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

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OCT 29 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: EPA ID#: 5F04456 and 6F04623 Chlorfenapyr: Application for Establishing Permanent Tolerance and Registration of Chlorfenapyr (Pirate®, AC 303,630) for use in/on Cotton Seed, Meat, Milk and Citrus

DP BARCODE No.: D212558, D222007 & D228742
Submission #: S481410 & S497246
P.C.#: 129093

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10/10/96

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10/10/96 KB 10/25/96

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I. CONCLUSIONS:

The data base supports the permanent tolerance and Section 3 registration of chlorfenapyr for the use on/in cotton seed, meat, milk and citrus. All reviewed studies are acceptable.

The Health Effects Division RfD Peer Review Committee (07/18/96) has established an RfD of 0.003 mg/kg/day, based on rat subchronic neurotoxic NOEL of 2.6 mg/kg/day

(1)

and applying an Uncertainty Factor (UF) of 100 to account for interspecies and intraspecies variability and additional Modifying Factor (MF) of 10 for lack of understanding of the cause/relationship for the nervous system lesions and its toxicity to the young. The NOEL = 2.6 mg/kg/day and the LOEL = 13.6 mg/kg/day, based on spinal nerve myelinopathy (M), decreased mean body weights, body weight gains and feed efficiency in both sexes, absolute feed consumption (F) and water consumption (M). This NOEL Of 2.6 mg/kg/day is supported by the rat chronic toxicity NOEL of 2.9 mg/kg/day and mouse chronic toxicity NOEL of 2.8 mg/kg/day. In the rat study slight to moderate non-neoplastic centrilobular to midzonal or diffuse hepatocellular enlargement were seen in both sexes at the LOEL of 15 mg/kg/day. In the mouse study decreased body weight gains and CNS lesions (M & F) and dermal lesions (M) were seen at the LOEL of 16.6 mg/kg/day. The chemical was reviewed by the Health Effects Division Cancer Peer Review Committee on 09/25/96. Preparation of the document is in progress.

Although acute (81-8S) and subchronic (82-7SS) neurotoxicity studies in rats are acceptable and the data are adequate for section 3 registration of the chlorfenapyr, the HED RfD Committee recommended that the Registrant conduct a confirmatory study elucidating the cause/relationship of CNS lesions to the neurotoxicity potential of the chemical. The study should follow the §83-6 guideline format for conducting developmental neurotoxicity, with several modifications. The modifications include treating the male and female rats for 90 days prior to mating and dams would go off treated feed 10 days post-delivery as is in the standard 83-6 protocol. In addition, males would be used assist in determining the nature/cause of CNS vacuoles seen in rats and mice. This would facilitate in characterizing the toxicity of chlorfenapyr. It is strongly recommended that the protocol and dose selection rational should be submitted to TB-I for review prior to initiation of the study.

II. ACTION REQUESTED:

American Cyanamid Company, has submitted applications for establishing permanent tolerances and registrations of chlorfenapyr tech. (PIRATE® Insecticide-Miticide/AC 303,630) and two-end use products, PIRATE Insecticide-Miticide/AC 303,630 3SC and ALERT™ Insecticide-Miticide/AC 303,630 2SC for use on/in cotton seed, meat and milk and citrus.

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Following are the list of studies submitted in support of section 3 registration of chlorfenapyr (DERs are attached):

Guideline #	Study Type	MRID #
81-1	Acute Oral toxicity	43492824 - 43492828
81-8SS	Acute Neurotoxicity - rat	43492829
82-1a	90-Day feeding - mouse	43492830
82-2	28-Day Dermal - rabbit	43492831
82-2	28-Day Dermal - rabbit	43492832
82-7SS	Subchronic Neurotoxicity - rat	43492833
83-1(b)	Chronic toxicity - dog	43492834
83-4	2-Generation reproduction - rat	43492836
83-5	Chronic toxicity/Carcinogenicity - rat	43492837
83-5	Chronic toxicity/Carcinogenicity - mouse	43492838
84-2	Gene mutation (Ames)	43492840
84-2	Gene mutation (Ames)	43492841
84-2	Gene mutation (Ames)	43492842
84-2	Structural chromosomal aberration	43492843
84-2	Structural chromosomal aberration	43492839
85-1	General metabolism	43492844

III. **PRODUCT INFORMATION:** Chlorfenapyr (Pirate®, AC303,630)
PC CODE: 129093
Updated: October 2, 1996

Chlorfenapyr [4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile] is an insecticide-miticide for use on cotton, vegetables, citrus and ornamentals. It is manufactured by American Cyanamid Company and proposed to be sold under the trade names of PIRATE 3SC Insecticide-Miticide or AC303,630 3SC Insecticide-Miticide (32% a.i.) and ALERT 2SC Insecticide-Miticide or AC303,630 2SC Insecticide-Miticide (21% a.i.). The Sponsor's proposed mode of action of the chemical is by uncoupling pests' mitochondrial oxidative phosphorylation through an electrochemical gradient. The chemical is not known to develop cross-resistance in insects/mites to other classes of insecticides/miticides including the carbamates, organophosphates, pyrethroids, cyclodienes, organochlorines and benzophenylurea compounds.

Chlorfenapyr is a white solid with a melting point of 100-101°C. It is soluble 0.12

mg/ml in deionized water and 7.09 g/100 ml in methanol at 25°C.

The Chemical Abstracts Registry Number (CAS No.) is 122453-73-0

The chemical structure of chlorfenapyr is:

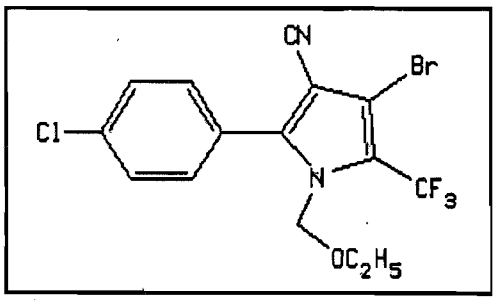

Figure 1 Chlorfenapyr (Pirate)

IV DATA REQUIREMENTS:

Updated: 10/02/96

Technical: Chlorfenapyr (AC 303,630, Pirate® Insecticide-Miticide)
Use Pattern: Terrestrial food use
Action Type: Permanent Tolerance

Guideline #	Study	Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Yes
81-3	Acute Inhalation Toxicity	Yes	Yes
81-4	Primary Eye Irritation	Yes	Yes
81-5	Primary Dermal Irritation	Yes	Yes
81-6	Dermal Sensitization	Yes	Yes
81-8	Acute Neurotoxicity (rodent)	Yes	Yes
82-1(a)	Subchronic Oral (rodent)	Yes	Yes
82-1(b)	Subchronic Oral (non-rodent)	Yes	Yes
82-2	21-Day Dermal	Yes	Yes
82-7SS	Subchronic Neurotoxicity - rat	Yes	Yes
83-1(a)	Chronic Toxicity (rodent)	Yes	Yes
83-1(b)	Chronic Toxicity (non-rodent)	Yes	Yes
83-2(a)	Carcinogenicity - rat	Yes	Yes

Guideline #	Study	Required	Satisfied
83-2(b)	Carcinogenicity - mouse	Yes	Yes
83-3(a)	Teratology (rodent)	Yes	Yes
83-3(b)	Teratology (non-rodent)	Yes	Yes
83-4	2-Generation reproduction (rodent)	Yes	Yes
83-6	Developmental neurotoxicity - rat	Yes a	No
84-2	Gene mutation (Ames)	Yes	Yes
84-2	Gene mutation (mammalian)	Yes	Yes
84-2	Structural chromosomal aberration	Yes	Yes
84-2	Other genotoxic effects	No	Yes
85-1	General metabolism	Yes	Yes

a Confirmatory study

Formulation: PIRATE® Insecticide-Miticide/AC 303,630 3SC

Guideline #	Study Type	Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Yes
81-3	Acute Inhalation Toxicity	Yes	Yes
81-4	Primary Eye Irritation	Yes	Yes
81-5	Primary Dermal Irritation	Yes	Yes
81-6	Primary Dermal Sensitization	Yes	Yes

Formulation: ALERT™ Insecticide-Miticide/Ac 303,630 2SC

Guideline #	Study type	Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Yes
81-3	Acute Inhalation Toxicity	Yes	Yes a
81-4	Primary Eye Irritation	Yes	Yes
81-5	Primary Dermal Irritation	Yes	Yes
81-6	Dermal Sensitization	Yes	Yes b

a Satisfied by 3SC Formulation
b Satisfied by Chlorfenapyr technical and 3SC Formulation

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V. TOXICOLOGY PROFILE Updated: 10/10/96

Chlorfenapyr tech., Metabolites and Formulations

Guideline#, Study Identification and Classification	Results
TECHNICAL	
81-1 Acute Oral Toxicity - Rats MRID#: 42770207/42884201 Study #:T-0417 7/20/1992 HED Doc.#: 010651 **Chlorfenapyr - Technical** **Acceptable**	**LD_{50} (95% C.I.) = 441 (195 - 832) mg/kg, males** **LD_{50} (95% C.I.) = 1152 mg/kg, females** **LD_{50} (95% C.I.) = 626 (274 - 1085) mg/kg, combined** TOXICITY CATEGORY: **II**, based on most sensitive sex
81-1 Acute Oral Toxicity - Mouse MRID#: 43492828 American Cyanamid, USA Report#: A93-20.02; 12/07/94 **Chlorfenapyr/AC 303,630** **Acceptable**	In an acute oral toxicity study, groups of 5 albino mice/sex (Crl:CD-1(ICR)BR strain) were given single oral doses of AC 303,630 Technical (purity 94.5%, Lot No. AC 7504-59A) at 35, 70, or 140 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 2 hours postdosing, then daily (≥24 hours) for the remainder of the study. **Oral LD_{50} Males = 45 (37-56) mg/kg (95% C.I.)** **Females = 78 (41-152) mg/kg (95% C.I.)** **Combined = 55 (37-80) mg/kg (95% C.I.)** AC 303,630 Technical is classified as **TOXICITY CATEGORY I** based on the LD_{50} in males. Mortality occurred at 8-24 hours postdosing in 17/18 decedents. Clinical observations were limited to decreased activity during the first 2 hours postdosing of the mice treated at 140 mg/kg. No significant treatment-related effect on body weight was observed in surviving animals. Gross necropsy of decedent mice revealed a single occurrence of a bright red-colored lung. Gross necropsy revealed no visible lesions in animals sacrificed after 14 days. This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino mouse.
81-2 Acute Dermal Toxicity - Rabbits MRID#: 42770208 Study #:T-0406 7/20/1992 HED Doc.#: 010651 **Chlorfenapyr Tech.** **Acceptable**	**LD_{50} > 2000 mg/kg (Limit Dose)** TOXICITY CATEGORY: **III**
81-3 Acute Inhalation Toxicity - Rats MRID#: 42770209 Study (American Cyanamid)#:91-8351 3/25/1993 HED Doc.#: 010651 **Chlorfenapyr Tech.** **Acceptable**	**Doses 0, 0.34, 0.71, 1.8 or 2.7 mg/l in SD rats.** **LC_{50} (95% C.I.) = 0.83 (0.48 - 1.4) mg/l, (males)** **LC_{50} (95% C.I.) = > 2.7 mg/l, females]** **LC_{50} (95% C.I.) = 1.9 (1.1 - 3.3) mg/l, combined** TOXICITY CATEGORY: **III**, based on most sensitive sex

Guideline#, Study Identification and Classification	Results
81-4 Primary Eye Irritation - Rabbits MRID#: 42770210 Study #:T-0404 7/20/1992 HED Doc.#: 010651 **Chlorfenapyr Tech. Acceptable**	Corneal opacity (4/6), iritis (2/6) and conjunctivitis (6/6) present at 48 hours. At 72 hours iritis was resolved. All rabbits were normal by Day-7. TOXICITY CATEGORY: III
81-5 Primary Dermal Irritation - Rabbits MRID#: 42770211 Study #:T-0405 7/20/1992 HED Doc.#: 010651 **Chlorfenapyr Tech. Acceptable**	Non-irritating. TOXICITY CATEGORY: IV
81-6 Dermal Sensitization - Guinea Pigs MRID#: 42770212 Study #:T-0439 3/26/1993 HED Doc.#: 010651 **Chlorfenapyr Tech. Acceptable**	**Not a skin sensitizer** (Closed-Patch Repeated Insult)
81-8 Acute Neurotoxicity - rat MRID#: 43492829 Pharmaco LSR Inc., NJ. Study# 93-4510; 08/15/94 **Acceptable**	In an acute neurotoxicity study, AC 303,630, (94.5% ai, Lot No. AC 7504-59-A) was dissolved in 0.5% carboxymethylcellulose and administered once, via gastric intubation in a dosing volume of 10 ml/kg/dose, to 60 Sprague-Dawley CD rats (10/sex/group) at dose levels of 0, 45, 90, and 180 mg/kg. All rats were observed for 2 weeks following dosing. The rats were evaluated for reactions in functional observational and motor activity measurements pretest and on study days 1, 8, and 15. In addition, five rats per group were examined for neuropathologic lesions. Two males and two females in the 180 mg/kg dose group died within 7 hours of dosing, possibly as a result of accidental injury during treatment. Surviving rats in this dose group exhibited changes in gait, locomotion, and arousal, and 20-30% of the males and females were lethargic on the day of treatment. In the 90 mg/kg dose group, 20% of the males were lethargic on the day of treatment. No dose-related effects on body weights, food consumption, neurobehavioral observations, or gross or histological post mortem examinations were noted. **The LOEL is 90 mg/kg, based on lethargy of the rats on the day of treatment. The NOEL is 45 mg/kg.** This acute neurotoxicity study is classified as **Acceptable** and satisfies the guideline requirement for an acute neurotoxicity screening study in rodent (81-8SS).

Guideline#, Study Identification and Classification	Results
82-1(a) Subchronic Feeding (90-Day) - rat MRID#: 42770219 American Cyanamid, USA Study# T-0316; 4/93 HED Doc.#: 010949 **Guideline**	In a sub-chronic oral toxicity study, technical AC 303,630 (Lot. # AC7171-141A; 93.6% a.i.) was administered in feed to 20/sex/dose Crl:CD® (SD) rats at dose levels of 0, 150, 300, 600, 900 or 1200 ppm (measured intake of 0, 11.7, 24.1, 48.4, 72.5 or 97.5 mg/kg/day, respectively) for 90 days. At 600 ppm, males had an decreased body weight gain (14%) and increased relative liver weights (19%), while females exhibited decreased hemoglobin (14.9%) and increased absolute/relative liver weights (16.8%/21.6%). At 900 ppm, body weight gain (25%/21%) and feed consumption in males/females, RBC numbers, %HCT and %HGB in females were decreased. At the same dose level, platelets, ALK in males, absolute/relative liver weights (18.3%/33.1%) in females, relative liver weights (15%) in males and absolute/relative spleen weights in males and females increased. At 1200 ppm, male rats exhibited decreased activity, ataxia, anorexia, chromodacryorrhea and dark brown material around nose. Additionally, in males/females, body weight gains (37%/24%), feed consumption, RBC numbers, %HCT and %HGB decreased and platelet counts, BUN in males, ALK levels in males/females, absolute/relative liver (25.9%/44.8%) and splenic weights in females and absolute/relative splenic weights and relative liver (47%) weights in males were increased. **The LEL of 600 ppm (48.4 mg/kg/day) is based on decreased body weight gain and increased relative liver weight in males and decreased HGB and increased absolute/relative liver weights in females. The NOEL is 300 ppm (24.1 mg/kg/day).** This study is **core-guideline** and **satisfies** guideline requirement for a 82-1(a) study in the rat.
82-1(a) Subchronic Oral - mice MRID#: 43492830 American Cyanamid, USA Study #s T-0219 and T-0302; 03/04/94 **Acceptable**	In a subchronic toxicity study, AC 303,630 (Pirate; 93.6% a.i.; Lot No. AC 7171-141A) was administered to 20 albino mice/sex/dose at dietary dose levels of 40, 80, 160, or 320 ppm (average 7.1, 14.8, 27.6, or 62.6 mg/kg/day, respectively, for males; 9.2, 19.3, 40.0, or 78.0 mg/kg/day, respectively, for females) for 91 days. Male mice fed AC 303,630 at 80 ppm, and male and female mice fed AC 303,630 at 160 or 320 ppm exhibited a toxic response to the test compound. Two mice died prior to the termination of the study; one male and one female dosed at the 320 ppm level died after only 2 days of feeding. In male mice, hepatic cell hypertrophy was observed in 30% of the animals in the 80 ppm treatment group, 65% in the 160 ppm treatment group, and 95% in the 320 ppm treatment group. Male mice in the 160 or 320 ppm treatment groups had increased relative liver and spleen weights. Male mice in the 320 ppm treatment group had a 26% lower body weight gain, and increased hematocrit values and RBC counts compared to the controls. In female mice, hepatic cell hypertrophy occurred in 20% of the animals in the 160 ppm treatment group and 50% in the 320 ppm treatment group. Female mice in the 320 ppm treatment group had a 29% lower body weight gain, increased WBC counts, and increased relative liver weights compared to the controls. Spongiform encephalopathy was noted in the brain and myelin of the spinal cord of 90-95% of both males and females receiving the 320 ppm treatment level. No other significant treatment-related changes in ophthalmology, hematology, blood chemistry, or organ weights and morphology were observed during the study; urinalysis was not conducted. **The LOEL is 80 ppm (14.8 mg/kg/day) for male mice and 160 ppm (40.0 mg/kg/day) for female mice, based on hepatic cell hypertrophy in ≥20% of the test animals at this treatment level. The NOEL is 40 ppm (7.1 mg/kg/day).** This subchronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a subchronic oral study (§82-1a) in rodents.
82-1(b) Subchronic Feeding in Dogs (90-Day) MRID#: 42770220 Study (American Cyanamid)#:971-92-118 4/8/1993 HED Doc.#: 010651 **Minimum**	Doses is beagles: 0, 60, 120 or 247 ppm (0, 2.16, 4.23 or 6.1 mg/kg/day) in feed. The 247 ppm was based on concentration of AC 303,630 in the diet of 300 ppm from Day 1 - 14, 240 ppm from Day 15 - 25 and 200 ppm from Day 25 - 93 (5.2, 5.9 and 7.2 mg/kg/day, respectively). **NOEL = 120 ppm (4.23 mg/kg/day)** **LOEL = 247 ppm (6.1 mg/kg/day)**, based on reduced body weight gain and feed efficiency and emaciation.

Guideline#, Study Identification and Classification	Results
82-2 28-Day Dermal - rabbit MRID#: 43492831 Bio/dynamics Inc., NJ. Lab. Project ID#: 92-2162; 10/13/93 **Acceptable**	In a repeated dose dermal toxicity study, AC 303,630 (Pirate; 94.5% a.i., Lot No. AC 7504-59A) was applied to the shaved skin of six New Zealand White rabbits/sex/dose at dose levels of 0, 100, 400, or 1000 mg/kg, 6 hours/day, 5 days/week for 4 weeks. Rabbits of both sexes in the 400 and 1000 mg/kg treatment groups exhibited statistically significant and concentration-related increases in serum cholesterol (60-95%) and relative liver weights (22-43%), and suffered from cytoplasmic vacuolation of the liver. The vacuolation of the liver was minimal to slight for male and female rabbits in the 400 mg/kg treatment groups (4 of 12 animals), and minimal to moderately severe for the 1000 mg/kg treatment groups (8 of 11 animals). In addition, female rabbits in the 1000 mg/kg treatment group exhibited a 97% increase in serum alanine aminotransferase ($p < 0.05$) concentrations. No differences were observed between rabbits in the 100 mg/kg treatment groups and the control groups. **The LOEL is 400 mg/kg for both sexes, based on changes in liver chemistry and morphology. The NOEL is 100 mg/kg.** This subchronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a repeated dose dermal toxicity study (§82-2) in rabbits.
82-7 One-year dietary neurotoxicity - rat MRID#: 43492833 Argus Res. Labs., PA. Study #: 101-019; 05/10/94 **Acceptable**	In a one-year dietary neurotoxicity study, AC 303,630 (Pirate; 94.5% ai, Lot No. AC 7504-59-A) was administered in the diet at 0, 60, 300, or 600 ppm (52-week average 0, 2.6, 13.6, or 28.2 mg/kg/day, respectively, for males; 0, 3.4, 18.0, or 37.4 mg/kg/day, respectively, for females) to Sprague-Dawley CD BR VAF/Plus rats (25/sex/group) for 52 weeks, followed by a 16-week recovery period during which the remaining rats were fed the control diet. The rats were evaluated for reactions in functional observational battery followed by motor activity measurements 1 week before the test diets were provided; 4, 8, 13, 26, 39, and 52 weeks after the first day of exposure; and 13 weeks after the cessation of treatment. A portion of the rats in each treatment group were sacrificed for neuropathological examination following 13 or 52 of exposure, or 16 weeks of recovery. In the 600 ppm dose group, both sexes exhibited statistically significant decreases in average body weights, body weight gains, absolute and relative feed consumption, feed efficiency, and water consumption (males only). Neurohistological examination of males sacrificed after 13 weeks of exposure revealed myelin sheath swelling in the spinal nerve roots (5/5), compared to 2/5 in the controls. At 52 weeks, a more generalized myelinopathic process consisting of vacuolar myelinopathy (6/10), vacuolation (6/10), and/or mild myelin sheath swelling (9/10), was found. This process was not associated with myelin or axon degeneration and was not evident in rats sacrificed after 16 weeks of recovery. In the 300 ppm dose group, both sexes exhibited decreases in average body weights, body weight gains, feed efficiency, absolute feed consumption (females only) and water consumption (males only) at various times during the exposure period and body weight gains were reduced (non-significantly) for males during recovery. The myelinopathic observations described in the 600 ppm group males was also found in the 300 ppm group of rats after 13 and 52 weeks exposure but were less severe and at a lower incidence. In the 60 ppm dose group rats, minimum myelin sheath swelling was seen in the Gasserian ganglia of one male at 52 weeks and spinal nerve roots of 3/5 males (compared to 2/5 controls) after 13 weeks of exposure. The toxicologic importance of these findings is equivocal since swelling in the spinal nerve roots was absent in the 60 ppm group after 52 weeks. Neuropathological changes were confined to males; females were not affected. **The LOEL is 300 ppm (13.6 mg/kg/day) based on the presence of myelinopathic alterations in the 300 ppm group male rats, decreased average body weights, body weight gains, feed efficiency, absolute feed consumption (females) and water consumption (males). The NOEL is 60 ppm (2.6 mg/kg/day).** This one-year dietary neurotoxicity study is classified **Acceptable** and satisfies the guideline requirement for a neurotoxicity study (82-7SS) in rats. Although, the sponsor put 83-1a on the cover of the study, the study only satisfies the 82-7SS requirement and was not meant to be a chronic rat study.

Guideline#, Study Identification and Classification	Results
83-1(b) Chronic Toxicity - dog MRID#: 43492834 Pharmaco LSR Inc., NJ. Lab. Project ID#: 92-3107; 08/31/94 **Acceptable**	In a chronic toxicity study, AC 303,630 (Pirate; 94.5% ai; Lot No. AC 7504-59A) was administered to beagle dogs (5-6 dogs/sex/dose) in the diet at dose levels of 60, 120, or 240 ppm (2.1, 4.0, or 8.7 mg/kg/day, respectively, for males; 2.3, 4.5, or 10.1 mg/kg/day, respectively, for females) for 52 weeks. Body weights and body weight gains were depressed in both sexes treated at 240 ppm, with more pronounced differences observed in the females. Body weights and body weight gains of both sexes treated at 60 or 120 ppm were comparable to those of the controls. No treatment-related effects were observed on the survival, clinical signs, ophthalmology, hematology, clinical chemistry or urinalysis parameters, organ weights or gross and microscopic pathology at any dose level. **The LOEL is 8.7 mg/kg/day (240 ppm), based on decreased body weights and body weight gains. The NOEL is 4.0 mg/kg/day (120 ppm).** This chronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a chronic oral study (§83-1b) in dogs.
83-3(a) Developmental Toxicity - rat MRID#: 42770221/428884202 Argus Res. Labs., Study# 971-90--177; 7/93 HED Doc.#: 010949 **Guideline**	In a developmental toxicity (teratology) study, 25 timed-pregnant rats per dose group of Crl:CD®BR VAF/Plus® (SD), received either 0, 25, 75 or 225 mg/kg/day by oral gavage from gestation day 6 through 16, inclusive. The test compound (Lot # AC 7504-59A, Purity 94.5%) in 0.5% carboxymethylcellulose was administered in 10 mL/kg body weight. Maternal toxicity was noted in the form of a dose-related decrease in body weight gain in the mid (21.2%; 6-12 days) and high (23.4%; 6 - 16 days) dose groups, a dose-related decrease in relative feed consumption in the mid (6.3%) and high (12.2%) dose groups and a decrease in water intake in the high (12.9%) dose group; the body weight gain, relative feed intake and water consumption rebounded to control levels in both groups during the post-dosing (16 - 20 days) period. Therefore, the **Maternal Toxicity LEL = 75 mg/kg/day, and the Maternal Toxicity NOEL = 25 mg/kg/day, based on reduced body weight gain, reduced relative feed intake and reduced water consumption.** Developmental toxicity was not observed either in the form of maternal cesarean section observations or fetal external, visceral or skeletal malformations and variations. Therefore, the **Developmental Toxicity LEL is greater than 225 mg/kg/day and the NOEL is greater than or equal to 225 mg/kg/day.** **The study is classified as Core - Guideline Data and satisfies the requirement (§ 83-3 a) for a developmental toxicity (teratology) study in rats.**
83-3(b) Developmental Toxicity - rabbit MRID#: 42770222 Study (American Cyanamid)#:971-90-179 3/2/1993 HED Doc.#: 010651 **Minimum**	Doses of 0, 5, 15 or 30 mg/kg/day administered by gavage in 0.5% carboxymethylcellulose to pregnant New Zealand White rabbits from Days 7 to 19 of gestation, inclusive. **Maternal NOEL: 5 mg/kg/day** and **LOEL: 15 mg/kg/day,** based upon reduced body weight gain during treatment. **Developmental NOEL: > 30 mg/kg/day.**

Guideline#, Study Identification and Classification	Results
83-4 2-Generation Reproduction - rat MRID#s: 434292836 (main), 434292835 (Range-finding) Pharmaco LSR Inc., NJ. Study# 90-3638; 08/08/94 **Acceptable**	In a 2-generation reproduction study, AC 303,630, (94.5% ai; Lot No. AC 7504-59A) was administered continuously in the diet to Sprague Dawley CD rats (30/sex/dose) at concentrations of 0, 60, 300, or 600 ppm (0, 5, 22, or 44 mg/kg/day, respectively, based on body weight and food consumption during pre-mating periods) for two successive generations (1 litter/generation). P_1 and F_1 males were mated after approximately 16 and 23 weeks of treatment, respectively. P_1 females were fed the test diets for approximately 19 weeks; mating was initiated at 10 weeks. F_1 pups were weaned on the same test diet fed their parents. F_1 females were fed the test diets for approximately 23 weeks; mating was initiated at 11 weeks. In the 600 ppm male treatment group, the pre-mating weight gains of P_1 and F_1 animals were 11% and 12% lower, respectively, than for control animals ($p < 0.05$). In the 600 ppm female treatment group, the pre-mating weight gains of P_1 and F_1 females were 9% and 15% lower, respectively, than control animals (significant only in the F_1 generation). Mean weights of F_1 and F_2 pups in the 600 ppm treatment group at weaning were 12% and 14% lower, respectively, than for control animals. Pup deaths during lactation days 0-4 were significantly higher in the F_2 litters from the 600 ppm treatment group. In the 300 ppm treatment group, mean body weight and body weight gains in P_1 males during the pre-mating period were 7 and 11% lower, respectively, than control animals. The mean body weight gains of F_1 males, and of P_1 and F_1 females were similar to the controls. The mean lactational weight gain of F_1 and F_2 pups in the 300 and 600 ppm treatment groups were significantly lower than the controls, although the mean weights of pups at birth were comparable to controls. At weaning, the mean weights of F_1 and F_2 pups in the 300 and 600 ppm groups were significantly lower (6 and 13%, respectively) than controls; this is considered a reproductive effect. No changes in reproductive performance were seen in either males or females of the parental generations. At 60 ppm, there were no adverse effects on the parental generations, there were no neonatal effects of toxicological importance, and there were no effects on reproductive performance. **The LOEL for systemic toxicity was 22 mg/kg/day (300 ppm), based on pre-mating effects on parental weight gain; the NOEL was 5 mg/kg/day (60 ppm). The LOEL for reproductive toxicity was 22 mg/kg/day (300 ppm), based on decreased lactational weight gains; the NOEL was 5 mg/kg/day (60 ppm). No effects on reproductive performance were seen at any dose; reproductive indices, gestational indices, and parturition data were not affected.** The study is **acceptable** and fulfills the guideline requirements (OPPTS 870.3800, §83-4) in rats for a 2-generation reproductive study.
83-5 Chronic/Oncogenicity - rat MRID#: 434292837 (main), 434292836 (range-finding) Hazleton Washington, Inc., Lab. Project ID#: HWA 362-206; 08/23/94 **Chronic toxicity- Acceptable** **Carcinogenicity- Acceptable**	In a chronic/oncogenicity toxicity study, Pirate (94.5% ai, Lot No. AC 7504-59A) was administered to 65 Crl:CD BR rats/sex/dose in the diet at dose levels of 0, 60, 300, or 600 ppm (0, 2.9, 15.0, or 30.8 mg/kg/day, respectively in males; 0, 3.6, 18.6, or 37.0 mg/kg/day, respectively in females) for 104 weeks. Chronic toxicity observed in males and females at 300 and 600 ppm included slight to moderate non-neoplastic centrilobular to midzonal or diffuse hepatocellular enlargement (3/65 control, 1/65 low-, 17/65 mid and 47/65 high-dose in males) and (6/65 control, 1/65 low-, 18/65 mid-, and 54/65 high-dose in females). At the 300 and 600 ppm levels in both sexes, there were significant increases in mean liver-to-body weight ratios at 12 months (14-30%) and in 600 ppm rats at 24 months. **The LOEL for systemic toxicity is 300 ppm (15.0 and 18.6 mg/kg/day for males and females, respectively) based on liver toxicity, and the NOEL is 60 ppm (2.9 and 3.6 mg/kg/day for males and females, respectively) based on liver toxicity.** There was an increased incidence of malignant histiocytic sarcoma in male rats in the 600 ppm group (4/65, 6.2%) compared to controls (0/65, 0%). Rats in this study probably could have tolerated higher dosing due to the low mortality at 600 ppm; however, there were non-neoplastic lesions in the liver and significantly decreased body weight gains in treated groups. This study is classified as **acceptable** and satisfies the guideline requirements for a carcinogenicity study (83-2) and for a chronic toxicity study (83-1) in rats.

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Guideline#, Study Identification and Classification	Results
83-5 Chronic/Oncogenicity - mice MRID#: 43492838 (main), 43492830 (range-finding) Bio Res. Labs., Quebec, Canada Lab. Project ID#: 84580; 08/22/94 **Chronic toxicity- Acceptable** **Carcinogenicity- Acceptable**	In a chronic toxicity/oncogenicity study, Pirate (94.5% a.i., Lot No. AC-7504-59A) was administered to 65 male and 65 female Swiss Crl:CD-1(ICR)BR mice/sex/dose in the diet at dose levels of 0, 20, 120, or 240 ppm (0, 2.8, 16.6, or 34.5 mg/kg/day, respectively, in males; 0, 3.7, 21.9, or 44.5 mg/kg/day, respectively, in females) for 80 weeks. Chronic toxicity observed in males and females at 120 and 240 ppm included decreased body weight gains, non-neoplastic brain vacuolation primarily in the white matter of the corpus callosum, tapetum, hippocampus, and cerebellum. Body weight gains decreased in males and females of 23 and 21%, respectively, at 240 ppm and 11 and 12%, respectively, at 120 ppm, by the end of study. The incidence of brain vacuolation in males was 4/65 control, 14/64 mid-, and 49/65 high-dose, and in females it was 10/65 control, 28/65 mid-, and 58/65 high-dose. Males and females at 240 ppm also exhibited vacuolation of the spinal cord and optic nerve. Treatment-related gross pathological changes, including skin ulceration and scabbing, occurred in males and females at the 240 ppm level, and scabbing occurred in males at 120 ppm. **The LOEL for systemic toxicity is 120 ppm (16.6 and 21.9 mg/kg/day in males and females, respectively) based on decreased body weight gains, brain toxicity and scabbing of the skin (males), and the NOEL is 20 ppm (2.8 and 3.7 mg/kg/day for males and females, respectively).** At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls. Survival in females was depressed by 40% in the 240 ppm treatment group. Dosing was considered adequate based on decreased body weight gain in males and females. This chronic/oncogenicity study in mice is **acceptable** for oncogenicity and satisfies the guideline requirement for a carcinogenicity study (83-2) in mice. The study is **acceptable** for chronic toxicity (83-1) although clinical chemistry and urinalysis data are missing.
84-2 Gene Mutation- Ames MRID#: 42770223 American Cyanamid # 91-02-001; 03/24/93 HED Doc.#: 010651 **Acceptable**	**Negative** for reverse mutation in S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 and E. coli strain WP2 uvrA- exposed up to cytotoxicity (50 µg/plate, +/- S9)
84-2 Gene Mutation - in mammalian cells (CHO/HGPRT) MRID#: 42770224 American Cyanamid # 91-05-001; 03/25/93 HED Doc.#: 010651 **Acceptable**	In two independently conducted trails, Pirate™ was exposed to chinese ovary cells at nonactivated doses of 2.5 - 250 µg/mL or S9-activated doses of 5 - 500 µg/mL. S9 fraction was derived from Aroclor 1254-induced rat livers. Compound was delivered in DMSO. Not mutagenic up to 500 µg/mL and above with and without S9 activation in preliminary range-finding study. Test article precipitated in the test system at 250 - 500 µg/mL with S9 and 100 - 250 µg/mL without S9 activation. Relative survival (RS) at the highest dose yielding was 36.7% or 40.1% at 250 µg/mL in the nonactivated trials or 23.9% or 38.5% at 250 µg/mL in the S9-activated trials. The positive controls were adequate. The study is upgraded from **Unacceptable** to **Acceptable.** The study satisfies the guideline requirement for a gene mutation study (84-2).
84-2 Structural chromosome aberration - in vivo mouse MRID # 42770225 American Cyanamid #: 91-18-001; 03/17/93 HED Doc#s: 010651 & 010986 NEW Doc#:------ **Acceptable**	**Negative** for micronucleus induction in bone marrow cells of male and female CD-1 mice 24, 48, and 72 hours after the single oral gavage administration of 7.5, 15, or 30 mg/kg (males) or 5, 10, or 20 mg/kg (females) CL 303,630. The study is **Acceptable** and satisfies the requirements for Structural Chromosomal Aberration Assay (84-2).

Guideline#, Study Identification and Classification	Results
84-2 Structural chromosome aberration - in vitro CHO cells MRID#: 43492843 American Cyanamid, USA Study#: 92-11-001; 06/06/94 **Acceptable**	In a mammalian cell chromosome aberration assay (MRID 43492843), Chinese Hamster ovary (CHO) cell cultures were exposed to AC 303,630 (Pirate; 94.5% ai) in dimethylsulfoxide at concentrations of 6.25, 12.5, 25, or 50 μg/mL with metabolic activation (S-9 mix), or 12.5, 25, 50, or 100 μg/mL without metabolic activation. The high dose was selected so that AC 303,630 was tested to cytotoxic concentrations but sufficient cells remained for evaluation, and the low and intermediate doses were the concentrations corresponding to 12.5, 25, and 50% of the high dose. Cell cultures with metabolic activation were harvested 6, 18, or 42 hours following the termination of exposure (12, 24, or 48 hours following the start of exposure). Cell cultures without activation were harvested approximately 2 hours following the termination of exposure (12, 24, or 48 hours following the start of exposure). Because of cytotoxicity at 50 μg/mL with activation, the activated cultures exposed to AC 303,630 at 6.25, 12.5, and 25 μg/mL were evaluated for aberrant and polyploid cells. The nonactivated cultures exposed at 25, 50, and 100 μg/mL were evaluated. AC 303,630 had no significant effect on the occurrence of aberrant chromosomes at any harvest time in cultures with or without metabolic activation. Analysis of data for polyploidy showed a statistically significant effect at 6.25 μg/mL with activation at the 24-hour harvest only. This effect was not dose-related, since polyploidy values for the 12.5 and 25 μg/mL treatments were similar to the vehicle control, and the data in general exhibited a statistically nonsignificant and negative trend. When statistical analysis of the polyploids was done excluding endoreduplication, no statistical significance was found. **AC 303,630 caused no statistically significant increases in the proportion of aberrant or polyploid chromosomes in Chinese Hamster ovary cells compared to solvent control values.** Positive controls induced the appropriate response. This study is classified as **acceptable** and satisfies the guideline requirement for *in vitro* cytogenetic mutagenicity studies (84-2).
84-2 Structural chromosome aberration - in vitro CHL cells MRID#: 43492839 Huntingdon Res. Ctr., UK Lab. Project #: MCI 206/941465; 05/23/94 **Acceptable**	In a mammalian cell chromosome aberration assay, Chinese Hamster Lung (CHL) cell cultures were exposed to MK-242 technical (Pirate; 93.8% ai) in dimethylsulfoxide at concentrations of 0.9, 1.8, 3.5, 7.0, 14.1, 28.1, 56.3, 112.5, 225, 450, 900, or 1800 μg/mL for 6 hours with metabolic activation (rat S-9 mix), or for 6, 24, or 48 hours without metabolic activation. At final concentrations of 112.5-225 μg/mL and above, a precipitate formed in the tissue culture medium. Cells were harvested at 24 or 48 hours after the initiation of treatment, and the proportion of mitotic cells per 1000 cells was determined. In general, metaphase analysis was conducted on cells from three dose levels for each activation/exposure time combination; the high dose was the concentration that resulted in a >50% depression in the mitotic index compared to the solvent control, and the low and intermediate doses were the concentrations corresponding to 25 and 50% of the high dose. **MK-242 technical caused no statistically significant increases in the proportion of aberrant or polyploid chromosomes in Chinese Hamster lung cells compared to solvent control values.** Positive controls induced the appropriate response. This study is classified as **acceptable** and satisfies the guideline requirement for *in vitro* cytogenetic mutagenicity studies (84-2).
84-2 Repair in vitro (UDS) MRID #: 42770226 Microbiological#: T9775.380025 02/23/93 HED Doc.#: 010651 **Acceptable**	**Negative** for inducing unscheduled DNA synthesis in primary rat hepatocyte cultures exposed up to severely toxic concentrations (≥ 30 μg/ml).

Guideline#, Study Identification and Classification	Results
85-1 Metabolism - rat MRID#: 43492844 American Cyanamid, USA Study# MET 94-021; 10/28/94 **Acceptable**	In a metabolism study, [2-pyrrole-^{14}C] or [phenyl-^{14}C] pirate was administered to 5 HSD:Sprague-Dawley/SD rats/sex/dose by oral gavage at dose levels of 20 mg/kg/day as a single dose or following a 14-day pre-treatment with non-radioactive pirate, or at 200 mg/kg as a single dose. Low recoveries of the radioactive dose in urine and tissues indicate limited absorption of pirate by rats. The radioactivity in urine from the high dosed rats was about half that from the single and multiple-low dosed rats. More than 80% of the doses were eliminated in the feces. Most of the radioactivity was eliminated in the feces and urine within 48 hours of dosing. After 7 days, 89-121% of the dosed radioactivity was recovered. At sacrifice, female rats had greater (about twice) recovery of radioactivity in the carcass, blood, and fat at all doses than did males. The highest recovery of radioactivity from a single organ was from the liver (0.15-0.48% of dose). Metabolite extraction and identification accounted for 72-91% of the radioactive doses. The parent was the major radioactive compound found in excreta, accounting for approximately 40-70% of the administered doses. Minor amounts of eight primary and conjugated metabolites and four unidentified isolated components were detected, each at less than 10% of the dosed radioactivity. Liver and kidney contained several primary and conjugated metabolites and only minor levels of the parent compound ($\leq$8.3% of the radioactivity in the sample). Based on the metabolites identified, the major deposition route of orally administered pirate is fecal excretion of unaltered parent compound. Other pathways include cleavage of the ethoxymethyl side-chain, followed by de-alkylation and ring hydroxylation, and some degree of conjugation of the de-alkylated, ring-hydroxylated metabolite. The two rings of the molecule are not cleaved. Metabolites are excreted primarily in urine; accumulation in tissues is minimal. This metabolism study in the rat is classified **acceptable** and satisfies the guideline requirement for a metabolism study (85-1) in the rat.

