UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SUBJECT: Tolerance petition for residues of the plant growth regulant ((2-chloroethyl) trimethylammonium chloride) in or on sugarcane at 3.0 ppm; in meat, fat, and meat byproducts of cattle, goats, hogs, horses, sheep and poultry at 0.10 ppm, and in milk at 0.05 ppm.

Food additive tolerance for residues of ((2-chloroethyl) trimethylammonium chloride) in molasses at 15.0 ppm

DATE: JUL 16 1976

FROM: Toxicology Branch

TO: Robert Taylor
Product Manager No. 25

Petition No. 6F1759
6H5127

PC 016101

Related Petition No.: 4G-1461

Product Name: CYCOCEL

Chemical Name: ((2-chloroethyl) trimethylammonium chloride)

Other Names: (1) Chlor-Choline-Chloride
(2) CCC
(3) N-trimethyl-B-chloroethylammonium chloride
(4) Chlormequat chloride
(5) CL38555

Structural Formula: $ClCH_2CH_2N^+(CH_3)_3Cl^-$

Empirical Formula: $C_5H_{13}Cl_2N$

Color and State: White crystalline solid

Solubility: Water soluble 74% at 20°C

Recommendations:

TOX recommends that the proposed tolerances for Cycocel NOT be granted for the following reasons. TOX notes that in some cases deficiencies may be corrected rather than repeating these studies.

I. Dog, Two Year Feeding Study

BASF, 4/17/67

A. A signed pathologist's report must be submitted. A grading system should be used with detailed reporting.

B. The pathologist should give special attention to the two animals that died in group III (Animal No. 162 and 169). The explanation (Pg. 2 of the study) is not acceptable.

C. The study must be statistically analyzed.

D. Animals were poorly assigned to test groups. Initial mean group animals weights varied 3 Kg. This hindered evaluation of the study.

E. At least 4 males and 4 females should have been used per test level.

F. The value of this study is in question. If points A, B, C, and D can be corrected, this study will be acceptable. See review. or rationale presented

II. Mouse, 78-week Feeding Study

Huntingdon Research Centre, May 5, 1971

A. This is not a valid carcinogenicity study because only one test level was employed. Furthermore a mouse strain was used which apparently has an extremely high incidence of tumors. The oncogenic evaluation in the mouse must be repeated using another strain. For the present study the following questions should be resolved:

1. The normal incidence of lung tumors is stated to be 26.1% in background data submitted for this strain (Toxicology and applied Pharmacology 30, 337-359 (1974)). This does not coincide with the normal range of 18-38% stated to exist. Explanation is required. Note: Incidence in CCC treated males was 38% (double control values of 20%).

2. The pathologist's report is not signed.

3. The pathologist failed to report findings consistently for all tissues as specified in the procedure. On the average, the report only contained histological reports for approximately 3 organs per animal and in many cases only one organ was listed. The report does list normal findings, so this is not an explanation. See review.

III. Rat, 2-year Feeding Study

A. The pathologist's report was not signed and no grading system was used.

B. The study did not include brain, heart (etc.) organ weights.

C. Pathology results as stated do not agree with tables presented.

D. There is no demonstrated effect level.

E. The study contained no statistical evaluations.

F. Background data for their "own strain" is required. See review.

IV. Rat, 3-Generation Reproduction Study

A. The "giant cells" referred to should be characterized more accurately.

B. Slides from the 2-year rat feeding study must be reviewed for evidence of these "giant cells".

V. Mutagenicity Studies Must be Submitted

VI. Rat, Teratology

A. The incidence of gastroschisis in control animals was zero, while 3 incidences were found in the 50 mg/kg cycocel group. If this is within normal limits for the rat, background data and statistical evaluation will be necessary to determine this.

Note: Other teratology studies submitted are not valid because they are in summary form.

The following studies are noted but not acceptable as fulfilling and data requirements.

I. Rat, 90-day Feeding

Oettel & Frohberg 10/12/64

A. Number of animals tested per level (10) is inadequate.

B. Dose levels were changed after several weeks of testing.

C. Blood chemistry, pathology, organ weight data, etc. inadequate or totally absent. See review.

II. Cat, 6-month Feeding

Oettel & Hofmann, 10/16/64

A. No pathology report.

B. No clinical/chemistry data

C. Number of animals (4) is inadequate.

D. Test dosage, route, and time interval were varied at the end of the study. See review.

In addition, TOX recommends against Registration for the following reasons:

I. 20-day Dermal Study Cycocel 4L

A. The vehicle must be identified

B. Gross and histopathology reports as written are inadequate.

C. An untreated control group must be used.

II. The following studies must be submitted for registration of this product:

Acute oral (rat) - Formulation
Acute dermal - Formulation
Acute inhalation - Technical
Acute eye irritation - Formulation
Acute eye irritation - Technical

Note: Atropine acts synergistically with CCC and the toxicity is therefore significantly increased. The label "Note to physicians" should include some comment.

FORMULATION: Cycocel 4L Plant Growth Regulant

Active Ingredient	% by Wt.
Chlorocholine Chloride Aqueous Concentrate 65%	71.2

Inert Ingredients

[redacted]

Note: Confidential Statement of Formula does not total to 100%.

Inerts cleared under §180.1001(c).

Acute Studies, 2-chloroethyl Trimethylammonium Chloride, technical, Report No. 62-14, November 23, 1962.

Rat, Acute Oral

The product was administered to 5 animals per test level as a dispersion (0.2g/ml) in an aqueous solution of 0.2% agar and 0.1% tween 80. Animals receiving dosages of 1.25 g/kg or higher showed signs of Cholinergic stimulation prior to death. Observationf for 7 days.

