambient temperatures for at least 7 days. Homogeneity of the test material in the diets was acceptable; all analytical values were 89-103 percent of nominal.

Clinical Observations and Mortality: One male control and one female receiving 100 ppm died during the 1-year study. The death of the female was due to an intestinal ulcer while the cause of death of the male was undetermined. The deaths were not considered to be related to ingestion of NC-302. No compound-related changes in clinical condition or behavior were reported.

Body Weight: No significant differences in body weight or weight gain were noted during the study (Table 1). The group mean body weight and net weight gain of females showed a dose-related depression while the body weight and net weight gain of males was more variable. Body weight gain in the male animals was reported to be erratic throughout the study. However, the time of the noted decrease in body weight gain was consistent, but unrelated to food consumption, in all male treatment groups. The body weight gain of the control females was in excess of that of the treated groups. However, this trend was present prior to study initiation. Since there were no statistically significant differences among treated and control groups, the author concluded that NC-302 did not produce an adverse effect on the body weight of dogs over the 1-year study.

Food Consumption: The daily ration of feed was limited to 400 g for both male and female dogs. During the major portion of the study period, both males and females consumed the food allotment. On this basis, this study was not designed to properly evaluate differences in food consumption. Food consumption did not differ markedly in any group throughout the study.

Clinical Chemistry Measurements: There were no significant changes in hematologic or clinical chemistry values in control or dosed animals at -1, 13, 26, or 52 weeks of the study. Plasma CPK showed a dose-related increase in activity in males and females when compared with control animals at each scheduled analysis (Table 2). However, this trend in activity was reported in the CPK analysis prior to study initiation and therefore was not considered to be of biological significance. Urinary parameters were similar in control and dosed groups.

TABLE 1. Selected Mean Body Weight Data for Dogs Fed NC-302 for 1 Year

Dietary Concentration (ppm)	Group Mean Body Weight (kg ± SD) at Week					Net Weight Gain (kg ± SD) for Weeks		
	1	13	26	39	52	1-39	39-52	1-52
Males								
0	11.28 ±0.3	11.95 ±0.6	12.13 ±0.7	11.94 ±1.0	12.20 ±1.0	0.66 ±1.08	0.26 ±0.11	0.92 ±1.05
25	11.10 ±0.7	12.28 ±0.6	12.13 ±0.7	12.33 ±0.8	12.94 ±0.8	1.23 ±0.95	0.61 ±0.27	1.84 ±0.95
100	10.89 ±0.3	11.65 ±0.7	11.55 ±0.8	11.55 ±1.1	12.14 ±1.1	0.66 ±1.26	0.59 ±0.43	1.25 ±1.30
400	11.25 ±0.4	12.20 ±0.4	11.80 ±0.7	11.83 ±0.8	12.35 ±0.7	0.58 ±0.68	0.52 ±0.32	1.10 ±0.58
Females								
0	10.07 ±1.4	11.13 ±1.2	11.85 ±1.5	11.84 ±1.4	12.32 ±1.5	1.77 ±0.94	0.47 ±0.31	2.25 ±1.00
25	9.37 ±1.4	10.41 ±1.4	10.94 ±1.5	11.01 ±1.3	11.35 ±1.4	1.64 ±0.36	0.34 ±0.11	1.98 ±0.37
100	9.33 ±0.8	10.03 ±0.5	10.56 ±0.7	10.60 ±0.5	10.83 ±0.6	1.24 ±0.70	0.23 ±0.26	1.47 ±0.87
400	9.73 ±1.2	10.53 ±1.1	10.78 ±1.0	10.81 ±1.2	10.81 ±0.9	1.07 ±0.52	0.57 ±0.34	1.65 ±0.54

TABLE 2. Creatine Phosphokinase (CPK) Data for Dogs Fed NC-302 for 1 Year

Dietary Level (ppm)	Mean CPK Activity (IU/L ± SD) at Week			
	0	13	26	52
Males				
0	192 ±24	176 ±39	114 ± 9	129 ±22
25	204 ±39	192 ±51	128 ±12	130 ±21
100	236 ±26	196 ±35	135 ±20	141 ±35
400	249 ±73	201 ±54	164 ±61	169 ±42
Females				
0	141 ±19	147 ±36	129 ±53	121 ±43
25	199 ±63	165 ±18	122 ±11	105 ±28
100	234 ±111	164 ±32	137 ±22	138 ±54
400	186 ±32	202 ±50	141 ±26	163 ±44