Guideline#, Study Identification and Classification	Results
CHLORFENAPYR METABOLITES	
81-1 Acute Oral Toxicity - Rats MRID#: 43492824 American Cyanamid, USA Report#: A94-38.01; 06/14/94 **Metabolite - AC 303, 268** **Acceptable**	In an acute oral toxicity study, groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 303,268 Technical (purity 100.3%, Lot No. 8979-44B) at 7.8, 15.6, 23.4, 31.25, 62.5, 125, or 250 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing. **Oral LD_{50} Males = 27.0 mg/kg** **Females = 29.4 mg/kg** **Combined = 28.7 (16.8-30.7) mg/kg (95% C.I.)** AC 303,268 Technical is classified as **TOXICITY CATEGORY I** based on the LD_{50} for both sexes. Mortality (1-8 hours postdosing) occurred in 39/40 animals tested at 31.25 mg/kg and above. Clinical observations included prostrate with or without hind legs extended, increased activity when aroused, writhing, and protruding testes. In general, surviving animals showed no clinical signs and no treatment-related effect on body weight throughout the 14-day observation period; one surviving animal dosed at 31.25 mg/kg exhibited prostration with hind legs extended, but returned to normal within 24 hours. Gross necropsy of decedent animals revealed pronounced striations of the muscle tissue, and tonus. Less prevalent abnormalities included dark and/or mottled liver, and salivation. Gross necropsy of animals sacrificed after 14 days revealed no visible lesions. This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.
81-1 Acute Oral Toxicity - Rats MRID#: 43492825 American Cyanamid, USA Report#: A94-70; 06/14/94 **Metabolite -AC 312,094** **Acceptable**	In an acute oral toxicity study, groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 312,094 Technical (purity 96.3%, Lot No. AC 8698-67A) at 5,000 mg/kg (limit dose). The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing. **Oral LD_{50} Males = >5,000 mg/kg** **Females = >5,000 mg/kg** **Combined = >5,000 mg/kg** AC 312,094 Technical is classified as **TOXICITY CATEGORY IV** based on the LD_{50} in both sexes. Mortality occurred in one male rat at 4 days postdosing and in one female at 6 days. Clinical observations were limited to decreased activity in male rats, which subsided by 24 hours postdosing. No treatment-related effect on body weight was observed in 14-day survivors, and gross necropsy of decedent and animals sacrificed after 14 days revealed no visible lesions. This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.

Guideline#, Study Identification and Classification	Results
81-1 Acute Oral Toxicity - Rats MRID#: 43492826 American Cyanamid, USA Report#: A94-215; 08/11/94 **Metabolite - AC 322,250** **Acceptable**	In an acute oral toxicity study, groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 322,250 (purity 89%, Lot No. AC 9014-97A) at 1,250 (females only), 2,500 (females only), or 5,000 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing. **Oral LD_{50} Males = >5,000 mg/kg** **Females = 2500 (1581-3954) mg/kg (95% C.I.)** AC 322,250 is classified as **TOXICITY CATEGORY III** based on the LD_{50} in females. In male rats, one death occurred at 3 days postdosing. In female rats, mortality occurred from 8 hours to 5 days postdosing in 7/10 animals tested at 2,500 and 5,000 mg/kg. At 1,250 and 2,500 mg/kg (females only), both decedent and 14-day survivors exhibited no clinical signs throughout the 14-day study period. At 5,000 mg/kg, the major clinical observation in both sexes was diarrhea, which generally subsided in males by 24 hours postdosing; one female rat also exhibited decreased activity, ptosis, and diuresis prior to death. No treatment-related effect on body weight was observed in 14-day survivors. Gross necropsy of decedent animals revealed a low frequency of dark spleen, distended stomach, and test material- and gas-filled stomach. Gross necropsy of animals sacrificed after 14 days revealed no visible lesions. This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.
81-1 Acute Oral Toxicity - Rats MRID#: 43492827 American Cyanamid, USA Report#: A94-252; 10/20/94 **Metabolite - AC 325,195** **Acceptable**	In an acute oral toxicity study, groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 325,195 (purity 89%, Lot No. AC 9014-93B) at 312.5 (males only), 625, 1,250, 2,500, or 5,000 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing. **Oral LD_{50} Males = 776 (448-1,329) mg/kg (95% C.I.)** **Females = 1367 (755-2,364) mg/kg (95% C.I.)** AC 325,195 is classified as **TOXICITY CATEGORY III** based on the LD_{50} in both sexes. Mortality occurred in 26/30 animals tested at 1,250 mg/kg and above; the majority of deaths occurred at $\leq$24 hours postdosing. Clinical observations in decedent animals included salivation, decreased activity, hyperthermia, chromodacryorrhea, dyspnea, ptosis, brown material around nose, red material in urine, dehydration and prostration. Observations in surviving animals, which subsided by 9 days postdosing, included ptosis, diuresis, and brown material around nose. No treatment-related effect on body weight was observed in surviving animals. Gross necropsy of decedent animals revealed white foci on the liver and spleen and discoloration of the spleen, which corresponded microscopically to hepatocellular necrosis and/or infarcted areas and diffuse hemorrhage, respectively. Other observations included vascularized stomach, distended stomach, test material- and gas-filled stomach, and gas-filled cecum (females only). Gross necropsy of animals sacrificed after 14 days revealed no gross pathological changes. This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.

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Guideline#, Study Identification and Classification	Results
84-2 Gene Mutation - Ames MRID#: 43492840 American Cyanamid, USA Study#: 9402001; 08/12/94 **Metabolite CL 303,268** **Acceptable**	In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella typhimurium or Escherichia coli WP2 uvrA- were exposed to CL 303,268 (100.3% a.i.) in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 303,268 at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, or 5.0 µg/plate (+/-S9). E. coli WP2 uvrA- was tested with CL 303,268 at concentrations of 10, 25, 50, 100, and 250 µg/plate (+/-S9). CL 303,268 (100.3% a.i.) was tested up to cytotoxic concentrations with the S. typhimurium strains and the limit of solubility, 250 µg/plate, with E. coli WP2 uvrA-. The positive controls induced the appropriate responses in the corresponding strains. CL 303,268 failed to induce a genotoxic response in any of the tester strains with the exception of a borderline positive result for the TA100 strain at the 1.0 µg/plate dose level. As the result was equivocal and a genotoxic response was not found in any of the other tester strains, **CL 303,630 was determined to not be mutagenic under the conditions of the submitted study.** This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.
84-2 Gene mutation - Ames MRID#: 43492841 American Cyanamid, USA Study#: 9402002; 08/12/94 **Metabolite CL 312,094** **Acceptable**	In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella typhimurium and Escherichia coli WP2 uvrA- were exposed to CL 312,094 (96.3% a.i.), in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 or E. coli WP2 uvrA- were evaluated with CL 312,094 at concentrations of 25, 50, 100, 250, 500, or 1000 µg/plate (+S9) and at 5, 10, 25, 50, 100, or 250 µg/plate (-S9). CL 312,094 (96.3% a.i.) was tested up to the limit of solubility. It was not cytotoxic at these concentrations to any of the S. typhimurium strains or the E. coli WP2 uvrA-. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.** This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.
84-2 Gene Mutation - Ames MRID#: 43492842 American Cyanamid, USA Study#: 9402003; 08/19/94 **Metabolite CL 322,250** **Acceptable**	In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella typhimurium or Escherichia coli WP2 uvrA- were exposed to CL 322,250 (89% a.i.), in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. S. typhimurium strains were tested with CL 322,250 at concentrations of 100, 250, 500, 1000, or 2500 µg/plate (+S9) and 50, 100, 250, 500, or 1000 µg/plate (-S9). E. coli WP2 uvrA- was tested with CL 322,250 at concentrations of 250, 500, 1000, 2500 or 5000 µg/plate (+/- S9). CL 322,250 (89% a.i.) was tested up to cytotoxic concentrations and the limit concentration, 5000 µg/plate. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.** This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.
PIRATE® Insecticide-Miticide/AC 303,630 3SC (32% a.i.)	
81-1 Acute Oral Toxicity in Rats MRID #:42770214 Study #:T-0515 1/18/93 HED Doc.#: 010651 **Acceptable.**	LD_{50} (95% C.I.) = 626 (274-1085) mg/kg, combined LD_{50} (95% C.I.) = 283 (101-502) mg/kg, males LD_{50} (95% C.I.) = 999 (431-1821) mg/kg, females Decreased activity, salivation, ataxia, hyperthermia, protruding testes, prostration and mortality were observed at all levels. Grossly, congested and mottled livers and pronounced striations of abdominal muscles were observed. Weight gains of the survivors were not effected. TOX. CATEGORY: II, based on most sensitive sex

Guideline#, Study Identification and Classification	Results
81-2 Acute Dermal Toxicity in Rabbits MRID 42770214 Study #:T-0515 1/18/93 HED Doc.#: 010651 **Acceptable**	LD_{50} (95% C.I.) = 1782 [1112 - 2856] mg/kg, males LD_{50} (95% C.I.) > 2000 mg/kg, females Nasal discharge (1/5), excessive lacrimation (1/5) and diarrhea (1/5) were observed at the 1000 and 4000 mg/kg. Two of five rabbits in the 4000 mg/kg and 3/5 rabbits in the 2000 mg/kg dose died within 48 hours of treatment. Necropsy of the surviving was unremarkable. **TOX. CATEGORY**: II, based on most sensitive sex
81-3 Acute Inhalation Toxicity in Rats MRID 42770215 Cyanamid #:971-92-109 3/8/93 HED Doc.#: 010651 **Acceptable**	Doses 0, 0.84, 1.9 or 2.6 mg/l in SD rats. LC_{50} (95% C.I.) = 1.3 (0.86 - 2.1) mg/l, males LC_{50} (95% C.I.) = 2.4 (1.6 - 3.5) mg/l, females LC_{50} (95% C.I.) = 2.1 (1.5 - 2.9) mg/l, combined sexes Clinical signs during exposure were labored breathing and excessive salivation at all doses; eye closure at the two high doses; and gasping and decreased activity at the highest dose. Among survivors, in addition to the aforementioned, rales, dried brown material on face and fur, matted coat, wet fur and yellow ano-genital staining were observed. At necropsy, red discoloration in lungs of some deceased animals was noticed. **TOX. CATEGORY**: III, based on most sensitive sex
81-4 Primary Eye Irritation in Rabbits MRID #: 42770216 Study #: T-0513 12/4/92 HED Doc.#: 010651 **Acceptable**	Slight-to-moderate conjunctivitis (6/6) was observed at one and 24 hours; had resolved by 48 hours. **TOX. CATEGORY**: III
81-5 Primary Dermal Irritation in Rabbits MRID 42770217 Study #T-0514 1/18/93 HED Doc.#: 010651 **Acceptable**	Slightly irritating to rabbit skin. A very slight (5/6)-to-moderate (1/6) erythema and slight (1/6) edema at 1 and slight (3/6) erythema at 24 hour post-dosing were observed. At 48 hour examination 1/6 exhibited slight erythema which resolved by 72 hours. **TOX. CATEGORY**: IV
81-6 Dermal Sensitization in Guinea Pig MRID 42770218 Study #:T-0530 3/5/93 HED Doc.#: 010651 **Acceptable**	Not a sensitizer

Guideline#, Study Identification and Classification	Results
82-2 28-Day Dermal - rabbit MRID#: 43492832 Bio/dynamics, Inc., NJ. Lab. Project ID#: 92-2163; 03/18/94 **Acceptable**	In a repeated dose dermal toxicity study, AC 303,630 (Pirate; 33.3% a.i., Lot No. AC 8053-87A) was applied to the shaved skin of six New Zealand White rabbits/sex/dose at dose levels of 0, 100, 400, or 1000 mg/kg 6 hours/day, 5 days/week for 4 weeks. No treatment-related effects were observed. No animals died during the study. There were no significant differences in body weights or body weight gains by study termination. No treatment-related effects were observed in hematology, blood chemistry factors, the eyes, or urinalysis; there were no changes in organ weight or morphology. **The LOEL is >1000 mg/kg for rabbits. The NOEL is 1000 mg/kg for rabbits.** This subchronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a repeated dose dermal toxicity study (82-2) in rabbits.
ALERT® Insecticide-Miticide/AC 303,630 2SC (21% a.i.)	
81-1 Acute Oral Toxicity in Rats MRID #:43268204 Study #:T-0588 6/9/94 HED Doc.#: 011245 **Acceptable**	LD_{50} (95% C.I.) = 560 (410-890) mg/kg, males LD_{50} (95% C.I.) = 567 (281-988) mg/kg, females Decreased activity, salivation, writhing and abnormal posture. Necropsy was unremarkable in surviving animals. In dead animals, grossly, dark and molted liver, pronounced striations of abdominal wall, tetany, salivation, pale intestinal tracts, dark lungs and diarrhea were observed. **TOX. CATEGORY: III**
81-2 Acute Dermal Toxicity in Rabbits MRID 43268205 Study #:T-0592 6/9/94 HED Doc.#: 011245 **Acceptable**	LD_{50} (95% C.I.) > 2000 mg/kg, males and females Nasal discharge and lacrimation were observed. There were no deaths. Grossly, red foci in kidneys, pale colored kidneys and pale lungs were observed only in males. **TOX. CATEGORY: III**
81-3 Acute Inhalation Toxicity in Rats HED Doc.#: 011245 **Waived**	Data requirements satisfied by AC 303,630 3SC Formulation (32% a.i.). **TOX. CATEGORY: III**
81-4 Primary Eye Irritation in Rabbits MRID #: 43268206 Study #: T-0593 3/12/94 HED Doc.#: 011245 **Acceptable**	Slight (5/6)-to-moderate (1/6) redness of conjunctivae, and slight ocular discharge were present at 1 hour. All signs of irritation had resolved by 24 hours. The mean conjunctival (redness + chemosis + discharge; range 2 - 20) score for this evaluation was 3.0. The overall eye irritation score was 1 (range 0 - 110) and was considered practically non-irritating. **TOX. CATEGORY: IV**
81-5 Primary Dermal Irritation in Rabbits MRID 43268207 Study #T-0594 5/12/94 HED Doc.#: 011245 **Acceptable**	Slight erythema (3/6) was observed at 1 hour and persisted in 1 rabbit at 24 hours. All signs of irritation had resolved by 48 hours. **TOX. CATEGORY: IV**

Guideline#, Study Identification and Classification	Results
81-6 Dermal Sensitization in Guinea Pig HED Doc.#: 011245 Waived	Data requirements satisfied by Chlorfenapyr tech. and AC 303,630 3SC Formulation (32% a.i.). Not a sensitizer

VI. DATA GAPS:

The toxicity data supports establishment of permanent tolerance and Section 3 registration of chlorfenapyr on/in cotton seed, meat and milk and for use in/on citrus; however, a confirmatory developmental neurotoxicity study (§83-6) elucidating the characteristics of the CNS and dermal lesions observed in chronic toxicity/carcinogenicity study (§83-5) in rats and mice and subchronic neurotoxicity study (§82-7SS) in rats is required (see Section X, Subsection B).

VII. ACTION BEING TAKEN TO OBTAIN ADDITIONAL INFORMATION OR CLARIFICATION:

The sponsor should be notified of the issues discussed under Section VI and Section X.

VIII. ENDPOINTS USED FOR RISK ASSESSMENT:

The Health Effects Division, Toxicology Endpoint Selection Committee considered the toxicity data available for this chemical at a meeting held on July 23, 1996. Based upon a review of the toxicology data base for this chemical listed above, toxicity endpoints and dose levels of concern have been identified for use in risk assessment corresponding to the categories below.

Dermal absorption: There are no dermal absorption studies. A dermal absorption value of 5% was calculated based on route-to-route extrapolation using the maternal NOEL of 5 mg/kg/day from the rabbit developmental toxicity study (MRID#: 42770222) and the systemic NOEL of 100 mg/kg/day in the 28-day dermal toxicity study in rabbits (MRID#: 43492831). This dermal absorption value will be used ONLY for the chronic exposure risk assessment since an oral study was selected for this scenario. A dermal absorption factor is not needed for the short- and intermediate- term exposure risk assessments since a 28-day dermal toxicity study was used for these scenarios.

Acute Dietary Endpoint (One Day): In a acute neurotoxicity study (MRID#: 43492829), lethargy was observed at 90 mg/kg/day. The dose and endpoint for risk assessment is a NOEL of 45 mg/kg/day, based on neurotoxicity. This risk assessment IS required.

Short Term Occupational or Residential Exposure: In a 28-day rabbit dermal toxicity

study (MRID#: 43492831), systemic toxicity was observed as increased cholesterol levels and liver weights, and increased incidence and severity of hepatocellular morphologic changes at 400 mg/kg/day. The dose and endpoint for risk assessment is a NOEL of 100 mg/kg/day based on increased cholesterol levels, liver weights and hepatocellular morphologic changes. This risk assessment IS required.

Intermediate-Term occupational or Residential Exposure: In a 28-day rabbit dermal toxicity study (MRID#: 43492831), systemic toxicity was observed as increased cholesterol levels and liver weights, and increased incidence and severity of hepatocellular morphologic changes at 400 mg/kg/day. The dose and endpoint for risk assessment is a NOEL of 100 mg/kg/day based on increased cholesterol levels, liver weights and hepatocellular morphologic changes. This risk assessment IS required.

Long-Term Occupational or Residential Exposure: In a chronic toxicity/carcinogenicity mouse study (MRID#: 43492838) brain vacuolation and skin scabbing were observed at 120 ppm (16.6 mg/kg/day in males and 21.9 mg/kg/day in females). The dose and endpoint for risk assessment is a NOEL of 20 ppm (2.8 and 3.7 mg/kg/day, for males and females respectively), based on brain and skin lesions. Since this oral study was used for dermal exposure scenario, the dermal absorption factor of 5% must be used in risk assessment. This risk assessment IS required.

Inhalation Exposure (Any Time Period): Based on the combined LC_{50} of 1.9 mg/L (MRID#: 42770209), chlorfenapyr is placed in tox. Cat. III. Therefore, risk via the inhalation route is not a concern at this time. Therefore, a separate risk assessment for the inhalation route is NOT required.

VIII. REFERENCE DOSE (RfD):

The HED RfD/Peer Review Committee on July 18, 1996, has established an RfD of 0.003 mg/kg/day, based on rat subchronic neurotoxic NOEL of 2.6 mg/kg/day and applying an Uncertainty Factor (UF) of 100 to account for interspecies and intraspecies variability and additional Modifying Factor (MF) of 10 for lack of understanding of the cause/relationship for the nervous system lesions and its toxicity to the young. The NOEL = 2.6 mg/kg/day and the LOEL = 13.6 mg/kg/day, based on spinal nerve myelinopathy (M), decreased mean body weights, body weight gains and feed efficiency in both sexes, absolute feed consumption (F) and water consumption (M). This NOEL of 2.6 mg/kg/day is supported by the rat chronic toxicity NOEL of 2.9 mg/kg/day and mouse chronic toxicity NOEL of 2.8 mg/kg/day. In the rat study slight to moderate non-neoplastic centrilobular to midzonal or diffuse hepatocellular enlargement were seen in both sexes at the LOEL of 15 mg/kg/day. In the mouse study decreased body weight gains and CNS lesions (M & F) and dermal lesions (M) were seen at the LOEL of 16.6 mg/kg/day. The chemical was reviewed by the Health Effects Division Cancer Peer Review Committee on 09/25/96. Preparation of the document is in progress.

IX. PENDING REGULATORY ACTIONS:

The Toxicology Branch is unaware of any pending regulatory actions against this pesticide.

X. TOXICOLOGY ISSUES:

A. In the rat chronic toxicity/carcinogenicity study there were increased trends in the incidence of hepatocellular adenomas, hepatocellular adenomas and/or carcinomas combined, malignant histiocytic sarcomas and testicular interstitial cell tumors in Crl:CD BR males rats. In female rats significant increasing trends in endometrial stromal polyps. Significant difference is pair-wise comparison of fibroadenomas at low dose and carcinomas at the mid-dose existed for females rats. There was no evidence of tumorigenic potential in CD-1 mice. To discuss these findings the HED Cancer Peer Review Committee (CPR) met on September 25, 1996. The preparation of the CPR document is in progress.

B. The chronic toxicity/carcinogenicity study in mice suggest a compound-related effect on the CNS and skin lesions. In addition, the acute neurotoxicity study in the rat also revealed myelinopathic alterations. Although the data is adequate to establish a permanent tolerance and Section 3 registration of the chemical, the HED RfD Peer Review Committee recommended that an additional MF of 10 be used until the potential for developmental neurotoxicity is determined and the lesions are better characterized. The Sponsor should conduct a mechanistic study to determine the cause/relationship of CNS/myelinopathic alterations to neurotoxicity (including developmental). An Ad Hoc Committee to the RfD/Peer Review Committee met on October 09, 1996 and made the following recommendations:

> To elucidate the cause/relationship of CNS lesions to neurotoxicity (including developmental) potential of the chemical, a confirmatory developmental neurotoxicity study (§83-6) is required. The Sponsor should follow the §83-6 guideline format for conducting a developmental neurotoxicity study, with several modifications. The modifications include treating the males and females for 90 days prior to mating and dams would go off treated feed 10 days post-delivery as is in the standard 83-6 protocol. In addition, males would be used to assist in determining the nature/cause of CNS lesions seen in rats and mice. This would facilitate characterizing the toxicity of chlorfenapyr. It is strongly recommended that the protocol and dose selection rationale should be submitted to TB-I for review and comments prior to initiation of the study.

DATA EVALUATION RECORD

PIRATE Metabolite

Study Type: 81-1; Acute Oral Toxicity - Rats

Work Assignment No. 1-1A (MRID 43492824)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
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Primary Reviewer:
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Signature: Christie E. Padova
Date: 10-19-95

Secondary Reviewer:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 10/19/95

Project Manager:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 10/19/95

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date: 10/19/95

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer:W. Greear, M.P.H. William B. Greear, Date 5/14/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer:M. Copley, D.V.M. Marion Copley, Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS 870.1100 [§81-1]

DP BARCODE: D212558 SUBMISSION CODE:
P.C. CODE: 129093 TOX. CHEM. NO.: NONE

TEST MATERIAL (PURITY): AC 303,268 Technical (100.3%)

SYNONYMS: None specified.

CITATION: Lowe, C. (1994) Oral LD_{50} study in albino rats with AC 303,268 Technical. American Cyanamid Company, Princeton, NJ. Report Number A94-38.01 (Amendment #1). June 14, 1994. MRID 43492824. Unpublished.

SPONSOR: American Cyanamid Company, Princeton, NJ.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43492824), groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 303,268 Technical (purity 100.3%, Lot No. 8979-44B) at 7.8, 15.6, 23.4, 31.25, 62.5, 125, or 250 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing.

Oral LD_{50} Males = 27.0 mg/kg
Females = 29.4 mg/kg
Combined = 28.7 (16.8-30.7) mg/kg (95% C.I.)

AC 303,268 Technical is classified as **TOXICITY CATEGORY I** based on the LD_{50} for both sexes.

Mortality (1-8 hours postdosing) occurred in 39/40 animals tested at 31.25 mg/kg and above. Clinical observations included prostrate with or without hind legs extended, increased activity when aroused, writhing, and protruding testes. In general, surviving animals showed no clinical signs and no treatment-related effect on body weight throughout the 14-day observation period; one surviving animal dosed at 31.25 mg/kg exhibited prostration with hind legs extended, but returned to normal within 24 hours. Gross necropsy of decedent animals revealed pronounced striations of the muscle tissue, and tonus. Less prevalent abnormalities included dark and/or mottled liver, and salivation. Gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 303,268 Technical
   Description: White powder
   Lot/Batch #: AC 8979-44B
   Purity: 100.3% a.i.
   CAS #: Not provided

2. Vehicle: Carboxymethyl cellulose:sterile distilled water (0.5%, w:v)

3. Test animals: Species: Albino rat
   Strain: Crl:CD(SD)BR
   Age: 7-9 weeks
   Weight: 151-186 g males; 137-180 g females
   Source: Charles River Laboratories, Kingston, NY
   Acclimation period: ≥7 days
   Diet: Purina Rodent Laboratory Chow, ad libitum
   Water: Tap water, ad libitum

B. STUDY DESIGN and METHODS:

1. In life dates: January 11 to February 17, 1994

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of AC 303,268 Technical by gavage. The rats were observed for clinical signs and mortality at 0-1, 1-2, 2-4, and 4-8 hours after dosing and then daily for the remainder of the study; body weights were recorded at 0 (prior to dosing), 7, and 14 days. After 14 days, surviving rats were sacrificed, and all animals were necropsied and examined for gross pathological changes.

TABLE 1. Doses, mortality/animals treated

Dose, mg/kg	Males	Females	Combined
7.8	0/5	0/5	0/10
15.6	0/5	0/5	0/10
23.4	0/5	0/5	0/10
31.25	5/5	4/5	9/10
62.5	5/5	5/5	10/10
125	5/5	5/5	10/10
250	5/5	5/5	10/10

3. Statistics: The oral LD_{50} was calculated using the D.J. Finney Probit Analysis method (1952).

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 1. Mortality occurred from 1-8 hours postdosing in 39/40 animals tested at 31.25 mg/kg and above.

Oral LD_{50} Males = 27.0 mg/kg
Females = 29.4 mg/kg
Combined = 28.7 (16.8-30.7) mg/kg (95% C.I.)

B. Clinical observations: At 31.25, 62.5, and 125 mg/kg, clinical observations included prostrate with or without hind legs extended, increased activity when aroused, writhing, and protruding testes. At 250 mg/kg, animals died within 2 hours and no clinical signs were observed. In general, surviving animals showed no clinical signs throughout the 14-day observation period; one surviving animal dosed at 31.25 mg/kg exhibited prostration with hind legs extended, but returned to normal within 24 hours.

C. Body Weight: No treatment-related effect on body weight was observed in surviving animals.

D. Necropsy: Gross necropsy of decedent animals revealed pronounced striations of the muscle tissue, and tonus. Less prevalent abnormalities included dark and/or mottled liver, and salivation. Gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

E. Deficiencies: Vehicle controls were not included for this study. Since the vehicle [carboxymethyl cellulose:sterile distilled water (0.5%, w:v)] was 99.5% water, and since clinical and gross necropsy observations were normal for the three lowest concentrations of AC 303,268 tested, this deficiency is minor and should have no effect on the classification of the toxicity of AC 303,268 Technical.

DATA EVALUATION RECORD

PIRATE Metabolite

Study Type: 81-1; Acute Oral Toxicity - Rats

Work Assignment No. 1-1B (MRID 43492825)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
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Primary Reviewer:
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Date: 10-19-95

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Signature: William J. Spangler
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Project Manager:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 10/19/95

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 10/19/95

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EPA Reviewer:W. Greear, M.P.H. William R. Greear, Date 5/14/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer:M. Copley D.V.M. M. Copley, Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS 870.1100 [§81-1]

DP BARCODE: D212558
P.C. CODE: 129093
SUBMISSION CODE:
TOX. CHEM. NO.: NONE

TEST MATERIAL (PURITY): AC 312,094 Technical (96.3%)

SYNONYMS: 2-(p-Chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile

CITATION: Lowe, C. (1994) Oral LD_{50} study in albino rats with AC 312,094 Technical. American Cyanamid Company, Princeton, NJ. Report Number A94-70. June 14, 1994. MRID 43492825. Unpublished.

SPONSOR: American Cyanamid Company, Princeton, NJ.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43492825), groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 312,094 Technical (purity 96.3%, Lot No. AC 8698-67A) at 5,000 mg/kg (limit dose). The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing.

Oral LD_{50} Males = >5,000 mg/kg
Females = >5,000 mg/kg
Combined = >5,000 mg/kg

AC 312,094 Technical is classified as **TOXICITY CATEGORY IV** based on the LD_{50} in both sexes.

Mortality occurred in one male rat at 4 days postdosing and in one female at 6 days. Clinical observations were limited to decreased activity in male rats, which subsided by 24 hours postdosing. No treatment-related effect on body weight was observed in 14-day survivors, and gross necropsy of decedent and animals sacrificed after 14 days revealed no visible lesions.

This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data

Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 312,094 Technical
   Description: Powder (not further described)
   Lot/Batch #: AC 8698-67A
   Purity: 96.3% a.i.
   CAS #: Not provided

2. Vehicle: Carboxymethyl cellulose:sterile distilled water (0.5%, w:v)

3. Test animals: Species: Albino rat
   Strain: Crl:CD(SD)BR
   Age: 7-9 weeks
   Weight: 166-177 g males; 146-165 g females
   Source: Charles River Laboratories, Kingston, NY
   Acclimation period: ≥7 days
   Diet: Purina Rodent Laboratory Chow, ad libitum
   Water: Tap water, ad libitum

B. STUDY DESIGN and METHODS:

1. In life dates: February 3-17, 1994

2. Animal assignment and treatment: Following an overnight fast, 5 rats/sex were given a single dose of AC 312,094 Technical at 5,000 mg/kg (limit dose) by gavage. The dose level was selected using information obtained from the results of similar compounds. The rats were observed for clinical signs and mortality at 0-2, 2-8, and 8-24 hours after dosing and then daily for the remainder of the study; body weights were recorded at 0 (prior to dosing), 7, and 14 days. After 14 days, surviving animals were sacrificed, and all animals were necropsied and examined for gross pathological changes.

3. Statistics: Not applicable to this study.

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality occurred in one male rat at 4 days postdosing and in one female at 6 days.

Oral LD_{50} Males = >5,000 mg/kg

Females = >5,000 mg/kg
Combined = >5,000 mg/kg

B. Clinical observations: Clinical observations were limited to decreased activity in male rats, which subsided by 24 hours postdosing.

C. Body Weight: No treatment-related effect on body weight was observed in 14-day survivors.

D. Necropsy: Gross necropsy revealed no visible lesions in decedent or animals sacrificed after 14 days.

E. Deficiencies: Vehicle controls were not included for this study. Since the vehicle [carboxymethyl cellulose:sterile distilled water (0.5%, w:v)] was 99.5% water, and since mortality was <50% at the acute limit dose of 5,000 mg/kg, this deficiency is minor and should have no effect on the classification of the toxicity of AC 303,268 Technical.

DATA EVALUATION RECORD

PIRATE Metabolite

Study Type: 81-1; Acute Oral Toxicity - Rats

Work Assignment No. 1-1C (MRID 43492826)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
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2275 Research Boulevard
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Primary Reviewer:
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Date: 10-19-95

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Date: 10/19/95

Project Manager:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 10/19/95

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 10/19/95

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EPA Reviewer:W. Greear, M.P.H. William B. Greear, Date 5/14/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer:M. Copley, D.V.M. M Copley, Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS 870.1100 [§81-1]

DP BARCODE: D212558
P.C. CODE: 129093
SUBMISSION CODE:
TOX. CHEM. NO.: NONE

TEST MATERIAL (PURITY): AC 322,250 (89%)

SYNONYMS: 3-Bromo-5-(p-chlorophenyl)-4-cyanopyrrole-2-carboxylic acid

CITATION: Lowe, C. (1994) Oral LD_{50} study in albino rats with AC 322,250. American Cyanamid Company, Princeton, NJ. Report Number A94-215. August 11, 1994. MRID 43492826. Unpublished.

SPONSOR: American Cyanamid Company, Princeton, NJ.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43492826), groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 322,250 (purity 89%, Lot No. AC 9014-97A) at 1,250 (females only), 2,500 (females only), or 5,000 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing.

Oral LD_{50} Males = >5,000 mg/kg
Females = 2500 (1581-3954) mg/kg (95% C.I.)

AC 322,250 is classified as **TOXICITY CATEGORY III** based on the LD_{50} in females.

In male rats, one death occurred at 3 days postdosing. In female rats, mortality occurred from 8 hours to 5 days postdosing in 7/10 animals tested at 2,500 and 5,000 mg/kg. At 1,250 and 2,500 mg/kg (females only), both decedent and 14-day survivors exhibited no clinical signs throughout the 14-day study period. At 5,000 mg/kg, the major clinical observation in both sexes was diarrhea, which generally subsided in males by 24 hours postdosing; one female rat also exhibited decreased activity, ptosis, and diuresis prior to death. No treatment-related effect on body weight was observed in 14-day survivors. Gross necropsy of decedent animals revealed a low frequency of dark spleen, distended stomach, and test material- and gas-filled stomach. Gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 322,250
   Description: Cream, solid
   Lot/Batch #: AC 9014-97A
   Purity: 89% a.i.
   CAS #: Not provided

2. Vehicle: Carboxymethyl cellulose:sterile distilled water (0.5%, w:v)

3. Test animals: Species: Albino rat
   Strain: Crl:CD(SD)BR
   Age: 7-9 weeks
   Weight: 210-232 g males; 156-223 g females
   Source: Charles River Laboratories, Stoneridge, NY
   Acclimation period: ≥7 days
   Diet: Purina Rodent Laboratory Chow, ad libitum
   Water: Tap water, ad libitum

B. STUDY DESIGN and METHODS:

1. In life dates: June 3-29, 1994

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of AC 322,250 by gavage. The rats were observed for clinical signs and mortality at 0-1, 1-2, 2-4, and 4-8 hours after dosing and then daily for the remainder of the study; body weights were recorded at 0 (prior to dosing), 7, and 14 days. All decedents and 14-day survivors were sacrificed, necropsied, and examined for gross pathology.

TABLE 1. Doses, mortality/animals treated

Dose, mg/kg	Males	Females	Combined
1250	-/-	0/5	0/5
2500	-/-	3/5	3/5
5000	1/5	4/5	5/10

3. Statistics: The acute oral LD_{50} (for females) was calculated using the method of moving averages as described by C.W. Weil (1952).

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 1. In male rats, one death occurred at 3 days postdosing. In female rats, mortality occurred from 8 hours to 5 days postdosing in 7/10 animals tested at 2,500 and 5,000 mg/kg.

Oral LD_{50} Males = >5,000 mg/kg
Females = 2500 (1581-3954) mg/kg (95% C.I.)

B. Clinical observations: At 1,250 and 2,500 mg/kg (females only), both decedent and 14-day survivors exhibited no clinical signs throughout the study period. At 5,000 mg/kg, the major clinical observation in both sexes was diarrhea, which generally subsided in males by 24 hours postdosing; one female rat also exhibited decreased activity, ptosis, and diuresis prior to death.

C. Body Weight: No treatment-related effect on body weight was observed in surviving animals.

D. Necropsy: Gross necropsy of decedent animals revealed a low frequency of dark spleen, distended stomach, and test material- and gas-filled stomach. Gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

E. Deficiencies: Vehicle controls were not included for this study. Since the vehicle [carboxymethyl cellulose:sterile distilled water (0.5%, w:v)] was 99.5% water, and since clinical and gross necropsy observations were normal for the 1,250 and 2,500 mg/kg concentrations, this deficiency is minor and should have no effect on the classification of the toxicity of AC 322,250.

DATA EVALUATION RECORD

PIRATE Metabolite

Study Type: 81-1; Acute Oral Toxicity - Rats

Work Assignment No. 1-1D (MRID 43492827)

Prepared for

Health Effects Division
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U.S. Environmental Protection Agency
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Prepared by

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Project Manager:
William Spangler, Ph.D.

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Date: 10/19/95

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date: 10/19/95

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EPA Reviewer:W. Greear, M.P.H. William B Greear, Date 5/14/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer:M. Copley, D.V.M. M Copley, Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS 870.1100 [§81-1]

DP BARCODE: D212558 SUBMISSION CODE:
P.C. CODE: 129093 TOX. CHEM. NO.: NONE

TEST MATERIAL (PURITY): AC 325,195 (97%)

SYNONYMS: 2-(p-Chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-2-pyrrolidine-3-carbonitrile

CITATION: Lowe, C. (1994) Oral LD_{50} study in albino rats with AC 325,195. American Cyanamid Company, Princeton, NJ. Report Number A94-252. October 20, 1994. MRID 43492827. Unpublished.

SPONSOR: American Cyanamid Company, Princeton, NJ.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43492827), groups of 5 albino rats/sex (Crl:CD(SD)BR strain) were given single oral doses of AC 325,195 (purity 89%, Lot No. AC 9014-93B) at 312.5 (males only), 625, 1,250, 2,500, or 5,000 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 14 days postdosing.

Oral LD_{50} Males = 776 (448-1,329) mg/kg (95% C.I.)
Females = 1367 (755-2,364) mg/kg (95% C.I.)

AC 325,195 is classified as **TOXICITY CATEGORY III** based on the LD_{50} in both sexes.

Mortality occurred in 26/30 animals tested at 1,250 mg/kg and above; the majority of deaths occurred at ≤24 hours postdosing. Clinical observations in decedent animals included salivation, decreased activity, hyperthermia, chromodacryorrhea, dyspnea, ptosis, brown material around nose, red material in urine, dehydration and prostration. Observations in surviving animals, which subsided by 9 days postdosing, included ptosis, diuresis, and brown material around nose. No treatment-related effect on body weight was observed in surviving animals. Gross necropsy of decedent animals revealed white foci on the liver and spleen and discoloration of the spleen, which corresponded microscopically to hepatocellular necrosis and/or infarcted areas and diffuse hemorrhage, respectively. Other observations included vascularized stomach, distended stomach, test material- and gas-

filled stomach, and gas-filled cecum (females only). Gross necropsy of animals sacrificed after 14 days revealed no gross pathological changes.

This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the albino rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 325,195
   Description: Light yellow powder
   Lot/Batch #: AC 9014-93B
   Purity: 97% a.i.
   CAS #: Not provided

2. Vehicle: Carboxymethyl cellulose:sterile distilled water (0.5%, w:v)

3. Test animals: Species: Albino rat
   Strain: Crl:CD(SD)BR
   Age: 7-9 weeks
   Weight: 191-254 g males; 150-210 g females
   Source: Charles River Laboratories, Stoneridge, NY
   Acclimation period: ≥7 days
   Diet: Purina Rodent Laboratory Chow, ad libitum
   Water: Tap water, ad libitum

B. STUDY DESIGN and METHODS:

1. In life dates: May 20 to June 29, 1994

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of AC 325,195 by gavage. The rats were observed for clinical signs and mortality at 0-1, 1-2, 2-4, 4-8, and 8-24 hours after dosing and then daily for the remainder of the study; body weights were recorded at 0 (prior to dosing), 7, and 14 days. After 14 days, surviving animals were sacrificed, and all decedents and 14-day survivors necropsied and examined for gross pathological changes. Subsequent histopathological examinations were performed on tissues or organs with gross lesions from animals that died ≥24 hours postdosing.

TABLE 1. Doses, mortality/animals treated

Dose, mg/kg	Males	Females	Combined
312.5	0/5	-/-	0/5
625	2/5	0/5	2/10
1,250	4/5	3/5	7/10
2,500	5/5	4/5	9/10
5,000	5/5	5/5	10/10

3. Statistics: The acute oral LD_{50} was calculated using the D.J. Finney Probit Analysis method (1952).

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 1. Mortality occurred in 26/30 animals tested at 1,250 mg/kg and above; the majority of deaths occurred at ≤24 hours postdosing.

Oral LD_{50} Males = 776 (448-1,329) mg/kg (95% C.I.)
Females = 1367 (755-2,364) mg/kg (95% C.I.)

B. Clinical observations: Clinical observations in decedent animals included salivation, decreased activity, hyperthermia, chromodacryorrhea, dyspnea, ptosis, brown material around nose, red material in urine, dehydration and prostration. Observations in surviving animals included ptosis, diuresis, and brown material around nose; these effects subsided by 9 days postdosing.

C. Body Weight: No treatment-related effect on body weight was observed in surviving animals.

D. Necropsy: Gross necropsy of decedent animals revealed white foci on the liver and spleen and discoloration of the spleen, which corresponded microscopically to hepatocellular necrosis and/or infarcted areas and diffuse hemorrhage, respectively. Other observations included vascularized stomach, distended stomach, test material- and gas-filled stomach, and gas-filled cecum (females only). Gross necropsy of surviving animals revealed no gross pathological changes.

E. Deficiencies: Vehicle controls were not included for this study. Since the vehicle [carboxymethyl cellulose:sterile distilled water (0.5%, w:v)] was 99.5%

water, and since clinical and gross necropsy observations were normal for the 312.5 mg/kg (females) and 625 mg/kg (males) concentrations, this deficiency is minor and should have no effect on the classification of the toxicity of AC 325,195.

DATA EVALUATION RECORD

PIRATE

Study Type: 81-1; Acute Oral Toxicity - Mouse

Work Assignment No. 1-1E (MRID 43492828)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
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Dynamac Corporation
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Date: 10/19/95

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William Spangler, Ph.D.

Signature: William J. Spangler
Date: 10/19/95

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date: 10/19/95

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer:W. Greear, M.P.H., D.A.B.T. William B. Greear Date 5/14/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer:M. Copley, D.V.M., D.A.B.T. M Copley, Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Mouse
OPPTS 870.1100 [§81-1]

DP BARCODE: D212558 SUBMISSION CODE:
P.C. CODE: 129093 TOX. CHEM. NO.: NONE

TEST MATERIAL (PURITY): AC 303,630 Technical (94.5%)

SYNONYMS: Pirate; 4-Bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile

CITATION: Bradley, D. (1994) Oral LD_{50} study in albino mice with AC 303,630 Technical. American Cyanamid Company, Princeton, NJ. Report Number A93-20.02 (Amendment #2). December 7, 1994. MRID 43492828. Unpublished.