LD_{50} = 0.67 g/kg (0.48 - 0.94)

Note: Small test group size.

Rabbit, Acute Dermal

The test material was applied as an aqueous paste to 5 animals per test level and kept in contact with the skin for 24 hours by means of an impervious cuff. Animals receiving dosages of 1.25 g/kg or higher showed signs of Cholinergic stimulation. No skin irritation was observed.

LD_{50} = 0.44/kg (0.30 - 0.66)

Note: Test group size too small.

Rabbit, 14-day Repeated Dermal

Five animals per group received dosages of 200 mg/kg, 100 mg/kg and 50 mg/kg as a 12% aqueous solution. Applications were made daily 5 days per week, for two weeks. Animals were restrained during dosing (30-45 minutes). One week after the last dose, animals were sacrificed and given a thorough gross autopsy.

Results: At 200, 100 and 50 mg/kg, animals exhibited salivation and muscular fibrillations. At 200 mg/kg 2 animals died after the first dosage.

No signs of skin irritation were noted at any test level. No relevant gross pathology was observed.

Rabbit, Eye Irritation

Ten milligrams of the technical was instilled into the conjunctival sac of the left eye of 5 rabbits. No irritation was observed for 7 days.

Note: Dose level inadequate.

Rat, 29-day Feeding

The test material was incorporated into the diet at concentrations of 0.05%, 0.1%, and 0.2% and fed to albino rats (10 per level) for 29 days. No deaths or abnormal behavior was noted. No relevant gross pathology was noted. Decrease in body weight gain noted in several animals fed 0.2 g/kg. Twenty-nine day feeding 'NEL'=0.1 g/kg.

Acute Oral Studies, Cycocel technical 99% pure, Report No. 66-25, March 9, 1966.

Five animals were used per test level (except cat (2) and dog (2-4)). Four test levels were used per animal species.

Species	Oral LD_{50} (mg/kg)
Hamster	1070 (760-1500)
Mouse	1020 (770-1340)
Chick	920 (580-1450)
Rat	670 (480-940)
Guinea Pig	620 (470-810)
Rabbit	81 (47-138)
Cat & Dog	<50 (25-100)

Note: Highly toxic to cat and dog.

Dog (Beagle), acute oral

Cycocel 4L, Affiliated Medical Research, Inc., R.W. Fogelman D.V.M., July 14, 1974.

The compound was administered as a 25 mg a.i./ml by intubration to 4 groups of 4 beagle dogs. Observations were made for 14 days.

Calculated LD_{50} = 133 mg active ingredient/kg $\pm$ 31 mg

Rat, Acute Inhalation

Cycocel 4L, Affiliated Medical Research, Inc., R.W. Fogelman, D.V.M., June 14, 1974,

Concentrations of Cycocel 4L were calculated rather than determined by analysis of chemical samples. Six rats were exposed to 892.9 mg/l for 1 hour. Observations were made for 14 days. All animals were then autopsied and examined grossly.

Results: Mild depression was noted followed by complete recovery in 4 to 8 hours. No mortalities and no significant gross pathology was noted.

LC_{50} > 892.9 mg/l

Rabbit, 21-day dermal

Cycocel 4L, Food and Drug Research Laboratories, E. Cox., M.D., pathologist Aug. 13, 1975.

Thirty-two albino rabbits were randomly assigned into 4 groups of 4 males and 4 females each. The test material was diluted in the vehicle (as supplied (?)) into three concentrations such that 0.3m/per Kg supplied 13.4, 42.5 and 134 mg Cycocel 4L. Ten percent of the body surface (dorsal aspect) of all animals was clipped and skin of 2 animals per sex/group was abraded. The test material was applied under a gauze pad. Exposure lasted 6 hours/day and 5 days/week for 3 weeks. After each exposure, the excess material was removed by washing the skin.

Body weights were recorded initially and at termination. Blood and urine analysis were also conducted. Necropsy with gross examination was conducted at termination. All organs, and a segment of treated skin were preserved in formalin. Any grossly abnormal sites were examined microscopically.

Results: All test animals survived the study. Body weights were within normal limits.

Skin irritation during the study became increasingly severe with maximum scores attained on the 11-12th day and continuing to termination. Controls also reached maximum scores, and there were no differences between these scores and the test groups.

Hematology and urine data revealed no significant trends in the test groups.

Conclusions: It is impossible to discern any differences induced by this test compound versus controls. Control animals received "Blank Cycocel 4L (AC 2524-70-2) and this was described as a "vehicle". This "vehicle" must be identified. Furthermore, the prose form description of gross and microscopic findings is inadequate to determine differences between test and control groups. A more finite grading system should have been used. Another untreated control group should also have been used.

Rat, Chlorocholinechloride elimination

BASF Industrial Hygiene & Pharmacological Inst., 12/31/65.

All animals (10 male & 10 female Sprague-Dawley rats) received 330 mg chlorocholinechloride (8 ml/kg of 10% aqueous solution). Urine was obtained by squeezing it out of the bladder at 1/2, 1, 2, 4, 8, 12, 24, 30, 36, 48, 56 and 72 hours after administration. The fractions were tested for chlorocholinechloride content by thin layer chromatography. Four days after start of the test all surviving rats were killed.

Results: Two female and one male rat died 2 1/2 and 3 1/2 hours after administration. Remaining animals showed typical CCC poisoning but survived the four days. CCC elimination was highest at 1-8 hours. After 36 and 48 hours only traces of CCC were found while at 56 hours none was evident.