Organ Weight: The author reported that all absolute and relative organ weights were within the accepted ranges for the species with the exception of isolated cases of absolute and relative liver weights in one control, one 100-ppm female, and one 400-ppm male. A slight but nonsignificant increase in mean relative liver weights was reported in 100-ppm females and 25-, 100-, and 400-ppm males.

The recalculation of absolute organ weight and organ-to-body weight ratios and the evaluation of organ-to-brain weight ratios using analysis of variance (ANOVA) and Dunnett's T-test revealed significant differences in brain weight data for males and females (Table 3).

The absolute brain weight in 100-ppm males was found to be significantly decreased at $p < 0.05$ (ANOVA, Dunnett's T-test) (Table 3a). The decrease in the absolute brain weight of the 400-ppm males was slight but nonsignificant. The absolute brain weight in 400-ppm females as well as relative brain weights in 100- and 400-ppm females were found to be significantly increased at $p < 0.05$ (ANOVA, Dunnett's T-test) (Table 3b).

In addition to increased but nonsignificant relative liver weights in 25-, 100-, and 400-ppm males, as reported by the author, the liver-to-body weight ratios were found to increase with a nonsignificant dose-response trend (regression analysis). However, liver-to-brain weight ratios in males were found to increase with a significant dose-response trend ($p < 0.05$, regression analysis). Slightly decreased absolute weights were found in the heart and gonads of 25-, 100-, and 400-ppm males. Absolute brain weights in females were found to increase with a significant dose-response trend ($p < 0.05$, regression analysis).

Histology and Gross Pathology: No compound-related histological or gross pathological effect was observed in animals that died prior to 52 weeks or in animals sacrificed at 52 weeks.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Under the conditions of the study, NC-302 was not considered toxic when fed to beagle dogs for 52 weeks at dietary levels of 25, 100, or 400 ppm.

The author stated that there were no compound-related mortalities. One 100-ppm female, whose food consumption and body weight were decreased prior to death, was found dead in week 14. The cause of death was thought to be an intestinal ulcer that was confirmed histologically. A control male was found dead in week 34; the cause of death was not established.

The author reported no compound-related changes in body weight gain. However, weight gain in male animals was erratic and the mean weight gain of control females was greater than the historical values for beagles on chronic studies at these laboratories. The author attributed this mean body weight increase to three exceptional animals.