SPONSOR: American Cyanamid Company, Princeton, NJ.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43492828), groups of 5 albino mice/sex (Crl:CD-1(ICR)BR strain) were given single oral doses of AC 303,630 Technical (purity 94.5%, Lot No. AC 7504-59A) at 35, 70, or 140 mg/kg. The test substance was delivered in an aqueous solution of carboxymethyl cellulose (0.5%, w:v); there were no vehicle controls. Animals were observed for clinical signs and mortality for up to 2 hours postdosing, then daily (≥24 hours) for the remainder of the study.

Oral LD_{50} Males = 45 (37-56) mg/kg (95% C.I.)
Females = 78 (41-152) mg/kg (95% C.I.)
Combined = 55 (37-80) mg/kg (95% C.I.)

AC 303,630 Technical is classified as **TOXICITY CATEGORY I** based on the LD_{50} in males.

Mortality occurred at 8-24 hours postdosing in 17/18 decedents. Clinical observations were limited to decreased activity during the first 2 hours postdosing of the mice treated at 140 mg/kg. No significant treatment-related effect on body weight was observed in surviving animals. Gross necropsy of decedent mice revealed a single occurrence of a bright red-colored lung. Gross necropsy revealed no visible lesions in animals sacrificed after 14 days.

This acute oral study is classified **acceptable**, and satisfies the guideline requirement for an acute oral study (81-1) in the

albino mouse.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 303,630 Technical
   Description: Tan powder
   Lot/Batch #: AC 7504-59A
   Purity: 94.5% a.i.
   CAS #: 122453-73-0

2. Vehicle: Carboxymethyl cellulose:sterile distilled water (0.5%, w:v)

3. Test animals: Species: Albino mice
   Strain: Crl:CD-1(ICR)BR
   Age: 5-8 weeks
   Weight: 18.7-28.0 g males; 18.2-21.7 g females
   Source: Charles River Laboratories, Wilmington, MA
   Acclimation period: ≥7 days
   Diet: Purina Rodent Chow, ad libitum
   Water: Tap water, ad libitum

B. STUDY DESIGN and METHODS:

1. In life dates: September 2-16, 1992.

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Following a 4-hour fast, mice were given a single dose of AC 303,630 Technical by gavage. The mice were observed for clinical signs and mortality at 0-1 and 1-2 hours after dosing and then daily (≥24 hours) for the remainder of the study; body weights were recorded at 0 (prior to dosing), 7, and 14 days. After 14 days, surviving animals were sacrificed, and all animals were necropsied and examined for gross pathological changes. Subsequent histopathological examinations were performed on tissues or organs with gross lesions from animals that died ≥24 hours postdosing.

TABLE 1. Doses, mortality/animals treated

Dose, mg/kg	Males	Females	Combined
35	1/5	1/5	2/10
70	5/5	2/5	7/10
140	5/5	4/5	9/10

3. Statistics: The acute oral LD_{50} was calculated using the method of moving averages as described by C.W. Weil (1952).

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 1. Mortality occurred at 8-24 hours postdosing in 17/18 decedents.

Oral LD_{50} Males = 45 (37-56) mg/kg (95% C.I.)
Females = 78 (41-152) mg/kg (95% C.I.)
Combined = 55 (37-80) mg/kg (95% C.I.)

B. Clinical observations: Clinical observations were limited to decreased activity of the 140 mg/kg level mice during the first 2 hours postdosing.

C. Body Weight: No significant treatment-related effect on body weight was observed in surviving animals.

D. Necropsy: Gross necropsy of decedent mice revealed a single occurrence of a bright red-colored lung. Gross necropsy revealed no visible lesions in animals sacrificed after 14 days.

E. Deficiencies: In most cases, animals were not clinically observed prior to death. Mice were only observed up to 2 hours postdosing, then daily (≥24 hours) for the remainder of the study; 17/18 deaths occurred between 2-24 hours postdosing. Although this deficiency severely limits the clinical observation data, it does not affect the calculated LD_{50} values, or the subsequent classification of the toxicity of AC 303,630 Technical.

Vehicle controls were not included for this study. Since the vehicle [carboxymethyl cellulose:sterile distilled water (0.5%, w:v)] was 99.5% water, and based on the results of similar studies using albino rats (MRIDs 43492824-43492827), this deficiency is minor and

should have no effect on the classification of the toxicity of AC 303,630 Technical.

Due to problems associated with suspending the test material in water, the mice were dosed with a constant volume of 25 ml/kg, exceeding the limit of 20 ml/kg (2 ml/g) outlined in Subdivision F guidelines. This deficiency is considered minor and should have no impact on the results of this study.

DATA EVALUATION REPORT

PIRATE

Study Type: 81-8; Acute Neurotoxicity Feeding Study - Rats

Dynamac Study No. 101F (MRID 43492829)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Date: 1-17-96

Project Manager:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 1/17/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler by WJS
Date: 1/17/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: W. Greear, M.P.H., D.A.B.T. William B. Greear, Date 4/24/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, D.V.M., D.A.B.T. M Copley, Date 5/10/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Feeding Study - Rats
OPPTS Number: 870.6200 OPP Guideline Number: §81-8, 82-7, 83-1

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.9% ai)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Ponnock, K. (1994) An acute neurotoxicity study with AC 303,630 in rats. Pharmaco LSR Inc., Mettlers Road, East Millstone, NJ. Project 93-4510. August 15, 1994. MRID 43492829. Unpublished.

SPONSOR: American Cyanamid Company; Global Plant Industry Development; P.O. Box 400; Princeton, NJ 08543-0400.

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 43492829), AC 303,630, (94.5% ai, Lot No. AC 7504-59-A) was dissolved in 0.5% carboxymethylcellulose and administered once, via gastric intubation in a dosing volume of 10 ml/kg/dose, to 60 Sprague-Dawley CD rats (10/sex/group) at dose levels of 0, 45, 90, and 180 mg/kg. All rats were observed for 2 weeks following dosing. The rats were evaluated for reactions in functional observational and motor activity measurements pretest and on study days 1, 8, and 15. In addition, five rats per group were examined for neuropathologic lesions.

Two males and two females in the 180 mg/kg dose group died within 7 hours of dosing, possibly as a result of accidental injury during treatment. Surviving rats in this dose group exhibited changes in gait, locomotion, and arousal, and 20-30% of the males and females were lethargic on the day of treatment. In the 90 mg/kg dose group, 20% of the males were lethargic on the day of treatment. No dose-related effects on body weights, food consumption, neurobehavioral observations, or gross or histological post mortem examinations were noted. **The LOEL is 90 mg/kg, based on lethargy of the rats on the day of treatment. The NOEL is 45 mg/kg.**

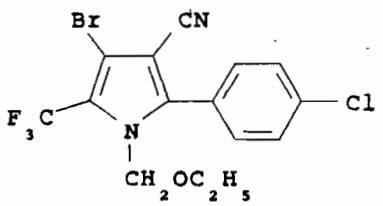
This acute neurotoxicity study is classified as **Acceptable** and satisfies the guideline requirement for an acute neurotoxicity screening study in rodent (81-8SS).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 303,630 (Pirate)
   Description: Tan solid
   Lot/Batch #: AC 7504-59-A
   Purity: 94.9% ai
   Stability of compound: Stable
   CAS #: Not provided
   Structure:



2. Vehicle: Carboxymethylcellulose, 0.5% aqueous solution.

3. Test animals: Species: Rat
   Strain: Sprague-Dawley Crl: CD BR VAF/Plus
   Age and weight at study initiation: 46 days of age; males 156.7-211.2 g, females 131.7-175.8 g
   Source: Charles River Breeding Labs., Stone Ridge, NY
   Housing: Individually housed in elevated stainless steel wire mesh cages
   Diet: Certified Rodent Diet No. 5002 - Meal (PMI Feeds, St. Louis, MO), ad libitum
   Water: tap water, ad libitum
   Environmental conditions:
   Temperature: 67-72 F
   Humidity: 40-78%
   Air changes: Not provided
   Photoperiod: 12-hour light/dark cycle
   Acclimation period: 14 days

B. STUDY DESIGN:

1. In life dates: Start: 2/28/94 End: 3/17/94

2. Animal Assignment

Animals (10/sex) were assigned to the test groups in Table 1 using a computerized random sort program.

TABLE 1. STUDY DESIGN.

Dose to Animal (mg/kg)	Animals Assigned	
	Male	Female
0	10	10
45	10	10
90	10	10
180	10	10

3. Dose selection rationale

Doses were selected on the basis of a preliminary study in which rats received single oral doses of 63 to 500 mg/kg (males) or 125 to 1000 mg/kg (females). Mortality occurred 2-4 hours after dosing in all males at doses of 250 mg/kg and above, and in 2 of 3 females at a dose of 250 mg/kg. Neurotoxic effects (decreased activity, rapid respiration, irregular gait, or excessive salivation) peaked at 1.5-3.5 hours. No autonomic effects or convulsions were observed. The highest non-lethal and lowest lethal doses were 125 and 250 mg/kg, respectively. The NOEL was 125 mg/kg in females and <63 mg/kg in males.

4. Dosing suspensions

Prior to the initiation of the study, batches of the 45 and 180 mg/kg suspensions were prepared. To establish the adequacy of the mixing procedure, three samples each were collected from the top, middle, and bottom portions of the suspensions and analyzed using LC. To establish the stability of AC 303,630 in suspension, aliquots of the 45 mg/kg suspensions were refrigerated, then analyzed 4 or 14 days after preparation.

Results:
Homogeneity Analysis: 81.6-100.6% of nominal
Stability Analysis: 85.6-94.4% of nominal
Concentration Analysis: 19 of 20 samples contained

within 85% of the nominal doses, and 18 of 20 were within 90% of the nominal doses (Data obtained from pages 402-404 in the study report).

The analytical data indicated that the dosing suspension preparation procedures were adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Dosing Regimen

Fasted (18 hours) animals were orally administered, via gastric intubation, a single dose of AC 303,630 suspended in 0.5% carboxymethylcellulose at a dosing volume of 10 mL/kg/dose.

6. Statistics

Body weight, body weight change, food consumption, and motor activity data were subjected to appropriate one-way analysis of variance (ANOVA) technique using the F distribution to assess significance. If this technique indicated significant differences among the means, Bartlett's test was used to determine the equality of variances. If the variances were equal, parametric procedures were used. If the variances were not equal, nonparametric procedures were used. Nonparametric procedures consisted of the Kruskal-Wallis test to detect differences in the means, which were then subjected to a summed ranked test (Dunn).

7. Validation (Positive Control) Data

The original study report (MRID 43492829) did not contain adequate description of the validation studies conducted to demonstrate proficiency of the testing laboratory in neurobehavioral and neuropathological evaluations. However, in a revised study report (MRID 44067401; revision date July 16, 1996), additional information was provided on a study using acrylamide. This study is briefly summarized in Appendix I to this DER and demonstrates proficiency of the testing laboratory in evaluation of several neurobehavioral and neuropathologic lesions.

C. METHODS

1. Observations

Animals were inspected twice daily (once in the morning and once in the afternoon) for signs of toxicity and mortality.

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2. Body weight

Animals were weighed pretest on the day of dosing, weekly during the study, and at study termination.

3. Food consumption

Food consumption for each animal was determined weekly, beginning one week prior to treatment.

4. Neurobehavioral Studies

Locomotor Activity - Locomotor activity of all animals was measured pretest and on days 1, 8, and 15 of the study using an automated Photobeam Activity System (San Diego Instruments). Each session consisted of twelve 5-minute intervals, and treatment groups were counterbalanced across test times.

Functional Observation Battery (FOB) - Assessments were made on study days 1, 8, and 15. Time of testing was counterbalanced across treatment groups, and evaluations were performed without the observers knowing the identity of the dose groups. The following parameters were evaluated:

Home Cage Observations
- Piloerection
- Posture
- Gait
- Tremors
- Convulsions
- Clonic movements
- Tonic movements

Manipulative Observations
- Ease of removal from cage
- Ease of handling
- Respiration
- Palpebral closure
- Pupil size
- Staining: eyes, oral, anal
- Lacrimation
- Salivation
- Vocalization

Response Observations
- Auditory response
- Approach response
- Touch response
- Pupil response
- Pain response

Open Field Observations
- Posture
- Gait
- Arousal
- Stereotypic and bizarre behavior
- Tremors
- Convulsions
- Circling
- Locomotion
- Rearing count
- Urination
- Defecation boluses

Neuromuscular tests
- Hindlimb extensor strength
- Hindlimb grip strength
- Forelimb grip strength
- Landing footsplay
- Righting reflex

5. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were

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subjected to gross pathological examination. This activity consisted of examining the external surfaces and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remaining carcass. Five rats/sex/group were randomly selected for neuropathology (anesthetized with an ip injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by paraformaldehyde in the same buffer). All remaining rats were exsanguinated following carbon dioxide anesthesia. The following tissues from the neuropathology animals were collected for histological examination:

brain (medulla/pons, cerebellar cortex and cerebral cortex)
spinal cord (intact; cervical, thoracic and lumbar segments)
sciatic nerve
tibial nerve
sural nerve
optic nerve

No organs were weighed.

II. RESULTS

A. Mortality

Two males and two females in the 180 mg/kg treatment group died 5-7 hours following dosing. The study author stated that these deaths were a result of accidental trauma during the dosing procedure.

B. Body weight and weight gain

No treatment-related effects on body weight or body weight gain were observed.

C. Food consumption and compound intake

No treatment-related effects on food consumption were observed.

D. Neurotoxicity

No overt signs of neurotoxicity were observed. There were indications of a few neurobehavioral changes at FOB testing on the day of dosing, particularly in the animals that received the 180 mg/kg dose. Table 2 summarizes the incidence of

selected findings that appear to be treatment-related.

TABLE 2. SIGNIFICANT FUNCTIONAL OBSERVATION BATTERY TESTING RESULTS.[a]

Observation	Dose level (mg/kg)			
	0	45	90	180
Male				
Impaired locomotion, day 1	1/10	0/10	1/10	5/10
Decreased arousal, day 1	1/10	0/10	1/10	5/10
Lying/flattened in cage in a.m.	0/10	0/10	4/10	8/10
Female				
Impaired locomotion, day 1	0/10	0/10	0/10	3/9
Decreased arousal, day 1	0/10	1/10	0/10	3/9
Lying/flattened in cage in a.m.	0/10	0/10	1/10	5/10

[a] Data obtained from Table T 6, pages 55-137, in the study report.

At a dose level of 180 mg/kg, changes in gait, locomotion and arousal were produced on the day of treatment. On the day following treatment, 5/10 males and 3/9 females exhibited impaired locomotion, and slightly decreased arousal was noted in 5/10 males and 3/9 females. These effects were not observed at days 7 or 14 of the study. On the morning immediately after treatment, 8/10 males and 5/10 females were observed either lying on their side or flattened in the cage. Forelimb and hindlimb grip strength for male rats appeared to be slightly reduced on days 1, 8, and 15; however, these decreases were not statistically significant and were noted at the pre-test observations, and therefore are not considered to be indicative of neurotoxicity.

At a dose level of 90 mg/kg, 4/10 males and 1/10 females were observed either lying on their side or flattened in the cage on the morning immediately after treatment. On day 1, combined sexes in the 90 mg/kg treatment group exhibited a statistically significant decrease in mean motor activity compared to the combined control group rats. Since this

effect was not significant in the 180 mg/kg treatment group rats, there was no evidence of a dose related response and the observed response is considered to have been incidental and not treatment related.

F. Sacrifice and Pathology

No treatment-related macroscopic or microscopic abnormalities were found in this study.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded the NOEL for this study was 45 mg/kg, "based on the increased mortality and changes in gait, locomotion, and arousal noted in the 180 mg/kg dose group and lethargy observed in both sexes of the 180 mg/kg dose group and in males of the 90 mg/kg dose group". The study author also concluded that AC 303,630 was not an acute neurotoxicant.

B. Reviewer's Discussion

The study was adequately conducted. A sufficient number of FOB parameters were evaluated, a standardized scoring protocol for those parameters was presented, and summary data tables for FOB parameters were supported by individual animal data.

Two males and two females in the 180 mg/kg dose group died within 7 hours of dosing. The study author stated that these animals were killed by dosing accidents, but provided no macroscopic evidence to substantiate this claim. Surviving rats in the 180 mg/kg treatment group exhibited changes in gait, locomotion, and arousal, and 20-30% were lethargic on the day of treatment. In the 90 mg/kg dose group, 2 of 10 males were lethargic on the day of treatment. No atypical behavior was observed with rats in the 45 mg/kg group. There were no treatment-related effects on body weights, food consumption, neurobehavioral observations, or gross or histological post mortem examinations. The LOEL is 90 mg/kg; the NOEL is 45 mg/kg.

IV. STUDY DEFICIENCIES

No significant study deficiencies were identified. [Note: the laboratory historical positive control data as provided in the original study report were inadequate. Although

there were no indications of neurotoxicity produced or a positive control group in this study, recent historical positive control data generated at Pharmaco LSR (Appendix M) indicated neurobehavioral changes after 9 or 13 weeks of dosing with an unidentified compound but no effects after 3 weeks of dosing and only a few changes after 5 weeks of dosing. These historical data are inadequate to validate the sensitivity of the tests in the present study. Additional information on the time of testing (how many hours or days after dosing), the type of chemical used as a positive control, the type of instrumentation for measuring locomotion, etc. should be submitted with historical data. Results with various types of neurotoxicants are also useful.

The Registrant submitted a revised study report (MRID 44067401)) which contained a more detailed report of a positive control study evaluating the effects of repeated oral administration of acrylamide on the FOB parameters, motor activity and neuropathology (see Appendix M of the study report). TB-I considers this information adequate to validate the sensitivity of the tests in the Pirate study.]

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APPENDIX I
POSITIVE CONTROL STUDY ON ACRYLAMIDE

Study conducted between June 17 - October 19, 1993
Study title/author/completion date/number not given (report contained in Appendix M of MRID 44067401)

SUMMARY: Ten Crl:CD®BR rats/sex were administered 10 mg acrylamide/kg/day by gavage in distilled water (5 ml/kg body wt) daily for at least 90 days. Negative control animals (10/sex) received no treatment or vehicle gavage. Functional observational battery (FOB) and motor activity testing were performed pretest and at weeks 5, 9 and 13. Neurohistopathological examinations of all treated and 5 negative control animals were performed following in situ perfusion.

FOB evaluation: Repeated treatment with acrylamide caused reduced fore-and hind-limb grip strength, impaired righting reflex (landing on side or back), increased landing foot splay distance, impaired locomotion and abnormal gait (ataxia or hind-limbs splayed or dragging) in both sexes. Animals showed a progressive increase in the incidence and severity of many findings with time. By week 13, essentially all animals showed most symptoms and pronounced decreases in forelimb grip strength (26-30%), decreased hindlimb grip strength (44-71%) and increased landing foot splay distance (135-137%) were observed, whereas at week 5, only a few animals were affected and changes in the quantitative measurements were considerably less marked.

Motor activity: Statistically significantly decreased motor activity (mean activity counts) was observed at week 13 in both males (57% less than controls) and females (44% less than controls) in animals treated with acrylamide. Motor activity at week 9 was also significantly decreased in both sexes but only the decrease in males (23%) and not females (2%) was considered biologically significant. Activity of treated animals was generally reduced throughout each testing session compared to controls at the same intervals.

Neurohistopathology: Examination of neural tissues revealed significant lesions in peripheral nerves of both treated males and females. The sciatic, sural and tibial nerves showed moderate to severe segmental degeneration of myelin affecting all treated animals. No negative control animals were reported to be affected with this or any other degenerative lesion.

Conclusions: The findings of this study were typical of neurobehavioral and structural changes caused by repeated administration of acrylamide. Ability to identify several types of neurobehavioral/pathological effects was demonstrated.

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DATA EVALUATION RECORD

PIRATE

Study Type: 82-1a; Subchronic Oral Toxicity Study
With Rangefinding - Mice

Dynamac Study No. 101G (MRID 43492830)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: W. Greear, M.P.H., D.A.B.T. William R. Greear Date 4/30/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, D.M.V., D.A.B.T. M Copley Date 5/10/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [feeding-mice]
OPPTS Number: 870.3100 (rodent) OPP Guideline Number: §82-1a

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 93.6% ai)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Fischer, J.E. (1994) AC 300,630: 13-Week and 28-Day Dietary Toxicity Studies in the Albino Mouse. American Cyanamid Company; Agriculture Research Division; Princeton, NJ. Laboratory Project ID Study T-0219 and T-0302; Report No. AX91-2 and AX93-4. March 4, 1994. MRID 43492830. Unpublished.

SPONSOR: American Cyanamid Company; Global Plant Industry Development; P.O. Box 400; Princeton, NJ 08543-0400.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 43492830), AC 303,630 (Pirate; 93.6% a.i.; Lot No. AC 7171-141A) was administered to 20 albino mice/sex/dose at dietary dose levels of 40, 80, 160, or 320 ppm (average 7.1, 14.8, 27.6, or 62.6 mg/kg/day, respectively, for males; 9.2, 19.3, 40.0, or 78.0 mg/kg/day, respectively, for females) for 91 days.

Male mice fed AC 303,630 at 80 ppm, and male and female mice fed AC 303,630 at 160 or 320 ppm exhibited a toxic response to the test compound. Two mice died prior to the termination of the study; one male and one female dosed at the 320 ppm level died after only 2 days of feeding. In male mice, hepatic cell hypertrophy was observed in 30% of the animals in the 80 ppm treatment group, 65% in the 160 ppm treatment group, and 95% in the 320 ppm treatment group. Male mice in the 160 or 320 ppm treatment groups had increased relative liver and spleen weights. Male mice in the 320 ppm treatment group had a 26% lower body weight gain, and increased hematocrit values and RBC counts compared to the controls. In female mice, hepatic cell hypertrophy occurred in 20% of the animals in the 160 ppm treatment group and 50% in the 320 ppm treatment group. Female mice in the 320 ppm treatment group had a 29% lower body weight gain, increased WBC counts, and increased relative liver weights

compared to the controls. Spongiform encephalopathy was noted in the brain and myelin of the spinal cord of 90-95% of both males and females receiving the 320 ppm treatment level. No other significant treatment-related changes in ophthalmology, hematology, blood chemistry, or organ weights and morphology were observed during the study; urinalysis was not conducted. **The LOEL is 80 ppm (14.8 mg/kg/day) for male mice and 160 ppm (40.0 mg/kg/day) for female mice, based on hepatic cell hypertrophy in ≥20% of the test animals at this treatment level. The NOEL is 40 ppm (7.1 mg/kg/day).**

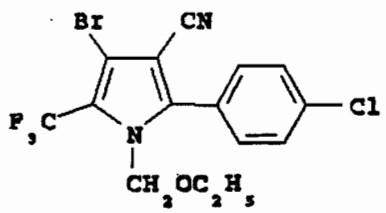
This subchronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a subchronic oral study (§82-1a) in rodents.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 303,630
   Description: white solid
   Lot/Batch #: AC 7171-141A
   Purity: 93.6% ai
   Stability of compound: Not provided
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Mice
   Strain: CD-1[Crl:CD(SD)]strain albino mice.
   Age and weight at study initiation: 6 weeks of age; body weight 23.6-32.6 g for males and 22.0-26.9 g for females
   Source: Wilmington, Massachusetts, facility of Charles River Breeding Laboratories, Inc.
   Housing: Individually housed in stainless steel, suspended, screen-bottomed cages
   Diet: Purina Certified Rodent Chow #5002, ad libitum

Water: source not specified, ad libitum
Environmental conditions:
Temperature: 72 ± 4 F (22 ± 2 C)
Humidity: 50 ± 20%
Air Changes: Not specified
Photoperiod: 12-hour light/dark cycle
Acclimation period: 7 days prior to testing

B. STUDY DESIGN:

1. In life dates Start: 10/10/90
End: 1/17/91

2. Animal assignment

Of 260 original mice (130/sex), 100 mice of each sex were selected for use on the basis of body weight. The selected mice were assigned to the test groups in Table 1 using a computerized randomization procedure.

TABLE 1: STUDY DESIGN[a]

Test Group	Conc. in Diet (ppm)	Nominal Dose to Animal (mg/kg/day)	Animals Assigned	
			Male	Female
Control	0	0	20	20
Low	40	6	20	20
Mid	80	12	20	20
Mid	160	24	20	20
High	320	48	20	20

[a] Dose levels were selected on the basis of a 28-day range-finding study in mice that was appended (pages 848-875) to this 90-day study. In the range-finding study, AC 303,630 was administered in feed at 160, 240, 320, 480, and 640 ppm. The NOEL was determined to be <160 ppm (<32 mg/kg/day).

3. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of test substance with 200 g of Purina Certified Rodent Chow #5002 by blending in a Waring Blender for 2 minutes. The treated feed was mixed with additional Rodent Chow, subsampled for concentration analysis, and stored at room

temperature in closed polyethylene containers until use.

Additional diet mix was treated at 40 and 320 ppm as described. Samples were collected from the top, middle, and bottom portions of the blender immediately posttreatment, and subsamples were immediately analyzed to determine homogeneity. The homogeneity of the 40 ppm treatment was retested after 9 months of frozen storage because the original analyses were "out of acceptable range (± 15%) of % nominal values". Also, samples of the diet mixes treated at 40 and 320 ppm were collected from the middle portion of the blender, stored in open feeders in the animal room at ambient conditions, and analyzed after 7 and 14 days to determine stability.

Results:

Homogeneity Analysis:

- Immediate posttreatment 40 ppm: 46.78-48.75 ppm (average 47.90 ppm, 119.77% nominal); reanalysis 40 ppm, 38.95-40.95 ppm (average 39.86 ppm, 99.67% nominal)
- Immediate posttreatment 320 ppm: 323.6-334.0 ppm (average 327.3 ppm, 102.3% nominal)

Stability Analysis:

- 7-day 40 ppm: 45.68 and 46.51 ppm (average 46.10 ppm, 115.3% nominal); reanalysis, 7-day 40 ppm, 39.62 and 39.85 ppm (average 39.74 ppm, 99.4% nominal)
- 14-day 40 ppm: 42.83 and 43.09 ppm (average 42.96 ppm, 107.4% nominal)
- 7-day 320 ppm: 313.7 and 314.2 ppm (average 314.0 ppm, 98.1% nominal)
- 14-day 320 ppm: 286.3 and 291.4 ppm (average 288.9 ppm, 94.2% nominal)

Concentration Analysis:

- For the 40 ppm treatment: 37.73-47.27 ppm (average 40.8 ppm, 102.1% nominal)
- For the 80 ppm treatment: 75.95-85.25 ppm (average 80.7 ppm, 100.9% nominal)
- For the 160 ppm treatment: 148.2-173.9 ppm (average 158.2 ppm, 98.9% nominal)
- For the 320 ppm treatment: 296.7-347.6 ppm (average 322.6 ppm, 100.8% nominal)

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

Standard one-way analysis of variance (ANOVA) was used to analyze body weights, body weight gains, food consumption, hematology, clinical chemistry, organ weights, and organ-body weight percentages for each sex. If the ANOVA was

significant, then a Dunnett's t-test was used for pairwise comparisons between the treated groups and the control.

C. METHODS:

1. Observations

Animals were observed daily for signs of toxicity and mortality. At least once each week, each animal was removed from its cage and carefully examined for abnormalities and clinical signs of toxic effects.

2. Body weight

Animals were weighed 1 day prior to study initiation, at study initiation, and weekly thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly during the exposure period. Food consumption values (g food/mouse/week) were calculated weekly for each animal. Compound intake values (mg/kg/day) were calculated weekly based on consumption, the number of days in the sampling interval, and the average body weight during the interval. Food efficiency was not determined.

4. Ophthalmoscopic examination

Corneal opacity was determined weekly during the exposure period.

5. Blood

Blood was collected from 10 mice/sex/dose level for hematology determinations and an 10 additional mice/sex/dose for clinical chemistry determinations at the termination of the experiment. Because of clotting prior to analysis, hematology was determined on only 8-9 mice each in the control and 80 ppm treatment groups, 6-7 mice in the 160 ppm treatment groups, and 3-5 mice in the 320 ppm treatment groups. The CHECKED (X) parameters were examined in all samples analyzed.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*		Total Cholesterol
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
			Serum protein electrophores
X	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
X	Gamma glutamyl transferase (also GGT, GGPT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was not collected during the study. Urinalysis is not required for subchronic toxicology studies.

7. Sacrifice and Pathology

All animals that died during the study, and the remainder which were sacrificed at the termination of the study were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

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	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*		Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*T
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	XX	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*T
X	Ileum*				
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
X	Rectum*				
XX	Liver*+	XX	Kidneys*+	XX	Adrenal gland*
X	Gall bladder*	X	Urinary bladder*		Lacrimal gland[T]
X	Pancreas*	XX	Testes*+	X	Mammary gland[T]
		X	Epididymides	X	Parathyroids*++
		X	Prostate	X	Thyroids*++
	RESPIRATORY	X	Seminal vesicle		
		XX	Ovaries*+		
X	Trachea*	XX	Uterus*		OTHER
X	Lung*	X	Vagina		
	Nose			X	Bone*
	Pharynx			X	Skeletal muscle*
	Larynx			X	Skin*
				X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations

1. Mortality - One male and one female mouse receiving the 320 ppm treatment died on day 2 of the study. One control female died on day 80. All other mice survived until the termination of the experiment.

2. Clinical Signs - In the 320 ppm treatment groups, one male mouse exhibited mild tremors, diuresis, and anorexia between days 12 and 19 of the study. No other mice exhibited obvious abnormalities that could be related to treatment.

B. Body weight and weight gain

Body weights of both the male and female mice in the 320 ppm treatment group were 26-29% lower (significance at $p < 0.05$) than mice in the control group (Table 2). The depressed weight gain was observed beginning in week 1 of the exposure period for the male group and week 5 for the female group. There were no significant differences in the body weights and

body weight gains of male and female mice in the 40, 80, or 160 ppm treatment groups and the control group.

TABLE 2. AVERAGE BODY WEIGHTS AND BODY WEIGHT GAINS OF MICE AT SELECTED INTERVALS 91 DAYS OF FEEDING[a]

Conc. in Diet (ppm)	Body Weights (g)				Total body weight gain at 13 weeks (g)
	0 Weeks	4 Weeks	8 Weeks	13 Weeks	
Male					
0	29.2	36.6	39.2	41.0	11.8
40	29.5	36.1	39.7	39.9	10.4
80	29.6	35.6	39.0	39.9	10.2
160	28.7	35.8	39.2	40.6	11.9
320	29.2	33.6*	37.0*	38.0*	8.7*
Female					
0	24.4	29.7	32.2	34.1	9.8
40	24.6	30.6	33.3	34.3	9.7
80	24.0	29.8	32.6	33.6	9.6
160	24.5	28.8	31.7	32.5	8.1
320	24.6	28.3	30.8	31.6*	7.0*

[a] Data obtained from Tables 5.3.1, 5.3.2, 5.3.3 and 5.3.4 pages 38-39 and 41-42, in the study report.
* Significantly different ($p < 0.05$) from the untreated control.

C. Food consumption and compound intake

1. Food consumption - Food consumption was comparable between the control and treatment groups. Weekly average food consumption during the 91-day feeding for the male mice was 42.0-51.8 g/mouse/week, and for the female mice was 43.2-62.7 g/mouse/week.

2. Compound consumption - Weekly dietary consumption of AC 303,603 by male and female mice is presented in Table 3.

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TABLE 3: CONSUMPTION OF AC 303,603 BY MICE DURING THE STUDY[a]

Conc. in Diet (ppm)	Nominal Dose to Animal (mg/kg/day)	Weekly Consumption [Average] (mg/kg/day)	
		Male	Female
40	6	6.1-8.9 [7.1]	8.0-10.9 [9.2]
80	12	13.2-17.7 [14.8]	16.9-21.6 [19.3]
160	24	24.5-33.1 [27.6]	35.7-44.9 [40.0]
320	48	55.8-77.1 [62.6]	69.9-86.9 [78.0]

[a] Data obtained from Table 5.2.3, page 37, in the study report.

D. Ophthalmoscopic examination

No treatment-related optical abnormalities were noted.

E. Blood work

1. Hematology - Male mice in the 320 ppm treatment group exhibited significant increases ($p < 0.05$) in the hematocrit and red blood cell counts compared to that of the control group (Table 4).

TABLE 4: AVERAGE HEMATOCRIT AND RBC VALUES IN CONTROL AND TREATED MICE FOLLOWING 91 DAYS OF FEEDING[a]

Conc. in Diet (ppm)	Hematocrit (% ± SD)	RBC (x $10^6/mm^3$ ± SD)
Males		
0	42.0 ± 4.5	7.9 ± 0.6
40	43.1 ± 2.4	8.1 ± 0.5
80	42.8 ± 2.3	8.0 ± 0.4
160	42.4 ± 1.9	8.1 ± 0.1
320	49.5 ± 2.0*	8.8 ± 0.3*
Females		
0	41.7 ± 3.2	8.2 ± 0.5
40	40.7 ± 5.3	7.7 ± 1.0
80	43.2 ± 2.5	7.9 ± 0.3
160	40.6 ± 1.6	7.7 ± 0.3
320	45.2 ± 3.4	8.3 ± 0.3

[a] Data obtained from Tables 5.4.1 and 5.4.2, pages 43-44, in the study report.
* Significantly different ($p < 0.05$) from the untreated control.

In addition, male mice in the 80, 160, or 320 ppm treatment groups had an average 12% more lymphocytes and 27% fewer neutrophiles than mice in the control groups; these differences were significant at the 80 and 160 ppm treatment levels, and were not concentration-dependent. WBC counts were not affected by treatment. No other significant differences were observed between the hematology of control and the 80, 160, or 320 ppm treatment groups, and no significant differences were observed between the hematology of the control and the 40 ppm treatment groups.

The average WBC count of female mice in the 320 ppm treatment group was significantly ($p < 0.05$) higher than that of the control group (6.5 ± 3.7 x $10^3/mm^3$ and 3.1 ± 2.0 x $10^3/mm^3$, respectively). Individual WBC counts for five treated mice at 320 ppm were 2.3, 3.1, 7.2, 9.8, and 10.1 x $10^3/mm^3$; the hematology of the remaining five mice could not be determined because the blood samples were unusable as a

result of clotting. An 8% increase in the hematocrit of female mice in the 320 ppm treatment group did not reach a level of statistical significance; RBC counts appeared to be unaffected. No other differences were observed between the hematology of female mice in the 320 ppm treatment group and the control group. No significant differences were observed between the hematology of female mice in the 40, 80, or 160 ppm treatment and control groups.

2. Clinical Chemistry - Male mice in the 320 ppm treatment group exhibited a statistically significant increase in sodium and decrease in albumin compared to that of the control group. At the termination of the study, sodium concentrations averaged 156.0 and 153.8 meq/L for the 320 ppm treatment and control groups, respectively, and albumin concentrations averaged 2.2 and 2.5 g/dL, respectively. No other significant differences were observed between the clinical blood chemistry of male mice in the 320 ppm treatment group and the control group, and no significant differences were observed between the clinical blood chemistry of male mice in the 40, 80, or 160 ppm treatment and control groups.

Female mice in the 320 ppm treatment group exhibited statistically significant increases in potassium and total protein compared to that of the control group. At the termination of the study, potassium concentrations averaged 10.6 and 8.0 meq/L for the 320 ppm treatment and control groups, respectively, and total protein concentrations averaged 5.9 and 5.4 g/dL, respectively. Female mice in the 160 ppm treatment group exhibited significant decreases in albumin compared to that of the control group. Albumin concentrations were 2.7 and 2.9 g/dL for the 160 ppm treatment and control groups, respectively. No other significant differences were observed between the clinical blood chemistry of female mice in the 160 or 320 ppm treatment groups and the control group, and no significant differences were observed between the clinical blood chemistry of female mice in the 40 or 80 ppm treatment and control groups.

F. Urinalysis

Urine was not collected during the study.

G. Sacrifice and Pathology:

1. Organ weight - Relative liver weights were greater in male and female mice from all treatment groups compared to those of the control groups; these increases were concentration-related, and were statistically significant ($p < 0.05$) for both the male 160, and the male and female 320 ppm treatment

groups (Table 5). Relative spleen weights were significantly greater in male mice in the 160 or 320 ppm treatment groups compared to those of the control groups (Table 5). Absolute spleen weights were significantly greater in male mice in the 160 ppm treatment group compared to those of the control group.

TABLE 5: AVERAGE RELATIVE LIVER AND SPLEEN WEIGHTS OF CONTROL AND TREATED MICE FOLLOWING 91 DAYS OF FEEDING[a]

Conc. in Diet (ppm)	Liver (% of body weight ± SD)	Spleen (% of body weight ± SD)
Male		
0	6.03 ± 0.58	0.32 ± 0.05
40	6.32 ± 0.67	0.34 ± 0.07
80	6.15 ± 0.50	0.36 ± 0.07
160	6.65 ± 0.95*	0.39 ± 0.08*
320	6.82 ± 0.51*	0.39 ± 0.06*
Female		
0	5.79 ± 0.58	0.46 ± 0.09
40	6.16 ± 0.53	0.47 ± 0.07
80	6.02 ± 0.70	0.45 ± 0.09
160	6.13 ± 0.51	0.47 ± 0.11
320	6.84 ± 0.68*	0.52 ± 0.10

[a] Data obtained from MRID 43492830, Tables 5.6.1 and 5.6.2, pages 47-48.
* Significantly different ($p < 0.05$) from the untreated control.

No other differences in the relative or absolute organ weights were observed between the mice in any treatment groups and the control groups.

2. Gross pathology - No pathological differences were observed between mice in the treatment and control groups. Tissue discoloration and other abnormalities occurred randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Microscopic changes characterized as

hepatic parenchymal cell hypertrophy were observed in the livers of ≥20% of the male mice in the 80, 160, or 320 ppm treatment groups, and in the livers of female mice in the 160 or 320 ppm treatment groups (Table 6). The hypertrophy was attributed to an increase in cytoplasmic volume, and was thought to have been produced by enzyme induction. Focal lymphoid cell infiltrate, focal/localized hepatic cell necrosis, hepatic cell coagulative necrosis were sporadic and did not appear to be related to pesticide ingestion. There was no evidence of bile retention, progressive degenerative change, proliferative change, or toxic necrosis.

TABLE 6: INCIDENCE OF HEPATIC CELL HYPERTROPHY IN THE LIVERS OF CONTROL AND TREATED MICE[a]

Conc. in Diet (ppm)	Affected Animals per Total	
	Males	Females
0	0/20	0/20
40	1/20	0/20
80	6/20	0/20
160	13/20	4/20
320	19/20[b]	10/20[b]

[a] Data obtained from Figure 1, page 56, in the study report.
[b] Only 19 mice of each sex in the 320 ppm treatment groups survived until study termination. Hepatic cell hypertrophy was not observed when two mice that died after 2 days of treatment were autopsied.

Microscopic changes characterized as spongiform(encephalo)myelopathies were observed in the white matter of the brain and myelin of the spinal cord (3 levels) of male and female mice at the 320 ppm treatment level, and in the myelin of the spinal cord of one male at the 160 ppm treatment level. Two mice, one male and one female in the 320 ppm treatment groups, who died on day 2 of the study exhibited no brain or spinal cord spongiform changes.

TABLE 7: INCIDENCE OF SPONGIFORM ENCEPHALOPATHY IN MICE IN THE 320 PPM TREATMENT GROUP[a]

Affected Tissue	Affected Animals per Total	
	Males	Females
Brain (3 levels)	19/20[b]	19/20[b]
Spinal cord/cervical	18/20	19/19
Spinal cord/thoracic	18/20	19/19
Spinal cord/lumbar	18/20	19/19
Peripheral nerve/sciatic	0/19	0/19
Optic nerve	0/20	0/20

[a] Data obtained from MRID 43492830, Figure 2, page 57.
[b] Only 19 mice of each sex survived until study termination. Spongiform encephalopathy was not observed when two mice that died after 2 days of treatment were autopsied.

All other tissue abnormalities, including those in the spleen, occurred randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in mice in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study authors concluded that the NOEL was 40 ppm (equivalent to 7.1 mg/kg/day in males, 9.2 mg/kg/day in females). On the basis of these study results, dose levels of 0, 20, 120, and 240 were chosen for use during the chronic/oncogenicity study with mice.

B. Reviewer's Discussion

Male mice fed AC 303,630 at 80 ppm, and male and female mice fed AC 303,630 at 160 or 320 ppm exhibited a toxic response to the test compound. Two mice, one male and one female, dosed at the 320 ppm level died after only 2 days of feeding, apparently as a result extreme sensitivity to AC 303,630. One male mouse in the 320 ppm treatment group exhibited mild tremors, diuresis, and anorexia during the

second week of the study, but recovered. All other mice survived without obvious symptoms until the termination of the study.

Body weight gains of both the male and female mice in the 320 ppm treatment group were 26-29% lower than mice in the control group. No other significant differences were observed between the body weights and body weight gains of the treatment and control groups. Food consumption was comparable between the control and treatment groups.