Conclusions: Chlorocholinechloride is eliminated by rats in approximatly 2 days. CCC was not shown to accumulate, but quantitative evidence was not submitted for review.

Rabbit & Cat, Detoxification of chlorocholinechloride (CCC) with atropine, Industrial Hygiene & Pharmacological Institute (Cyanamid), 12/31/65.

Pretreatment of atropine (50 mg/kg i.v.) for rabbits and (10 mg/kg p.o.) for cats gave some protection against poisoning symptoms of CCC administered at 100 mg in the rabbit or 10 mg CCC/kg in the cat.

Rabbits and cats receiving harmless doses of 50 mg or 10 mg/kg Atropine respectively, followed by normally non-lethal dosages of CCC were killed. Atropine therefore acts synergistically and significantly increases the toxicity of CCC. Increase of speed of injection of Atropine increased the onset of symptoms and death.

Rat, 90-Day Feeding, Chlorocholinechloride, Cyanamid Laboratory, Oettel and Frohberg, 10/12/64

a) Specimen C 520/389 purity grade approximately 98%
b) Test 73/24 purity grade approximately 96%

Twenty-five male and 25 female Sprague-Dawley (own breed?) were divided into 4 test and one control group of 5 male and 5 females each. Rats (5) received 100 g feed (powdered rat bread, Allied Mills) with doses of 0, 75, 150, 300 or 600 mg CCC per Kg rat. All animals were weighed 3 times per week, their mean weight calculated, and from this the substance concentration was computed.

Within the following 1-4 weeks, no symptoms were observed and dose levels were increased to 600, 1200, 2400, and 3600 mg/kg.

Results: During the first two months, male rats gained considerably more than females. During the third month, males showed considerable weight loss (from 325-260 grams).

Except for some suppressed weight gain, all animals in the 600 mg/kg group showed no toxic symptoms. Animals receiving 1200 mg/kg from the second week, lagged significantly behind controls in weight gain.

Five days after feeding rats in at 2400 and 3600 mg/kg, these groups developed diarrhea, in part fibrillary movements and spasmophilia and no longer gained weight; the rats of the 3600 mg/kg group even lost weight continuously. Six out of ten rats of the 2400 mg/kg group and all animals of the 3600 mg/kg group died 35-85 days after the start of the test.

Clinical-Chemical Results: At six weeks, 2 out of six rats (1200 ppm group) were alive. They showed hypoleukocytosis (900 and 3500), increase of the SGTP (63 and 155 units), one with blood urea increased 108 mg%. In the 2400 mg/kg group, blood urea increased in two animals (55 and 67 mg%).

Urinalysis results showed no deviations.

Necropsy

Necropsy and histological examination revealed no irregularities. Relative organ weight ratios of the liver and kidney did not differ significantly from controls.

Conclusions: The only conclusion that can be drawn is that chlorocholine-chloride when administered in the diet is less toxic than a single administration via stomach tube. The study is not acceptable as a 90-day study for the following reasons:

1. No. of animals tested per test level (10) is inadequate

2. Doses levels were changed after several weeks of testing.

3. Blood chemistry, hematology, pathology, and organ weight data were either totally inadequate or completely lacking in the test report.

Cat, 6 month feeding, chlorocholinechloride purified, Cyanamid Laboratory, Oettel and Hofmann, 10/16/64.

Four cats (2 castrated male and 2 female animals) received 1 mg/kg CCC in approximately 20 g raw coursely ground meat, 5 days per week for 6 months. All parameters monitored during this period (body weight, hematology, SGPT, Blood urea, liver function, and urine) were within normal limits.

After 6 months, the dose was increased to 5 mg CCC per Kg. After the 4th dose (females) and the 9th dose (males) pronounced symptoms of tremors etc. appeared.

After an interval of seven days, female cats were again given 5 mg/kg CCC in their diet and symptoms appeared after 5 daily doses. After 2 days, males were also given 5 mg/kg CCC for 3 successive days and one animal developed symptoms.

After symptoms disappeared, all cats were given 10 mg/kg CCC for 2 days. Pronounced symptoms developed in both females and one male after each dose.

Five mg/kg was then administered via stomach tube. All animals demonstrated severe symptoms but survived. They were then given 10 mg/kg via stomach tube and 3 of 4 died.

Conclusions: Daily doses of 1 mg/kg chlorocholinechloride in the diet for six months induced no observable symptomology. Cumulative effects not observed.

But, this study is totally unacceptable as a six month feeding study for the following reasons:

1. No pathology report accompanied the study.
2. No hematology, blood chemistry, or urinalysis data was submitted.
3. The number of animals used (4) is inadequate.
4. Test dosages were continued for a short period after six months. They were administered by means inconsistent with the earlier part of the study with dosage and time interval varied.

Dog, Two Year Feeding Study, Chloro-choline-chloride (CCC) BASF (Cyanamid), 4/17/67.

Forty-four pure-bred beagle dogs were divided into 4 experimental groups of 6 dogs (3 males and 3 females) and one control group of 20 dogs (10 males and 10 females). CCC was fed in the diet (350 g) at the following test levels:

Group I	100 ppm
II	300 ppm
III	1000 ppm
VI	1000 ppm + 700 ppm Choline-chloride

Random samples of feed were taken and concentrations checked using thin layer chromatography.

During the first month, feeding took place once daily at mid-day. Thereafter, it was twice daily at 10.00 hours (200 g) and 15.00 hrs. (150 g). At 16.00 hrs. uneaten food was re-weighed and consumption calculated. Body weights were monitored weekly. Clinical-chemistry examinations were carried out every 6 months. Urinary excretion of CCC was checked a total of 3 times.