TABLE 3a. Selected Weight Data for Male Dogs Fed NC-302 for 1 Year

Organ	Dietary Level (ppm) 0	25	100	400
	Mean Absolute Body and Organ Weights (g ± S.D.)			
Body	12090± 922	12800± 663	11992± 1208	12267± 700
Brain	84.0± 4.4	83.8± 4.0	75.9± 6.9*	81.4± 5.3
Liver	358.0± 20.7	396.2± 31.4	387.4± 63.1	435.9± 114.3
Heart	115.5± 11.1	112.5± 5.7	102.0± 14.4	111.3± 11.3
Gonads	23.1± 2.3	20.3± 4.1	20.6± 2.3	19.9± 2.6
Adrenals	1.435± 0.210	1.417± 0.208	1.437± 0.190	1.324± 0.164
Thyroids	0.875± 0.246	0.838± 0.056	0.857± 0.219	0.791± 0.071
Pituitary	0.081± 0.027	0.077± 0.014	0.074± 0.041	0.064± 0.007
Kidneys	59.68± 7.63	62.12± 5.77	58.16± 8.19	59.46± 6.99
Spleen	22.80± 3.47	24.64± 4.04	20.94± 6.13	22.57± 6.44
	Mean Organ-to-Body Weight Ratio (g/kg ± S.D.)			
Brain	6.993± 0.805	6.556± 0.381	6.373± 0.828	6.653± 0.580
Liver	29.808± 3.377	31.012± 2.675	32.228± 2.927	35.529± 9.123
Heart	9.554± 0.567	8.796± 0.444	8.517± 0.966	9.085± 0.882
Gonads	1.905± 0.075	1.593± 0.340	1.731± 0.288	1.629± 0.215
Adrenals	0.1184±0.0108	0.1115±0.0209	0.1203±0.0148	0.1084±0.0159
Thyroids	0.0717±0.0158	0.0657±0.0061	0.0710±0.0121	0.0645±0.0051
Pituitary	0.0068±0.0024	0.0061±0.0010	0.0061±0.0031	0.0053±0.0007
Kidneys	4.936± 0.519	4.855± 0.404	4.863± 0.628	4.856± 0.605
Spleen	1.879± 0.180	1.935± 0.376	1.733± 0.406	1.835± 0.507
	Mean Organ-to-Brain Weight Ratio (g/g ± S.D.)			
Liver	4.264± 0.094	4.745± 0.519	5.128± 0.835	5.316± 1.075
Heart	1.382± 0.186	1.345± 0.093	1.347± 0.164	1.377± 0.194
Gonads	0.276± 0.041	0.243± 0.050	0.272± 0.025	0.246± 0.034
Adrenals	0.0172±0.0035	0.0169±0.0027	0.0189±0.0019	0.0163±0.0023
Thyroids	0.0105±0.0035	0.0100±0.0009	0.0113±0.0027	0.0097±0.0009
Pituitary	0.0010±0.0003	0.0009±0.0002	0.0010±0.0005	0.0008±0.0001
Kidneys	0.715± 0.117	0.744± 0.095	0.776± 0.164	0.733± 0.102
Spleen	0.274± 0.056	0.294± 0.046	0.279± 0.092	0.277± 0.078

* Significantly different from control value ($p \leq 0.05$), as calculated by our reviewers using analysis of variance and Dunnett's T-test.

TABLE 3b. Selected Weight Data for Female Dogs Fed NC-302 for 1 Year

Organ	Dietary Level (ppm) 0	25	100	400
	Mean Absolute Body and Organ Weights (g ± S.D.)			
Body	12300± 1491	11183± 1320	10770± 655	11075± 813
Brain	75.6± 3.6	77.0± 5.8	80.6± 3.4	82.8± 5.4*
Liver	425.2± 109.2	358.9± 54.6	433.0± 112.8	383.1± 48.6
Heart	106.5± 13.7	99.6± 4.7	96.3± 5.0	102.5± 10.8
Gonads	1.424± 0.365	1.430± 0.559	1.426± 0.677	1.186± 0.164
Adrenals	1.550± 0.244	1.530± 0.293	1.331± 0.098	1.643± 0.256
Thyroids	0.819± 0.116	0.714± 0.174	0.747± 0.117	0.774± 0.153
Pituitary	0.071± 0.018	0.071± 0.023	0.067± 0.010	0.072± 0.015
Kidneys	54.77± 7.22	48.08± 6.47	49.59± 5.98	52.49± 10.18
Spleen	27.28± 8.15	27.15± 5.51	23.14± 1.56	24.39± 4.81
	Mean Organ-to-Body Weight Ratio (g/kg ± S.D.)			
Brain	6.223± 0.855	6.993± 1.161	7.512± 0.655*	7.509± 0.732*
Liver	34.645± 8.238	32.019± 2.281	40.145±10.007	34.611± 3.607
Heart	8.676± 0.666	9.001± 1.029	8.948± 0.336	9.261± 0.810
Gonads	0.1169±0.0326	0.1263±0.0434	0.1308±0.0571	0.1071±0.0121
Adrenals	0.1272±0.0242	0.1365±0.0207	0.1240±0.0121	0.1482±0.0184
Thyroids	0.0671±0.0108	0.0636±0.0120	0.0694±0.0111	0.0694±0.0092
Pituitary	0.0057±0.0010	0.0064±0.0022	0.0062±0.0009	0.0065±0.0011
Kidneys	4.478± 0.532	4.298± 0.206	4.641± 0.784	4.731± 0.739
Spleen	2.189± 0.442	2.420± 0.390	2.157± 0.218	2.209± 0.452
	Mean Organ-to-Brain Weight Ratio (g/g ± S.D.)			
Liver	5.650± 1.496	4.695± 0.877	5.424± 1.628	4.642± 0.621
Heart	1.414± 0.213	1.303± 0.151	1.198± 0.107	1.240± 0.133
Gonads	0.0189±0.0052	0.0187±0.0077	0.0179±0.0089	0.0143±0.0019
Adrenals	0.0205±0.0033	0.0201±0.0046	0.0165±0.0011	0.0199±0.0033
Thyroids	0.0109±0.0019	0.0094±0.0029	0.0093±0.0018	0.0093±0.0019
Pituitary	0.0009±0.0002	0.0009±0.0003	0.0008±0.0002	0.0008±0.0002
Kidneys	0.727± 0.108	0.630± 0.120	0.615± 0.070	0.635± 0.120
Spleen	0.363± 0.115	0.355± 0.081	0.288± 0.028	0.293± 0.050