Male mice in the 320 ppm treatment group exhibited significant increases in the hematocrit and red blood cell counts compared to that of the control group. An 8% increase in the hematocrit of female mice in the 320 ppm treatment group did not reach a level of statistical significance; RBC counts appeared to be unaffected. The average WBC count of female mice in the 320 ppm treatment group was significantly higher than that of the control group; individual counts varied from 2.3 to 10.1 x $10^3/mm^3$. The WBC counts of males were unaffected; significant differences in the relative concentrations of lymphocytes and neutrophiles in mice in the 80 or 160 ppm treatment groups were not concentration-dependent and were not expected to be treatment-related. No other significant differences were observed between the hematology of the treatment and control groups.

Male mice in the 320 ppm treatment group exhibited a statistically significant increase in sodium and decrease in albumin compared to that of the control group. Female mice in the 320 ppm treatment group exhibited statistically significant increases in potassium and total protein compared to that of the control group. Female mice in the 160 ppm treatment group exhibited significant decreases in albumin compared to that of the control group. These differences did not appear to be a direct result of treatment. No other significant differences were observed between the clinical blood chemistry of the treatment and control groups.

Relative liver weights were greater in male and female mice from all treatment groups compared to those of the control groups; these increases were concentration-related, and were statistically significant for both the male 160, and the male and female 320 ppm treatment groups. Microscopic AC 303,630 concentration-related changes characterized as hepatic parenchymal cell hypertrophy were observed in the livers of ≥20% of the male mice in the 80, 160, or 320 ppm treatment groups, and in the livers of female mice in the 160 or 320 ppm treatment groups. Relative spleen weights were significantly greater in male mice in the 160 or 320

ppm treatment groups compared to those of the control groups; no treatment-related tissue abnormalities were associated with the spleen.

Microscopic changes characterized as spongiform(encephalo)myelopathies were observed in the white matter of the brain and myelin of the spinal cord (3 levels) of all of the male and female mice in the 320 ppm treatment groups that survived until the termination of the study. The two mice in the 320 ppm treatment groups who died on day 2 of the study exhibited no brain or spinal cord spongiform changes. The study authors stated that these changes did not correlate with clinical signs of ataxia or decreased motor activity in the affected animals, and may have been "partly exaggerated by artifacts caused by immersion fixation technique[s]". In the 18-month chronic/oncogenicity study with mice, brain vacuolation was observed in 24-43% of the animals in the 120 ppm (16.6 mg/kg/day) treatment group and 75-90% of those in the 240 ppm (34.5 mg/kg/day) treatment group.

IV. Study deficiencies

No significant deficiencies were noted in this study.

The study authors failed to measure blood calcium, which is required by Subdivision F guidelines. This omission did not affect the interpretation of the study results.

DATA EVALUATION RECORD

PIRATE

Study Type: 82-2; Repeated Dose Dermal Toxicity - 28 Day Rabbit

Work Assignment No. 1-1H (MRID 43492831)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Signature: William J. Spangler
Date: 11/6/95

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 11/6/95

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: W. Greear, M.P.H.,D.A.B.T. William B. Greear Date 5/14/96
Review Section IV, Toxicology Branch I (7509C)

EPA Secondary Reviewer: M. Copley, D.V.M., D.A.B.T. MCopl, Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Repeated dose dermal toxicity - 28-day rabbit
OPPTS Number: 870.3200 OPP Guideline Number: §82-2

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.5% ai)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Blaszcak, D.L. (1993) A 28-Day Dermal Toxicity Study with AC 303,630 in Rabbits. Bio/dynamics, Inc., Mettlers Road, East Millstone, NJ, 08875-2360. Laboratory Project ID Study 92-2162. October 13, 1993. MRID 43492831. Unpublished.

SPONSOR: American Cyanamid Company; P.O. Box 400; Princeton, NJ, 08543-0400.

EXECUTIVE SUMMARY:

In a repeated dose dermal toxicity study (MRID 43492831), AC 303,630 (Pirate; 94.5% a.i., Lot No. AC 7504-59A) was applied to the shaved skin of six New Zealand White rabbits/sex/dose at dose levels of 0, 100, 400, or 1000 mg/kg, 6 hours/day, 5 days/week for 4 weeks.

Rabbits of both sexes in the 400 and 1000 mg/kg treatment groups exhibited statistically significant and concentration-related increases in serum cholesterol (60-95%) and relative liver weights (22-43%), and suffered from cytoplasmic vacuolation of the liver. The vacuolation of the liver was minimal to slight for male and female rabbits in the 400 mg/kg treatment groups (4 of 12 animals), and minimal to moderately severe for the 1000 mg/kg treatment groups (8 of 11 animals). In addition, female rabbits in the 1000 mg/kg treatment group exhibited a 97% increase in serum alanine aminotransferase ($p < 0.05$) concentrations. No differences were observed between rabbits in the 100 mg/kg treatment groups and the control groups. **The LOEL is 400 mg/kg for both sexes, based on changes in liver chemistry and morphology. The NOEL is 100 mg/kg.**

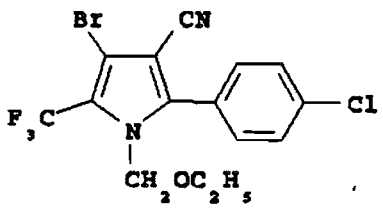
This subchronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a repeated dose dermal toxicity study (§82-2) in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 303,630
   Description: Tan solid
   Lot/Batch #: AC 7504-59A
   Purity: 94.5% ai
   Stability of compound: "Documentation of the stability of the test substance prior to study initiation was the responsibility of the sponsor."
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Rabbits
   Strain: New Zealand White
   Age and weight at study initiation: approximately 3.5 months; body weight range of 2.0 to 2.5 kg for males and 2.0 to 2.5 kg for females
   Source: Hazleton Research Products, Inc., Denver, Pennsylvania
   Housing: Individually housed in elevated stainless steel, wire mesh cages
   Diet: Purina Lab Rabbit Chow HF (Purina #5326) ad libitum
   Water: tap water ad libitum
   Environmental conditions:
      Temperature: 64-73 F (18-23 C)
      Humidity: 32-80%
      Air Changes: Not specified
      Photoperiod: 12 hour light/dark cycle
   Acclimation period: approximately 3 weeks prior to testing

B. STUDY DESIGN:

1. In life dates - For males - Start: 9/8/92 End:10/5/92
For females - Start: 9/9/92 End: 10/6/92

2. Animal assignment

Of 54 original rabbits (27/sex), 24 rabbits of each sex were selected for use on the basis of pretest ophthalmoscopic examinations and clinical laboratory data. The selected rabbits were assigned to the test groups in Table 1 using a computerized randomization procedure, so the body weight means for each group were comparable.

TABLE 1: STUDY DESIGN[a]

Test Group	Dose to Animal (mg/kg)	Animals Assigned	
		Male	Female
I Control	0	6	6
II Low	100	6	6
III Mid	400	6	6
IV High	1000	6	6

[a] Dose levels were selected on the basis of the acute dermal toxicity study. The results of the acute dermal toxicity study were not reported.

3. Preparation and treatment of animal skin

Approximately 24 hours before the initial exposure, and weekly thereafter, the hair of each rabbit was "closely clipped" from the dorsal surface and sides from scapular to pelvic area using electric clippers, so that 10-15% of the body surface was exposed. AC 303,630 was applied dry to gauze dressing, then moistened with 0.9% saline (1 mL saline/1 g AC 303,630). The treated dressings were then attached to the exposed skin of the rabbits using gauze dressing, an "impervious material", and nonirritating tape, and the rabbits were fitted with Elizabethan collars. The rabbits were treated for 6 hours/day on 5 days each week, at approximately the same time each day. Following each 6-hour exposure, the dressings were removed from the rabbits and the treated area "thoroughly cleansed" with soap and water.

Rabbits in the control group were exposed to pesticide-free, saline-moistened gauze dressings, but otherwise handled as described for the treated animals.

4. Statistics

The equality of means for data from the treatment groups was established using Bartlett's test of homogeneity of variances. If the variances were found to be equal, the data were analyzed by standard one-way ANOVA followed by Dunnett's t-test. If variances proved to be unequal, the data were analyzed by the Kruskal-Wallis test followed by Dunn's summed rank test. Trends related to the dose level were analyzed using either standard regression techniques with a test for trend and lack of fit, or by Jonckheere's test for monotonic trend to determine significance. Bartlett's test was conducted at the 1%, two-sided risk level; all other tests were conducted at the 5% and 1%, two-sided risk levels.

C. METHODS

1. Observations

Animals were observed twice daily for signs of mortality, and once daily for signs of toxicity and the presence of dermal irritation. The rabbits were evaluated once each week for dermal irritation using the Draize method.

2. Body weight

Animals were weighed prior to the initial treatment, weekly during treatment, and at study termination following fasting.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly beginning 1 week prior to the initial treatment. Mean daily diet consumption was calculated as g food/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on each rabbit prior to and at the termination of the study.

5. Blood

Blood was collected from, and hematology and clinical chemistry studies were performed on all rabbits prior to initiation of the study, and on all surviving animals at

study termination. Animals were fasted overnight prior to the collection of blood from the auricular artery. The CHECKED (X) parameters were examined in all samples analyzed.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume
X	Platelet count*		(MCV)Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total cholesterol
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
			Serum protein electrophores
X	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

6. Urinalysis*

Urine was collected from fasted animals at the termination of the study. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
	Volume	X	Ketones
	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Urinalysis is not required for repeated dose dermal toxicity studies.

7. Sacrifice and Pathology

All animals that died during the study, and the remainder which were sacrificed at the termination of the study were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta		Brain
	Salivary glands		Heart		Periph.nerve
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes		
	Duodenum		Spleen		Pituitary
	Jejunum		Thymus		Eyes (optic n.)
	Ileum				
	Cecum				
	Colon		UROGENITAL		GLANDULAR
	Rectum				
XX	Liver*+	XX	Kidneys*+	XX	Adrenal gland
	Gall bladder		Urinary bladder		Lacrimal gland
	Pancreas	XX	Testes*+		Mammary gland
			Epididymides		Parathyroids
			Prostate		Thyroids
	RESPIRATORY		Seminal vesicle		
			Ovaries		
	Trachea		Uterus		OTHER
X	Lung*				
	Nose				Bone*
	Pharynx				Skeletal muscle*
	Larynx			X	Skin*
				X	All gross lesions and masses*

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.
+ Organ weight required in repeated dose dermal toxicity studies.

II. RESULTS

A. Observations

1. Mortality - One female rabbit in the 1000 mg/kg treatment group died of accidental trauma on day 9 of exposure.

2. Clinical Signs - No rabbits exhibited obvious treatment-related abnormalities during the study.

B. Body weight and weight gain

There were no significant differences in the terminal body weights and body weight gains of male and female rabbits in the 100, 400, and 1000 mg/kg treatment groups and the control groups. Female rabbits in the 1000 mg/kg treatment group did not gain weight during the first 3 weeks of the experiment; however, this group gained weight during the fourth week and was similar in weight to the controls at study termination.

At the termination of the experiment, the average weight of each rabbit in the male control group was 2.4 kg, in the 100 mg/kg treatment group was 2.3 kg, in the 400 mg/kg treatment group was 2.3 kg, and in the 1000 mg/kg treatment group was 2.4 kg. At the termination of the experiment, the average weight of each rabbit in the female control group was 2.4 kg, in the 100 mg/kg treatment group was 2.4 kg, in the 400 mg/kg treatment group was 2.4 kg, and in the 1000 mg/kg treatment group was 2.4 kg.

C. Food consumption

In all treatment groups, food consumption was generally comparable to that of the control group. Daily average food consumption for the male control group was 48.1-53.6 g/kg/day, and for the treated groups was 47.8-54.1 g/kg/day. Daily average food consumption for the female control group was 49.7-52.3 g/kg/day, and for the treated groups was 49.7-54.1 g/kg/day.

D. Ophthalmoscopic examination

No abnormalities were observed between the treated and control groups at the termination of the study.

E. Blood work

1. Hematology - Female rabbits in the 1000 mg/kg treatment group exhibited a significant decrease ($p < 0.05$) in the red blood cell counts compared to that of the control group (Table 2).

TABLE 2: AVERAGE CONCENTRATION OF ERYTHROCYTES (RBC) IN CONTROL AND TREATED RABBITS AT STUDY TERMINATION[a]

Treatment Rate (mg/kg)	Erythrocytes (mil/uL ± SD)	
	Males	Females
0	5.90 ± 0.55	5.89 ± 0.24
100	6.20 ± 0.40	5.76 ± 0.23
400	6.08 ± 0.59	5.57 ± 0.32
1000	5.62 ± 0.47	5.37 ± 0.36*

[a] Data extracted from Appendix H, pages 96-97, in the study report.
* Significantly different ($p < 0.05$) from the untreated control.

No other significant differences were observed between the hematology of female rabbits in the 1000 mg/kg treatment group and the control group, and no significant differences were observed between the hematology of female rabbits in the 100 or 400 mg/kg treatment and control groups. No significant differences were observed between the hematology of male rabbits in the 100, 400, or 1000 mg/kg treatment and control groups.

2. Clinical Chemistry - Rabbits of both sexes in the 400 and 1000 mg/kg treatment groups exhibited a significant and concentration-related increases ($p < 0.05$ and < 0.01, respectively) in mean serum cholesterol concentrations compared to that of the control group at study termination (Table 3). The average glucose concentrations of male rabbits in the 100, 400, and 1000 mg/kg treatment groups were 11% lower than those of the control group; the decrease was significant ($p < 0.05$) only for the 400 mg/kg treatment group and was not correlated with concentration levels. Female rabbits in the 1000 mg/kg treatment group exhibited a significant increase ($p < 0.05$, respectively) in the mean serum alanine aminotransferase (ALT) concentration compared to that of the control group.

TABLE 3: AVERAGE SERUM CHOLESTEROL, GLUCOSE, AND ALANINE AMINOTRANSFERASE (ALT) CONCENTRATIONS IN CONTROL AND TREATED RABBITS AT STUDY TERMINATION[a]

Treatment Rate (mg/kg)	Cholesterol (mg/dL ± SD)	Glucose (mg/dL ± SD)	ALT (IU/L ± SD)
Male			
0	58 ± 18	143 ± 16	43 ± 15
100	59 ± 13	129 ± 5	54 ± 22
400	93 ± 29*	125 ± 11*	65 ± 35
1000	108 ± 9**	128 ± 8	46 ± 10
Female			
0	67 ± 13	134 ± 9	39 ± 13
100	66 ± 21	120 ± 8	61 ± 17
400	115 ± 38*	138 ± 16	57 ± 27
1000	131 ± 33**	132 ± 10	77 ± 14*

[a] Data extracted from Appendix J, pages 126-129 in the study report.
* Significantly different ($p < 0.05$) from the untreated control.
** Significantly different ($p < 0.01$) from the untreated control.

No other significant differences were observed between the clinical blood chemistry of male and female rabbits in the 400 or 1000 mg/kg treatment groups and the control group, and no significant differences were observed between the clinical blood chemistry of male and female rabbits in the 100 mg/kg treatment and control groups.

F. Urinalysis

No significant differences were observed between urine from the treated and control groups at the termination of the study.

G. Sacrifice and Pathology

1. Organ weight - Male and female rabbits in the 400 and 1000 mg/kg treatment groups exhibited significant increases in average and/or relative liver weights compared to that of

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the control group at study termination (Table 4).

TABLE 4: AVERAGE MEAN ABSOLUTE AND RELATIVE LIVER WEIGHTS OF CONTROL AND TREATED RABBITS AT STUDY TERMINATION[a]

Treatment Rate (mg/kg)	Absolute Liver Weight (g ± SD)	Relative Liver Weight (% body wt ± SD)
Male		
0	53.6 ± 4.1	2.28 ± 0.12
100	55.4 ± 4.6	2.38 ± 0.12
400	61.8 ± 6.6	2.80 ± 0.20**
1000	72.3 ± 7.6**	3.05 ± 0.16**
Female		
0	58.3 ± 3.5	2.51 ± 0.08
100	57.8 ± 6.4	2.46 ± 0.24
400	67.8 ± 7.1*	2.94 ± 0.17**
1000	78.2 ± 6.8**	3.38 ± 0.34**

[a] Data extracted from Appendix L, pages 168-170, in the study report.
* Significantly different ($p < 0.05$) from the untreated control.
** Significantly different ($p < 0.01$) from the untreated control.

No other differences in the relative or absolute organ weights were observed between the rabbits in the 400 and 1000 mg/kg treatment groups and the control groups. No differences in the relative or absolute organ weights were observed between the rabbits in the 100 mg/kg treatment groups and the control groups.

2. Gross pathology - Female rabbits in the 400 and 1000 mg/kg treatment groups (1/6 and 3/5, respectively) had discolored livers compared to that of the control group at study termination. No pathological differences were observed between female rabbits in the 100 mg/kg treatment group and the control group, or between male rabbits in any treatment group and the control group. Other abnormalities occurred randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Cytoplasmic vacuolation of the liver was observed in male and female rabbits in the 400 and 1000 mg/kg treatment groups (Table 5). These changes were described as being slight to moderately severe in the females in the 1000 mg/kg treatment group, and minimal or slight in other affected groups. The cytoplasmic vacuoles were different sizes, and the affected cells did not exhibit a consistent lobular pattern.

TABLE 5: INCIDENCE OF CYTOPLASMIC VACUOLATION OF THE LIVERS OF CONTROL AND TREATED RABBITS AT STUDY TERMINATION[a]

Treatment Rate (mg/kg)	Affected Animals per Total	
	Males	Females
0	0/6	0/6
100	0/6	0/6
400	1/6	3/6
1000	4/6	4/5

[a] Data extracted from Pathology Report Table IV, pages 249-250, in the study report.

All other tissue abnormalities occurred randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in rabbits in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the NOEL of AC 303,630 was 100 mg/kg for rabbits under the conditions of this study. The basis of this decision was the increased absolute and/or relative liver weights, changes in liver morphology, and increases in serum cholesterol observed in rabbits in the 400 or 1000 mg/kg treatment groups.

B. Reviewer's Conclusions

Rabbits in the 100 mg/kg treatment groups appeared to be unaffected by the test substance. Rabbits of both sexes in the 400 or 1000 mg/kg treatment groups exhibited significant

and concentration-related increases in serum cholesterol, increased liver weights, and changes in liver morphology. Male and female rabbits in the 1000 ppm treatment groups exhibited significant increases in average and relative liver weights compared to that of the control group at study termination. Four of the six males had minimal to slight cytoplasmic vacuolation of the liver; three of five females had discolored livers, and four of five had slight to moderately severe cytoplasmic vacuolation of the liver. Males in the 400 ppm treatment group exhibited a significant increase in relative liver weights, and one of six had minimal to slight cytoplasmic vacuolation of the liver. Females in the 400 mg/kg treatment group exhibited significant increases in average and relative liver weights, one of six had discolored livers, and three of six had minimal to slight cytoplasmic vacuolation of the liver.

During the study, no rabbits died of treatment-related causes and none exhibited obvious abnormalities. There were no significant differences in body weights or body weight gains by study termination. No significant, treatment-related differences other than those mentioned were observed between rabbits in the treated and control groups.

IV. Study deficiencies

No significant deficiencies were noted in this study.

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DATA EVALUATION RECORD

PIRATE

Study Type: 82-2; Repeated Dose Dermal Toxicity - 28 Day Rabbit

Work Assignment No. 1-1I (MRID 43492832)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
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Dynamac Corporation
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Primary Reviewer:
Kathleen Ferguson, Ph.D.

Signature: Kathleen Ferguson
Date: 11/6/95

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Date: 11/6/95

Project Manager:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 11/6/95

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date: 11/6/95

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: W. Greear, M.P.H., D.A.B.T. William B. Greear Date 5/14/96
Review Section IV, Toxicology Branch I (7509C)

EPA Secondary Reviewer: M. Copley, D.V.M., D.A.B.T. M Copley Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Repeated dose dermal toxicity - 28-day rabbit
OPPTS Number: 870.3200 OPP Guideline Number: §82-2

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 33.3% ai)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Blaszcak, D.L. (1994) A 28-Day Dermal Toxicity Study with AC 303,630 3SC in Rabbits. Bio/dynamics, Inc., Mettlers Road, East Millstone, NJ, 08875-2360. Laboratory Project ID Study 92-2163. March 18, 1994. MRID 43492832. Unpublished.

SPONSOR: American Cyanamid Company; P.O. Box 400; Princeton, NJ, 08543-0400.

EXECUTIVE SUMMARY:

In a repeated dose dermal toxicity study (MRID 43492832), AC 303,630 (Pirate; 33.3% a.i., Lot No. AC 8053-87A) was applied to the shaved skin of six New Zealand White rabbits/sex/dose at dose levels of 0, 100, 400, or 1000 mg/kg 6 hours/day, 5 days/week for 4 weeks.

No treatment-related effects were observed. No animals died during the study. There were no significant differences in body weights or body weight gains by study termination. No treatment-related effects were observed in hematology, blood chemistry factors, the eyes, or urinalysis; there were no changes in organ weight or morphology. **The LOEL is >1000 mg/kg for rabbits. The NOEL is 1000 mg/kg for rabbits.**

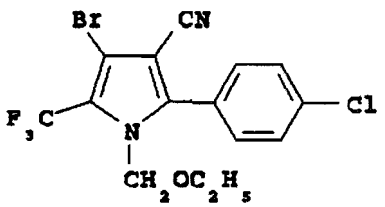
This subchronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a repeated dose dermal toxicity study (82-2) in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 303,630 3SC
   Description: tan liquid
   Lot/Batch #: AC 8053-87A
   Purity: 33.3% ai
   Stability of compound: "Documentation of the stability of the test substance prior to study initiation was the responsibility of the sponsor."
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Rabbits
   Strain: New Zealand White
   Age and weight at study initiation: approximately 3 months; body weight range of 2.1 to 2.4 kg for males and 2.0 to 2.5 kg for females
   Source: Hazleton Research Products, Inc., Denver, Pennsylvania
   Housing: Individually housed in elevated stainless steel, wire mesh cages
   Diet: Purina Certified Rabbit Chow No. 5325 (High Fiber) ad libitum
   Water: tap water ad libitum
   Environmental conditions:
   - Temperature: 60-74 F (16-23 C)
   - Humidity: 42-66%
   - Air Changes: Not specified
   - Photoperiod: 12 hour light/dark cycle

   Acclimation period: Approximately 3 weeks prior to testing

B. STUDY DESIGN:

1. In life dates - Start: 10/21/92 End: 11/17/92

2. Animal assignment

Of 62 original rabbits (31/sex), 24 rabbits of each sex were

selected for use on the basis of pretest ophthalmoscopic examinations and clinical laboratory data. The selected rabbits were assigned using a computerized randomization procedure to the test groups in Table 1 so the body weight means for each group were comparable.

TABLE 1: STUDY DESIGN[a]

Test Group	Dose to Animal (mg/kg)	Animals Assigned	
		Male	Female
I Control	0	6	6
II Low	100	6	6
III Mid	400	6	6
IV High	1000	6	6[b]

[a] Dose levels were selected on the basis of the acute dermal toxicity study. The results of the acute dermal toxicity study were not reported.
[b] One additional female was used to replace a female in the 1000 mg/kg treatment group that was found dead of accidental trauma on day 3.

3. Preparation and treatment of animal skin

Approximately 24 hours before the initial exposure, and weekly thereafter, the hair of each rabbit was "closely clipped" from the dorsal surface and sides from scapular to pelvic area using electric clippers, so that 10-15% of the body surface was exposed. AC 303,630 was applied directly to the exposed skin. The treated area was then covered with a porous gauze dressing, an "impervious material", and nonirritating tape, and the rabbits were fitted with Elizabethan collars. The rabbits were treated for 6 hours/day on 5 days each week, at approximately the same time each day. Following each 6-hour exposure, the dressings were removed from the rabbits and the treated area "thoroughly cleansed" with soap and water.

Rabbits in the control group were not treated with any substance, but were wrapped with gauze dressing, "impervious material", and nonirritating tape, and handled as described for the treated animals.

4. Statistics

The equality of means for data from the treatment groups was established using Bartlett's test of homogeneity of variances. If the variances were found to be equal, the data were analyzed by standard one-way ANOVA followed by Dunnett's t-test. If variances proved to be unequal, the data were analyzed by the Kruskal-Wallis test followed by Dunn's summed rank test. Trends related to the dose level were analyzed using either standard regression techniques with a test for trend and lack of fit, or by Jonckheere's test for monotonic trend to determine significance. Bartlett's test was conducted at the 1%, two-sided risk level; all other tests were conducted at the 5% and 1%, two-sided risk levels.

C. METHODS

1. Observations

Animals were observed once each day for signs of mortality, toxicity, and the presence of dermal irritation. The rabbits were evaluated once each week for dermal irritation using the Draize method.

2. Body weight

Animals were weighed prior to the initial treatment, weekly during treatment, and at study termination following fasting.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly beginning 1 week prior to the initial treatment. Mean daily diet consumption was calculated as g food/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on each rabbit prior to and at the termination of the study.

5. Blood

Blood was collected from, and hematology and clinical chemistry studies were performed on all rabbits prior to initiation of the study, and on all surviving animals at study termination. Animals were fasted overnight prior to the collection of blood from the auricular artery. The CHECKED (X) parameters were examined in all samples analyzed.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume
X	Platelet count*		(MCV)Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total cholesterol
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
			Serum protein electrophores
X	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

6. Urinalysis*

Urine was collected from fasted animals at the termination of the study. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
	Volume	X	Ketones
	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Urinalysis is not required for repeated dose dermal toxicity studies.

7. Sacrifice and Pathology

All animals that died during the study, and the remainder which were sacrificed at the termination of the study were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta		Brain
	Salivary glands		Heart		Periph.nerve
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes		
	Duodenum		Spleen		Pituitary
	Jejunum		Thymus		Eyes (optic n.)
	Ileum				
	Cecum				
	Colon		UROGENITAL		GLANDULAR
	Rectum				
XX	Liver*+	XX	Kidneys*+	XX	Adrenal gland
	Gall bladder		Urinary bladder		Lacrimal gland
	Pancreas	XX	Testes*+		Mammary gland
			Epididymides		Parathyroids
			Prostate		Thyroids
	RESPIRATORY		Seminal vesicle		
			Ovaries		
	Trachea		Uterus		OTHER
X	Lung*				
	Nose				Bone*
	Pharynx				Skeletal muscle*
	Larynx			X	Skin*
				X	All gross lesions and masses*

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.
+ Organ weight required in repeated dose dermal toxicity studies.

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II. RESULTS

A. Observations

1. Mortality - One male rabbit in the 100 mg/kg treatment group died of accidental trauma on day 17 of exposure. One female rabbit in the 1000 mg/kg treatment group died of accidental trauma on day 3 of exposure.

2. Clinical Signs - Several male and female rabbits in all treatment groups exhibited mild (barely perceptible to well formed) erythema during the study. The number of affected animals and the severity of the erythema increased through week 2, then decreased by week 3. By week 4, erythema was observed only in one female in the 400 mg/kg treatment group. One female in the control group exhibited mild erythema at week 3. In addition, one female in the 1000 mg/kg treatment group exhibited staining of the anogenital area on several occasions between days 10 and 22 which was attributed to dosing trauma. Two females in the 1000 mg/kg treatment group and one in the 400 mg/kg treatment group exhibited nasal discharges on days 19-21.

B. Body weight and weight gain

There were no significant differences in the terminal body weights and body weight gains of male and female rabbits in the 100, 400, and 1000 mg/kg treatment groups and the control groups. At the termination of the experiment, the average weight of male and female rabbits in the treatment and control groups was 2.3-2.4 kg.

C. Food consumption

In all treatment groups, food consumption was generally comparable to that of the control group. Daily average food consumption for the male control group was 48.9-53.3 g/kg/day, and for the treated groups was 48.0-54.2 g/kg/day. Daily average food consumption for the female control group was 52.1-55.9 g/kg/day, and for the treated groups was 51.1-55.4 g/kg/day.

D. Ophthalmoscopic examination

No abnormalities were observed between the treated and control groups at the termination of the study.

E. Blood work

1. Hematology - No significant differences were observed between the hematology of male and female rabbits in the 1000, 400, or 100 mg/kg treatment and control groups.

2. Clinical Chemistry - Mean blood urea nitrogen and creatinine concentrations were significantly ($p < 0.01$ and < 0.05, respectively) greater in male rabbits in the 1000 mg/kg treatment group compared to that of the control group (Table 2). These values, however, remained within normal ranges for rabbits.

TABLE 2: AVERAGE BLOOD UREA NITROGEN AND CREATINE CONCENTRATIONS IN CONTROL AND TREATED MALE RABBITS AT STUDY TERMINATION[a]

Treatment Rate (mg/kg)	Urea Nitrogen (mg/dL ± SD)	Creatine (mg/dL ± SD)
0	16.7 ± 2.1	1.2 ± 0.1
100	18.7 ± 1.0	1.1 ± 0.1
400	19.0 ± 2.3	1.1 ± 0.1
1000	21.2 ± 1.7**	1.4 ± 0.1*

[a] Data extracted from Appendix J, pages 134-135, in the study report.
* Significantly different from the untreated control at the 5% level.
** Significantly different from the untreated control at the 1% level.

No other significant differences were observed between the clinical blood chemistry of male rabbits in the 1000 mg/kg treatment group and the control group. No significant differences were observed between the clinical blood chemistry of female rabbits in the 1000 mg/kg treatment group or male and female rabbits in the 400 and 100 mg/kg treatment groups and the control groups.

F. Urinalysis

No significant differences were observed between urine from the treated and control groups at the termination of the study.

G. Sacrifice and Pathology

1. Organ weight – No differences in the relative or absolute organ weights were observed between the rabbits in any treatment group and the control group.

2. Gross pathology - All macroscopic abnormalities that were noted occurred randomly and sporadically in all study

groups.

3. Microscopic pathology

a) Non-neoplastic - Mild hyperkeratosis was observed in the treated skin of three males in the 1000 mg/kg treatment group and in one male in the 100 mg/kg treatment group. Mild acanthosis was observed in three males in the 1000 mg/kg treatment group; and mild to severe acanthosis was observed in one male in the 400 mg/kg treatment group and two males in the 100 mg/kg treatment group. Hyperkeratosis and/or acanthosis were observed in one female in the 1000 mg/kg treatment group and 2 females in the 100 mg/kg treatment group. No rabbits in the control group exhibited either hyperkeratosis or acanthosis.

All other tissue abnormalities occurred randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in rabbits in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the NOEL of AC 303,630 was 1000 mg/kg for rabbits under the conditions of this study. The basis of this decision was that AC 303,630 produced no conclusive adverse effects on the rabbits at the highest dose level (1000 mg/kg) examined.

B. Reviewer's Discussion

No treatment-related effects were observed at the 100, 400, or 1000 mg/kg treatment levels. There were no significant differences in body weights or body weight gains between the treated and control rabbits at study termination. No treatment-related effects were observed in hematology. Although blood urea nitrogen and creatinine concentrations increased in male rabbits with increasing dosage levels, these values remained within normal ranges. There were no other significant differences in blood chemistry factors between the treated and control rabbits. No treatment-related effects were observed in the eyes, or urine chemistry; there were no changes in organ weight or morphology.

B. Study deficiencies

No significant deficiencies were noted in this study.

DATA EVALUATION REPORT

PIRATE

Study Type: 82-7SS , A One-Year Dietary Neurotoxicity Study in Rats

Dynamac Study No. 101J/MRID 43492833

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: W. Greear, MPH, DABT William B. Greear, Date 5/21/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: L. Hansen, Ph.D. M Hansen, Date 6/6/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: One-Year Dietary Neurotoxicity Study in Rats
OPPTS Number: 870.6200 OPP Guideline Number: § 82-7SS[1]

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.5% ai)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Foss, J.A. (1994) A one-year dietary neurotoxicity study with AC 303,630 in rats. Argus Research Laboratories; 905 Sheehy Drive; Horsham, PA; 19044. Argus Research Laboratories, Inc. Number 101-019. May 10, 1994. MRID 43492833. Unpublished.

SPONSOR: American Cyanamid Company; P.O. Box 400; Princeton, NJ 08543-0400.

EXECUTIVE SUMMARY:

In a one-year dietary neurotoxicity study (MRID 43492833), AC 303,630 (Pirate; 94.5% ai, Lot No. AC 7504-59-A) was administered in the diet at 0, 60, 300, or 600 ppm (52-week average 0, 2.6, 13.6, or 28.2 mg/kg/day, respectively, for males; 0, 3.4, 18.0, or 37.4 mg/kg/day, respectively, for females) to Sprague-Dawley CD BR VAF/Plus rats (25/sex/group) for 52 weeks, followed by a 16-week recovery period during which the remaining rats were fed the control diet. The rats were evaluated for reactions in functional observational battery followed by motor activity measurements 1 week before the test diets were provided; 4, 8, 13, 26, 39, and 52 weeks after the first day of exposure; and 13 weeks after the cessation of treatment. A portion of the rats in each treatment group were sacrificed for neuropathological examination following 13 or 52 of exposure, or 16 weeks of recovery.

In the 600 ppm dose group, both sexes exhibited statistically significant decreases in average body weights, body weight gains, absolute and relative feed consumption, feed efficiency, and

[1] Although, the sponsor put 83-1a on the cover of the study, the study only satisfies the 82-7SS requirement and was not meant to be a chronic rat study.

water consumption (males only). Neurohistological examination of males sacrificed after 13 weeks of exposure revealed myelin sheath swelling in the spinal nerve roots (5/5), compared to 2/5 in the controls. At 52 weeks, a more generalized myelinopathic process consisting of vacuolar myelinopathy (6/10), vacuolation (6/10), and/or mild myelin sheath swelling (9/10), was found. This process was not associated with myelin or axon degeneration and was not evident in rats sacrificed after 16 weeks of recovery. In the 300 ppm dose group, both sexes exhibited decreases in average body weights, body weight gains, feed efficiency, absolute feed consumption (females only) and water consumption (males only) at various times during the exposure period and body weight gains were reduced (non-significantly) for males during recovery. The myelinopathic observations described in the 600 ppm group males was also found in the 300 ppm group of rats after 13 and 52 weeks exposure but were less severe and at a lower incidence. In the 60 ppm dose group rats, minimum myelin sheath swelling was seen in the Gasserian ganglia of one male at 52 weeks and spinal nerve roots of 3/5 males (compared to 2/5 controls) after 13 weeks of exposure. The toxicologic importance of these findings is equivocal since swelling in the spinal nerve roots was absent in the 60 ppm group after 52 weeks. Neuropathological changes were confined to males; females were not affected. **The LOEL is 300 ppm (13.6 mg/kg/day) based on the presence of myelinopathic alterations in the 300 ppm group male rats, decreased average body weights, body weight gains, feed efficiency, absolute feed consumption (females) and water consumption (males). The NOEL is 60 ppm (2.6 mg/kg/day).**

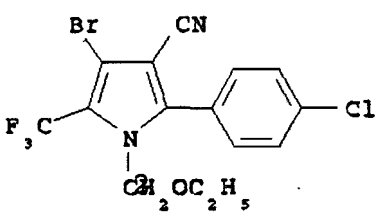
This one-year dietary neurotoxicity study is classified **Acceptable** and satisfies the guideline requirement for a neurotoxicity study (82-7SS) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AC 303,630
   Description: Tan solid
   Lot/Batch #: AC 7504-59-A
   Purity: 94.5% ai
   Stability of compound: Stable
   CAS #: Not provided
   Structure:



2. Vehicle: None

3. Test animals: Species: Rat
Strain: Sprague-Dawley Crl: CD BR VAF/Plus
Age and weight at study initiation: Approximately 8 weeks of age; males 195-275 g, females 128-184 g
Source: Charles River Breeding Labs., Portage, Michigan
Housing: Individually housed in suspended stainless steel wire-bottomed cages, ad libitum
Diet: Purina Certified Rodent Diet No. 5002 (Meal)
Water: Tap water deionized using a reverse osmosis membrane, then chlorinated prior to use, ad libitum
Environmental conditions: Temperature: 70-78 F
Humidity: 40-70%
Air changes: 10/hour (minimum)
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation period: 14 days

B. STUDY DESIGN

1. In life dates - Start: 11/18/91 End: 11/23/92

2. Animal assignment

Animals (100/sex) that "appeared to be in good health" were selected for use in the study, then assigned to the test groups in Table 1 using a computer-generated (weight-ordered) randomization.

TABLE 1: STUDY DESIGN.

Treatment Group	Conc. in diet[a] (ppm)	Nominal Dose to Animal (mg/kg/day)	Number of Animals Sacrificed at each Interval[b]					
			13 Weeks		52 Weeks		68 Weeks	
			M	F	M	F	M	F
Control	0	0	5	5	10	10	10	5
Low	60	4.5	5	5	5	5	5	5
Mid	300	22.5	5	5	5	5	5	5
High	600	45.0	5	5	10	10	10	5

[a] No information was provided in the report to justify the exposure levels selected for this study.
[b] The study was initiated with 25 rats/sex in each test group. Animals were sacrificed after 13 and 52 weeks of treatment, and after 16 weeks of posttreatment recovery.

3. Dosing preparation and analysis

The treated diet was prepared at least once each week or "as

100

needed" throughout the study period. AC 303,630 was mixed into small amounts of feed using a Hobart-type mixer, then the mixtures were blended into sufficient additional feed to obtain the desired concentrations. The treated feed was stored at room temperature in sealed plastic containers until use. The diet available to the rats was replaced "at least weekly" throughout the study. To confirm the concentrations of AC 303,630, samples were collected from each freshly prepared feed and frozen until analysis.

To establish the homogeneity and stability of the treated feeds prior to the initiation of dosing, batches of feed were treated with AC 303,630 at 60 or 600 ppm as described. Four samples (each approximately 200 g) were collected from each of six locations (the right and left side of the top, middle, and bottom portions) within each mixture. Two of the four sets of samples were immediately frozen for later analysis. The remaining two sets of samples were placed in standard food containers and stored at room temperature in the animal room for 7 or 14 days after preparation. The storage samples were kept frozen until analysis.

Results:
Homogeneity Analysis: 97-105% of targeted concentrations
Stability Analysis: 96-108% of the targeted concentrations
Concentration Analysis: 96-110% of the targeted concentrations at each preparation interval

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

Parametric data were subjected to Bartlett's test of homogeneity to estimate the probability that the dosage groups had different variances. If Bartlet's test was significant, the data were subjected to nonparametric analyses (Kruskal-Wallis Test, followed by the Dunn's test). If Bartlett's test was nonsignificant, the data were compared using Analysis Of Variance (ANOVA) testing. If the ANOVA was significant, the groups administered the test material were compared with the control group using Dunnett's Test.

Data from the motor activity test (repeated measurements within a session) were analyzed using an ANOVA with Repeated Measures. If the group effect was significant, the totals for the control and treated groups were compared using the Dunnett's Test. If the group X block interaction was significant, an ANOVA was used to evaluate the data at each measurement period, and a significant result was followed by

a comparison of the dosage groups using the Dunnett's Test.

FOB measurements having graded or count scores were analyzed using the Kruskal-Wallis and Dunn's tests. Clinical observation incidence data and descriptive and quantal FOB data were analyzed as contingency tables using the Variance Test for Homogeneity of the Bionomial Distribution.

5. Validation (Positive Control) Data: Six "positive control" neurotoxicity studies (#012-014, 012-015, 012-016, 012-017, 012-022 and 012-031) were conducted between September 1991 and July 1993) and the results were summarized in Appendix K of the study report. Brief summaries of these studies are provided in the Appendix to this DER. The studies provided adequate demonstration of the laboratory's ability to perform neurobehavioral/neuropathological evaluations.

C. METHODS:

1. Observations

Animals were inspected each morning for signs of toxicity and mortality throughout the study. Except for 7 days during which the animals were not checked for viability, all animals were also inspected once each afternoon.

2. Body weight

Animals were weighed pretest on day of dosing, once each week throughout the study, and at study termination.

3. Food consumption

Food consumption for each animal was determined weekly throughout the study beginning 1 week prior to treatment.

4. Neurobehavioral Studies

Functional Observational Battery (FOB) and Motor Activity - FOB and motor activity tests were conducted 1 week prior to treatment; after 4, 8, 13, 26, 39 and 52 weeks of treatment; and after 13 weeks of recovery. FOB was evaluated before motor activity. The following were evaluated:

Home Cage Observations
Piloerection
Posture
Gait
Tremors
Convulsions
Clonic movements
Tonic movements

Manipulative Observations
Ease of removal from cage
Ease of handling
Respiration
Palpebral closure
Pupil size
Staining (eyes, oral,anal)
Lacrimation
Salivation
Vocalization

Response Observations
Auditory response
Approach response
Touch response
Pupil response
Pain response

Open Field Observations
Posture
Gait
Arousal
Stereotypic and bizarre behavior
Tremors
Convulsions
Circling
Locomotion
Rearing count
Urination
Defecation boluses

Neuromuscular tests
Hindlimb grip strength
Forelimb grip strength
Landing footsplay
Righting reflex

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and gross lesions were examined histologically. Animals selected for neurohistopathologic examination were anesthetized and perfused *in situ* with neutral buffered 10% formalin. The head, vertebral column, and hindlimbs were dissected to expose the spinal cord and peripheral nerves and placed in formalin for 24 hours. The following tissues were dissected and trimmed for histological processing:

Gasserian ganglia
Spinal cord with dorsal root ganglia and nerve roots from cervical, thoracic and lumbar regions
Sciatic nerve
Tibial nerve
Fibular nerve
Sural nerves
Brain

The central nervous system tissues were embedded in paraffin, and the peripheral nerves were embedded in glycol

methacrylate. Saggital and coronal sections of the brain, horizontal and longitudinal sections of the cervical spinal cord, and cross sections of the thoracic and lumbar cord were prepared. Cross and longitudinal sections of the peripheral nerves were also prepared. Sections were stained with H & E, luxol fast blue, toluidine blue and Bielschowsky's technique.