At the conclusion of the study all animals were killed and the following organs were weighed and/or histologically examined:

brain	spleen
pituitary gland	kidneys
thyroid glands	suprarenal glands
lungs	ovaries or testes
heart	liver
trachea	uterus
stomach	pancreas
intestine (twice)	lymphatic gangleons
bladder	

Results: No animals in Group I or II elicited any symptoms of poisoning during the study.

In Group III animals (1000 ppm) a considerable flow of saliva (400 CM^3) and weakness of musculature in the hindlimbs was observed 2-3 hours after feeding and again 6-7 hours after feeding. One male and one female dog of the 1000 ppm group died and 38 days after the beginning of the experiment. It was then decided to divide the food ration into two meals.

Excess saliva flow was also noted in Group IV animals at the beginning of the study. After one year, this occured only occasionally.

One male of this group died after 533 days of a bite.

Conclusions:

1. Animals were poorly assigned to test groups, especially females. Mean group weights varied 3 Kg.

2. Mean weights of liver, kidney, spleen, and heart were elevated in female dogs (Group III). Mean spleen weights were elevated in male dogs (Group III). Mean spleen to body weight ratio was also elevated in Group III males.

   Liver and spleen to brain weight ratios were elevated in males of group III. Liver, kidney, spleen, and heart to brain weight ratios were also elevated for females of Group III. Individual liver, kidney and spleen to brain weight ratios were elevated in 1 of 2 females of Group III. One of three females in Group IV demonstrated an elevated kidney to brain weight ratio.

3. The study did not contain any statistical evaluation of the above values.

4. At least 4 males and 4 females should have been used per test level with 1 male and 1 female per test level sacrificed at one year.

5. A signed pathologist's report must be submitted with detailed explanation. Special attention should be given to the two animals that died in Group III (Animal No. 162 and 169). The explanation (Pg. 2 of the study) is not acceptable.

6. Because of the deficiecies listed above the validity of this study is in question. Some of these questions may be resolved.

Mouse, 18 Month Oral, CCC, Bionetics Research Laboratories, Litton Industries, Contract No. PH43-64-57 and PH43-67-735, J. Nat. Can Inst. 42:1101-1114, 1969.

"Specific pathogen-free" mice were obtained from Cumberland View Farms. Females of the C57BL/6 strain were mated with C3H/Anf or AKR males to obtain two F, hybrids used in this testing.

"Strain X" = (C57BL/6XC3H/Anf)F1
"Strain Y" = (C57BL/6XAKR) F1

Sample composition was confirmed by infrared spectroscopy, gas chromatography, or thin-layer chromatography.

The maximum tolerated doses were determined. In the case of CCC this was 21.5 mg/kg via stomach tube from days 7-28 of age. The vehicle used was 0.5% gelatin. After four weeks the compound was mixed in the diet at a concentration of 65 ppm. This level was calculated according to the weight and food consumption of the 4-week old mice so that again they would receive approximately the MTD (mg/kg). This same concentration was maintained throughout the 18 month test period.

Seventy-two animals, 18 animals of each sex of each strain, were used in this study. Each animal room contained an untreated control group and at least 1 positive control group.

The postmortem procedure included an external examination and a thorough examination of thoracic and abdominal cavities, with histologic examination of major organs and of all grossly visible lesions. The cranium was not dissected. The entire carcass and all internal organs were fixed and have been saved. Blood smears were made on all mice before they were killed, and then examined only in cases showing splenomegaly or lymphadenopathy.

From statistical analysis performed, conclusions were reached that there was no significant evidence of differences among the (5) negative control groups and they were lumped together. Positive controls (Urethan, Amitrol, Aramite, Dihydrosafrole, Isosafrole, and Safrole) and experimental groups were compared with the grouped negative controls. The relative risk (a measure of the tumor incidence among the treated mice as compared to the controls) was performed with four tumor groupings: hepatomas, pulmonary tumors, lymphomas, and total mice with tumors. Also, the significance test of each sex-strain subgroup and their various combinations by the Mantel - Haenszel procedure, the combined relative risk uses, and the weighted, geometric mean with the 1/2 corrections were used. In another test, all positive controls were lumped together and the corrected relative risk was calculated for each experimental group and compared to the positive control baseline.

Results: CCC was listed in Table 4 (those compounds showing an elevation of tumor incidence in an uncertain range). Additional statistical evaluation and/or experimentation was recommended.

A summary pathology report was obtained from Dr. Richard K. Bates in response to a telephone inquiry. It indicates the concern to be hepatoma in males. TOX concurs that this study is not in any way conclusive. (See Tables, pages 15 and 16)

Mouse, 78-week Feeding Study, Cycocel, Huntingdon Research Center, Wheldon et al., May 5, 1971.

Technical grade Cycocel (98.5% pure) was supplied by Badische Anilin & Soda-Frabik AG, Germany.

Two hundred and eight CFLP mice (a hysterectomy derived strain of Swiss origin) was obtained from Carworth Europe, England, aged 26 days, and allocated to treatment groups as follows:

Group	Treatment	No. of Mice	
		M	F
1	Control (untreated)	52	52
2	Cycocel, 1000 ppm	52	52

Equal numbers of animals from within each weight-range were randomly allocated to each of the two treatment groups. Mice were housed four per cage and these were placed in a random manner in the animal room to equilibrate environmental influences.

All animals had free access to water and powdered Spillers Small Animals Diet (autoclaved).

All mice were examined daily for toxic effects, behavioral changes, etc. All mice found dead, or sacrificed, were subjected to detailed macroscopic examination. Where possible, tissue samples were preserved.