* Significantly different from control value ($p \leq 0.05$), as calculated by our reviewers using analysis of variance and Dunnett's T-test.

There were no effects of dosing on behavior, general health, or food and water consumption. Hematologic values were within the normal range. The authors reported no compound-related changes in clinical chemistry parameters. The dose-related increase in plasma CPK in test animals relative to control animals was reported in the CPK analysis prior to study initiation.

The author reported that the slight, but nonsignificant, increase in mean relative liver weights for 100-ppm females and all male-treated animals might be considered a possible effect of treatment. However, the author also reported that all absolute and relative organ weights were within normal ranges for this species with the exception of isolated cases of elevated absolute and relative liver weights in one control, one 100-ppm female, and one 400-ppm male. These changes were not accompanied by any unusual histological effects in these organs; hence, they were not considered related to dosing. There were no compound-related gross or histopathological changes.

The author concluded that NC-302 was well tolerated by beagle dogs at dietary concentrations of 25, 100, or 400 ppm for 52 weeks and considered 400 ppm to be the NOEL.

B. A quality assurance statement was signed and dated April 16, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The results and conduct of the study were acceptable; however, several study deficiencies were noted:

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1. The number of animals tested per group (six dogs/sex/group) were inadequate for a 52-week subchronic feeding study. This limited the statistical power of the study when comparing body weight or organ weight data.

2. The body weight data were erratic, with the mean weight gain of the control males exhibiting the lowest and the mean weight gain of the control females exhibiting the highest relative weight gain. Inadequate numbers of animals per group limited the validation of these data.

5 weeks considered as Chron study

3. The only organ weight parameter that was reported to have been statistically evaluated was the liver-to-body weight ratio. The use of the Kruskal-Wallis test and Wilcoxon's rank sum test to determine significance between differences in liver-to-body weight ratios was questioned by the reviewer. Both of these analyses are intended to be used with nonhomogeneous data. There was no indication that a statistical test was performed for homogeneity in order to substantiate that these data were not normally distributed or that any other organ weight parameter was statistically analyzed. For these reasons, absolute organ

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weights, organ-to-body weight ratios, and organ-to-brain weight ratios were statistically evaluated using the analysis of variance (ANOVA) and Dunnett's T-test. Significant differences were found in brain weight data for males and females as specified in the organ weight results (Table 3). In addition, regression analyses were used to evaluate dose-response trends. A significantly increased dose-response trend ($p < 0.05$) was found in the liver-to-brain weight ratios in males and the absolute brain weights in females as indicated in the organ weight results. However, no compound-related histological or gross pathological effect was found in conjunction with these organ weight changes; therefore, we assess that the toxicologic importance of the changes was equivocal. Due to the inadequate number of animals tested per group, we cannot make an independent evaluation of organ weight changes.

We agree with the author's assessment that there were no toxicologically important changes in mortality, clinical observations, or clinical laboratory findings. This study does not provide a basis to establish a LOEL for NC-302. Therefore, the NOEL for NC-302 in dogs is 400 ppm, the highest dose tested.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol, CBI pp. C163-C182.

APPENDIX A

Protocol

ASSURE 005030

Page_____ is not included in this copy.

Pages 14 through 34 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

_X_ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.