II. RESULTS

A. Observations

1. Mortality - No treatment-related mortality occurred during the study. Two 60 ppm males were found dead and one was sacrificed moribund; a control male was also sacrificed moribund.

2. Clinical signs - There were no treatment-related clinical signs of toxicity observed in this study.

B. Body weight and weight gain

Tables 2 and 3 summarize mean body weights and weight gains at selected study intervals. In the 600 ppm dose group, average body weights were significantly reduced for males on study days 43-365 and for females on days 36, 43, 57, 64, 85, and 92 of exposure. Body weight gains were significantly reduced for males on days 1-8, 8-15, 22-29, 50-57, 78-85, and 92-99 of exposure and days 1-8 of the recovery period and for females on days 22-29 of exposure.

In the 300 ppm dose group, average body weights were significantly reduced for males on days 29-169,and females on days 162-176, 190, 204, 295, and 302 of exposure. Body weight gains were significantly reduced for males on days 1-8, 8-15, 22-29, 50-57, and 78-85 of exposure and days 1-8 of the recovery period and for females on days 155-162 of exposure.

TABLE 2. MEAN BODY WEIGHTS AT SELECTED INTERVALS.[a]

Weeks	Males				Females			
	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
1	232.1	231.4	233.2	234.2	160.0	158.9	161.3	157.8
4	395.1	391.1	376.6*	381.2	222.6	222.8	217.0	208.7
8	479.9	475.1	451.8*	453.9*	245.8	249.1	239.2	230.8*
13	532.9	530.2	500.5*	501.7*	300.8	298.8	267.3	267.5*
52	726.4	720.8	664.8	635.2*	376.5	385.8	339.0	353.2
Recovery Groups								
1	690.3	724.6	679.7	651.9	374.4	384.9	349.9	363.4
16	798.8	764.9	741.3	766.5	401.3	411.1	386.5	405.4

[a] Data obtained from Table B6, pages 128-131; Table C6, pages 421- 425); Table D4, pages 670-671; and Table E4, pages 724-725, in the study report.
* Significantly different from control, $p < 0.05$.

TABLE 3. MEAN BODY WEIGHT GAINS AT SELECTED INTERVALS.[a]

Weeks	Males				Females			
	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
1-4	163.0	159.7	143.4*	147.0*	62.6	63.9	55.7	50.9*
1-8	247.8	243.7	218.6*	219.7*	85.8	90.2	77.9	73.0
1-13	300.8	298.8	267.3	267.5*	105.0	111.1	97.1	91.0
1-52	492.5	488.4	433.4	400.3**	215.3	228.7	177.4	194.2
52-68	98.4	67.5	71.7	126.6	23.6	24.8	32.2	47.6

[a] Data obtained from Table B7, pages 132-135; Table C7, pages 425-428; Table D5, pages 672-673; and Table E5, pages 726-727, in the study report.
* Significantly different from control, $p < 0.05$.
** Significantly different from control, $p < 0.01$.

C. Food Consumption and Feed Efficiency

In the 600 ppm dose group, absolute feed consumption means (g/day) were statistically significantly decreased in males on days 1-8 of the exposure period and in females on days 1-29 of the exposure. Relative feed consumption means (g/kg/day) were statistically significantly decreased in males on days 1-8 and in females on days 8-15 of the exposure and recovery periods. Overall mean feed efficiency values were statistically significantly decreased in males (4.3% compared to 5.3% for

controls) and for females on days 22-29 of the exposure period.

In the 300 ppm dose group, absolute feed consumption means were statistically significantly decreased in females on days 22-29, 92-99 and 106-113, but overall food consumption was similar to control consumption.

D. Test Article Intake

Average dosages of test article consumed during the highest week of exposure (week 1) and throughout the one-year exposure period are summarized in Table 4.

TABLE 4: TEST ARTICLE INTAKE.[a]

Treatment Group	Week 1		Study Average	
	Males	Females	Males	Females
60 ppm	5.8	6.2	2.6	3.4
300 ppm	28.4	29.6	13.6	18.0
600 ppm	55.1	58.3	28.2	37.4

[a] Data obtained from Table B1, pages 83-86, and Table C1, page 376-379, in the study report.

E. Neurotoxicity

There were no biologically significant treatment-related indications of neurotoxicity seen in this study, based on evaluation of all of the listed FOB parameters.

F. Sacrifice and Pathology

Gross pathology - There were no biologically significant treatment-related gross pathological lesions seen in this study.

Microscopic pathology - After 13 weeks of test article administration, 5/5 males in the 600 ppm treatment group had scattered individual and clusters of nerve fibers within the spinal nerve roots with a greater than "background" degree of myelin sheath swelling (grade minimal for 1 male and mild for the remaining 4 male rats). Myelin sheath degeneration was not present and the axons of the affected nerve fibers were intact. Minimal degrees of myelin sheath swelling were also present in 3 males each in the 300 and 60 ppm dose groups, in 5 males in the 600 ppm group and in 2 control males. After 52 weeks of test article administration,

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minimal to mild degrees of myelin sheath swelling were seen in the spinal nerve roots of 9/10 600 ppm males (mild grade for 7/9 of these rats) and 4/5 males at 300 ppm and in the sciatic nerve of 4/10 males in the 600 ppm group. In addition, vacuolar myelinopathy was found to be prominent within many white matter tracts of the brain (anterior commissure, cerebral peduncle, cerebral white matter, pyramids, corpus callosum, internal and external capsules, olfactory tract, olfactory bulb, fimbria, optic nerve (chiasm), stria medullaris, globus pallidus and cervical spinal cord.) in 600 ppm males. In most of the affected rats, sections of the spinal cord (particularly the deep portions of the dorsal funiculus) were involved. This vacuolar myelinopathy was similar to myelin sheath swelling but more generalized and of greater severity. However, it was not associated with myelin or axon degeneration and was found to be reversible. Focal vacuolation primarily in non-myelinated areas (neuropil)or areas of low myelination was increased in the hippocampus, fornix, and cerebral white matter of males at 600 ppm. These vacuoles are not as large as in vacuolar myelinopathy, were not widespread or well defined. Significant histopathologic lesions seen in the nervous system of male rats are summarized in Table 5.

TABLE 5. SUMMARY OF NEUROPATHOLOGICAL FINDINGS IN MALE RATS.[a]

Lesion/Site	Concentration in Diet (ppm)				Grade of Lesion
	0	60	300	600	
13 Weeks					
Number examined	5	5	5	5	--
Myelin Sheath Swelling					
Spinal Nerve Roots	2	3	3	5	(1,4,0,0,0)
52 Weeks					
Number examined	10	5	5	10	
Vacuolar Myelinopathy					
Anterior Commissure	0	0	1	7**	(3,0,3,0,1)
Cerebral Peduncle	0	0	2	8**	(0,1,3,3,1)
Cerebral White Matter	0	0	3*	5*	(3,1,1,0,0)
Pyramids	0	0	1	8**	(0,4,1,2,1)
Corpus Callosum	0	0	1	7**	(1,1,4,0,1)
Internal Capsule	0	0	1	8**	(1,3,2,1,1)
External Capsule	0	0	1	6**	(2,3,0,1,0)
Olfactory Tract	0	0	0	5*	(2,2,0,1,0)
Fimbria	0	0	1	7**	(2,1,3,1,0)
Optic Nerve (Chiasm)	0	0	1	7**	(0,3,2,2,0)
Stria Medullaris	0	0	1	6**	(1,2,2,1,0)
Globus Pallidus	0	0	0	4*	(0,0,2,2,0)
Olfactory Bulb	0	0	1	5*	(1,3,0,1,0)
Spinal Cord, Cervical	0	0	1	7**	(1,2,3,1,0)
Vacuolation					
Hippocampus	0	0	0	6**	(1,4,1,0,0)
Cerebral White Matter	0	0	1	3	(1,2,0,0,0)
Fornix	0	0	1	4*	(1,3,0,0,0)
Myelin Sheath Swelling					
Spinal Nerve Roots	1	0	4*	9**	(2,7,0,0,0)
Sciatic Nerve	0	0	0	4*	(1,3,0,0,0)
Pons	0	0	1	2	(2,0,0,0,0)
Spinal Cord, Thor.	0	0	0	2	(2,0,0,0,0)

[a] Data were obtained from Appendix J, Tables 1 and 3, pages 951-978, in the study report.
[b] The numbers in parentheses (_,_,_,_,_) represent the numbers of high-dose males with lesion grades of minimum, mild, moderate, marked, or severe, respectively.
* Significantly different from control, $p<0.05$.
** Significantly different from control, $p<0.01$.

No significant neuropathological findings were observed in dosed females. At 13 weeks, 1/5 control and 1/5 females at 600 ppm had minimal spinal root nerve myelin sheath swelling; at 52 weeks 1/10 control and 2/10 high-dose females had the same finding. Minimum myelin sheath swelling was also seen in

control and 600 ppm females in the cervical spinal cord (3/10,5/10), the Gasserian ganglia (5/10,4/10) and sciatic nerve (3/10,1/10). No vacuolar myelinopathy was reported for females. Minimum vacuolation was seen in the hypothalamus of 2/10 600 ppm females and in the cerebellar white matter of 1/10 control and 2/10 600 ppm females. Minimal myelin degeneration was found in the cervical spinal cord, Gasserian ganglia, sciatic, tibial, pons and sural nerves of 5, 2, 3, 1, 1 and 1 controls, respectively, compared to 5, 0, 1, 0, 1 and 1 600 ppm females, respectively. No degeneration was reported in any females at 60 or 300 ppm.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The study author concluded the NOEL was 60 ppm, because male and female rats in the 300 and 600 ppm had reduced body weights and reduced feed consumption, and male rats were found to have myelinopathic alterations in the central nervous system. After a 16-week recovery period, the myelinopathic alterations were found to be "completely reversible" and the body weights converged with the controls.

B. REVIEWER'S DISCUSSION

Results of this study indicated that the test article had no significant adverse effects on the FOB and Motor Activity tests for neurotoxicity. However, histopathologic lesions were noted with dose-related incidences in test article treated males after both 13 and 52 weeks. It is possible that the histologic lesions were not severe enough to elicit functional changes in the FOB testing. The reviewing pathologist considered a minimum grade of myelin sheath swelling to be a normal background alteration or possibly an artefact. None of the rats had a grade above mild.

IV. STUDY DEFICIENCIES

No major study deficiencies were identified.

APPENDIX - POSITIVE CONTROL STUDIES, ARGUS LABORATORIES

The following positive control (validation) studies were summarized in Appendix K of the study report (MRID 43492833). These studies were conducted between September, 1991 and July, 1993. Experimental details and results were presented but a discussion of the results was not included in these reports.

Overall, the studies showed detection of expected effects from the known neurotoxicants that were tested. Some variation in incidence or occurrence of certain findings is noted in studies testing the same chemical but these may be due to experimental variation, slight differences in timing of observations relative to peak effect or differences in vehicle used. However, in general, appropriate findings were reported. Interobserver reliability showed relatively consistent findings.

Protocol 012-014: Neurotoxicity Evaluation of Positive Control Substances in Crl:CD® VAF/Plus® Rats. This study evaluated functional observational battery (FOB) parameters and motor activity levels in male and female rats treated with known neurotoxicants. Four rats/sex/group were treated with one of the following compounds: (1) acrylamide, 40 mg/kg/day intraperitoneally in 1 ml/kg 0.9% saline for 9 consecutive days. FOB conducted on day 7 and 4 days after the last dosage; (2) IDPN, 200 mg/kg intraperitoneally in 1 ml/kg 0.9% saline for 3 consecutive days. FOB conducted 4 and 10 days after the last dosage; (3) carbaryl, 75 mg/kg once by gavage in 5 ml/kg corn oil. FOB conducted 1 hr post-dosing; (4) DDT, 75 mg/kg once by gavage in 5 ml/kg corn oil. FOB conducted 5½ hrs post-dosing; (5) triadimefon, 200 mg/kg by gavage in 5 ml/kg corn oil. FOB conducted 2 hrs post-dosing. In addition, two vehicle control groups were given 1 ml/kg 0.9% saline by intraperitoneal injection or 5 ml/kg corn oil by gavage. Motor activity testing apparently followed the FOB evaluation for each group. Neuropathology evaluations were also performed for animals exposed to acrylamide and to IDPN.

Rats treated with acrylamide showed decreased rearing, increased reaction to handling/removal, exaggerated movements (limbs splayed) which increased in severity with time, whole body tremors/spasms, drooping eyelids and increased landing foot splay which was more pronounced with time (at 4 days post-dosing, 86% greater than controls). At 4 days post-dosing, animals also showed abnormal respiration, uncoordinated air righting response and decreased response to visual stimulus. Forelimb grip strength was lower than controls but not significantly (35% and 30%, males and females) and although hindlimb grip strength was lower in females (34%), no decrease was observed in males. Motor activity was decreased in treated animals (more pronounced with time: at day 4 post-dosing, 58% and 53% less than controls, males and females). Microscopic evaluation revealed degeneration of

the sciatic nerve (minimal to moderate) and its branches in 2 rats.

Rats treated with IDPN showed stereotyped/bizarre behavior, ataxia (slight), and impaired air righting response. Head bobbing and retropulsion were reported in the daily clinical observations. At the second testing period, non-significant decreases in forelimb grip strength (30%, males and 20%, females) and increased landing foot splay (29%) were also observed. Motor activity was slightly but not significantly lower in treated animals (21%-27% less than controls). Microscopic evaluation revealed localized axonal swellings within the dorsal root ganglia and adjacent spinal nerve roots, and in the trigeminal nerve fibers adjacent to the Gasserian ganglia in two animals, although the lesions within one of these animals were not of sufficient occurrence/severity to definitively ascribe them to treatment.

Rats treated with DDT showed whole body tremors (and 1 animal had twitches/tremors of the limbs), increased rearing (females only). Decreases in forelimb grip strength in females (18%) and hindlimb grip strength in males (19%) were not significant. Motor activity was slightly higher in both sexes (~20% less than controls) but not significantly.

Rats exposed to carbaryl showed whole body and/or limb twitches/tremors, increased urine pools, spastic (tip-toe) or duck-walk gait (moderate severity), excess lacrimation and salivation, decreased tail-pinch response, impaired air righting response and impaired visual placing response. A decrease in forelimb grip strength in females (25%) was not significant. Motor activity was sharply lower in males and females (69% and 78% less than controls, respectively).

Rats exposed to triadimefon showed increased rearing (females), but no other effects in the FOB. Motor activity was significantly higher in treated animals (265% and 341% greater than controls, males and females) due to sustained activity throughout the session.

Rats evaluated pretreatment showed normal FOB profiles.

Protocol 012-015: Neurotoxicity Evaluation of DDT in Crl:CD®BR VAF/Plus® Rats. This study evaluated the effects of DDT on FOB parameters at pretest and for 2 days following dosing. Four rats/sex/group were dosed once by gavage with vehicle only (corn oil) or 75 mg DDT/kg (dose volume was 1 mL/kg, reduced from protocol 012-014 due to minimal effects observed at higher dosing volume).

Treated animals showed numerous effects in the FOB, including unusual behavior, whole body tremors/spasms, decreased number of

rears, increased level of arousal, lack of tail pinch response, decreased forelimb grip strength in males (28% less than controls) and increased landing foot splay (27% greater than controls). Two deaths occurred in males on day 1 (day of dosing).

Protocol 012-016: Motor Activity Evaluation in Crl:CD®BR VAF/Plus® Rats Administered Chlorpromazine and d-Amphetamine (Positive Control Study). This study evaluated the effects of chlorpromazine and d-amphetamine on motor activity following a single intravenous dose in 0.9% saline (1 ml/kg). Fifteen rats/sex/group were administered vehicle only (0.9% saline), chlorpromazine at 1, 2 or 4 mg/kg or d-amphetamine sulfate at 0.5, 1.0 or 4 mg/kg. Motor activity was measured for 2 hrs beginning about 70 minutes post-dosing.

Rats treated with chlorpromazine showed dose-dependent decreases in total motor activity (males - 19%, 41% and 51% less than controls; females - 26%, 41% and 59% less than controls), as well as time spent in movement. Decreases were generally most pronounced during the first hour of testing.

Rats treated with d-amphetamine sulfate showed increased total motor activity that was dose-dependent at low and mid dose, but not at the high dose (males - 154%, 246% and 136% greater than controls; females - 270%, 364% and 203% greater than controls). Time spent in movement was also increased. Activities during the initial 10 to 20-minute intervals were similar in all groups but higher activity was sustained throughout the rest of the testing period in treated animals.

Protocol 012-017: Neurotoxicity Evaluation of Positive Control Substances in Crl:CD®BR VAF/Plus® Rats. This study evaluated the effects of acrylamide, DDT, IDPN and d-amphetamine on FOB parameters. Four rats/sex/group were treated with one of the following: (1) acrylamide, 40 mg/kg/day intraperitoneally in 0.9% saline, 1 ml/kg, 9 dosages. FOB conducted on day 7 and on days 4 and 12 after the last dose; (2) IDPN, 200 mg/kg/day intraperitoneally in 0.9% saline, 1 ml/kg, 3 dosages. FOB conducted 4 and 10 days after the last dose; (3) carbaryl, 200 mg/kg by single gavage dose in 0.5% methylcellulose, 5 ml/kg (dose was intended to be 40 mg/kg but concentration of dosing solution was accidentally 40 mg/mL instead of 8 mg/mL). FOB conducted 1 hr post-dosing; (4) DDT, 75 mg/kg by single gavage dose in corn oil, 1 ml/kg. FOB conducted 5½ hrs post-dosing; or (5) d-amphetamine, 4.0 mg/kg administered once by intraperitoneal injection in 0.9% saline, 1 ml/kg. FOB conducted 1 hr post-dosing. In addition, 2 vehicle control groups (1 ml/kg 0.9% saline, intraperitoneal injection and 5 ml/kg 0.5% methylcellulose, gavage) were evaluated.

Rats treated with acrylamide showed decreased rearing,

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increased reaction to handling, ataxia or exaggerated movement (splayed limbs) of increasing severity with time, twitches or tremors in limbs, increased landing foot splay, which increased with time (at day 12 post-dosing, 112% greater than controls), and in females, decreased forelimb grip strength (24% less than controls). At the day 4 and 12 post-dosing evaluations, abnormal respiration and impaired air righting response were also observed.

Rats treated with IDPN showed stereotyped or bizarre behavior, ataxia or exaggerated movement (slight to moderate; increasing severity with time), twitches or tremors in limbs, whole body tremors and impaired air righting reflex. Males showed non-significant decrease in forelimb grip strength at day 4 post-dosing (27% less than controls; 19% at day 10 post-dosing). In addition, at 10 days post-dosing, increased landing foot splay (45% greater than controls) was observed.

Rats treated with carbaryl showed decreased rearing, twitches/tremors in limbs (1 animal had whole body tremors), ataxia or exaggerated movement (slight), excess salivation and lacrimation, no pupillary response, impaired air righting response, abnormal respiration and decreased reaction to tactile stimulus and tail pinch. Urine/fecal staining was observed in 1 animal.

Rats treated with DDT showed unusual behavior, whole body and limb tremors/spasms, increased reaction to handling, decreased rearing, increased level of arousal (sudden startle), ataxia (slight to severe), and 1 animal attacked in reaction to auditory stimulus testing.

Rats treated with d-amphetamine showed unusual/stereotyped behavior, whole body tremors or spasms, increased rearing, piloerection, increased reaction to handling and removal (tense) and to tail pinch, and spastic or exaggerated movement (slight).

Protocol 012-022: Neurotoxicity Evaluation of Carbaryl in Crl:CD®BR VAF/Plus® Rats. This study evaluated the effects of carbaryl on FOB parameters at two dose levels (evaluated pretest and 1 hr post-dosing). Four rats/sex/group were dosed once by gavage with vehicle only (0.5% methylcellulose, aqueous), or 40 or 200 mg carbaryl/kg.

Treated animals showed a dose-related increase in the occurrence of symptoms typical of cholinesterase inhibition, including excessive salivation, lacrimation, limb tremors/twitches (1 animal had whole body tremors), decreased number of rears, sluggishness, abnormal respiration and ataxia (slight to severe; more severe at higher dose). Reduced reaction to visual and tactile stimuli, no reaction to tail pinch, impaired visual placing response, impaired righting response and unusual behavior were also observed. Urine and feces staining

was observed at 40 mg/kg. Animals evaluated pretreatment showed normal FOB profiles.

Protocol 012-031: Neurotoxicity Evaluation of Positive Control Substances in Crl;CD®BR VAF/PLUS® Rats. This study evaluated the effects of several known neurotoxic compounds on FOB parameters. Four rats/sex/group were treated with one of the following: (1) acrylamide, 45 mg/kg/day intraperitoneally in 0.9% saline, 1 ml/kg, 10 dosages. FOB conducted on day 8 and 4 days after the last dose; (2) IDPN, 250 mg/kg/day intraperitoneally in 0.9% saline, 1 ml/kg, 4 dosages. FOB conducted 4 and 10 days after the last dose; (3) carbaryl, 40 mg/kg by single gavage dose in 0.5% carboxymethylcellulose, 5 ml/kg. FOB conducted 1 hr post-dosing; (4) DDT, 75 mg/kg by single gavage dose in 0.5% carboxymethylcellulose, 5 ml/kg. FOB conducted 5½ hrs post-dosing; or (5) d-amphetamine, 4.0 mg/kg administered once by intraperitoneal injection in 0.9% saline, 1 ml/kg. FOB conducted 1 hr post-dosing. In addition, 2 vehicle control groups (1 ml/kg 0.9% saline, intraperitoneal injection and 5 ml/kg 0.5% carboxymethylcellulose, gavage) were evaluated.

Rats treated with acrylamide showed peripheral nerve toxicity that increased in severity and incidence between days 8 and 14. Parameters affected included decreased number of rears, ataxia progressing to splayed gait, abnormal gait of increasing severity with time, impaired air righting response, increased landing foot splay (32% greater than controls, day 14) and decreased fore- and hind-limb grip strength (forelimb 31% and 36% less than controls, males and females; hindlimb 40% and 49%, males and females, day 14). One male was sacrificed moribund on day 9.

Rats treated with IDPN showed bizarre behavior including head bobbing reported in the clinical observations, slight ataxia, uncoordinated air righting response, decreased auditory response and increased landing foot splay (23% greater than controls at day 14).

Rats treated with carbaryl showed decreased rearing, slight ataxia, possible salivation and lacrimation (only 1 animal each affected), reduced tactile and tail pinch responses and impaired righting reflex.

Rats treated with DDT showed unusual behavior, whole body tremors/spasms, decreased rearing, slight ataxia, lacrimation and reduced tactile reaction.

Rats treated with d-amphetamine showed increased rearing and one animal had a more energetic tail pinch reaction than normal.

Rats tested pretreatment showed normal FOB profiles.

Examination of Interobserver Reliability (Protocol 012-014). A

comparison of the observations made by each of two observers in the FOB of validation study Protocol 012-104 was performed. The two observers also performed the FOB evaluations in the 1-year study on Pirate. A total of 30 parameters were compared (several parameters including reaction to handling, pupillary response; grip strength, landing foot splay, rears, reaction to removal, home cage behavior, body weight and open field behavioral/postural alterations were not compared). The comparison showed good agreement between the two observers, with a median percentage agreement of 96% (range 74% to 100%).

Validation of Neuropathology. A section describing the experience of the veterinary neuropathologist for these studies, Dr. Robert Garnan, was provided.

DATA EVALUATION REPORT

PIRATE

Study Type: 83-1b; Chronic Oral Toxicity (Feeding) - Dogs

Dynamac Study No. 101K (MRID 43492834)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Review Section IV, Toxicology Branch I (7509C)

EPA Secondary Reviewer: M. Copley, D.M.V., D.A.B.T. M Copley, Date 5/15/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity [feeding] - dogs
OPPTS Number: 870.4100 (dog) OPP Guideline Number: §83-1b

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.5% ai)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Kelly, C. (1993) One year dietary study with AC 303,630 in purebred beagle dogs. Pharmaco LSR Inc., East Millstone, NJ. Laboratory Project ID 92-3107. August 31, 1994. MRID 43492834. Unpublished.

SPONSOR: American Cyanamid Company; Agricultural Research Division; P.O. Box 400; Princeton, NJ 08543-0400.

EXECUTIVE SUMMARY:

In a chronic toxicity study (MRID 43492834), AC 303,630 (Pirate; 94.5% ai; Lot No. AC 7504-59A) was administered to beagle dogs (5-6 dogs/sex/dose) in the diet at dose levels of 60, 120, or 240 ppm (2.1, 4.0, or 8.7 mg/kg/day, respectively, for males; 2.3, 4.5, or 10.1 mg/kg/day, respectively, for females) for 52 weeks. Body weights and body weight gains were depressed in both sexes treated at 240 ppm, with more pronounced differences observed in the females. Body weights and body weight gains of both sexes treated at 60 or 120 ppm were comparable to those of the controls. No treatment-related effects were observed on the survival, clinical signs, ophthalmology, hematology, clinical chemistry or urinalysis parameters, organ weights or gross and microscopic pathology at any dose level. **The LOEL is 8.7 mg/kg/day (240 ppm), based on decreased body weights and body weight gains. The NOEL is 4.0 mg/kg/day (120 ppm).**

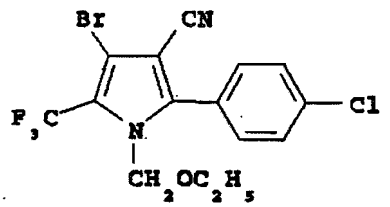
This chronic toxicity study is classified **acceptable** and does satisfy the guideline requirement for a chronic oral study (§83-1b) in dogs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AC 303,630
   Description: tan solid
   Lot/Batch #: AC 7504-59A
   Purity: 94.5% ai
   Stability: not provided
   CAS #: 122453-73-0
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Dog
   Strain: Beagle
   Age and weight at study initiation: 5-6 months of age; body weight 8.7-10.8 kg for males and 6.2-8.2 kg for females
   Source: Marshall Farms, U.S.A., Inc., North Rose, New York 14516.
   Housing: Individually housed in elevated metal grid cages
   Diet: Certified Canine Diet No. 5007. Each dog was provided with 400 g of food for approximately 22 hours daily except during the first 5 days of the acclimation period when food was presented for 6 hours per day.
   Water: ad libitum
   Environmental conditions:
   Temperature: 64-80 F (17.8-26.7 C)
   Humidity: 36-80%
   Air Changes: not specified
   Photoperiod: 12 hour light/dark cycle
   Acclimation period: 4 weeks

B. STUDY DESIGN

1. In life dates Start: 11/4/92 End: 11/10/93

2. Animal assignment

Twenty-one dogs of each sex were selected for the study on the basis of pretest physical examinations, ophthalmoscopic examinations or clinical laboratory data. The selected dogs were identified by ear tattoo, ear tag, and cage tag. The dogs were ranked by body weight and distributed into three blocks of five animals per sex for Groups I-III, and one block of six animals per sex for Group IV, as shown in Table 1.

TABLE 1: STUDY DESIGN[a]

Test Group	Conc. in Diet (ppm)	Nominal Dose to Animal (mg/kg/day)	Animals Assigned	
			Male	Female
I	0	0	5	5
II	60	1.5	5	5
III	120	3	5	5
IV	240	6	6	6

[a] Dose levels were selected on the basis of a 64-day range-finding study in beagle dogs that was appended in Volume 3 of this study. In the range-finding study, AC 303,630 was administered in the feed at 60, 120, 200, 300, 400, 500, 600, and 800 ppm or in capsules at 3, 5, 8, and 10 mg/kg/day. The NOEL was determined to be 200 ppm in the diet and 8 mg/kg/day via capsule administration based on clinical findings at 300 ppm and 10 mg/kg/day, decreased body weight at 300 and 400 ppm, and decreased food consumption at 400 ppm.

3. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of finely-ground test substance with Purina Certified Canine Diet #5007. Samples of the treated diet were collected weekly during the first 4 study weeks, then every 4 weeks during Study Weeks 5 through 52. The treated diet was stored at room temperature in a closed container until use.

Additional diet mix was treated at 60 or 240 ppm as described. Samples were collected from the top, middle, and bottom portions of the container immediately posttreatment, and subsamples were immediately analyzed to determine homogeneity. Samples of the diets treated

at 60 or 240 ppm were stored in standard food containers in the animal room at ambient conditions, and analyzed after 7 and 14 days to determine stability. Additional samples were stored in storage containers at room temperature for 7 and 14 days and analyzed to determine stability. Subsamples of the diets stored at room temperature were stored frozen for 7 or 14 days and analyzed to determine freezer storage stability.

Results:

Homogeneity Analysis:
- 0 day, 60 ppm: 54.9-57.2 ppm (91.5-95.3% nominal)
- 0 day, 240 ppm: 220-231 ppm (91.7-96.3% nominal)

Stability Analysis:
- Room temperature, feeder jar:
  - 7 day, 60 ppm: 51.9-53.9 ppm (86.5-89.8% nominal)
  - 14 day, 60 ppm: 52.4-56.7 ppm (87.3-94.5% nominal)
  - 7 day, 240 ppm: 214-228 ppm (89.2-95.0% nominal)
  - 14 day, 240 ppm: 219-234 ppm (91.3-97.5% nominal)
- Room temperature, food container:
  - 7 day, 60 ppm: 51.5 ppm (85.8% nominal)
  - 14 day, 60 ppm: 52.9 ppm (88.2% nominal)
  - 7 day, 240 ppm: 215-222 ppm (89.6-92.5% nominal)
  - 14 day, 240 ppm: 226-234 ppm (94.2-97.5% nominal)
- Freezer:
  - 7 day, 60 ppm: 55.3 ppm (92.2% nominal)
  - 14 day, 60 ppm: 55.3 ppm (92.2% nominal)
  - 7 day, 240 ppm: 225-229 ppm (93.8-95.4% nominal)
  - 14 day, 240 ppm: 234-245 ppm (97.5-102% nominal)

Concentration Analysis:
- 60 ppm: 55.4-61.7 ppm (96.3 $\pm$ 2.90% nominal)
- 120 ppm: 104-126 ppm (96.1 $\pm$ 3.96% nominal)
- 240 ppm: 216-237 ppm (96.7 $\pm$ 2.15 % nominal)

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

Body weight, body weight gain, food consumption, feed efficiency, hematology, clinical chemistry, terminal organ and body weights, and organ/body weight and organ/brain weight ratios for each sex were analyzed statistically. Mean values of all dose groups were compared to control values at each time interval. Statistical evaluation of equality of means was made by the appropriate one way analysis of variance (ANOVA), followed by a multiple comparison procedure if needed. Bartlett's test was performed to determine if groups had equal variance. Parametric procedures were used if the variances were equal; if not, nonparametric procedures

were used. The parametric procedures used were the standard one way ANOVA using the F distribution to assess significance. If significance among the means was indicated, Dunnett's test was used to determine which means were significantly different from the control. The Kruskal-Wallis test was used if a nonparametric procedure for testing equality of means was needed. A summed rank test [Dunn] was used if differences were indicated in order to determine which treatments differed from controls. A statistical test for trend in the dose levels was also performed. Standard regression technique with a test for trend and lack of fit was used in parametric cases. Jonckheere's test for monotonic trend was used in nonparametric cases. Bartlett's test was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

C. METHODS

1. Observations

Animals were observed at least twice daily for mortality and gross signs of toxicologic or pharmacologic effects. Detailed physical examinations were performed prior to the initiation of the study and weekly thereafter.

2. Body weight

Body weights were determined prior to study initiation, on day 0, weekly during treatment, and terminally after fasting.

3. Food consumption and compound intake

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency [(body weight change in kg x 1000/food consumption in g per unit time) X 100] and compound intake (mg/kg/day) values were calculated based on food consumption, the number of days in the sampling interval, and body weight gain during the interval.

4. Ophthalmoscopic examination

Ophthalmological examinations were performed prior to study initiation and at termination of the study.

5. Hematology and Clinical Chemistry

Blood was collected from the jugular vein of all test animals prior to study initiation and at 3, 6, and 12 months for hematology and clinical analyses. The test animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total and direct bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
			Serum protein electrophores
X	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Gamma glutamyl transpeptidase		

* Required for chronic studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was collected from animals at pretest and at Months 3, 6, and 12. Animals were fasted for collection of

freshly voided urine samples (approximately 4 hours) and were water deprived but not fasted for collection of 16-hour volumes. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific Gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilirubin
X	Osmolality		

* Required for chronic studies based on Subdivision F Guidelines.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
X	Rectum*				
XX	Liver*+	XX	Kidneys*+	XX	Adrenal gland*
X	Gall bladder*	X	Urinary bladder*		Lacrimal gland[T]
X	Pancreas*	XX	Testes*+	X	Mammary gland[T]
		X	Epididymides	XX	Parathyroids*++
		X	Prostate	XX	Thyroids*++
	RESPIRATORY		Seminal vesicle		
		X	Ovaries*+		OTHER
X	Trachea*	X	Uterus*		
X	Lung*	X	Oviducts	X	Bone*
	Nose	X	Vagina	X	Skeletal muscle*
	Pharynx			X	Skin*
	Larynx			X	All gross lesions and masses*

* Required for chronic studies based on Subdivision F Guidelines
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

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II. **RESULTS**

A. Observations

1. Mortality - All animals survived the duration of the study.

2. Clinical Signs - One male in the 240 ppm treatment group exhibited salivation during Study Weeks 1-4, which was considered to be treatment-related. No other clinical signs were considered to be treatment-related.

B. Body weight

Mean body weights and body weight gains for both sexes in the 60 and 120 ppm treatment groups were comparable to the respective control weights and weight gains throughout the study. Mean body weights for both sexes in the 240 ppm treatment groups were lower than the corresponding control weights, reaching statistical significance ($p < 0.05$) in the females only, beginning at Week 13 and at various intervals thereafter. Mean body weight gains for the 240 ppm males and females were 31 and 25% of the respective control body weight gains (Table 2).

Final mean body weights for males were 11.2 kg for the control group, 10.8 kg for the 60 ppm treatment group, 12.0 kg for the 120 ppm treatment group, and 10.1 kg for the 240 ppm treatment group. Mean body weight gains for males were 1.6 kg for the control group, 1.3 kg for the 60 ppm treatment group, 2.3 kg for the 120 ppm treatment group, and 0.5 kg for the 240 ppm treatment group (Table 2).

Final mean body weights for females were 8.8 kg for the control group, 8.3 kg for the 60 ppm treatment group, 8.1 kg for the 120 ppm treatment group, and 7.3 kg for the 240 ppm treatment group. Mean body weight gains for females were 1.6 kg for the control group, 1.3 kg for the 60 ppm treatment group, 1.1 kg for the 120 ppm treatment group, and 0.4 kg for the 240 ppm treatment group (Table 2).

TABLE 2. MEAN BODY WEIGHT CHANGE (kg) FROM WEEK 0 VALUES FOR MALE AND FEMALE BEAGLE DOGS FED AC 303,630 FOR ONE YEAR.[a]

Test Group (ppm)	Body Weight Change (kg)					Total Weight Gain (% of Control)
	WEEKS 0-4	WEEKS 0-12	WEEKS 0-24	WEEKS 0-30	WEEKS 0-52	
MALES						
0	0.5	0.6	0.8	1.2	1.6	--
60	0.6	1.1	1.5	1.5	1.3	81
120	0.6	1.2	1.5	1.8	2.3	144
240	-0.3	0.2	0.8	1.0	0.5	31
FEMALES						
0	0.1	0.8	1.3	1.4	1.6	--
60	0.3	0.9	1.0	1.0	1.3	81
120	0.5	0.8	1.1	1.2	1.1	69
240	-0.6	-0.1	0.3	0.5	0.4	25

[a] Data calculated from information provided in Appendix E, pages 69-78, in the study report.

C. Food consumption and compound intake

1. Food consumption - Food consumption in all treatment groups was generally comparable to that of the respective control groups. Sporadic instances of individual low values observed in the 240 ppm treatment groups during Study Weeks 1-2 may have been due to the reduced palatability of the treated food.

2. Compound consumption - Average consumption of AC 303,630 was slightly higher for treated females compared to the corresponding treated males (Table 3).

TABLE 3. AVERAGE CONSUMPTION OF AC 303,603 IN BEAGLE DOGS DURING ONE YEAR DIETARY FEEDING STUDY.[a]

Dose Level (ppm)	Test Substance Intake (mg/kg/day)			
	Males		Females	
	Mean	Range	Mean	Range
60	2.1	1.9-2.3	2.3	2.0-2.9
120	4.0	3.3-4.5	4.5	3.7-5.1
240	8.7	7.7-9.9	10.1	8.0-12.0

[a] Data obtained from page 30 in the study report.

3. Food efficiency - No significant differences were observed in the mean food efficiency values for either sex from any of the treatment groups compared to the corresponding control groups.

D. Ophthalmoscopic examination

One male from the 120 ppm treatment group exhibited peripapillary and intravitreal hemorrhage with retinal elevation above the optic disk at the termination of the study. The author stated that this effect was "possibly secondary to trauma or inflammation", and that "the lack of bilaterality and dose relationship make it unlikely that this finding was treatment-related" [page 29]. No other optical abnormalities were observed in any of the treated animals.

E. Blood work

1. Hematology - No treatment-related effects were observed in the hematology parameters for males in any of the treatment groups. The increased mean white blood cell count noted for the 120 ppm males at 12 months was not considered to be treatment-related, although it was significantly ($p < 0.05$) different compared to the controls, since it was not dose- or time-related, and was within normal biologic ranges.

No treatment-related effects were observed in the hematology parameters for females in any of the treatment groups. The decreased mean red blood cell count for the 240 ppm females at 3 months was not considered to be treatment-related, although significantly ($p < 0.05$) different compared to the control value, since it was not

time- or dose-related, and was within normal biologic ranges.

2. Clinical Chemistry - No treatment-related effects were observed in the clinical chemistry parameters for either sex in any of the treatment groups. Increased mean glucose in the 120 ppm males at 12 months, and increased mean creatinine in the 120 and 240 ppm males at 6 and 12 months compared to the controls were not considered to be treatment-related; although statistically significant ($p < 0.05$), they were within normal biologic ranges and did not correspond to organ weight or histopathological data to indicate specific tissue injury. Increased creatinine levels were not observed in the corresponding treated females.

   Females in the 120 ppm treatment group exhibited increased mean glucose and decreased mean globulin compared to the controls at 12 months; these differences were not considered to be treatment-related; although statistically significant ($p < 0.05$), they were not dose- or time-related, and were within normal biologic ranges.

F. Urinalysis

No treatment-related effects were observed in the urinalysis parameters of any of the treatment groups.

G. Sacrifice and Pathology

1. Organ weight - No treatment-related effects were observed in the absolute or relative organ weights of any of the treatment groups. Although the mean relative adrenal gland weight for males from the 120 ppm treatment group was significantly ($p < 0.05$) lower than the control weight, it was not dose-related; also, the absolute adrenal gland weight was not significantly different compared to the control weight (Table 4).

TABLE 4.. TERMINAL MEAN ADRENAL GLAND WEIGHTS AND ORGAN/BODY WEIGHT RATIOS OF CONTROL AND TREATED MALE DOGS.[a]

Test Group (ppm)	Terminal Body Weight (kg)	Adrenal Weight	
		Absolute (g)	Relative (organ wt/body wt)
0	11.4	1.715 ± 0.285	1.50 ± 0.14
60	10.9	1.566 ± 0.437	1.44 ± 0.39
120	12.2	1.240 ± 0.165	1.03 ± 0.17*
240	10.3	1.387 ± 0.268	1.35 ± 0.24

[a] Data obtained from Appendix M, page 555, in the study report.
* Significantly ($p < 0.05$) different from the untreated control.

No treatment-related effects were observed in the organ weights of females from any of the treatment groups. Mean relative brain and liver weights for females in all treatment groups were higher than the control weights, but were statistically significant ($p < 0.05$) for females in the 240 ppm treatment group only, which had a significantly ($p < 0.05$) depressed mean body weight compared to the control females (Table 5). Absolute brain and liver weights for females in all treatment groups were not significantly different from the corresponding control weights, and were not dose-related.

TABLE 5. TERMINAL MEAN BRAIN AND LIVER WEIGHTS AND ORGAN/BODY WEIGHT RATIOS OF CONTROL AND TREATED FEMALE DOGS[a].