Body weights were recorded initially and then at weekly intervals for 16 weeks and at monthly intervals thereafter.

Samples of the following tissues from all mice were preserved for histological examination:

adrenals
brain
caecum
colon
eye
heart
gall bladder
ileum
mammary gland (female)
kidneys
liver
lungs
marrow
mesenteric lymph node
pineal body
pituitary
prostate
pancreas
spleen
stomach
testes or ovaries
thymus
thyroid and parathyroids
urinary bladder
uterus

PROJECT #16 CARCINOGENESIS ASSAY STUDIES P.N. No. 66/815

Compound Name CCC

Compound No. 156-G, Oral

Date Killed 5-11-67

Date Completed 6-17-68

	B6C3F1		B6AKF1	
	Male	Female	Male	Female
No. mice at start	18	18	18	18
No. mice surviving 18 months	18	18	18	14
No. mice missing (no necropsy or tissue missing)	0	0	0	1
No. mice died during experiment	0	0	0	3
No. mice negative (killed and died)	8	12	12	12
No. mice died with tumors	0	0	0	1
No. mice killed with tumors	8	1	5	3
No. mice killed or died, other diseases	2	5	1	1
Tumors				
phatic Leukemia	0	0	0	0
Reticulum Cell Sarcoma, Type A	2	0	0	1
Reticulum Cell Sarcoma, Type B	0	0	0	0
Pulmonary Adenoma	0	0	1	0
Pulmonary Carcinoma	0	0	0	0
Hepatoma	5	0	5	0
Hepatic Carcinoma with Pulmonary metastases	0	0	0	0
Mammary Carcinoma	0	0	0	0
Carcinoma, skin	0	0	0	0
Other types				
bcutaneous fibrosarcoma	0	0	0	1
stric papilloma	0	0	0	1
~~Angioma~~	1	0	0	0
Leiomyoma, uterus	0	1	0	1
Total Number of Tumors	8	1	6	4
Common other Lesions				
Follicular hyperplasia, any site	3	2	0	0
Lymphoid infiltrate, any site	3	1	0	1
~~Focal pneumonia~~	2	1	2	0
Osteogenesis, spleen	2	0	0	0
Pyometra	0	1	0	0
~~Focal necrosis, any site~~	2	0	0	0
Focal gastritis	0	1	0	0
Cystic endometritis	0	1	0	0
Focal hepatitis	0	1	0	0

Signed: Borge M. Ulland

Borge M. Ulland, D.V.M.

L Fm. #449

PROJECT #16 CARCINOGENESIS ASSAY STUDIES P.M. No. 66/615

Compound Name CCC

Compound No. 156-F, Subcutaneous

Date Killed 10-27-66

Date Completed 6-28-68

	B6C3F1		B6AKF1	
	Male	Female	Male	Female
No. mice at start	18	18	18	18
No. mice surviving 18 months	5	17	12	11
No. mice missing (no necropsy or tissue missing)	12	0	4	7
No. mice died during experiment	1	1	2	0
No. mice negative (killed and died)	5	13	12	5
No. mice died with tumors	0	1	0	0
No. mice killed with tumors	0	0	0	0
No. mice killed or died, other diseases	1	4	2	6
Tumors				
Lymphatic Leukemia	0	0	0	0
Reticulum Cell Sarcoma, Type A	0	1	0	0
Reticulum Cell Sarcoma, Type B	0	0	0	0
Pulmonary Adenoma	0	0	0	0
Pulmonary Carcinoma	0	0	0	0
Hepatoma	0	0	0	0
Hepatic Carcinoma with Pulmonary Metastases	0	0	0	0
Mammary Carcinoma	0	0	0	0
Carcinoma, skin	0	0	0	0
Other types				
—				
Total Number of Tumors	0	1	0	0
Common other Lesions				
Follicular hyperplasia - any site	0	0	0	1
Lymphoid infiltrate - any site	0	0	0	3
Focal pneumonia	1	2	2	2
Hyperkeratosis, stomach	0	1	0	0
Fatty metamorphosis, liver	0	1	0	0
Cystic endometritis	0	0	0	1

Signed: Borge M. Ulland, D.V.M.

Fm. #449

The urinary bladder from all mice dying or killed after 52 weeks was distended in situ with fixative and examined under low power.

The following tissues were preserved but not processed:

aorta	fat	trachea
bone	mammary gland (males)	tongue
second eye	skeletal muscle	
	skin	

Microscopic Examinations

Initially restricted to:

1. All mice with macroscopic lesions
2. Urinary bladder from all mice dying or killed after 52 weeks
3. Tissues from 10 males and 10 females or each group sacrificed at week 78.

The evaluation was later extended to all tissues showing gross or macroscopic treatment-related changes and to all mice at week 78.

Statistical Analysis

Differences between the treated and control groups were assessed by a 2X2 contingency test, used as a one-tailed test, computing the exact probability of the tumor distribution.

Tumor incidences were quoted from an 80-week study of 239 male and 236 female untreated controls to establish a profile for the CFLP mouse under standard conditions.

RESULTS: No overt signs to treatment were noted. Numbers of mortalities did not vary from controls.

After 24 weeks, the treated mice showed a gradual retardation of growth. At termination, body weights of treated mice (surviving to the final weeks) were 6% below those of controls.

Macroscopic Pathology

Pathology representing or contributing to cause death among decedents show no change in the treated versus control groups.

Numbers of mice with macroscopic lung tumors were, in both decedents and mice killed at termination, higher than controls. Microscopically it was determined that number and size of lung tumors tended to be

slightly higher in treated males, "but in tumor multiplicity there was no significant differences between treated and untreated animals."

The 52 week sacrifice revealed the presence of amorphours masses in the bladder lumen in some male mice. This was considered "the result of accessory sex gland secretion."

Histopathology:

The numbers of mice lost due to cannibalism or autolysis was less than 6%.

Lung:

Chronic respiratory disease was evident in both controls and treatment groups. It was characterized by perivascular and peribronchiolar lymphocytic cuffing, bronchiolar exudation, focal increase in cellularity of lung parenchyma, and occasionally adenomatosis and interlobular adhesions.

Liver:

Variation in hepatocyte size and cytoplasmic vacuolation (variable in degree and distribution) was common to control and treatment groups.

Small foci of lymphoid cells were present in both control and treatment groups.

Kidney:

The presence of atrophic tubules, vacuolation of tubular epithelium, and interstitial, and interstitial aggregates of lymphoid cells was found in control and treated animals.

Urinary bladder:

No primary tumors were observed with the exception of a haemangioma in a control male. At post mortem one animal contained deposits of a lympho-reticular tumor.

Other changes were observed in other organs occasionally and in both controls and treated animals.

Neoplastic histopathology:

The incidence of liver tumors in mice treated with Cycocel was less than that among control mice. The majority of liver tumors were derived from parenchymal cells (i.e., hepatomata) with the exception of one malignant liver cell tumor in a control male, which displayed pneumonic metastases. Remaining tumors were haemangiosarcomata.

Lung tumors:

Lung tumors incidence in treated females was comparable with that of control females. In the Cycocel treated males, there were more tumors 20/52 versus 10/51 in control males.

There was no evidence of multiplicity of tumors; both treated and control males had an average of 1.1 tumors per mouse. The study classified tumors in Cycocel grade 1 and 2 (1) Non-invasive (2) Locally invasive and sometimes extending into airway. Thirty percent (3/10) control and 40% 8/20 treated mice bore tumors larger than 6 mm diameter.

The study concluded that this tumor increase fell within normal ranges for the CFLP mouse (18-38%) lung tumors.

NOTE: Background data for the CFLP mouse has been received and reviewed. A normal incidence of 26.1% for lung tumors in controls was determined.

Pituitary tumors:

Two pituitary tumors (4.1% incidence) were recorded among treated females, with none found in controls. It was noted that in a control profile study of 236 female mice, seven pituitary tumors were found with an expected range of 0 to 7.5%. These two tumors were concluded to be within the normal range.

Conclusions:

Only one test level (1000 ppm) was employed in this study. This in itself, negates the validity of the study in fulfilling TOX data requirements.

Background data for the CFLP strain was requested in order to evaluate tumor incidence (especially pulmonary). The petitioner submitted a review article, Tumors in Control Mice: Literature Tabulation, Sanford P. Sher, Toxicology and Applied Pharmacology 30, 337-359 (1974). See Table attached.

This table was taken from background data submitted by Cyanamid in response to a telephoned inquiry. It is from a Review Article, Tumors in Control Mice: Literature Tabulation, Sanford P. Sher, Toxicology and Applied Pharmacology 30,337-339 (1974)

TABLE 1

STRAIN AGE LABORATORY DIET	DURATION	NO. OF MICE M F	NO. OF MICE WITH TUMORS M F	NO. OF MICE WITH MALIGNANT TUMORS M F	NO. OF MICE WITH LYMPHOMAS OR LEUKEMIA M F	NO. OF MICE WITH MAMMARY TUMORS M F	NO. OF MICE WITH PULMONARY TUMORS M F	NO. OF MICE WITH HEPATIC TUMORS M F	NO. OF MICE WITH MISC. TUMORS M F	COMMENTS	REFERENCES
E. (CONTINUED											
Carworth CF-1 (CFLP) Huntingdon Res.Centre England	80 wk	238 236 survival 77% 66%	163 131 68.5% 55.5%		25 46 10.5% 19.5%	0 5[a] 2.1%	62 37 26.1% 15.7%	51 19 21.4% 8.1%	4 pituitary 8 11 testes - inter- stit. - ovary 2 - uterus 3 2 stomach 1 0 spleen[e,f] 2 1 pancreas[b] 0 1 kidney[b] 0 2 harderian 3 gland[b,d] 2 sub q 4 mediast.	Based on exam. of 474 mice kept as controls in 80 wk study in CFLP mouse derived from CF-1.	Carworth Farms, unpublished data

STRAIN AGE LABORATORY DIET	DURATION	NO. OF MICE M	 F	NO. OF MICE WITH TUMORS M	 F	NO. OF MICE WITH MALIGNANT TUMORS M	 F	NO. OF MICE WITH LYMPHOMAS OR LEUKEMIA M	 F	NO. OF MICE WITH MAMMARY TUMORS M	 F	NO. OF MICE WITH PULMONARY TUMORS M	 F	NO. OF MICE WITH HEPATIC TUMORS M	 F	NO. OF MICE WITH MISC. TUMORS M	 F	COMMENTS	REFERENCES
																0 muscle[f]	1		
																1 odonotoma	0		
																1 osteoma	0		
																25	24		
																10.5%	10.2%		

[a] adenoma & carcinoma, [b] adenoma, [c] carcinoma, [d] papilloma, [e] angioma, [f] sarcoma

Lung tumor incidence in treated males was 38% (20/52) versus 20% (10/51) in controls. This increase was rationalized as being within the "normal range of 18-38%." Since however the background data submitted indicated a normal incidence of 26.1% in males, this "range rationale" is not accepted, especially since this is based upon a single study. If on the other hand, it is based upon a number of completely different studies, then the rationale would be acceptable. Data reviewed cannot support this conclusion.