Test Group (ppm)	Final Body Wt. (kg)	Brain Weight		Liver Weight	
		Absolute (g)	Relative (organ wt/body wt)	Absolute (g)	Relative (organ wt/body wt)
0	8.7	72.0	8.27	229.5	2.64
60	8.6	73.7	8.67	261.4	3.07
120	8.3	76.2	9.30	265.4	3.22
240	7.3*	73.9	10.19*	252.0	3.46*

[a] Data obtained from Appendix M, pages 558-559, in the study report.
* Significantly ($p < 0.05$) different from the untreated control.

2. Gross pathology - No treatment-related effects were observed in the gross pathology of any of the treatment groups.

3. Microscopic pathology

a) Non-neoplastic - No non-neoplastic alterations attributable to treatment were observed in any of the treatment groups.

In several dogs in the 120 and 240 ppm treatment groups, the stomachs had an increased severity in the number and size of follicles containing lymphoid cells compared to the controls (Table 6). Since all of the test animals, including the controls, exhibited lymphoid alterations in the stomach, the increased severity observed in the 120 and 240 ppm treatment groups probably reflects the irritancy effect of the test substance, rather than a treatment-related effect. The study author stated that "Lymphoid cell populations are a normal finding in the gastrointestinal tract of dogs and represent "gut associated lymphoid tissue (GALT)"." [page 35] The author concluded that "the variable severity represents individual animal variation of the naturally-occurring presence of lymphofollicular tissue" which has been associated with infection of one or more types of spiral shaped bacteria, i.e., *Helicobacter felis* and/or .

Gastrospirillum hominis, believed to be part of the natural gastric flora in dogs. [page 5] The cause of the increased lymphoid follicular cell population in the stomach of dogs in the 120 and 240 ppm treatment groups "was not determined". [page 35]

TABLE 6. SEVERITY RATINGS OF THE LYMPHOID CELL POPULATION IN THE STOMACHS OF BEAGLE DOGS.[a,b]

Dose Group (ppm)	Blind Reading #1				Blind Reading #2			
	Severity Rating (Dogs per severity rating)							
	1	2	3	4	1	2	3	4
Males								
0	4	--	1	--	3	2	--	--
60	1	1	2	1	1	3	1	--
120	--	1	2	2	1	--	3	1
240	--	1	2	3	--	1	2	3
FEMALES								
0	3	--	2	--	2	2	1	--
60	--	3	2	--	--	2	2	1
120	1	--	2	2	1	--	2	2
240	--	1	3	2	1	--	1	4

[a] Data obtained from Table 2, page 34, in the study report.
[b] Severity Rating: 1= minimal; 2= mild; 3= moderate; 4= marked.

b) Neoplastic - No neoplastic tissue was observed in any of the test animals.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the NOEL for beagle dogs is 120 ppm (4.0 mg/kg/day for males; 4.5 mg/kg/day for females), based on depressed body weights in the 240 ppm treatment groups. The LOEL is 240 ppm (8.7 mg/kg/day for males; 10.1 mg/kg/day for females). The study author concluded that the lymphoid alterations noted in the

stomachs of several dogs in the 120 and 240 ppm treatment groups were not treatment-related since they were within the normal physiological range in the stomachs of all dogs.

B. Reviewer's Discussion

Dietary administration of AC 303,630 to male and female beagle dogs at 60, 120 or 240 ppm for 52 weeks did not cause any adverse effects on the survival, clinical signs, ophthalmology, hematology, clinical chemistry or urinalysis parameters, organ weights or gross and microscopic pathology. Mean body weights and body weight gains were lower for both sexes in the 240 ppm treatment groups, with more pronounced differences observed for the females. Food consumption and food efficiency values for all treated animals were generally comparable to those of the controls. The stomachs of several dogs in the 120 and 240 ppm treatment groups exhibited increased severity in the number and size of follicles containing lymphoid cells compared to the controls. Since all of the test animals, including the controls, exhibited lymphoid alterations, the increased severity observed in the 120 and 240 ppm treatment groups probably reflects the irritancy effect of the test substance, rather than a treatment-related effect.

IV. **STUDY DEFICIENCIES**

No significant deficiencies that would affect the acceptability of the study were noted.

The relative humidity values deviated occasionally from the desired range; however, no adverse effect on animal health was apparent [page 28].

DATA EVALUATION REPORT

PIRATE

Study Type: 83-4; Two-Generation Reproduction Study with Rangefinding - Rats

Dynamac Study No. 101L (MRIDs 43492835/36)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: W. Greear, M.P.H., D.A.B.T. William B. Greear, Date 4/26/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, D.V.M., D.A.B.T. M Copley Date 5/1/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - 2 Generation Study in Rats
OPPTS Number: 870.3800 OPP Guideline Number: §83-4

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.5% ai)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Schroeder, R.E. (1994) A two-generation (one-litter) reproduction study with AC 303,630 in rats. Pharmaco LSR Inc., Mettlers Road, East Millstone, NJ. Project 90-3638. August 8, 1994. MRID 43492836. Unpublished.

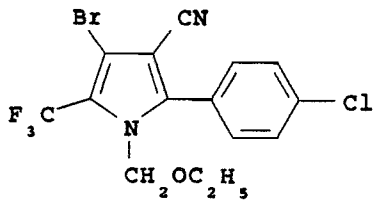
SPONSOR: American Cyanamid Company; Global Plant Industry Development; P.O. Box 400; Princeton, NJ 08543-0400.

EXECUTIVE SUMMARY:

In a 2-generation reproduction study (MRID 434292836), AC 303,630, (94.5% ai; Lot No. AC 7504-59A) was administered continuously in the diet to Sprague Dawley CD rats (30/sex/dose) at concentrations of 0, 60, 300, or 600 ppm (0, 5, 22, or 44 mg/kg/day, respectively, based on body weight and food consumption during pre-mating periods) for two successive generations (1 litter/generation). P_1 and F_1 males were mated after approximately 16 and 23 weeks of treatment, respectively. P_1 females were fed the test diets for approximately 19 weeks; mating was initiated at 10 weeks. F_1 pups were weaned on the same test diet fed their parents. F_1 females were fed the test diets for approximately 23 weeks; mating was initiated at 11 weeks.

In the 600 ppm male treatment group, the pre-mating weight gains of P_1 and F_1 animals were 11% and 12% lower, respectively, than for control animals ($p < 0.05$). In the 600 ppm female treatment group, the pre-mating weight gains of P_1 and F_1 females were 9% and 15% lower, respectively, than control animals (significant only in the F_1 generation). Mean weights of F_1 and F_2 pups in the 600 ppm treatement group at weaning were 12% and 14% lower,

Structure:



2. Vehicle: None

3. Test animals: Species: Rats
Strain: Sprague Dawley Crl: CD BR
Age at start of dosing: (P_1) 44 days, (F_1) 29-47 days
Weight at start of dosing:
(P_1) Males: 142-211 g, Females: 132-189 g
(F_1) Males: 94-279 g, Females: 79-219 g
Source: Charles River Laboratories, Inc., Portage, MI
Housing: Suspended, stainless steel cages with wire mesh bottoms
Diet: Purina Certified Rodent Chow #5002, ad libitum
Water: Tap water, ad libitum
Environmental conditions:
Temperature: 60-78 F
Humidity: 21-96%
Air changes: Not stated but conforms to NIH 1985 Guidelines (DHHS Pub. 85-23)
Photoperiod: 12-hour light/dark cycle
Acclimation period (P_1): 2 weeks

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure

Prior to mating, the estrous cycling of females was evaluated daily for 2 weeks. Initially, one male and one female were housed together for 10 consecutive days. The female was checked each morning for evidence of mating (microscopic observation of sperm and/or copulation plug in the vagina). Gestation Day 0 was designated on the day evidence of mating was observed. After mating, the females were removed from the males and housed individually in stainless steel cages with solid bottoms. The cages were supplied with hardwood shavings bedding for the gestation and lactation periods. Females not mating within the initial 10-day period were randomly redistributed to a proven fertile male within the same treatment group for a second 10-day period. F_1 animals were mated in the same manner as the P_1 animals. Sibling matings within the F_1 generation were avoided.

2. Study schedule

The study was initiated on 8/4/92 and completed on 5/26/93. P_1 animals were started on test diets at 6 weeks of age (study day 1) and were dosed for at least 71 days before mating. F_1 pups were delivered between study days 90-128 and received the same dietary dose levels as the parents until sacrificed. There was a maximum of 3 weeks difference in age for the parental F_1 generation. Mating occurred at least 78 days after weaning (between study days 215-226).

P_1 and F_1 males were mated 1:1 for two successive intervals, each lasting up to 10 days, following 113-114 days (P_1) or 162-164 days (F_1) on the test diet. Prior to mating, P_1 females were treated for 135-136 days (treated August 4-December 16-17 and mated October 13-22 and, if needed, October 23-November 1) and F_1 females were treated for 165 days (treated December 22-June 4 and mated March 9-18 and, if needed, March 19-28). At the weaning of each litter (Day 21 of lactation), the F_1 generation pups were removed from their dams and separated by sex. On Day 28 postpartum, 30 pups/sex/dose were randomly selected to become the F_1 parental generation. Where possible, at least one male and one female from each litter were selected. Selected pups were housed 1-2/sex/cage and fed the same test diet fed their parents until the last F_1 litter was weaned (3 weeks) and the F_1 pre-mating treatment was initiated. The same procedures were used for the F_2 pups, except that they were housed individually until they were sacrificed.

Significant events and days of study are summarized as follows:

Dates	Study Week	Event
8/4/92	1	P_1 Pre-mating treatments initiated
9/29/92	7.5	P_1 Estrous Typing Starts
9/29/92	9.4	P_1 Estrous Typing Ends
10/14/92	9.7	P_1 Mating Starts
11/01/92	12.3	P_1 Mating Ends
11/05/92	12.9	F_1 Pup Deliveries Start
11/23/92	15.4	F_1 Pup Deliveries End
12/3-12/21		F_1 Pups selected at 28 days of age
12/22/92	19.6	F_1 Pre-mating treatments initiated
2/23/93	28.7	F_1 Estrous Typing Starts
3/08/93	30.4	F_1 Estrous Typing Ends
3/10/93	30.7	F_1 Mating Starts
3/21/93	32.3	F_1 Mating Ends
3/31/93	33.7	F_2 Pup Deliveries Start
4/19/93	36.4	F_2 Pup Deliveries End
5/26/93	41.7	F_2 lactation period ends

3. Animal assignment

Animals were randomly assigned to the test groups in Table 1 using a computerized sorting program.

TABLE 1. STUDY DESIGN[a]

Test Group	Dose in Diet (ppm)	Animals Assigned			
		P_1 Males	P_1 Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low (LDT)	60	30	30	30	30
Mid (MDT)	300	30	30	30	30
High (HDT)	600	30	30	30	30

[a] Diets were administered from the beginning of the study until sacrifice. P_1 dosing was weeks 1-10 for males and weeks 1-16 for females. F_1 dosing was weeks 20-31 for males and 32-43 for females.

4. Dose selection rationale

In a pilot reproduction study (MRID 434292835), AC 303,630 was administered continuously in the diet to albino rats (10/sex/dose) at 0, 60, 300, or 600 ppm (0, 4, 22, or 45 mg/kg/day, respectively) for one generation. P_1 rats were mated after approximately 10 weeks on the test diet, and continued on treatment during the mating and post-mating periods until sacrificed. P_1 body weights and food consumption were recorded weekly throughout the study; F_1 pup body weights were collected on the day of birth, then weekly. Litters were evaluated for live and dead pups at birth, and litter size was recorded on days 4, 7, 14, and 21 of lactation. Rats administered AC 303,630 at 600 ppm exhibited a 12.8-15.1% depression in body weight gain over the pre-mating period, reduced pup survival index for through day 4 of lactation, and reduced pup weights throughout lactation compared to the controls. At the 300 ppm dietary level, rats had a 12.6% reduction in weight gain over the pre-mating period as compared to the P_1 controls. In the 60 ppm dietary treatment group, no adverse effects were indicated in mortality, physical observations, growth, food consumption, reproductive performance, or gross

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postmortem observations for the P_1 rats or growth and survival endpoints in the pups. The study author concluded that the LOEL was 300 ppm (22 mg/kg/day), based on reduced mean body weight gain of the P_1 rats during the pre-mating period, and the NOEL was 60 ppm (4 mg/kg/day).

5. Dosage preparation and analysis

The treatment diet was prepared weekly by mixing appropriate amounts of test substance into standard laboratory diet, and was stored at room temperature until use. Samples of the 60, 300, and 600 ppm diets were collected weekly throughout the study. Samples were analyzed weekly during the first four weeks of the study, then once every 4 weeks for the duration of the study. "A random sample selection was used to determine which dose level was assayed for a given week and for which week during the month the control diet was assayed to ensure that each dose level was assayed at least once during each month."

To determine the homogeneity of the treated feed, samples of the 60 and 600 ppm diets prepared on study days 0 and 21 were collected from the top, middle, and bottom of the mixer for analysis.

To determine the stability of the test substance in diet, AC 303,630 mixed into standard laboratory diet was stored at room temperature for 21 days either in the food containers in the animal room or in polyethylene containers; subsamples were analyzed at 0 and 21 days. Also, the stability of AC 303,630 during 7 and 14 days of storage was established in the range-finding reproductive study (MRID 43492835) submitted with this definitive study.

Results (Data from Appendix Y in the study report) -
Homogeneity Analysis: 89.0-98.2% of nominal
Stability Analysis (room temperature):
0 days: 95.1-96.6% of nominal
21 days: 92.6-96.7% of nominal
Concentration Analysis:
60 ppm: 54.7-61.5 ppm (97.4 ± 2.66% of nominal)
300 ppm: 272-304 ppm (97.3 ± 2.00% of nominal)
600 ppm: 554-616 ppm (97.4 ± 2.26% of nominal)

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. Parental animals: Adult animals were observed twice daily

for mortality and clinical signs of toxicity. Detailed physical examinations including palpation for masses was conducted weekly throughout the study for the adult generations. Body weights and food consumption data were recorded weekly during the study; for females these parameters were also recorded on gestation days 0, 7, 14 and 20 and on lactation days 0, 4, 7, 14 and 21 (body weight only). Estrous cyclicity and parturition observations, gross postmortem examinations (including a count of uterine implantation scars, when present) and selected histomorphological observations were also recorded.

2. Litter observations: The following litter observations (X) were made:

TABLE 2. P_1/F_1 LITTER OBSERVATIONS

Observation	Observation Interval (Lactation Day)					
	Day 0	Day 4[a1]	Day 4[a2]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X

[a] On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded.
[1] pre-cull
[2] post-cull

On either Day 21 of lactation (F_1) or Day 20 postpartum (F_2), one male and one female pup, where possible, from each litter were sacrificed and examined grossly for external and internal abnormalities.

3. Postmortem observations:

1) Parental animals: All surviving parental P_1 and F_1 males were sacrificed approximately 3 weeks after mating. All surviving parental P_1 and F_1 females were sacrificed shortly after the pups were weaned. These animals were subjected to postmortem examinations as follows:

Gross necropsy consisted of external and internal

examinations including the cervical, thoracic, and abdominal viscera.

Physical development of pups was assessed by recording the day of lactation for pinnae unfolding, hair growth, tooth eruption, eye opening, vaginal opening and preputial separation.

The following tissues from animals in the control and high dose treatment groups were prepared for microscopic examination. No organs were weighed.

Coagulating Gland
Epididymides
Lesions (none reported)
Ovaries
Pituitary
Prostate
Seminal vesicles
Testes
Uterus
Vagina/cervix

2) Offspring: The F_1 offspring that were not selected as parental animals and all F_2 offspring were sacrificed several days after weighing at 28 days of age. All animals were subjected to macroscopic examination only. No animals were subjected to microscopic examinations.

D. DATA ANALYSIS

1. Statistical analyses

All data collected were subjected to routine appropriate statistical procedures.

2. Indices

Reproductive indices: The following reproductive indices were calculated for each treatment group of rats from breeding and parturition records of animals in the study:

mating index (females) = number of females showing evidence of mating (ie., plug/sperm/pregnancy/uterine implantation scars at gross postmortem exam)/number of females

mating index (males) = number of males for which mating was confirmed in at least one female/number of males

gestation index = number of females that delivered

litters containing viable pups/number of pregnant females

pregnancy index = number of females showing evidence of pregnancy (ie., parturition/uterine implantation scars at gross postmortem examination)/number of females

fertility index (males) = number of males mated with at least one female for which pregnancy was evident/number of males

pup live birth index = total pups born alive/total pups born

pup viability index = total pups alive on day 4 (pre-cull)/total pups born alive

pup weaning index = total pups alive on day 21 (weaning)/total pups alive Day 4 (post-cull)

litter survival index = number of litters with live pups at day 21/number of litters with live pups at birth

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

pup live birth index

pup viability index

pup weaning index

3. Historical control data

Recent historical control data for the period 1987-1991 are presented in Table 3.

TABLE 3. HISTORICAL CONTROL DATA.[a]

Historical Data		Litters		
		All	F_1	F_2
Mating females		84-100	96-100	90-100
Indices males		70.8-100	76.7-100	72-92
Pregnancy rate (%)		71.4-100	83.3-100	71.4-100
Male fertility index (%)		76.2-100	87-100	76.2-100
Mean gestation length (days)		21.9-22.6	21.9-22.3	22.0-22.6
Mean number of live pups/litter	0 Days	10.8-14.4	11.6-14.4	10.8-14.2
	4 Days	10.6-14.1	11.0-14.1	10.6-13.5
Mean pup weight (g)	0 Days	5.6-6.6	5.9-6.5	5.6-6.6
	4 Days	8.5-10.6	8.5-10.6	8.6-10.6
	21 Days	36.3-56.1	37.7-56.1	36.3-51.8
Pup survival indices (%)	0-4 Days	88.4-99.4	94.6-99.4	88.4-98.8
	4-21 Days	92.9-100	93.7-100	92.9-100
Litter survival indices (%)		86.4-100	92.6-100	86.4-100
Sex distribution ratio (M/F)	0 Day Lactation	0.8-1.3	0.8-1.3	0.8-1.3
	4 Day Lactation	0.8-1.4	0.8-1.4	0.9-1.2
	21 Day Lactation	0.9-1.2	0.9-1.1	0.9-1.1

[a] Data obtained from Appendix Z, pages 1729-1738, Tables I through IV, in the study report.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

Mortality consisted of one F_1 male in the 60 ppm group and two F_1 males in the control group. The causes of death were not readily apparent from the gross postmortem findings. These deaths were not considered to be treatment-related.

No treatment-related increases in clinical signs were observed at any dose level in the parental generations. The most frequently occurring signs were alopecia, red swollen ears, and maloccluded incisors. These are common problems in laboratory rats.

2. Body weight and food consumption

Pre-mating body weights, weight gains, and food consumption are summarized in Tables 4a and 4b.

TABLE 4a. BODY WEIGHT AND FOOD CONSUMPTION OF P_1 GENERATION - PRE-MATING.[a]

Observations/study week	Dose Group			
	0 ppm	60 ppm	300 ppm	600 ppm
[P_1] Generation Males - Pre-mating				
Mean body weight (g) Week 10	475.6	456.5	443.3*	444.4*
Mean weight gain (g) Weeks 0-10	283.3	266.6	251.8**	252.3**
Mean food consumption (g/animal/day) Weeks 1-10	58.6	57.9	59.0	56.6**
[P_1] Generation Females - Pre-mating				
Mean body weight (g) Week 10	296.4	296.5	287.8	285.2
Mean weight gain (g) Weeks 0-10	126.9	129.1	121.0	115.7
Mean food consumption (g/animal/day) Weeks 1-10	69.8	68.4	66.6*	65.9*

[a] Data extracted from Tables T 3, T 4, and T 6, pages 102-114 and 125-132, in the study report.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

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TABLE 4b. BODY WEIGHT AND FOOD CONSUMPTION OF F_1 GENERATION - PRE-MATING.[a]

Observations/study week	Dose Group			
	0 ppm	60 ppm	300 ppm	600 ppm
[F_1] Generation Males - Pre-mating				
Mean body weight (g) Week [#31]	560.1	551.2	530.9	505.9**
Mean weight gain (g) Weeks [#20-#31]	333.3	321.1	319.5	293.6**
Mean food consumption (g/animal/day) Weeks [#20-#31]	52.7	54.5*	55.5**	55.8**
[F_1] Generation Females - Pre-mating				
Mean body weight (g) Week [#31]	303.5	291.8	275.9**	267.8**
Mean weight gain (g) Weeks [#20-#31]	128.2	127.5	121.2	109.3*
Mean food consumption (g/animal/day) Week [#20-#31]	65.9	68.2	67.4	68.7

[a] Data extracted from Tables T 3, T 4, and T 6, pages 102-114 and 125-132, in the study report.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

For the P_1 males, mean body weights and body weight gains were 5-7 and 11% lower, respectively, for animals treated at 300 and 600 ppm compared to the controls. No important decreases in food consumption were observed; overall food consumption by the 600 ppm males was 98% of controls. For the P_1 females, mean body weights at pretest were comparable in all groups; a nonsignificant decrease in weight gain (9%) was observed at 600 ppm, suggesting a possible effect. Food consumption was significantly decreased (5%) at 300 and 600 ppm when compared to controls.

For the F_1 males receiving 300 ppm, mean body weights at the beginning of the formal pretreatment period were slightly lower than controls (7%), but weight gains over the 11 week period were comparable to control gains. At 600 ppm, weight gains between weeks 20-31 were significantly lower (12%)

than in controls. Food consumption tended to be higher by dosed groups. For the F_1 females selected for mating, the mean body weights (28 days of age) were 6%, 12%, and 10% lower than controls in the 60, 300, and 600 ppm groups, respectively. The mean weight gains over the 11 week pretreatment period were significantly lower (15%) only at 600 ppm. The apparent differences in mean body weights observed in the 60 and 300 ($p<0.01$) ppm group were the result of pup selection and not considered related to dosing. Food consumption was significantly increased in the dosed F_1 males. During the mating and post-mating periods (weeks 11-16), body weight in the males receiving 600 ppm were significantly depressed in both generations.

Body weight gain data for females are less accurate indicators of toxic effects during the gestation and lactation periods compared to during non-pregnancy. Individual weight changes during lactation in control females ranged from -35 to +62 g. Similar changes were seen for individual females in the dosed groups.

Body weights for the 300 ppm group P_1 females were significantly depressed on lactation day 14, and body weights for the P_1 females in the 600 ppm group were significantly depressed at gestation days 7 and 14 and at lactation days 14 and 21. Body weights for the 60 ppm group F_1 females were significantly depressed on gestation days 14 and 20 and on lactation days 4-21. Body weights for the 300 and 600 group F_1 females were significantly depressed on gestation days 0-20 and on lactation days 0-20. The body weight depressions in the 600 ppm dose group were substantial. Mean weight gain for the 600 ppm group F_1 females was significantly decreased on days 0-20 of gestation. Food consumption was significantly increased in the 600 ppm F_1 females during days 14-20 of gestation.

3. Test Substance Intake

Based on food consumption, body weight, and dietary analysis results, the doses expressed as mean daily mg test substance/kg body weight during the pre-mating periods (9.7 weeks for P_1 rats and 11.1 weeks for F_1 rats) are presented in Table 5. The values for both generations are considered to be representative of the test substance intake for the pre-mating, mating, and post-mating phases of the entire study.

TABLE 5. TEST SUBSTANCE INTAKE DURING PRE- and POST-MATING TREATMENT PERIODS (MEAN MG/KG BODY WEIGHT/DAY).[a]

Male			Female		
60 ppm	300 ppm	600 ppm	60 ppm	300 ppm	600 ppm
P_1 Generation - Pre-mating					
4.5	22.2	44.0	5.0	24.5	48.3
P_1 Generation - Post-mating					
3.3	16.3	31.3	g[b] 4.9 l[b] 8.8	g 23.4 l 42.3	g 46.3 l 81.4
F_1 Generation - Pre-mating					
4.4	22.5	44.6	5.1	25.6	50.7
F_1 Generation - Post-mating					
2.8	14.3	29.6	g 4.7 l 8.6	g 23.6 l 41.8	g 47.7 l 82.9

[a] Data extracted from Table T 26, page 215, in the study report.
[b] g = gestation, l = lactation

4. Reproductive function

a. Estrous cycle length and periodicity: No significant adverse effects were observed on estrous cycle length or periodicity (Appendix D). Most of the P_1 and F_1 rats showed evidence of normal cycling during the 14-day evaluation period prior to mating. Results from the evaluation of vaginal smears in P_1 and F_1 rats indicated no significant abnormalities.

b. Sperm measures: No sperm parameter observations made in this study. There were no indications of treatment-related male fertility abnormalities during the study.

c. Sexual maturation (F_1): No significant treatment-related effect on sexual maturation was observed. Although mean vaginal opening time was increased in the 600 ppm group F_1 and F_2 pups, it was not considered biologically significant because the increase was very small and no preputial separation occurred.

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5. Reproductive performance

No significant treatment-related effects on reproductive performance were observed (Tables 6a and 6b), except for a significant increase in the median gestation interval of P_1 females in the 600 ppm group. This was not seen in the F_1 treatment groups.

TABLE 6a. REPRODUCTIVE PERFORMANCE OF P_1 GENERATION.[a]

Observation	Dose Group			
	0 ppm	60 ppm	300 ppm	600 ppm
P_1 Generation				
Mean precoital interval (days)	71	71	71	71
MALES				
Mated	28	30	26	29
Fertile	26	28	26	27
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	0
FEMALES				
Number mated	30	30	29	30
Number fertile	28	28	28	28
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	0
Median gestation interval (days)	21.9	22.0	22.0	22.2*
Number of litters	27	28	27	28

[a] Data extracted from Tables T 18 and T 19, pages 179-189, and Appendix E in the study report.
* Statistically different from controls, $p<0.05$

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TABLE 6b. REPRODUCTIVE PERFORMANCE OF F_1 GENERATION.[a]

Observation	Dose Group			
	0 ppm	60 ppm	300 ppm	600 ppm
F_1 Generation				
Mean precoital interval (days)	78	78	78	78
MALES				
Mated	24	29	27	28
Fertile	20	26	26	27
Fertility not determined	0	0	0	0
Intercurrent deaths	1	1	0	0
FEMALES				
Number mated	27	30	30	30
Number fertile	23	26	29	29
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	1
Median gestation interval (days)	22.0	22.0	22.1	21.9
Number of litters	23	25	29	29

[a] Data extracted from Tables T 18 and T 19, pages 179-189, and Appendix E in the study report.

5. Parental postmortem results

a) Organ weights

Organ weights were not taken in this study.

b) Pathology

1) Macroscopic examination: The report noted no observations which were related to the administration of the test substance.

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2) Microscopic examination: The report noted no observations were related to the administration of the test substance.

B. OFFSPRING

1. Viability and clinical signs: Mean litter size and viability results from pups during lactation are summarized in Tables 7a and 7b.

No significant treatment-related effect on mean litter size and viability were observed (Table 7a and 7b) except for the viability index of F_2 pups in the 600 ppm group which was significantly lower than controls.

TABLE 7a. MEAN LITTER SIZE AND VIABILITY OF F_1 GENERATION.[a]

Observation	Dose Group			
	0 ppm	60 ppm	300 ppm	600 ppm
F_1 Generation Pups				
Mean litter size				
Day 0	13.6	14.9	14.8	13.5
Day 4[b]	13.4	14.6	14.1	13.5
Day 4[c]	7.8	8.0	8.0	7.9
Day 14	7.8	8.0	7.8	7.8
Day 21	7.7	8.0	7.8	7.7
Number live pups				
Day 0	366	417	399	377
Day 4[b]	361	410	380	364
Day 4[c]	210	224	216	213
Day 14	210	223	211	210
Day 21	209	223	211	209
Number deaths				
Days 0-4	5	7	19	13
Days 4-21	11	4	15	15
Survival indices				
Viability index	98.8	98.5	95.3	97.0
Weaning index	99.5	99.6	97.7	98.1

[a] Data extracted from Appendices L and Q in the study report.
[b] Before standardization (culling).
[c] After standardization (culling).

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TABLE 7b. MEAN LITTER SIZE AND VIABILITY OF F_2 GENERATION.[a]

Observation	Dose Group (ppm)			
	0 ppm	60 ppm	300 ppm	600 ppm
F_2 Generation Pups				
Mean litter size				
Day 0	14.2	13.7	13.6	13.0
Day 4[b]	13.7	12.7	12.6	11.6
Day 4[c]	8.0	7.8	7.9	7.8
Day 14	7.8	7.7	7.8	7.8
Day 21	7.8	7.7	7.8	7.8
Number live pups				
Day 0	327	342	394	377
Day 4[b]	315	318	366	326
Day 4[c]	184	196	229	218
Day 14	180	185	226	218
Day 21	180	185	226	218
Number deaths				
Days 0-4	12	24	28	51
Days 4-21	9	12	9	16
Survival indices				
Viability index	96.2	93.5	93.5	86.5*
Weaning index	97.8	98.5	98.7	100.0

[a] Data extracted from Appendices L and Q in the study report.
[b] Before standardization (culling).
[c] After standardization (culling).
* Statistically different from controls, $p<0.05$

2. Body weight: Over the 21 days of lactation, weight gains in 300 ppm pups were 9.5% (F_1) and 13.6% (F_2) lower than in controls and at 600 ppm were 13.6% (F_1) and 15.3% (F_2) lower than controls. Selected mean pup weight and litter weight data are presented in Tables 8 and 9, respectively.

TABLE 8. MEAN BODY WEIGHT (GRAMS) OF F_1 AND F_2 PUPS.[a]

Day of lactation	Dose Group			
	0 ppm	60 ppm	300 ppm	600 ppm
F_1 Generation				
Day 0	6.3	6.3	6.1	6.1
Day 4[b]	10.5	10.0	9.3*	9.5*
Day 4[c]	10.4	10.0	9.3*	9.5*
Day 7	17.4	16.7	15.4**	15.3**
Day 14	35.7	33.8	31.2**	30.3**
Day 21	54.1	52.9	48.9**	47.4**
F_2 Generation				
Day 0	5.9	6.0	6.0	5.8
Day 4[b]	9.4	9.5	9.2	8.9
Day 4[c]	9.4	9.5	9.3	8.8
Day 7	15.7	15.4	15.2	14.0**
Day 14	33.2	31.8	30.8*	27.9**
Day 21	51.9	50.2	48.8*	44.8**

[a] Data extracted from Table T 21, pages 193-194, in the study report.
[b] Before standardization (culling)
[c] After standardization (culling)
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

3. Offspring postmortem results

a) Organ weights

No offspring organ weights were recorded.

b) Pathology

1) Macroscopic examination: No macroscopic findings were reported for the pups.

2) Microscopic examination: Pups were not examined for microscopic findings.

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III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

Low Dose (60 ppm) - No adverse effects were evident for parental or neonatal parameters and no adverse effects of treatment were indicated on reproductive performance.

Mid Dose (300 ppm)- Reproductive performance was not adversely affected. Parental toxicity was seen as a reduction in mean weights and weight gain for the P_1 males during the pre-mating treatment period and the only significant effect seen in neonates was decreased mean body weights during lactation and subsequent Day 28 pup weights for both the F_1 and F_2 litters.

High Dose (600 ppm) - Reproductive performance was not adversely affected. Parental toxicity was seen as a reduction in mean weights and weight gain for both sexes of the parental animals for both generations and body weight and body weight gain continued to be depressed for the P_1 and F_1 males during the mating and post-mating period. Mean weight gain for the high dose F_1 parental females over the 20-Day gestation period was reduced. No adverse effect at this treatment level was evident from reproductive indices, gestation indices or parturition data. Neonatal animals had significantly reduced pup weights during lactation and at Day 28 and reduced pup survival over Day 0-4 of lactation (F_2 litters). No adverse effects were seen in gross postmortem evaluations or the histomorphological evaluations of reproductive tissues, pituitary glands or gross lesions.

A NOEL of 60 ppm (approximately 5 mg/kg/day) was established for the test article in this study based on a lack of effects on parental toxicity, growth and development of offspring, fertility or any other aspect of reproductive function.

B. REVIEWER'S DISCUSSION

With the exception of depressed pup body weights, no adverse effects of AC 303,630 on reproductive parameters were observed in this study. All reproductive parameters in the treated rats were not different from the negative controls or recent historical control data for the period 1987-1991. These data indicated values for mating indices (84-100% for females and 70.8-100% for males), pregnancy rates (71.4-100%), male fertility indices (76.2-100%) [Appendix Z, Table I], gestation lengths (21.9-22.6 days), parturition data (day 0 live pups/litter 10.8-14.4, day 0 total pups/litter 11.1-14.8)(day 4 pre-cull pups/liter 10.6-14.1), and mean

pup body weights (day 0 5.6-6.6 g, day 4 pre-cull 8.5-10.6 g, day 21 36.3-56.1 g) [Appendix Z, Table II], pup survival indices (days 0-4, 88.4-99.4%; days 4-21, 92.9-100%) and litter survival indices (86.4-100%) [Appendix Z, Table III] and pup sex distribution data (M/F day 0, 0.8-1.3; day 4, pre-cull 0.8-1.4) [Appendix Z, Table IV].

Female F_1 rats in the 60 ppm treatment group exhibited slight (5.5-7.6%), statistically significant decreases in mean body weights on Days 14 and 20 of gestation and Days 4, 7, 14, and 21 of lactation (Tables T 12 and T 15 in the study report). There were also lesser but not significant absolute body weight decreases in P_1 female rats in this treatment group during gestation (less than 1.8%) and lactation (1.4-1.9%; Tables T 12 and 15). Mean 28-Day body weights of F_1 (selected) and F_2 pups in this dose group were also reduced and the F_1 body weight means were moderately (10.3%) and statistically significantly reduced (Table T 22). In addition, female rats in this treatment group increased their mean daily intake of test article by approximately the same amount during lactation (P_1 from 4.9 to 8.8 mg/kg/day; F_1 from 4.7 to 8.6 mg/kg/day; Table T 26). Thus, while the amount of test article ingested during lactation remained the same for each generation, the F_1 dams and F_2 pups showed a greater depression in body weight that was not detected in the earlier generation animals. However, except for possibly the 600 ppm treatment group body weight gains were not importantly different in control and dosed groups during gestation and lactation, even though there were significant differences in mean body weights at some intervals for most dosed groups.

At day 21, the mean weights of the 60, 300, and 600 ppm treatment group F_1 pups were 6, 10, and 13% lower, respectively, than the controls. These means were statistically significant for the 300 and 600 ppm groups (M and F combined).

Data on pages 108-109 of the study report indicate that body weights for the 60 ppm F_1 females at week 20 were 6.3% and at weeks 28-31 were 4-5% lower than the controls but not statistically significant. During weeks 21-27, body weights for these animals were 6-7% lower and significant.

Gestation mean body weights for the 60 ppm F_1 rats (Table 12 in the study report) were 7% decreased at days 14 and 20 and lactation mean body weights (Table T 15 in the study report) were 6-7% decreased.

The October 22, 1993 draft guidelines for Reproductive Toxicity Studies reference absolute body weights in the

parental animals as sensitive indicators of systemic toxicity (pages 36-37) and weight of the surviving pups (page 61) as an important measurement of reproductive toxicity. However, in the absence of other effects on reproduction, the depressed pup weights are considered to be indicative random results of day 4 selections.

IV. STUDY DEFICIENCIES

No significant deficiencies were noted in this study.

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DATA EVALUATION REPORT

PIRATE

Study Type: 83-5; A Chronic Dietary Toxicity and Oncogenicity Study with AC 303,630 in Rats.

Dynamac Study No. 101M/MRID 43492837

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Review Section IV, Toxicology Branch I (7509C)

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MC, Date 10/8/96

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic/oncogenicity (feeding) - rat
OPPTS Number: 870.4300 OPP Guideline Number: §83-5

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.5% ai)

SYNONYMS: CL 303,630; pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Trutter, J.A. (1994) A Chronic Dietary Toxicity and Oncogenicity Study with AC 303,630 in Rats. Hazelton Washington, Inc. Laboratory Project ID HWA 362-206. August 23, 1994. MRID 43492837 & 434292836. Unpublished.

SPONSOR: American Cyanamid Company; Agricultural Research Division; P.O. Box 400; Princeton, NJ 08543-0400.

EXECUTIVE SUMMARY:

In a chronic/oncogenicity toxicity study (MRID 43492837), Pirate (94.5% ai, Lot No. AC 7504-59A) was administered to 65 Crl:CD BR rats/sex/dose in the diet at dose levels of 0, 60, 300, or 600 ppm (0, 2.9, 15.0, or 30.8 mg/kg/day, respectively in males; 0, 3.6, 18.6, or 37.0 mg/kg/day, respectively in females) for 104 weeks.

Chronic toxicity observed in males and females at 300 and 600 ppm included slight to moderate non-neoplastic centrilobular to midzonal or diffuse hepatocellular enlargement (3/65 control, 1/65 low-, 17/65 mid-, and 47/65 high-dose in males) and (6/65 control, 1/65 low-, 18/65 mid-, and 54/65 high-dose in females). At the 300 and 600 ppm levels in both sexes, there were significant increases in mean liver-to-body weight ratios at 12 months (14-30%) and in 600 ppm rats at 24 months. **The LOEL for systemic toxicity is 300 ppm (15.0 and 18.6 mg/kg/day for males and females, respectively) based on liver toxicity, and the NOEL is 60 ppm (2.9 and 3.6 mg/kg/day for males and females, respectively) based on liver toxicity.**

There was an increased incidence of malignant histiocytic sarcoma in male rats in the 600 ppm group (4/65, 6.2%) compared to controls (0/65, 0%). Rats in this study probably could have tolerated higher dosing due to the low mortality at 600 ppm; however, there were non-

neoplastic lesions in the liver and significantly decreased body weight gains in treated groups.

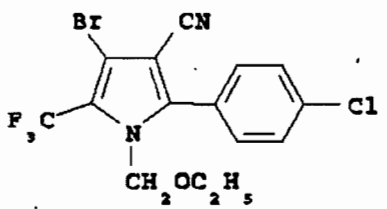
This study is classified as acceptable and satisfies the guideline requirements for a carcinogenicity study (83-2) and for a chronic toxicity study (83-1) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AC 303,630
   Description: Tan-colored solid powder
   Lot/Batch #: AC-7504-59A
   Purity: 94.5% ai
   Stability of compound: Purity varied from 94.3% to 95.2% when eight samples were tested during the period, January 13, 1992 to October 12, 1993. Average overall purity was 94.7 $\pm$ 0.35.
   CAS #: Not available
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Albino Rat
   Strain: Sprague Dawley Crl:CD BR
   Age and weight at study initiation: Approximately 6 weeks of age; body weight range 168-248 g for males and 150-208 g for females
   Source: Charles River Laboratories, Inc., Raleigh, North Carolina
   Housing: Individually housed in wire-mesh stainless steel cages
   Diet: Purina Certified Rat Chow #5002, ad libitum
   Water: Tap water, ad libitum

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Environmental conditions:
Temperature: 72 ± 6° F
Humidity: 50 ± 20%
Air changes: Not specified
Photoperiod: 12 Hour light/dark cycle
Acclimation period: 2 Weeks prior to treatment

B. STUDY DESIGN

1. In life dates - Start: October 15, 1991; end: October 18, 1993

2. Animal assignment

All animals were weighed and examined for health and ophthalmologic suitability before being accepted into the randomization pool. Animals accepted into the pool were assigned to the test groups in Table 1 using a computerized weight-randomization program, Bartlett's test, and one-way analysis of variance (ANOVA) to obtain homogeneity of group means and variances for body weight.

TABLE 1. STUDY DESIGN FOR 104 WEEK FEEDING STUDY IN RATS.

Test Group	Conc. in Diet (ppm)	Mean Compound Consumption (mg/kg/day)		Number Animals Assigned			
				Total Study 24 Months		Interim Sac. 12 Months	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	65	65	10	10
Low (LDT)	60	2.9	3.6	65	65	10	10
Mid (MDT)	300	15.0	18.6	65	65	10	10
High (HDT)	600	30.8	37.0	65	65	10	10

Data obtained from Table 8, pages 139-144, in the study report.

3. Dose Selection

The author indicated that dietary levels were selected based on findings from previous studies. However, no explanation for a range finding study or other basis was included in this submission. A 2-generation reproduction study in rats with

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AC 303,630 (MRID 434292836) used the same dietary concentrations as this study.

4. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of test substance with about 200 g of Purina Certified Rat Chow #5002. This premix was then added to the remaining amount of basal feed and further subjected to thorough mixing. Fresh diets were stored at room temperature in standard animal food containers.

Homogeneity was tested prior to initial treatment and at week 54 on aliquots from each of six areas of typical batch-size formulations for 60 and 600 ppm. Stability was assessed on six samples similarly obtained as those for homogeneity and analyzed at 7 and 16 days of storage at room temperature. A sample of each test formulation (60, 300, and 600 ppm) from weeks 1-4 and of a randomly selected dietary level for each week thereafter was analyzed in duplicate for concentration of test material.

Results:

Homogeneity Analysis: The range of prestudy 60 ppm values was 58.59-59.62 ppm with a mean percent of target of 98.6%. The range of prestudy 600 ppm values was 586.5-607.0 ppm with a mean percent of target of 99.8%.