In control females, lung tumor incidence was 16% (8/50) versus 22.4% (11/49) in the Cycocel treated group. This increase is not considered enough to be significant but it is noted.

A major problem in the study is the failure of the pathologist to report findings consistently for all organ tissues at termination as specified in the procedure. As an example, the report contained no pathologists observations for lung and liver in 9 control males and 10 treated. In the case of females, for example, no histopathology observations were noted for the liver in 14 control and 20 treated mice at termination. On the average, the report contained histological observations for 3 organs per animal and in many cases only one organ was listed per animal. It cannot be accepted that those organs were not listed because they were within normal limits because the report also lists normal findings.

The pathologist's report is not signed.

The questions raised previously must be answered even though this study will not satisfy tolerance data requirements. It cannot be acceptable because (1) only one test level was used and (2) the naturally high lung tumor incidence in controls makes interpretation of lung tumors in the test nearly impossible.

Rat, 2-year feeding study, chlor-choline-chloride, BASF Institute for Industrial Hygiene and Pharmacology, 12/9/66 (Translation).

Two hundred male and 200 female Sprague-Dawley (own strain) were used in this study. They were stated to be "particularly sensitive" to CCC (Oettel et al, Sept. 1966) (?). At the beginning rats weighed about 100 g. Each control and each test group was composed of 50 male and 50 female animals.

The test feed was prepared by mixing CCC with 1% aqueous (traganth(?)) suspension in Ultra-Turrex (?) and then mixed with finely ground rat bread (Lab Blox of Allied Mills, Chicago). Control rats received bread to which 10,000 ppm cellulose had been added in the same manner.

	CCC	Cellulose
Experimental Group I	500 ppm +	9,500 ppm
" " " " II	1000 ppm +	9,000 ppm
Control Group I		10,000 ppm
Control Group II		10,000 ppm

Two to three rats were caged together. During the first year, one piece of rat bread was given to the animals five days per week and two on two days per week. The average consumption per animal was calculated to be 13.5g of feed per day.

In the second year, one piece of rat bread was given four days per week and two pieces on three days. Average consumption based upon this regi= ment was calculated to be about 14.5g of feed per day. Water was given ad libitum and 10g of carrots per animal were given per animal twice a week.

In "some of the animals" of the experimental and control groups the following parameters were determined initially, between day 95-125 days 365-400, and on the 700th day (10 male and 10 female per group):

hemoglobin	Urinalysis
leucocyte count	pH
erythrocyte count	protein
hematocrit value	sugar
differential	urobilinogen
blood urea	sediment
SGPT	

All animals that died during the study first 400 days were autopsied and tissues histologically examined. Five female and five male animals were killed and examined after 400 days of the study. Absolute and relative organ weights of the liver and kidney were determined.

All animals that died during the remainder of the study and all animals living to the end of the study were killed, autopsied and the absolute and relative organ weights of the liver and kidneys were obtained. The following organs were histologically examined:

Heart	CNS
lungs	cerebrum & Cerebellum
liver	pons
kidneys	any tumors
suprarenal gland	testicles or ovary
spleen	thyroid gland
stomach	intestines

RESULTS:

No symptoms of poisoning were evident. Increase in body weight in experimental groups was the same as controls. Clinical/Chemical tests revealed no abnormal changes.

The death rate of treated animals was comparable to that of controls. Absolute and relative organ weights of liver and kidneys were comparable with controls.

Autopsies and histological examination revealed changes in the respiratory organs sometimes causing death. In 64 control and 65 experimental animals respiratory "changes" were noted and very simply grouped together as "chronic inflation of the respiratory organs."

This reviewer totalled all changes categorized as above in tables 77-84. These totals do not agree with totals for "chronic inflammation of the respiratory as stated in the report (above).

	Males	Females
EXP. G.1 (500 ppm)	13	21
EXP. G.2 (1000 ppm)	10	13
Total	23	34
Control G.1	12	18
Control G.2	15	12
Total	27	30

An explanation for these differences is in order even though no dose-response is evident.

Incidence of otitis as stated in the 'results' does not agree with tables 77-84.

Otitis incidence (as determined from tables)

	Males	Females
(500 ppm) EXP G.1	10	12
(1000 ppm) EXP G.2	5	16
Control G.1	12	12
Control G.2	6	7

Glomerulosclerosis observed in control and treated animals could be considered age related, but it is not graded. Spleen hemosiderosis was found in 142 experimental and 94 control rats but again there was no grading system. (Note: experimental groups were lumped together!)

Alveolar carcinoma of the thyroid was stated to occur 81 times in control versus 80 times in experimental rats. (No grading system). None of these tumors broke through the thyroid gland capsule or metastasized. Mammary gland tumors also occurred frequently in controls and test animals.

Background data should be available for this particular strain. The study results attempt to negate any importance of these tumors based upon a 33% incidence (thyroid gland carcinomes) in Long Evans rats. Clarification on these points are needed.

Conclusions:

The following deficiencies must be corrected before this study is acceptable:

1. The pathology report was not signed and no grading system was used.
2. The study did not include brain, heart (etc.) organ weights.
3. Pathological results as stated do not agree with tables presented.
4. There is no demonstrated effect level and only two test levels were used.

5. The study contained no statistical evaluations.

6. The rats used in this study are stated to be Sprague-Dawley, "own strain" and they are "particularly sensitive to CCC." Background data for this strain should be submitted.

Rat, 3-Generation Reproduction Study, CC, F. Leuschner, A. Leuschner, W. Schwerdtfeger, Laboratory of Pharmacology and Toxicology, Cyanamid, April 14, 1976 (Translation from German).

The review of D. L. Ritter, 4/18/76, PP No. 4G1461 is noted.

Note: The histopathology report of Professor Dr. H. Otto (only carried out for 2nd litter F_3 generation) indicate that apart from incidental findings 4/10 males of group V (900 ppm CCC, infeed) and 2/10 in group III (300 ppm CCC infeed) had "giant cells" detectable in the testes. He concluded that "The importance of these findings must be left open." Furthermore, he noted that nothing was observed in group I (100 ppm CCC infeed) or group VII (controls). He also concluded that "The detectable giant cells could be a sign of suppressed development at the state of spermiogenesis."

Conclusions about this study cannot be drawn until the existence and description of these "giant cells" is clarified further. Also, slides in the 2 year rat study must be reviewed for the existence of "giant cells."

The average daily intake of CCC

	PPM Diet	P generation		
M	100	(I)	8.4 mg CCC/kg ±	1.9 mg (6)
F	100	(II)	12.2 mg CCC/kg ±	4.7 mg (6)
M	300	(III)	24.8 mg CCC/kg ±	4.9 mg (6)
F	300	(IV)	39.1 mg CCC/kg ±	15.8 mg (6)
M	900	(V)	76.8 mg CCC/kg ±	17.3 mg (6)
F	900	(VI)	118.4 mg CCC/kg ±	49.2 mg (6)

	PPM Diet	F_1 generation		
M	100	(I)	8.0 mg CCC/kg ±	2.5 mg (6)
F	100	(II)	11.7 mg CCC/kg ±	4.5 mg (6)
M	300	(III)	24.5 mg CCC/kg ±	8.3 mg (6)
F	300	(IV)	34.4 mg CCC/kg ±	13.8 mg (6)
M	700	(V)	71.1 mg CCC/kg ±	21.6 mg (6)
F	900	(IV)	104.0 mg CCC/kg ±	35.9 mg (6)

	PPM Diet	F_1 generation		
M	100	(I)	8.3 mg CCC/kg ±	3.0 mg (6)
F	100	(II)	11.8 mg CCC/kg ±	4.9 mg (6)
M	300	(III)	24.3 mg CCC/kg ±	7.5 mg (6)
F	300	(IV)	33.9 mg CCC/kg ±	15.4 mg (6)
M	900	(V)	82.5 mg CCC/kg ±	23.6 mg (6)
F	900	(VI)	99.1 mg CCC/kg ±	42.0 mg (6)

	PPM Diet	F_3 generation
M	100	(I) 13.4 mg CCC/kg ± 2.4 mg (6)
F	100	(II) 13.0 mg CCC/kg ± 1.4 mg (6)
M	300	(III) 39.9 mg CCC/kg ± 6.7 mg (6)
F	300	(IV) 38.4 mg CCC/kg ± 3.4 mg (6)
M	900	(V) 115.2 mg CCC/kg ± 15.1 mg (6)
F	100	(VI) 118.8 mg CCC/kg ± 16.1 mg (6)

Rat, Teratology Study, Cycocel Food and Drug Research Laboratories, Inc., D. E. Bailey, K. Morgareidge, March 6, 1975.

One hundred and fifty female albino rats (FDRL-Wistar derived) were distributed into 5 groups of 30 animals each. Dosage levels were 1000, 2500 and 5000 ppm for the first week when symptoms of maternal toxicity were observed at the highest test level (250 mg/kg = 5000 ppm). The highest test level was then dropped and a low level added. Dosage levels were then as follows:

Test Material	Level mg/kg	Level ppm
Vehicle (sham)	0	0
Aspirin (P.C.)	250	5000
Cycocel	25	500
Cycocel	50	1000
Cycocel	125	2500

Each animal was individually housed. Rats were mated in a ratio of 1 female to 1 male and observation of the vaginal sperm plug was considered Day 0. The test material was administered by intubation from days 5 to 15 as an aqueous solution yielding 1.0 ml/kg body weight.

Animals were observed daily for behavior food intake. Body weights were recorded on Days 0, 6, 11, 15 and 20 of gestation. On day 20 all dams were subjected to caesarean and numbers of Corpora Lutea, implant sites, resorption sites, live and dead fetuses and sex and body weights of pups were recorded.

Each fetus was examined for external congential abnormalities. One third of each litter underwent visceral examination (Wilson sections). The remaining 2/3 were cleared and stained with alizarin-red S and examined for skeletal abnormalities.

RESULTS:

Pregnancy rates, body weights of dams, nidation, fetal survival and skeletal maturation were unaffected by Cycocel at any test level. No significant soft tissue anomolies were evident from the Wilson sections.

Aspiring, the positive control, exhibited an increase in resorption sites, decreased number of live fetuses, live fetus weight, and ossification retardation.

Three incidences of gastroschisis were found in the 50 mg/kg Cycocel group from 3 different dams and one incidence in the 125 mg/kg group. There was no incidence of gastroschisis in the control of 25 mg/kg group. The positive control (Aspirin, 250.0 mg/kg) exhibited 3 instances of gastroschisis.

CONCLUSIONS:

The incidence of gastroschisis may be within normal limits (as stated in the study for the rat), but controls in this study showed a zero incidence. Background data with statistical evaluation demonstrating that this incidence is actually within normal limits must be submitted.

Other teratology studies submitted in this petition are not acceptable (as stated in review of D. L. Ritter 4/18/76, PP No. 4G1461) because of the summary form in which they were submitted.

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