Stability Analysis: Based on the means of 6 samples/dose/time period, there was a 3.8 and 5.0% decrease in concentration at 7 and 14 days, respectively, for the 60 ppm diets and a decrease of 4.0 and 4.3% at 7 and 14 days, respectively, for the 600 ppm diets.

Concentration Analysis: The week 1 range for 60 ppm was 59.03-59.10 ppm with a mean percent of target of 98.5%. The week 1 range for 300 ppm was 301.4-301.5 ppm with a mean percent of target of 101%. The week 1 range for 600 ppm was 592.1-596.6 ppm with a mean percent of target of 99.1%. The overall mean concentration for 60 ppm (sample size 74) was 59.1 ± 1.15 ppm with a mean percent of target of 98.5%. For 300 ppm (sample size 76), the mean was 296.5 ± 4.36 ppm with a mean percent of target of 98.8%, and for 600 ppm (sample size 74), the mean was 592.8 ± 15.36 ppm with a mean percent of target of 98.8%.

The analytical data indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Animals received fresh diet weekly.

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6. Statistics

Body weight, body weight gain, total food consumption, total food efficiency, hematology including cell morphology changes, serum chemistry, urinalysis, organ weights, and organ weight/terminal body weight ratios were analyzed for equality of means using the appropriate one-way analysis of variance (ANOVA) followed by the Dunnett's test for comparing treatment means with controls. All analyses were evaluated at the 5.0% level with group comparison at the two-tailed probability level if trend analyses were not significant. Cumulative survival data were analyzed using the National Institute package trend analysis of survival at the 5.0% two-tailed probability level. Variance homogeneity was tested by Levene's method for individual observations and by Box's method for analyses of food efficiency summary statistics.

Initially, all tumor incidences were analyzed by the unadjusted Cochran-Armitage test for trend and Fisher's exact test for group comparisons. Tumors judged to be incidental (not cause of death) were analyzed by the survival adjusted logistic prevalence method or the exact prevalence method, as appropriate. For histiocytic sarcoma and malignant lymphocytic lymphoma in males, which were assigned cause of death, life table analyses were performed taking the time of death as a surrogate for their onset. Also, female mammary carcinoma and fibroadenoma incidences were analyzed by life-table techniques.

C. METHODS

1. Observations

Animals were inspected once daily, 7 days a week for signs of toxicity and twice daily for mortality. In addition, a detailed clinical examination was performed each time the animals were weighed.

2. Body weight

Animals were weighed on a weekly basis from week 1 through the start of week 15, once every two weeks from week 15 through 27, and once monthly thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Daily food consumption was recorded weekly for weeks 1-14, once every two weeks from week 14-26, and monthly thereafter. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (mg/kg/day) values were calculated for all food-intake measurements.

4. Ophthalmoscopic examination

Eyes of each animal were subjected to an indirect ophthalmoscopic examination prior to treatment and at week 52 and 104 of treatment using 1% Mydriacyl as the mydriatic agent.

5. Blood

Blood was collected during weeks 13, 26, 52-53, 78, and 104 from fasted animals (10/sex/group) that had been randomly selected prior to the study. Blood was extracted by orbital sinus venipuncture following CO_2/O_2 inhalation anesthesia. The CHECKED (X) parameters in Table 2 and Table 3 were examined.

a. Hematology

TABLE 2. HEMATOLOGICAL PARAMETERS EVALUATED.

X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for carcinogenicity studies (only on Cont. and HDT unless effects are observed based on Subdivision F Guidelines).
Data obtained from Table 10A, pages 149-161, in the study report.

b. Clinical Chemistry*

TABLE 3. CLINICAL CHEMISTRY PARAMETERS EVALUATED.

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)		
	Serum aspartate amino-transferase (also SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X			

* Not required for carcinogenicity studies based on Subdivision F Guidelines.
Data obtained from Table 11A, pages 172-184, in the study report.

6. Urinalysis*

Urine was collected in individual urine collection cages from animals during the overnight fast prior to collecting blood for hematology and clinical chemistry. The CHECKED (X) parameters in Table 4 were examined.

TABLE 4. URINE PARAMETERS EXAMINED.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein		Urobilinogen

* Not required for carcinogenicity studies based on Subdivision F Guidelines.
Data obtained from Table 12, pages 191-222, in the study report.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues in Table 5 were collected for histological examination. The (XX) organs, in addition, were weighed. Organ/body and organ/brain weight ratios were determined on 10 rats/sex/dose only at the scheduled sacrifices (12 months and 24 months).

TABLE 5. TISSUES COLLECTED FOR PATHOLOGICAL EVALUATION.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3
X	Stomach*	X	Lymph nodes*	X	levels)*
X	Duodenum*	XX	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*		Eyes (optic n.)*
X	Ileum*				
X	Cecum*		UROGENITAL	XX	GLANDULAR
X	Colon*	XX	Kidneys*+		Adrenal gland*
X	Rectum*	X	Urinary bladder*	X	Lacrimal gland
X	Liver*+	XX	Testes*+	XX	Mammary gland*
X	Gall bladder*	XX	Epididymides	XX	Parathyroids*++
	Pancreas*	X	Prostate		Thyroids*++
X		X	Seminal vesicle		
	RESPIRATORY	XX	Ovaries*+	X	OTHER
	Trachea*	X	Uterus*	X	Bone*
X	Lung*	X	Vagina	X	Skeletal muscle*
X	Nose	X	Cervix	X	Skin*
X	Pharynx	X	Oviducts	X	All gross lesions and
X	Larynx			X	masses*
					Zymbal's gland
					Harderian gland

* Required for carcinogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.
Data obtained from Tables 14A-B and 15A-D, pages 264-361, in the study report.

II. RESULTS

A. Observations

1. Mortality - No significant increase in mortality occurred in either sex of the treated groups compared to their respective controls. Table 6 presents survival ratios for selected weeks. At 12 months the survival rate, adjusted for interim sacrifice and accidental deaths, ranged from 95-100% in males and 96-100% in females. At 24 months the survival rate among all treated groups ranged from 56%-68% in males and 35-58% in females. The highest survival rates for both sexes at 24 months were in the 600 ppm groups. The survival rates are greater than the 25% required by the guidelines at 24 months.

TABLE 6. SURVIVAL IN RATS FOR SELECTED WEEKS.

Week	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
	Males				Females			
52	62/65 95%	65/65 100%	64/64 100%	61/64 95%	64/65 98%	65/65 100%	63/65 97%	65/65 100%
78	52/55 95%	46/55 84%	52/54 96%	43/53 81%	42/54 78%	48/54 89%	46/55 84%	53/55 96%
91	47/55 85%	42/55 76%	44/54 81%	42/53 79%	34/54 63%	35/54 65%	36/55 65%	44/55 80%
104	33/54 61%	36/55 65%	30/54 56%	36/53 68%	19/53 36%	18/52 35%	24/55 44%	32/55 58%

Data obtained from Table 2A, pages 84-93, in the study report.

2. Clinical Signs - Treatment-related signs of toxicity were not observed. Commonly seen clinical signs included hunched posture, malocclusion, foreskin paraphimosis, anorexia, hypoactivity, few and soft feces, chromodacryorrhea, rough hair coat, tail sores, and urine stains.

B. Body weight

Table 7 presents the mean body weights for both sexes at selected weeks. Statistically significant ($p \leq 0.05$) decreases in body weight were observed at 600 ppm in both sexes starting at week 3 of the study. In both sexes of the 300 ppm group, mean body weight depressions frequently reached statistical significance ($p \leq 0.05$) from week 4 onwards in females and week 8 onwards in males. By week 53 mean body weights were reduced significantly in males by 5% at 300 ppm and 9% at 600 ppm, and in females by 6% at 300 ppm and 13% at 600 ppm. However, by week 105 only females exhibited statistically significant decreases in mean body weight compared to controls. Their mean body weight reductions were 17% at 300 ppm and 18% at 600 ppm. The mean body weights of both sexes at 60 ppm were generally comparable to the controls throughout the study.

Table 8 summarizes data on body weight gains. Statistically significant ($p \leq 0.05$) dose-related decreases in weekly body weight gains occurred frequently in the 300 and 600 ppm groups of both sexes during the first year of treatment. These same treatment groups in the last half of the study exhibited only occasional decreases in

mean body weight gain. Cumulative mean body weight gains relative to controls were significantly reduced statistically through week 52 in both sexes at 300 ppm and 600 ppm. In males this reduction reached 6% at 300 ppm and 12% at 600 ppm, and in females it reached 10% at 300 ppm and 22% at 600 ppm. By the end of the study cumulative mean body weight gain reductions were statistically significant only in females with a 26% reduction at 300 ppm and 29% reduction at 600 ppm.

TABLE 7. MEAN BODY WEIGHT IN RATS AT SELECTED WEEKS.

Week	Mean Body Weight (g)							
	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
	Males				Females			
1	221	217	219	218	175	171	172	173
4	368	362	363	353*	244	241	237*	234*
8	479	464*	462*	449*	288	282	279	271*
13	548	538	532	511*	314	307	308	295*
25	631	617	607*	585*	363	353	348*	329*
40	693	667	648*	622*	399	388	379*	356*
53	708	693	675*	645*	432	417	404*	375*
79	748	727	687*	660*	470	465	441	412*
92	709	698	668*	646*	508	488	464*	420*
105	636	624	600	593	471	468	392*	384*

Data obtained from Table 4, pages 113-119, in the study report.
* Statistically different from controls at p ≤0.05.

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TABLE 8. CUMULATIVE BODY WEIGHT GAINS (PERCENT OF CONTROL).

Week	Cumulative Mean Body Weight gain (g) in Rats and (Percent of Control)							
	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
	Males				Females			
1-4[a]	181 –	176 (97)	174 (96)	164 (91)	83 –	81 (98)	78 (94)	72 (87)
1-8[a]	275 –	266 (97)	263 (96)	246 (89)	120 –	114 (95)	112 (93)	106 (88)
1-13[a]	339 –	333 (98)	318 (94)	295 (87)	145 –	144 (99)	134 (92)	127 (88)
1-52[b]	487 –	476 –	456* (94)	428* (88)	258 –	246 –	231* (90)	202* (78)
1-104[b]	419 –	410 (98)	381 (91)	378 (90)	296 –	298 –	218* (74)	210* (71)

Data obtained from Table 5, pages 120-125, in the study report.
[a] Calculated by reviewers from weekly weight gains; not statistically analyzed.
[b] Calculated by study author.
* Statistically different from controls at $p \leq 0.05$.

C. Food consumption and compound intake

1. Food consumption - Weekly measurements of food consumption did not reveal meaningful differences in males in any treatment group. Food consumption in females at the 600 ppm level tended to be slightly lower than controls throughout the study and occasionally reached statistical significance ($p \leq 0.05$). Weekly food consumption in females for weeks 1-52 and 1-104 were significantly decreased ($p \leq 0.05$). Overall food consumption was similar in all groups of males but was about 7% decreased in 600 ppm females.

2. Compound consumption (time-weighted average) - Average consumption of AC 303,630 for weeks 1-104 at 60, 300, and 600 ppm levels was 2.9, 15.0, and 30.8 mg/kg/day, respectively, for males and 3.6, 18.6, and 37.0 mg/kg/day, respectively, for females.

3. Food efficiency - Throughout the study, both sexes at 300 ppm and 600 ppm occasionally showed statistically significant depressions in food efficiency, and average food

efficiency did not show any consistent trends in the treatment groups compared with controls. Total mean food efficiency in both sexes was significantly ($p \leq 0.05$) decreased statistically in the 600 ppm group during weeks 1-4 and in both the 300 ppm and 600 ppm groups during weeks 1-13 of the study.

D. Ophthalmoscopic examination

Although ocular lesions were noted in controls and treated animals, no dose-related or time-dependent relationships could be established. The lesion most frequently observed at 52 and 104 weeks was chromodacryorrhea in control and treatment groups of both sexes. Only male rats at week 104 exhibited an increased incidence of cataracts (11, 14, 30, and 22%) at 0, 60, 300, and 600 ppm, respectively. Historical Charles River mean incidence data (published in 1991) for cataracts in males at 24 months is 24/313 or 7.7% (range 0-18.8%).

E. Blood work

1. Hematology - Treatment-related effects consistent with anemia were observed in 600 ppm males during weeks 13, 26, and 52 of treatment. Table 9 summarizes mean values for erythrocyte count (RBC), hematocrit (HCT), hemoglobin (HGB), and reticulocyte count (RETIC) in control and 600 ppm males. Compared to concurrent controls, the values at 600 ppm for RBC and HGB were decreased 6-9% and HCT values were decreased 8-11% at weeks 13, 26, and 52; HCT also was decreased 6% at 78 weeks ($p \leq 0.05$). In 600 ppm females, significant decreases were only seen for RBC (7%) and HCT (6%) at week 13. Values for concurrent controls were very close to age matched reference doses for laboratory historical controls. The percentage of reticulocytes was increased in 600 ppm males throughout the study and differed significantly from controls at 26 and 52 weeks; reticulocyte percentages were significantly increased in 600 ppm females only at week 13. There were no significant differences in the hematology parameters between control and treated groups for both sexes at week 104. No treatment-related differences in differential leukocyte counts were observed in any of the treatment groups.

TABLE 9. SELECTED MEAN HEMATOLOGY DATA FOR MALE RATS.

Parameter/ Unit	Treatment Group (ppm)	Week 13	26	52	78	104
RBC (M/μl)	0	8.89	8.65	8.60	7.96	6.80
	600	8.27*	8.10*	7.85*	7.80	7.10
HCT (%)	0	46.3	44.6	44.2	41.9	35.9
	600	42.6*	41.2*	39.5*	39.5*	37.0
HGB (g/dl)	0	15.9	15.7	15.5	15.0	13.2
	600	14.8*	14.7*	14.1*	14.5	13.6
RETIC (% RBC)	0	2.1	1.8	1.3	3.0	2.9
	600	2.8	3.3*	3.0*	3.1	3.7

Data obtained from Table 10A, pages 149-161, in the study report.
* Statistically different from controls at p ≤0.05

2. Clinical Chemistry - Treatment-related effects were observed on total cholesterol and serum globulin. Table 10 summarizes the mean total cholesterol and serum globulin data. Mean total cholesterol in females receiving 600 ppm was elevated (p ≤0.05) 31-43% over concurrent controls at weeks 13, 26, 52, and 78. At week 104 total cholesterol was elevated 61% over concurrent controls; however, the author did not consider this to be statistically significant. Females in the 300 ppm group showed elevated total cholesterol over concurrent controls of 38% (p ≤0.05) at 78 weeks and 65% at 104 weeks.

The mean values for globulin, compared to concurrent controls, at 13 weeks increased (p ≤0.05) by 19% in 300 ppm females and 31% in 600 ppm females. Significantly elevated mean globulin values persisted in females at week 26 by 20% in 300 ppm and 35% in 600 ppm groups. In 600 ppm females significant increases in mean globulin levels also were seen at 52 weeks (26%) and 78 weeks (33%) but not at 104 weeks. In 600 ppm males significant increases in mean globulin were seen only at 26 weeks (18%) and at week 52 (29%). Statistically significant decreases in the mean albumin/ globulin ratios occurred in 300 ppm females at weeks 13 and 26; in 600 ppm females at weeks 13, 26, and 78; in 300 ppm males at weeks 52 and 78; and in 600 ppm males at weeks 26, 52, and 78.

TABLE 10. TOTAL CHOLESTEROL AND SERUM GLOBULIN DATA IN RATS.

Dietary Concentration (ppm)		Week 13	26	52	78	104
Total Cholesterol (mg/dL)						
Males	0	74	83	98	124	167
	60	70	76	94	81*	128
	300	69	70	82	103	145
	600	77	82	95	140	162
Females	0	85	104	113	105	107
	60	86	95	120	120	129
	300	91	108	132	145*	177
	600	111*	142*	162*	142*	172
Serum Globulin (g/dL)						
Males	0	1.8	2.2	2.1	2.6	2.9
	60	1.8	2.2	2.4	2.8	3.0
	300	1.8	2.3	2.5	2.9	3.3
	600	2.0	2.6*	2.7*	3.0	3.3
Females	0	1.6	2.0	2.3	2.4	3.3
	60	1.6	1.9	2.1	2.6	2.8
	300	1.9*	2.4*	2.6	2.8	3.4
	600	2.1*	2.7*	2.9*	3.2*	3.3

Data obtained from Table 11A, pages 172-184, in the study report.
* Statistically increased from controls at $p \leq 0.05$

Other serum chemistry parameters, such as elevated urea nitrogen in 300 ppm and 600 ppm males at week 52 and in 600 ppm males at week 78, and decreases in total bilirubin in males at all tested dose levels in week 78 were noted. However, their relatively low-magnitude, inconsistent occurrence over time, and lack of dose-response relationships indicate that they are incidental to the administration of AC 303,630.

F. Urinalysis

No treatment-related changes were observed in the urine values. Protein, bilirubin, and other urine parameters were comparable to those of controls for all weeks tested (13, 26, 52, 78, and 104).

G. Sacrifice and Pathology

1. Organ weight - Slight to moderate increases in absolute liver weights occurred at 12 and 24 months. Table 11 summarizes the liver weight data. At 12 months absolute liver weights were increased 7-13% compared to controls in males receiving 300 ppm and 600 ppm, respectively, and liver-to-body weight ratios were significantly increased at 300 and 600 ppm. Liver weights in females at 12 months were similarly increased by 8% at 300 ppm and 12% at 600 ppm, and liver-to-body weight ratios were significantly increased at 300 and 600 ppm. At terminal sacrifice, absolute liver weights were increased 12% compared with controls in 600 ppm males, and liver-to-body weight ratios were increased significantly in both sexes receiving 600 ppm.

TABLE 11. MEAN ABSOLUTE AND RELATIVE LIVER WEIGHTS IN RATS.

Parameter/Units	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
	Males				Females			
12 Months								
Liver Wt. Abs. (g)	16.0	17.1	17.3	18.0	10.2	10.5	11.0	11.4
Rel. to B. Wt. (%)	2.34	2.54	2.67*	2.88*	2.53	2.70	3.03*	3.28*
Term. B. Wt. (g)	683	672	648	627	404	391	364	349
24 Months								
Liver Wt. Abs. (g)	16.3	15.8	17.0	18.2	12.7	12.7	11.4	12.8
Rel. to B. Wt. (%)	2.65	2.67	2.94	3.30*	2.81	2.82	3.09	3.55*
Term. B. Wt. (g)	618	604	584	566	454	451	374*	365*

Data obtained from Table 14A-B, pages 264-283, in the study report.
* Statistically different from controls at $p \leq 0.05$.

Other increases in organ-to-body weight ratios that achieved a level of significance included the brain in 600 ppm males at 12 months and in 300 and 600 ppm females at 24 months, testes/epididymides in 600 ppm males at 12 months, and heart in 300 and 600 ppm females at 24 months. These increases in organ-to-body weight ratios are associated with decreased body weights and do not appear to be of toxicological importance.

2. Gross pathology - No increased incidence of gross pathological changes were observed in the organs of treated

rats compared with the controls following unscheduled deaths or at interim and terminal sacrifices.

3. Microscopic pathology

a) Non-neoplastic - Statistically significant ($p \leq 0.05$), treatment-related centrilobular to midzonal hepatocellular enlargement occurred in both sexes treated at the 300 ppm and 600 ppm levels. These changes occurred in rats at the interim sacrifice and also as diffuse enlargements in rats incurring unscheduled deaths and at terminal sacrifice. Table 12 summarizes the overall incidence and severity of liver lesions in rats.

TABLE 12. INCIDENCE AND SEVERITY OF TREATMENT-RELATED NON-NEOPLASTIC LESIONS IN RATS.

	Number Lesions/Animals Examined							
	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
Hepatocellular Enlargement	Males				Females			
Interim Sacrifice								
Centrilobular to Midzonal								
Minimal	0/10	1/10	2/10	4/10	1/10	0/10	2/10	3/10
Slight	0/10	0/10	2/10	5/10	0/10	0/10	0/10	7/10
Unscheduled Deaths								
Centrilobular to Midzonal								
Minimal	0/23	0/19	0/26	1/20	0/36	0/38	0/31	1/25
Slight	0/23	0/19	1/26	5/20	0/36	0/38	1/31	8/25
Diffuse								
Slight	1/23	0/19	3/26	3/20	0/36	1/38	1/31	8/25
Moderate	0/23	0/19	0/26	0/20	2/36	0/38	1/31	0/25
Terminal Sacrifice								
Centrilobular to Midzonal								
Slight	2/32	0/36	8/29	9/35	2/19	0/17	10/24	16/30
Moderate	0/32	0/36	0/29	0/35	0/19	0/17	0/24	6/30
Diffuse								
Slight	0/32	0/36	1/29	19/35	1/19	0/17	2/24	4/30
Moderate	0/32	0/36	0/29	1/35	0/19	0/17	1/24	1/30
Total	3/65 5%	1/65 2%	17/65* 26%	47/65* 72%	6/65 9%	1/65 2%	18/65* 27%	54/65* 83%

Data obtained from Table 15A-D, pages 284-361, and Appendices 13A-C, in the study report.
* Significantly different from control at $p \leq 0.05$.

At interim sacrifice the combined incidence of minimal/slight centrilobular to midzonal hepatocellular enlargement in the 0, 60, 300, and 600 ppm groups were 0, 10, 40, and 90%, respectively, in males, and 10, 0, 20, and 100%, respectively, in females. At terminal sacrifice centrilobular to midzonal as well as diffuse hepatocellular enlargement of slight or moderate severity were described. The combined incidence of these lesions in the 0, 60, 300, and 600 ppm groups was 6, 0, 31, and 83%, respectively, in males and 16, 0, 54, and 90%, respectively, in females.

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In the unscheduled deaths, hepatocellular (diffuse) enlargement was slight or moderate in 11 rats at 600 ppm and 5 rats at 300 ppm, and hepatocellular enlargement (centrilobular or midzonal) was minimal or slight in 15 rats at 600 ppm and 2 rats at 300 ppm. The combined incidence of these lesions in the 0, 60, 300, and 600 ppm groups was 4, 0, 15, and 45%, respectively, for males and 6, 3, 10, and 68%, respectively, for females.

Additional lesions frequently observed in numbers comparable to controls were chronic inflammation of the liver (83-92% of all rat groups), peribronchial/perivascular lymphoid infiltration in the lungs (97-100%) of all rat groups, chronic progressive nephropathy in the kidneys (92-98% of male and 83-95% of female groups), and degenerative cardiomyopathy in the heart (26-94% of male and 31-74% of female groups).

b) Neoplastic - Table 13 presents tumor incidence data. Females in the 600 ppm group exhibited a statistically significant ($p \leq 0.05$) increase in endometrial stromal polyps compared to controls when analyzed by Fisher's exact test. The incidence, which was 7.7%, is above the historical rate of 4.8% but below the maximal spontaneous incidence rate of 13.3% for the laboratory. Also, there was a relatively high number of females at risk in this group compared with controls because survival was significantly increased in 600 ppm females. When these incidence data were analyzed using the exact prevalence method to compare two groups with heterogeneous survival rates, the increase was not statistically significant. These benign proliferative lesions were not considered treatment related because they did not exceed the historical spontaneous incidence rate and when adjusted for survival, they are not statistically significant.

Treatment-related increased tumor incidence was observed at the 600 ppm dose. Males in the 600 ppm group showed a statistically significant ($p \leq 0.05$) increase of malignant histiocytic sarcoma over controls by life-table analysis. The overall incidence of malignant histiocytic sarcoma in this 600 ppm group (6.2%) is slightly above the upper maximum range of historical incidence (5.6%) for the laboratory, but it was within the maximal historical incidence of 7.1% for the Charles River Laboratories (February 1992). Histiocytic sarcoma was the cause of death in all rats in which it was observed.

Males in the 600 ppm group exhibited a slightly increased incidence of malignant lymphocytic lymphoma (5/65 or 7.7%) compared to controls, but the increase was not statistically

significant by Fisher's exact test or life-table methods. The maximum range of historical spontaneous tumor incidence for lymphocytic lymphoma at the laboratory is 5/70 or 7.1% and at Charles River Laboratories, historical incidence is 2.9%.

TABLE 13. TUMOR INCIDENCE IN RATS (ALL DISPOSITIONS).

Tumors	Number Lesions/Organs Examined							
	Concentration in Diet (ppm)							
	0	60	300	600	0	60	300	600
	Males				Females			
Uterus Endometrial Stromal Polyp	—	—	—	—	0/65 —	0/45 —	0/39 —	5/65* 7.7%
Mammary Carcinoma	—	—	—	—	11/65 17%	12/52 23%	18/52 35%	16/64 25%
Mammary Fibroadenoma	—	—	—	—	28/65 43%	37/52 71%	24/52 46%	19/64 30%
Liver Hepatocellular Adenoma	0/65 —	0/65 —	3/65 4.6%	3/65 4.6%	1/65 1.5%	0/65 —	0/65 —	0/65 —
Liver Hepatocellular Adenomas/Carcinomas	3/65 4.6%	0/65 —	5/65 7.7%	5/65 7.7%	1/65 1.5%	0/65 —	0/65 —	0/65 —
Malignant Histiocytic Sarcoma (multiple sites)	0/65 —	1/65 1.5%	1/65 1.5%	4/65* 6.2%	2/65 3.1%	0/65 —	0/65 —	0/65 —
Malignant Lymphocytic Lymphoma	1/65 1.5%	2/65 3.1%	0/65 —	5/65 7.7%	2/65 3.1%	1/65 1.5%	2/65 3.1%	0/65 —
Testis Benign Interstitial Cell Tumor	3/65 4.6%	1/22 4.5%	3/32 9.4%	7/65 10.8%	—	—	—	—

Data obtained from Table 15A-D, pages 284-361, and Appendices 13A-C, in the study report.
* Significantly different from control at $p \leq 0.05$; Fisher exact test.

The slightly increased incidences of liver adenomas in the 600 ppm male group (0/65 in controls; 3/65 at 600 ppm) and combined adenomas/carcinomas (3/65 in controls; 5/65 or 7.7% at 600 ppm) were not statistically significant. The incidence of combined hepatocellular adenoma/carcinoma at 600 ppm is comparable to the maximal historical spontaneous incidence at the laboratory based on two studies (4/50 or 8% and 5/70 or 7.1%) and below the maximal incidence published by Charles River Laboratories (27.3%). Thus, these tumors are not considered treatment related.

Animals in the 600 ppm group showed a slight increase in the benign interstitial cell tumor of the testis, an effect that appeared to increase with dose (4.6, 4.5, 9.4, and 10.8% for 0, 60, 300, and 600 ppm, respectively), but the effect was not statistically significant (pair wise or trend).

Although the incidence of mammary carcinomas was slightly elevated in treated females, the effect did not reach statistical significance by the Fisher's exact test and life-table analysis. Also, treated females showed a decreased incidence of mammary fibroadenoma at 600 ppm. Thus, the increased incidence of mammary carcinomas was not considered treatment related.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the NOEL for chronic toxic effects through 24 months was 60 ppm (2.9 and 3.6 mg/kg/day, respectively, for males and females), and the LOEL was 300 ppm, based on hepatocellular enlargement in the 300 and 600 ppm treatment groups. The NOEL for oncogenic effects through 24 months was 600 ppm (30.8 and 37.0 mg/kg/day for males and females, respectively).

B. Reviewer's Discussion

Male and female Crl:CD BR rats were fed AC 303,630 (Pirate; 94.5% ai) at 0, 60, 300, or 600 ppm for 24 months. Average calculated test substance consumption for the 60, 300, and 600 ppm groups was: 0, 2.9, 15.0, or 30.8 mg/kg/day, respectively, for males; and 0, 3.6, 18.6, or 37.0 mg/kg/day, respectively, for females.

Mortality among treated animals was comparable to that of controls (Table 6). Most of the deaths occurred during the last 12 months of the study. Pituitary adenoma/carcinoma and mammary fibroadenoma/carcinoma, which occurred with an incidence comparable to controls, were the most frequent causes of death.

Mean body weights and body weight gains of both sexes in the 300 and 600 ppm groups showed statistically significant, treatment-related decreases during the first year of the study (Tables 7 and 8). By the last week of the study, only females showed statistically significant decreases in mean body weights and body weight gain. Mean body weight gain was reduced in females by about 26% at 300 ppm and 29% at 600 ppm over the 104-week study. Average food consumption did not

show consistent trends in males and was only occasionally depressed statistically in females. Although both sexes at 300 ppm and 600 ppm demonstrated significantly diminished food efficiencies during weeks 1-13 of the study, no consistent trends were observed in either sex during the 104 week study.

There were significant decreases in erythrocyte count, hemoglobin concentration, and hematocrit in 600 ppm males at weeks 13, 26, and 52 and hematocrit at week 78 of treatment (Table 9). Males in the 600 ppm group also showed statistically significant elevations in mean reticulocyte values at weeks 26 and 52 and absolute reticulocyte levels at week 52. These findings are consistent with anemia and appear to be treatment related. Anemia, similar to that observed in males, did not occur in females, although females in the 600 ppm group showed a significant decrease in mean erythrocyte count and hematocrit and elevated mean reticulocyte levels at week 13. None of the hematological changes persisted beyond week 78 in males and week 13 in females, suggesting that the animals may have adapted to the effect. The increases noted in serum globulin and total cholesterol during weeks 13, 26, 53, and 78 may be attributed to treatment (Table 10). No treatment-related effects were observed on clinical signs, gross pathology, and urinalysis.

The increased incidence of cataracts in males receiving 300 or 600 ppm AC 303,630 may not be toxicologically important. Females in all groups exhibited a very low incidence of cataracts.

The treatment-related increases observed in the liver-to-body weight ratios of both sexes at 300 ppm and 600 ppm are consistent with the increased incidences of histopathological lesions in the livers of these groups (Table 11). The increased mean organ-to-body weight ratio for the testes/epididymides at 600 ppm corresponds to histopathological evidence of an increased incidence of benign testis interstitial cell tumors. The increased mean brain-to-body weight ratio in males at 600 ppm did not correspond with any significant histopathological changes.

Non-neoplastic microscopic pathological studies revealed treatment-related centrilobular to midzonal hepatocellular enlargement, sometimes characterized as diffuse enlargement, in both sexes at 300 ppm and 600 ppm (Table 12). The severity of this lesion ranged from slight to minimal in animals at interim sacrifice and those incurring unscheduled deaths. Diffuse hepatocellular enlargement in unscheduled deaths was characterized as slight or moderate, but was usually of slight severity. At terminal sacrifice hepatocellular enlargement was described as slight or moderate.

Microscopic evaluations showed a variety of spontaneous neoplasms typical of those found in rats of this strain. Among them were pituitary adenomas/carcinomas and mammary fibroadenomas/carcinomas. These neoplasms occurred more frequently than any other neoplasms but in comparable numbers with controls and were the most common cause of death.

The statistically significant increased incidence of malignant histiocytic sarcoma compared with controls in 600 ppm males is below the maximum historical rate reported by the Charles River Laboratories but is slightly above the upper maximum range of historical incidence for the laboratory (Table 13). This neoplasm corresponds with the increased incidence of non-neoplastic hepatic lesions and increased mean organ-to-body weight ratios in the liver of males at the 300 ppm and 600 ppm level. The slight increase in another lymphoreticular system neoplasm, malignant lymphocytic lymphoma, in males at 600 ppm does not appear to be treatment related because it is not significantly different from controls.

The incidence of hepatic adenomas, carcinomas, and combined adenomas/carcinomas are not significantly increased statistically and thus are not likely to be treatment related. Similarly, the slight increase in benign interstitial cell tumor of the testis was not statistically significant but may correspond to the significantly increased mean organ-to-body weight ratio of the testis/epididymis observed at the 600 ppm level.

The benign endometrial stromal polyps that were statistically increased in the uterus of females at 600 ppm when analyzed by the Fisher's exact test are below the maximal spontaneous incidence rate for the laboratory and are not considered treatment related.

No explanation for the dose levels used in this study were presented. However, a 2-generation reproduction study in rats with AC 303,630 (MRID 434292836) used the same dietary concentrations as this study. Although mortality was not increased statistically, suggesting that the animals might have tolerated a higher dose, the decreased body weight and body weight gain, increased liver-to-body weight ratios, and the significant occurrence of non-neoplastic lesions in the liver at 300 ppm and 600 ppm indicate that these dose levels may be adequate to characterize chronic toxicity and carcinogenic potential.

IV. STUDY DEFICIENCIES

This chronic/oncogenicity study (83-5) is acceptable for carcinogenicity and satisfies the guideline requirements for a

carcinogenicity study (83-2) in rats. The study is considered acceptable for chronic toxicity (83-1) because it provides scientifically valid information that is fully documented and clearly addresses the study objectives as outlined in Subdivision F.

DATA EVALUATION RECORD

PIRATE

Study Type: 83-5; A Chronic Dietary Toxicity and Oncogenicity Study with AC 303,630 in Mice

Work Assignment No. 101N (MRID 43492838)

Prepared for
Health Effects Division
Office of Pesticide Programs
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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: W. Greear, M.P.H. _____, Date 10/9/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, D.V.M. _____, Date 10/9/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic/oncogenicity OPPTS 870.4300 [§83-5] [Feeding - Mice]

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.5% a.i.)

SYNONYMS: CL 303,630, pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Bernier, L. (1994) A Chronic Dietary Toxicity and Oncogenicity Study with AC 303,630 in Mice. Bio-Research Laboratories Ltd. (Senneville, Quebec). Laboratory Project ID 84580, August 22, 1994. MRID 43492838. Unpublished study submitted by American Cyanamid Company, Princeton, NJ.

SPONSOR: American Cyanamid Company; Agricultural Research Division; P.O. Box 400; Princeton, NJ 08543-0400.

EXECUTIVE SUMMARY:

In a chronic toxicity/oncogenicity study (MRID 43492838), Pirate (94.5% a.i., Lot No. AC-7504-59A) was administered to 65 male and 65 female Swiss Crl:CD®-1(ICR)BR mice/sex/dose in the diet at dose levels of 0, 20, 120, or 240 ppm (0, 2.8, 16.6, or 34.5 mg/kg/day, respectively, in males; 0, 3.7, 21.9, or 44.5 mg/kg/day, respectively, in females) for 80 weeks.

Chronic toxicity observed in males and females at 120 and 240 ppm included decreased body weight gains, non-neoplastic brain vacuolation primarily in the white matter of the corpus callosum, tapetum, hippocampus, and cerebellum. Body weight gains decreased in males and females of 23 and 21%, respectively, at 240 ppm and 11 and 12%, respectively, at 120 ppm, by the end of study. The incidence of brain vacuolation in males was 4/65 control, 14/64 mid-, and 49/65 high-dose, and in females it was 10/65 control, 28/65 mid-, and 58/65 high-dose. Males and females at 240 ppm also exhibited vacuolation of the spinal cord and optic nerve. Treatment-related gross pathological changes, including skin ulceration and scabbing, occurred in males and females at the 240 ppm level, and scabbing occurred in males at 120 ppm. **The LOEL for systemic toxicity is 120 ppm (16.6 and 21.9 mg/kg/day in males and females, respectively) based on decreased body weight gains, brain vacuolation and scabbing of**

the skin (males), and the NOEL is 20 ppm (2.8 and 3.7 mg/kg/day for males and females, respectively).

At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls. Survival in females was depressed by 40% in the 240 ppm treatment group. Dosing was considered adequate based on decreased body weight gain and brain lesions in males and females.

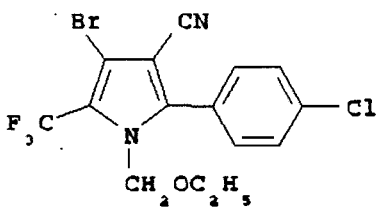
This chronic/oncogenicity study in mice is **acceptable** for oncogenicity and satisfies the guideline requirement for a carcinogenicity study (83-2) in mice. The study is **acceptable** for chronic toxicity (83-1) although clinical chemistry and urinalysis data are missing.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: AC 303,630
   Description: beige powder
   Lot/Batch #: AC-7504-59A
   Purity: 94.5% a.i.
   Stability of compound: Storage stability was tested prior to study initiation and every 12 weeks between February 20, 1992 and August 9, 1993 and revealed a purity range of 94.8%-94.4% a.i.
   CAS #: Not available



2. Vehicle and/or positive control: None

3. Test animals: Species: Albino Mouse
   Strain: Swiss Crl:CD®-1(ICR)BR
   Age and weight at study initiation: 6 Weeks (± 1 day) of age; body weight range 20.6-27.0 g for males and 17.2-21.9 g for females
   Source: Charles River, St. Constant, Quebec, Canada
   Housing: Individually housed in mesh-bottomed stainless steel cages
   Diet: PMI Feeds, Inc. Certified Rodent Show #5002 ad libitum

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Water: Tap water-softened, purified by reverse osmosis, and sterilized by ultraviolet light ad libitum
Environmental conditions:
Temperature: Target 22 ± 3° C
Humidity: 50 ± 20%
Air changes: Not specified
Photoperiod: 12 Hour light/dark cycle
Acclimation period: 2 Weeks prior to treatment

B. STUDY DESIGN:

1. In life dates: - start: December 9, 1991; end: July 5, 1993

2. Animal assignment:

All animals were weighed and assigned to the test groups in Table 1 using a computer-based randomization procedure that ensured homogeneity of group means and variances for body weight. The animals, replaced within the first 2 weeks of treatment included five due to an unclear tail tattoo, a female control and a female in the 20 ppm group sacrificed because the author did not believe they would survive to terminal sacrifice, and one 240 ppm female found dead. None of the data on these animals were included in the results of the study.

TABLE 1. STUDY DESIGN FOR 80 WEEK FEEDING STUDY IN MICE

Test Group	Conc. in Diet (ppm)	Mean Compound Consumption (mg/kg/day)		Number Animals Assigned			
				Total Study 80 Weeks		Interim Sac. 52 Weeks	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	65	65	10	10
Low (LDT)	20	2.8	3.7	65	65	10	10
Mid (MDT)	120	16.6	21.9	65	65	10	10
High (HDT)	240	34.5	44.5	65	65	10	10

Data extracted from Study No. 84580 (MRID 43492838) Table No. 10, p 84.

3. Dose Selection:

The author did not include a rationale for the selected dose levels. The animals in this study may have been able to tolerate a higher dosing. However, the 13-week dietary toxicity study of AC 303,630 in CD-1 mice (MRID

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43492830) showed depressions of mean body weight gains (significant at $p < 0.05$) that reached 26% in males and 29% in females in the highest (320 ppm) treatment group compared to controls while food consumption remained generally comparable to that of controls. Also, significant incidences of spongioform(encephalo)myelopathies were observed on microscopy in males and females in the 320 ppm treatment group.

4. Diet preparation and analysis:

Diet was prepared weekly by mixing appropriate amounts of test substance with a small quantity of pre-weighed powdered PMI Feeds, Inc. Certified Rodent Show #5002 in a mortar. The remaining pre-weighed basal diet was added to this mix and blended in a Hobart blender for 15 minutes. Fresh diets were stored in the animal rooms at room temperature in closed polyethylene containers.

Homogeneity and stability were tested on aliquots from the top, middle, bottom, left, and right in each diet mix at 20 and 240 ppm. Homogeneity was assessed prior to animal treatment. Stability was assessed after 7 and 14 days of storage at room temperature in the animal room. Concentration analyses were conducted weekly from week 1-14 and thereafter whenever food and body weights were measured. They were reported weekly for the first 4 weeks and thereafter for weeks 7, 9, 13, 20, 22, 29, 36, 37, 38, 44, 46, 50, 53, 60, 63, 68, 72, 73, and 77.

Results -

Homogeneity Analysis: The range of prestudy 20 ppm values was 18.27-21.13 ppm which averaged 19.41 ppm (average nominal of 97.1%). The range of prestudy 240 ppm values was 229.35-250.54 ppm which averaged 242.66 ppm (average nominal of 101.1%).

Stability Analysis: The 7-day 20 ppm range of values was 18.46-21.11 ppm which averaged 19.32 ppm (average nominal of 96.6%). The 7-day 240 ppm range of values was 243.91-247.51 ppm which averaged 245.71 ppm (average nominal of 102.4%).

The 14-day 20 ppm range of values was 17.75-19.27 ppm which averaged 18.44 ppm (average nominal of 92.2%). Analysis of other samples of the 20 ppm mix showed a range of 18.92-20.52 ppm which averaged 19.72 (average nominal of 98.6%). The 14-day 240 ppm range of values was 221.03-233.95 ppm which averaged 225.94 ppm (average nominal of 94.1%). Analysis of other samples of the 240 ppm mix at 14 days showed a range of 226.16-

233.94 ppm which averaged 230.05 ppm (average nominal of 95.9%).

Concentration Analysis: The week 1 range for 20 ppm was 19.28-21.17 ppm which averaged 20.23 ppm (average nominal of 101.2%). The week 1 range for 120 ppm was 118.34-125.24 ppm which averaged 121.79 ppm (average nominal of 101.5%). The week 1 range for 240 ppm was 243.91-247.51 ppm which averaged 245.71 ppm (average nominal of 102.4%). Table 2 presents the average percent nominal concentration for these and other selected weeks.

TABLE 2. AVERAGE PERCENT NOMINAL CONCENTRATION

Study Week	Average Percent Nominal Concentration AC 303,630 in Diet		
	20 ppm	120 ppm	240 ppm
1	101.2	101.5	102.4
2	101.3	98.3	97.1
3	98.5	105.0	110.1
4	85.8	97.5	100.4
13	101.2	100.6	103.6
53	94.1	92.1	96.4
77	92.2	91.9	92.6

Data extracted from Study No. 84580 (MRID 43492838) Appendix No. 22, pp 1925-1941.

The analytical data indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. However, analyzed concentrations differed from nominal by more than 5% at 5/23, 3/23, and 3/23 intervals at the low-, mid-, and high-dose, respectively.

5. Animals received fresh diet weekly.

6. Statistics: Body weight, body weight gain, food consumption, food efficiency, hematology, and organ weight data were analyzed for homogeneity of group variances using Bartlett's test. Heterogeneous group variances were analyzed using the Kruskal-Wallis test, and the significance of intergroup differences was

assessed using the Wilcoxon's test. Homogeneous group variances for mean body weights, body weight gains, food consumption, and food efficiency were analyzed for homogeneity of slopes. If the slopes were determined to be homogeneous, they were analyzed using a covariance analysis. Intergroup differences were determined with a t-test on the least square means (using week 0 or week-1 data as covariates). When no pretreatment data were available or when slopes were heterogeneous ($p = 0.05$), the data were subjected to an analysis of variance (ANOVA) and intergroup differences were assessed using the Dunnett's test.

Non-neoplastic and neoplastic lesions were selected for analysis if at least one or more treated group had an absolute occurrence of at least five for the lesion being considered and the incidence of that lesion in a treated group was at least 5% higher than that of the control group. To compare the distribution of selected lesions at the 80 week termination, the Cochran-Armitage test for trend and Fisher's exact method for group differences were used. The Statistical Analysis System (SAS) "Chronic" procedure using life table analyses were applied to mortality and tumor data.

C. METHODS:

1. Observations:

Animals were inspected by cageside observation twice daily, 7 days a week for signs of toxicity and mortality. In addition, a detailed clinical examination was performed weekly on each animal.

2. Body weight:

Animals were weighed individually on a weekly basis during the last week of the acclimatization period and during the first 14 weeks of treatment. They were weighed biweekly from weeks 14 to 26, inclusive, and once monthly thereafter.

3. Food consumption and compound intake:

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day weekly from weeks 1-14, inclusive. Thereafter it was determined whenever body weights were measured. Food efficiency (body weight gain in g/food consumption in g per mouse per week X 100) and compound intake (mg/kg/day) values were calculated for the first 13 weeks

as time-weighted averages from the food consumption and body weight gain data.

4. Ophthalmoscopic examination:

Eyes were examined in all animals prior to treatment, in animals found dead or sacrificed in moribund condition, and in all animals sacrificed at 52 and 80 weeks, but the method of examination was not specified.

5. Blood was collected for hematology from fasted animals scheduled for interim sacrifice and from 10 selected mice/sex/group at 80 weeks. However, blood samples could not be obtained from three animals at interim sacrifice and one animal at terminal sacrifice. In addition, blood smears were obtained at necropsy from all animals sacrificed in moribund condition during the study. The CHECKED (X) parameters in Table 3 were examined.

a. Hematology

TABLE 3. HEMATOLOGICAL PARAMETERS EVALUATED

X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements	X	Red Cell Morphology
	(Thromboplastin time)	X	Mean Platelet Volume
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for carcinogenicity studies (only on Cont. and HDT unless effects are observed based on Subdivision F Guidelines).
Data extracted from Study No. 84580 (MRID 43492838) Table No. 11, pp 85-98.

b. Clinical Chemistry was not evaluated.

6. Urinalysis was not conducted.

7. Sacrifice and Pathology:

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues in Table 4 were collected for histological examination. The (XX) organs, in addition, were weighed. Organ weights were assessed in all animals killed at interim sacrifice and in the 10 mice/sex/group selected for hematological evaluations at the end of the study.

Histological examination was performed on a full complement of tissues from all animals in the control and 240 ppm groups scheduled for interim and terminal sacrifices and from all animals in all dose groups that died or were sacrificed moribund.

TABLE 4. TISSUES COLLECTED FOR PATHOLOGICAL EVALUATION

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	XX	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+	XX	Adrenal gland*
X	Rectum*	X	Urinary bladder*	X	Lacrimal gland
XX	Liver*+	X	Testes*+	X	Mammary gland*
X	Gall bladder*	X	Epididymides	X	Parathyroids*++
X	Pancreas*	X	Prostate	X	Thyroids*++
		X	Seminal vesicle		
	RESPIRATORY	XX	Ovaries*+		OTHER
X	Trachea*	X	Uterus*	X	Bone*
XX	Lung*	XX	Gonads	X	Skeletal muscle*
X	Nose			X	Skin*
	Pharynx			X	All gross lesions and masses*
	Larynx				

* Required for carcinogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.
Data extracted from Study No. 84580 (MRID 43492838) Tables 16-27, pp 123-278.

II. RESULTS

A. Observations:

1. Toxicity - The clinical signs that occurred with slightly greater frequency in treated than in control groups involved mainly the pinna, skin, and fur and included redness, scabbing, swelling, flaking, or missing pinnas; skin ulcerations and scabs, mainly on the pinna but also on the cervical and urogenital regions; alopecia and thinning of the fur; wet or ungroomed fur in various body regions; dehydration; and a weak, thin body condition. Treated mice that died before the end of the study exhibited these signs slightly more frequently than the animals scheduled for sacrifice. At interim sacrifice, only an increased incidence of pinna changes was observed compared with controls in treated males. At terminal sacrifice, treated males in the 240 ppm group exhibited

an increased frequency of mainly skin ulcerations and scabs, and pinna changes.

2. Mortality - Overall mortality was statistically increased compared with controls in the 240 ppm female group. Most of the deaths in this group occurred after the first year of treatment. At 15 months the survival rate in males and females was 82 - 89%, thus exceeding the guideline requirement of not less than 50% survival. At termination, survival rates ranged in males from 71% to 78% and in females from 60% to 80%, far exceeding the guideline requirement of not less than 25%. Excluding the 10 mice/sex/group selected for sacrifice at 52 weeks, the distribution of survival within each test group over 80 weeks and at termination is presented in Table 5.

   Historical data presented by the author for the same laboratory included three 18-month oncogenicity studies conducted between 1988 and 1991 with CD-1 mice. Survival in control females in these studies was 70% (week 78), 75% (week 80), and 80% (week 78).

TABLE 5. SURVIVAL RATE IN MICE

Week	Percent Survival of Original Group Size							
	Concentration in Diet (ppm)							
	0	20	120	240	0	20	120	240
	Males				Females			
16	100	100	100	100	100	100	100	100
52	96	95	93	91	93	89	85	95
64	89	85	89	87	87	82	84	82
72	80	80	84	82	85	78	80	67
80	75	76	71	78	80	71	73	62
Termination	75	75	71	78	80	71	73	60*

* Statistically different from controls when analyzed using life-table analysis.
Data extracted from Study No. 84580 (MRID 43492838) Table No. 2, p 51.

B. Body weight:

At randomization (week -1) mean body weights of treated animals were all comparable to those of controls, but on day 0, prior to initiating treatment, males in the 20 and

240 ppm groups exhibited a slight but statistically significant decrease in mean body weight (Table 6). Throughout the study this difference increased between controls and the 240 ppm treated males. Among the females, statistically significant decreases in mean body weights were observed occasionally from week 1 in animals receiving 240 ppm and from week 16 in animals receiving 120 ppm. By week 30 in 120 ppm females and week 34 in 240 ppm females the decrease in mean body weight was statistically significant compared with controls on almost all occasions through the end of the study.

TABLE 6. MEAN BODY WEIGHT IN MICE

Week	Mean Body Weight (g)							
	Concentration in Diet (ppm)							
	0	20	120	240	0	20	120	240
	Males				Females			
0	27.0	26.5*	26.8	26.1***	21.1	20.7	20.8	21.0
2	30.0	29.4	29.5	28.4***	23.2	22.6	22.7	22.5***
4	32.0	31.6	31.8	30.5	24.9	24.3	24.3	24.1***
6	33.4	33.1	33.5	32.2	25.9	25.6	25.4	25.1*
8	34.7	34.3	34.7	33.2**	26.7	26.2	26.5	26.2
13	36.3	36.0	36.3	34.6*	28.1	27.9	28.0	27.7
16	37.4	36.9	37.2	35.5*	29.6	29.3	28.5*	28.6*
34	40.3	39.0	39.5	37.0***	32.4	31.9	30.6**	30.5***
54	42.5	40.6	41.7	38.3***	34.8	33.9	32.3**	32.8
62	42.7	40.6	41.7	38.2***	35.1	33.6	32.6*	33.1*
70	43.3	41.4	41.6	37.4***	35.7	35.1	33.0*	33.3*
80	43.1	40.9	42.0	37.2***	35.5	35.4	33.2*	33.6*

* Statistically different from controls at p <0.05).
** Statistically different from controls at p <0.01).
*** Statistically different from controls at p <0.001).
Data extracted from Study No. 84580 (MRID 43492838) Table No. 6, p 62-67.

Treatment with AC 303,630 resulted in a lower growth rate overall that was statistically significant (p <0.05) in both sexes receiving 240 ppm and in females receiving 120 ppm. Between weeks 0 and 13 mean weight gains in males at 240 ppm were 84.0% of control (calculated by reviewers). Overall mean body weight gain in males was 23% lower at 240 ppm than that of controls by the end of the study. Similarly, in

females at study termination, mean body weight gain was 12 and 21% lower in the 120 ppm and 240 ppm groups, respectively, compared with the control. Table 7 presents mean body weight gain at selected intervals for both sexes.

TABLE 7. MEAN BODY WEIGHT GAIN IN MICE

Week	Mean Body Weight Gain (g)							
	Concentration in Diet (ppm)							
	0	20	120	240	0	20	120	240
	Males				Females			
-1 - 0	3.2	2.6	2.9	2.3**	1.7	1.3	1.3	1.5
1 - 2	1.3	1.2	1.2	1.1	0.8	0.6	0.7	0.7
3 - 4	0.8	1.0**	1.1***	1.0*	0.6	0.6	0.6	0.6
4 - 5	0.6	0.8	0.8	0.6	0.2	0.5*	0.8***	0.7***
5 - 6	0.8	0.7	0.8	1.1**	0.8	-.9	0.4**	0.4*
7 - 8	0.7	0.6	0.7	0.7	0.6	0.1**	0.6	0.5
12 - 13	0.4	0.5	0.4	0.3	-0.2	0.2**	0.4***	0.5***
30 - 34	0.2	-0.3	0.1	-0.2	0.7	1.1	0.6	-0.1**
52 - 54	-0.02	-0.4	0.2	-0.8***	0.2	-1.0**	-0.3*	-0.3
58 - 62	0.4	0.6	0.6	0.4	0.8	0.4	0.7	0.6
66 - 70	0.2	0.5	0.1	-0.8***	0.3	0.3	-0.01	0.4
78 - 80	-0.4	-0.3	-0.3	0.05	-0.4	0.3	0	-0.2
0 - 80	15.0 —	13.5 (90%)	13.3 (89%)	11.5** (77%)	15.0 —	15.7 (105%)	13.2* (88%)	11.9* (79%)

* Statistically different from controls at p <0.05).
** Statistically different from controls at p <0.01).
*** Statistically different from controls at p <0.001).
Data extracted from Study No. 84580 (MRID 43492838) Table No. 7, p 70-75.

C. Food consumption and compound intake:

1. Food consumption - Prior to initiating treatment, males scheduled to receive 20 ppm or 240 ppm exhibited a statistically significant decrease in mean food consumption compared with controls. This decrease, though minimal, persisted in males throughout the first 13 weeks of treatment but was not statistically significant. The overall mean decrease in males during weeks 0-13 in the 240 ppm group was 3.4%. Mean food consumption overall during weeks 13-26 in males was 2.6%

and 3.6% lower than controls at 120 and 240 ppm, respectively. During week 12-13 mean food consumption in males was 36.5 g/mouse for the control, 36.9 g/mouse in the 20 ppm group, 36.8 g/mouse in the 120 ppm group, and 36.4 g/mouse in the 240 ppm group. During week 79-80, food consumption in males was 32.1 g/mouse for the control, 31.4 g/mouse in the 20 ppm group, 29.8 g/mouse in the 120 ppm group, and 28.2 g/mouse in the 240 ppm group.

In females at all dose levels, weekly food consumption was comparable or slightly higher than the control levels. The dosed groups exhibited an overall increase of 5-8% during weeks 1-13. From week 33 onwards, females receiving 120 ppm or 240 ppm AC 303,630 had comparable or slightly lower weekly food consumption values than controls.

Thus, AC 303,630 was associated with a marginal decrease in food consumption between weeks 0 and 13 in males receiving 240 ppm (average 96.6% of control) and between weeks 13 and 26 in males receiving 120 ppm (97.7% of control) and 240 ppm (96.5% of control). In females, overall food consumption during weeks 1-13 was 5-8% greater in the dosed groups than in controls.

2. Compound consumption (time-weighted average) - The average AC 303,630 consumption for male mice during the 80-week treatment period was 2.8 mg/kg/day (range 2-4) in the 20 ppm group, 16.6 mg/kg/day (range 12-22) in the 120 ppm group, and 34.5 mg/kg/day (range 26-44) in the 240 ppm group. For female mice, compound consumption was 3.7 mg/kg/day (range 3-5) in the 20 ppm group, 21.9 mg/kg/day (range 16-28) in the 120 ppm group, and 44.5 mg/kg/day (range 32-60) in the 240 ppm group.

3. Food efficiency - Although food efficiency in females was variable (ranging from -0.21 to 2.90% in the 240 ppm group compared with -0.63 to 4.12% in controls) during the first 13 weeks of the study, there were no indications that food efficiency was impaired consistently in either males or females.

D. Ophthalmoscopic examination - No treatment-related ophthalmoscopic abnormalities were noted.

E. Blood work:

1. Hematology - There were no important effects on hematology parameters; nearly all values were within the normal range. At interim sacrifice, males in the 240 ppm treatment group exhibited a statistically significant

($p \leq 0.05$) increase in mean corpuscular hemoglobin concentration (MCHC). Their MCHC was 34.1 ± 0.69 g/dl compared with control levels of 32.9 ± 1.13 g/dl. No other changes in erythrocyte indices including mean corpuscular volume, mean corpuscular hemoglobin, and red cell distribution width (RDW) were observed. Also, this effect did not reach statistical significance in mice sacrificed at the end of the study. However, at terminal sacrifice, males in the 240 ppm group exhibited a statistically significant ($p < 0.01$) increase in RDW (16.2 ± 3.1% compared with 13.4 ± 0.75% in controls).

Females at the interim sacrifice exhibited no unusual hematological effects. At terminal sacrifice, mean platelet volume was significantly depressed ($p < 0.05$) but only in the 20 ppm and 120 ppm treatment groups. Also, at terminal sacrifice, the mean monocyte level in the differential white blood cell count of the 240 ppm female group showed a statistically significant ($p < 0.001$) decrease.

2. Clinical Chemistry - Clinical chemistry was not measured.

F. Urinalysis - Urine was not collected.

G. Sacrifice and Pathology:

1. Organ weight - At terminal sacrifice, absolute kidney weights of males at 240 ppm were significantly ($p < 0.01$) decreased (19%), but the kidney to body weight ratio was not affected suggesting that the effect was related to the decrease in mean body weight. No significant effects on the kidney weights were observed at 12 months in the 240 ppm males. Absolute liver weights in the 240 ppm females were slightly increased (non-significantly) at 12 months, but not at termination. Also at 12 months liver to body weight ratios in females were increased slightly (not significant) in the 240 ppm group and increased significantly ($p < 0.05$) at termination at 120 ppm but not significantly at 240 ppm. None of the effects on organs weights were considered of toxicological importance since no microscopic findings correlated with the changes.

2. Gross pathology - Males, and to a lesser extent females, in the 240 ppm treatment group exhibited a slightly increased incidence of skin ulceration and scabbing, and males at 120 ppm exhibited an increased incidence of skin scabbing. The majority of the animals that had skin ulcerations and scabbing were those that died preterminally or were sacrificed in a moribund condition. These pathological indices were not elevated in mice

sacrificed at 52 weeks. Carcass emaciation and digesta discoloration occurred only slightly more often in males in the 240 ppm group when data were combined from all unscheduled and scheduled deaths.

3. Microscopic pathology -

a) Non-neoplastic - The statistically significant and treatment-related non-neoplastic lesions that occurred when lesions from all deaths were combined, including those sacrificed and those found dead or dying were the following:

- Increased incidence of vacuolation of the brain, characterized (but not defined) as slight to severe, occurred in males and females of the 120 and 240 ppm treatment groups. The incidence of vacuolation of the brain in almost all preterminal animals occurred after the first year of treatment. At interim sacrifice 1/10, 0/10, 0/10, and 6/10 of the males and 1/10, 0/10, 3/10, and 6/10 of the females from groups 0, 20, 120, or 240 ppm, respectively, exhibited brain vacuolation. The incidence of this finding in all other animals (unscheduled deaths or terminally sacrificed) was 3/55, 3/55, 14/55, and 43/55 in males and 9/55, 5/55, 25/55, and 52/55 in females from groups 0, 20, 120, or 240 ppm, respectively. In 240 ppm animals, vacuolation was generally observed in the white matter of the corpus callosum, tapetum, hippocampus, and cerebellum. Table 8 presents the overall incidence of selected non-neoplastic lesions in mice.

- Vacuolation was observed less frequently in the spinal cord, particularly thoracic and cervical areas, of preterminal and terminally sacrificed males and females. The incidence of this finding in the thoracic and cervical cord reached statistical significance in males and females at 240 ppm. Males at this dose level also exhibited a statistically significant increase in vacuolation of the lumbar cord. In the majority of 120 ppm animals, central nervous system vacuolation was limited mainly to the brain.

- A statistically significant increased incidence of vacuolation of the optic nerve occurred in male and female mice at the 240 ppm treatment level.

Vacuolation of the central nervous system was attributed to AC 303,630 because the incidence was statistically significant and dose-related. Other non-neoplastic lesions with incidences that reached statistical significance at the 240 ppm level but may not be related

to treatment include increased bone marrow myelopoiesis in males and histiocytosis of the lung and lacrimal gland amyloidosis in females.

TABLE 8. NON-NEOPLASTIC LESIONS IN MICE

Site and Lesion	Number Lesions/Animals Examined							
	Concentration in Diet (ppm)							
	0	20	120	240	0	20	120	240
	Males				Females			
Brain: Vacuolation White Matter	4/65	3/65	14/65*	49/65*	10/65	5/65	28/65*	58/65*
Spinal Cord: Vacuolation								
Cervical Cord	0/65	0/65	2/65	20/65*	1/65	0/65	0/65	23/65*
Thoracic Cord	0/65	1/65	2/65	17/64*	2/65	0/65	1/65	16/65*
Lumbar Cord	0/65	0/65	2/65	11/65*	0/65	0/65	0/65	3/65
Optic Nerve: Vacuolation	0/63	0/64	0/62	12/65*	0/65	0/65	1/62	14/64*
Skin: Dermatitis	9/65	12/65	11/65	21/65	3/65	1/65	6/65	9/65
Bone Marrow: Increased Myelopoiesis	17/65	5/14	5/16	27/65*	8/65	2/16	2/15	7/65
Lung: Histiocytosis	5/65	4/65	9/65	9/65	2/65	3/65	0/65	10/65*
Lacrimal Gland: Amyloidosis	0/65	0/14	3/16	0/65	0/65	0/16	1/15	5/65*

* Significantly different from control at $p < 0.05$.
(Statistical analysis was not conducted for skin dermatitis)
Data extracted from Study No. 84580 (MRID 43492838) Table No. 27, p 253-278.

b) Neoplastic - The number of treated males and females with one or more benign, malignant, or combined [benign + malignant] neoplasms was similar to that observed in their respective untreated controls. The incidence of neoplasms did not show statistical significance when compared with controls and was viewed by the authors as low for CD-1 mice. The highest incidence of tumors occurred in the lungs, but the incidence was similar to controls. Table 9 presents the incidence of neoplastic lesions of the liver and lungs.

TABLE 9. INCIDENCE OF NEOPLASTIC LESIONS OF LIVER AND LUNGS

Organ/Neoplasm	Number Lesions							
	Concentration in Diet (ppm)							
	0	20	120	240	0	20	120	240
	Males				Females			
	(65) *	(65)	(65)	(65)	(65)	(65)	(65)	(65)
Liver:								
Hepatocellular Adenoma	8	10	4	2	0	1	1	0
Hepatocellular Carcinoma	1	3	0	0	0	0	0	0
	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)
Lung:								
A/B Adenoma	16	12	13	10	7	7	8	9
A/B Carcinoma	2	1	3	4	1	2	0	1

* Number of tissues examined.
Data extracted from Study No. 84580 (MRID 43492838) Table No. 23, p 190-194.

III. DISCUSSION

A. Male and female CD-1 mice were fed AC 303,630 (Pirate; 94.5% a.i.) at 0, 20, 120, or 240 ppm for 18 months. Average calculated test substance consumption for the 20, 120, and 240 ppm groups were: 2.8, 16.6, and 34.5 mg/kg/day, respectively, for males and 3.7, 21.9, and 44.5 mg/kg/day, respectively, for females. The slightly higher weekly food consumption in females and the slightly lower food consumption in males compared with their respective controls at all dose levels may have contributed to the relatively higher test substance consumption in females compared to males.

Overall mortality in females at 240 ppm increased compared with that of controls. However, most of the deaths occurred during the last 8 months of the study. It is not clear from the evidence why mortality was significantly higher in females than males, but the cause of death was not associated with the incidence of neoplastic tumors. The death rate in females may have been related to the depressed body weight gains, which were reduced by approximately 12 and 21% at 120 and 240 ppm, respectively, and the increased incidence of central nervous system vacuolation, particularly in the brain at 120 and 240 ppm. Although females ingested more compound per kilogram body weight per

day than males, body weight gain in 240 ppm males was depressed 23%, and males in the 120 and 240 ppm groups had statistically significant vacuolation of the central nervous system as well. In a subchronic study (MRID 43492830) of 20 CD-1 mice/sex/dose fed AC 303,630 (Pirate 93.6% a.i.) at 0, 40, 80, 160, or 320 ppm for 91 days, male mice appeared more sensitive than females although only one male and one female died before terminal sacrifice.

Mean body weights and body weight gains were depressed for male mice at 240 ppm and in female mice at 120 and 240 ppm compared to their respective controls. The differences began early in the study for mean body weights and generally increased with time. Food consumption was only slightly decreased in males. Food efficiency, calculated for the first 13 weeks of the study, did not show any consistent indications of impairment in either sex. Thus, the changes in mean body weights and body weight gains in males and females appear to be treatment related.

The most common clinical signs—pinna, skin, and fur changes—were observed primarily in unscheduled deaths, and although they were found only slightly more frequently in treated males and females than in controls, they support the histological observation (not statistically evaluated) of dermatitis in 240 ppm animals. The increased frequency of dehydration, thin bodies, and weakness in preterminal females at the 240 ppm treatment level may be associated with the higher mortality rate in females. No treatment-related effects were observed on hematological parameters.

No treatment-related changes were observed in absolute or relative organ weights. The depressed kidney weights in males at 240 ppm were not supported by gross or histopathological findings. This effect may have been related to the depressed terminal body weights as evidenced by the similarities of relative kidney to body weight ratios between control and 240 ppm males. The slightly increased liver to body weight ratios in females at interim and terminal sacrifice reached statistical significance only in 120 ppm females by the end of the study. However, since no histopathological changes were correlated with these organ weight changes, the effect is not considered treatment related. In the 91-day study of AC 303,630 (MRID 43492830), both sexes at 320 ppm showed a relative liver weight increase that was statistically significant when compared to controls. However, relative liver weight changes were not significant at or below 160 ppm in females, and microscopic hepatic cell changes in females did not occur in the 0, 40 or 80 ppm groups in the 91-day study.

Treatment-related gross pathological changes, including skin ulceration and scabbing, occurred in males and females at the 240 ppm level, and scabbing occurred in males at 120 ppm. This finding is supported by microscopic evaluations, which revealed a tendency for increased incidence of dermatitis (not statistically evaluated) in males and females at 240 ppm. This finding also is supported by the clinical observations of pinna, skin, and fur changes exhibited mainly by animals that had unscheduled deaths.

Microscopic evaluation revealed a variety of non-neoplastic lesions, but only slight to severe vacuolation of the central nervous system was attributed to dietary administration of AC 303,630. The statistically significant increase in the incidence of vacuolation of the brain compared with controls was observed in both sexes at 120 and 240 ppm. Vacuolation of the brain was first seen at interim sacrifice at 240 ppm. Microscopic changes also were observed in the 91-day study (MRID 43492830) as spongioform(encephalo)-myelopathies in the brain and myelin of the spinal cord of male and female mice treated with AC 303,630 at 320 ppm. Statistically significant vacuolation also occurred, though less frequently, in the spinal cord (mainly cervical and thoracic areas) and optic nerve of 240 ppm males and females. **The LOEL for systemic toxicity is 120 ppm (16.6 and 21.9 mg/kg/day in males and females, respectively) based on decreased body weight gains, brain toxicity and scabbing of the skin (males), and the NOEL is 20 ppm (3 and 4 mg/kg/day for males and females, respectively).**

The increased incidence of bone marrow myelopoiesis in 240 ppm males and lung histiocytosis and lacrimal gland amyloidosis in 240 ppm females do not appear to be associated with treatment. These degenerative or inflammatory microscopic changes are considered to be biological variations typically observed in aging mice. They were not dose-related and were not associated with organ weight changes.

No treatment-related neoplastic lesions were observed. Although the animals probably could have tolerated a higher dose, the depression in body weight gain in a subchronic mouse study (MRID 43492830) was 26% in males and 29% in females, and in this study, they were 23% in males and 21% in females.

B. Study deficiencies - This chronic/oncogenicity study (83-5) in mice is acceptable for carcinogenicity and satisfies the guideline requirements for a carcinogenicity study (83-2). The study for chronic toxicity (83-1) is **acceptable**, although clinical chemistry and urinalysis data were not

collected, but the study provided scientifically valid information that addresses the study objectives as outlined in Subdivision F.

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DATA EVALUATION RECORD

PIRATE

Study Type: 84-2; *In vitro* Chromosome Aberration Assay in Chinese Hamster Lung (CHL) Cells

Work Assignment No. 1-1O (MRID 43492839)

Prepared for

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Disclaimer

This Data Evaluation Repcrd may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Review Section III, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, DVM, DABT, Date 5/10/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: In vitro mammalian chromosome aberrations in Chinese hamster lung (CHL) cells
OPPTS Number: 870.5375 OPP Guideline Number: §84-2

DP BARCODE: D212558 SUBMISSION CODE: S481410
P.C. CODE: 129093 TOX. CHEM. NO.: NONE

TEST MATERIAL (PURITY): MK-242 technical (Pirate; 93.8% ai)

SYNONYMS: 4-Bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile

CITATION: Adams, K. (1994) AC 303,630 (MK-242) Analysis of metaphase chromosomes obtained from CHL cells cultured in vitro. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, PE18 6ES, England. Laboratory Project ID: MCI 206/941465. May 23, 1994. MRID 43492839. Unpublished.

SPONSOR: American Cyanamid Company

EXECUTIVE SUMMARY:

In a mammalian cell chromosome aberration assay (MRID 43492839), Chinese Hamster Lung (CHL) cell cultures were exposed to MK-242 technical (Pirate; 93.8% ai) in dimethylsulfoxide at concentrations of 0.9, 1.8, 3.5, 7.0, 14.1, 28.1, 56.3, 112.5, 225, 450, 900, or 1800 µg/mL for 6 hours with metabolic activation (rat S-9 mix), or for 6, 24, or 48 hours without metabolic activation. At final concentrations of 112.5-225 µg/mL and above, a precipitate formed in the tissue culture medium.

Cells were harvested at 24 or 48 hours after the initiation of treatment, and the proportion of mitotic cells per 1000 cells was determined. In general, metaphase analysis was conducted on cells from three dose levels for each activation/exposure time combination; the high dose was the concentration that resulted in a >50% depression in the mitotic index compared to the solvent control, and the low and intermediate doses were the concentrations corresponding to 25 and 50% of the high dose. **MK-242 technical caused no statistically significant increases in the proportion of aberrant or polyploid chromosomes in Chinese Hamster lung cells compared to solvent control values.** Positive controls induced the appropriate response.

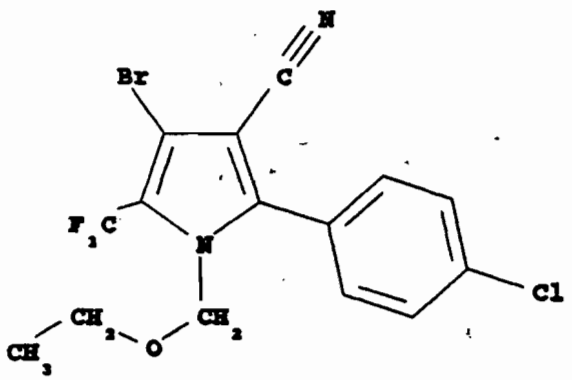
This study is classified as **acceptable** and satisfies the guideline requirement for <u>in vitro</u> cytogenetic mutagenicity studies (84-2).

<u>COMPLIANCE</u>: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. <u>MATERIALS</u>

1. <u>Test Material</u>: MK-242 technical (Pirate)
   Description: Tan powder
   Lot/Batch #: AC 7504-59A
   Purity: 93.8% ai
   Stability of compound: Expiration date of batch reported by sponsor to be December 31, 1994 (after study completion)
   CAS #: None
   Structure:



   Solvent used: Dimethylsulfoxide
   Other comments: Dosing solutions were not analyzed to determine the actual concentrations or the stability of MK-242.

2. <u>Control Materials</u>
   Negative: Culture medium (Eagle's Minimum Essential Medium; MEM)
   Solvent/final concentration: Dimethylsulfoxide (5 µl/mL)
   Positive:
   Activation: Cyclophosphamide (10 or 15 µg/mL in distilled water) for clastogenicity
   Nonactivation: Mitomycin C (0.1 or 0.2 µg/mL in distilled water) for clastogenicity; Carbendazim (12 or 3 µg/mL in distilled water) for polyploidy

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Other comments: Dimethylsulfoxide was not used as the solvent for the positive control chemicals.

3. Activation
S-9 was derived from:

X	Aroclor 1254	X	Induced	X	Rat	X	Liver
	Phenobarbital		Non-induced		Mouse		Lung
	None				Hamster		Other
	Other				Other		

The S-9 mix prepared by the study laboratory consisted of the S-9 fraction (5%, v:v), $MgCl_2$ (8 mM), Na_2HPO_4 buffer (pH 7.4, 100 mM), glucose-6-phosphate (5 mM), NADP (2 mM), and NADPH (2 mM) .

4. Test compound concentrations used
Cytotoxicity was determined concurrent with the cytogenetic assay, rather than in preliminary tests. All activation/exposure time combinations were tested at MK-242 concentrations of 0.9, 1.8, 3.5, 7.0, 14.1, 28.1, 56.3, 112.5, 225, 450, 900, and 1800 µg/mL.

5. Test cells
Chinese hamster lung (CHL) cells, strain JCRB 0030, were grown and subcultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum.

Properly maintained? **Yes**
Cell line or strain periodically checked for Mycoplasma contamination? **No**
Cell line or strain periodically checked for karyotype stability? **No**

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay
A preliminary cytotoxicity assay was not performed, rather, the cytotoxicity assay was conducted concurrent with the cytogenetic assay. CHL cells suspended in the MEM culture solution were incubated at 37 C in a humid atmosphere containing 5% carbon dioxide for 24 hours. The cells were then exposed to MK-242 using the following activation/exposure/harvest treatment combinations:

Activated conditions (solution containing 1.25 mL S-9):
Set 1: 6 hours of exposure, harvest 24 hours after the start of exposure

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Nonactivated conditions:
Set 2: 6 hours of exposure, harvest 24 hours after the start of exposure
Set 3: 24 hours of exposure, harvest 24 hours after the start of exposure (immediately following the termination of exposure)
Set 4: 48 hours of exposure, harvest 48 hours after the start of exposure (immediately following the termination of exposure)

For each set, two cell cultures were treated with each dose level, and four were treated with the solvent control. For Set 1 (activated), two cultures were treated with cyclophosphamide at concentrations of 10 or 15 μg/mL. For Set 2, two cultures each were treated with mitomycin C at 0.2 μg/mL, cyclophosphamide at 10 μg/mL, and carbendazim at 12 μg/mL. For Set 3, two cultures each were treated with mitomycin C at 0.2 μg/mL and carbendazim at 12 μg/mL. For Set 4, two cultures each were treated with mitomycin C at 0.1 μg/mL and carbendazim at 3 μg/mL.

The proportion of mitotic cells per 1000 cells was determined for all treatment combinations except the positive controls.

2. Cytogenetic Assay

Metaphase analysis was conducted on cells from three or four dose levels for each activation/exposure/harvest treatment combination in the cytotoxicity assay. For Sets 1, 3, and 4, the high dose was the highest analyzable concentration that resulted in a >50% depression in the mitotic index compared to the solvent control, and the low and intermediate doses were the concentrations corresponding to 25 and 50% of the high dose. For Set 2, which was a similar to Set 1 but nonactivated, metaphase analysis was conducted on cells from the same three dose levels analyzed for Set 1, and also from the highest analyzable concentration that resulted in a >50% depression in the mitotic index compared to the solvent control.

a. Cell treatment

The cytotoxicity samples selected for cytogenetic assay were:

Activated conditions:
Set 1: 6 hours of exposure to 3.5, 7.0, or 14.1 μg/mL; harvest 24 hours after the start of exposure
Nonactivated conditions:

Set 2: 6 hours of exposure to 3.5, 7.0, 14.1 or 225 μg/mL; harvest 24 hours after the start of exposure
Set 3: 24 hours of exposure to 1.8, 3.5, or 14.1 μg/mL (7.0 μg/mL cultures provided insufficient metaphase figures of suitable quality); harvest 24 hours after the start of exposure
Set 4: 48 hours of exposure to 1.8, 3.5, or 7.0 μg/mL; harvest 48 hours after the start of exposure

b. Spindle inhibition
Inhibitor used/concentration: Colcemid at 0.1 μg/mL
Administration time: 2 hours before the end of the incubation period (ie., 22 or 46 hours following the start of exposure)

c. Cell harvest
Cells were harvested 0 or 18 hours after the termination of treatment (refer to section B.2.a). The cells were detached from the flasks by trypsinization, swollen in a 0.07 M KCl solution, then fixed with methanol:glacial acetic acid (3:1) for 2-3 hours.

d. Slide preparation
Cell suspensions were placed onto cold microscope slides and air-dried. The slides were placed in buffered distilled water (pH 6.8), and the cells were stained with Giemsa (1:9, Giemsa:buffered distilled water). The stained slides were air-dried and mounted in DPX.

e. Metaphase analysis
No. of cells examined per MK-242 dose: **200**
No. of cells examined in the solvent control: **400**
No. of cells examined in the cyclophosphamide positive control: **200**
No. of cells examined in the mitomycin C positive control: **100**
Scored for structural aberrations: **Yes**
Scored for numerical polyploidy: **Yes**
Coded prior to analysis: **Yes**

f. Evaluation criteria
A positive response was claimed if a "clear treatment-related and statistically significant increase" was observed in the number of cells containing chromosome aberrations, or in the number of polyploid cells. Evidence of either a dose relationship or reproducibility of the study results

was required. (The evaluation criteria were not further defined.)

g. Statistical analysis
Data were evaluated for statistical significance at $p < 0.001$ and < 0.01, using Fisher's Test.

II. REPORTED RESULTS

MK-242 technical formed a precipitate in the culture medium at concentrations of 112.5 - 225 µg/mL and above, and produced a heavy precipitate at a concentration of 1800 µg/mL. The study sponsor determined that 1800 µg/mL should be the highest concentration used in testing.

A. Cytotoxicity assay
Cytotoxicity, as indicated by a depression of the mitotic index, was assessed concurrent with the cytogenetic assay. In Set 1 (activated, 6-hour exposure, 24-hour harvest), MK-242 concentrations of 14.1 and 28.1 µg/mL reduced the mitotic index to 39 and 12% of the solvent control value, respectively. In Set 2 (nonactivated, 6-hour exposure, 24-hour harvest), MK-242 concentrations $\leq$28.1 µg/mL had no effect on the mitotic index; and concentrations of 225 and 450 µg/mL reduced the mitotic index to 35 and 32% of the solvent control value, respectively. In Set 3 (nonactivated, 24-hour exposure and harvest), MK-242 concentrations of 3.5 and 14.1 µg/mL reduced the mitotic index to 40 and 45% of the solvent control value, respectively. In Set 4 (nonactivated, 48-hour exposure and harvest), MK-242 concentrations of 7.0 and 14.1 µg/mL reduced the mitotic index to 33 and 13% of the solvent control value, respectively. Data are presented in Attachment 1 (study report pages 20-23).

B. Cytogenetic assay
CHL cells were unaffected by exposure to MK-242 at concentrations up to cytotoxic levels (mitotic index <50% of solvent control) for up to 48 hours, either in the presence and absence of activation (S-9 mix). Exposed cells were found to contain a maximum of 4% aberrant cells and 4% polyploid cells, which was statistically comparable to untreated and solvent control cultures. All positive control compounds induced statistically significant increases ($p < 0.001$) in numbers of aberrant or polyploid cells. Data are presented in Attachment 2 (study report pages 24-29).

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

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The study author concluded that MK-242 technical caused no statistically significant increases in the proportion of aberrant or polyploid cells, in either the presence of S-9 mix or in its absence at any treatment time. MK-242 technical showed no evidence of clastogenic or polyploidy-inducing activity in this in vitro cytogenic test system.

B. Reviewer's Discussion

We agree with the study author that MK-242 technical was neither clastogenic nor polyploid-inducing in cultured CHL cells over the dose ranges that included cytotoxic levels (7.0 to 225 µg/mL without activation, and 14.1 µg/mL with activation) and exposure times up to 48 hours (at least two cell cycles). The solvent (dimethylsulfoxide) and untreated controls had comparable low frequencies of chromosome aberrations and polyploidy. The sensitivity of the assay system to detect damage to chromosomes and induction of polyploidy was adequately demonstrated by the results obtained with the positive controls (mitomycin C, carbendazim without activation, and cyclophosphamide with activation). We conclude, therefore, that the results of this study provided sufficient evidence to consider MK-242 to have no effect on this in vitro test system.

IV. STUDY DEFICIENCIES

Stability analysis of the MK-242 technical was not available. However, the sponsor stated that the batch of MK-242 used for the study was stable for the study period. This is considered to be a minor deficiency and would not be expected to compromise this in vitro mutagenicity study.

ATTACHMENT 1

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CHLORFENAPYR

Page _____ is not included in this copy.

Pages 208 through 218 are not included in this copy.

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.

✓ 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.

DATA EVALUATION REPORT

PIRATE Metabolite

Study Type: 84-2; *Salmonella typhimurium* and *Escherichia coli*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 101P (MRID 43492840)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Sandra Daussin, B.S.

Signature: Sandy Daussin by WJS
Date: 1/16/96

Secondary Reviewer:
Steven Brecher, Ph.D.

Signature: Steven Brecher by WJS
Date: 1/16/96

Project Manager:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 1/16/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler by WJS
Date: 1/16/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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[CL 303,268] **SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)**
(Pirate metabolite)

EPA Reviewer: Irving Mauer, Ph.D. Date 02/16/96
Review Section IO, Toxicology Branch 1 (7509C)
EPA Secondary Reviewer: Marion Copley, DVM, DABT, Date 2/21/96
Review Section 4, Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella typhimurium and Escherichia coli/mammalian activation gene mutation assay
OPPTS Number: 870.5265 & 870.5100 OPP Guideline Number: [§84-2]

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): CL 303,268 (pirate metabolite and impurity, 100.3% active ingredient)

SYNONYMS: 4-Bromo-2-(p-chlorophenyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile

CITATION: Mulligan, E. (1994) Microbial Mutagenicity Plate Incorporation Assay of CL 303,268. American Cyanamid Company, Genetic Toxicology Laboratory, Princeton, NJ. Study No. 9402001. August 12, 1994. MRID 43492840. Unpublished.

SPONSOR: American Cyanamid Co., Princeton, NJ

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 43492840), strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella typhimurium or Escherichia coli WP2 uvrA- were exposed to CL 303,268 (100.3% a.i.) in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 303,268 at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, or 5.0 µg/plate (+/-S9). E. coli WP2 uvrA- was tested with CL 303,268 at concentrations of 10, 25, 50, 100, and 250 µg/plate (+/-S9).

CL 303,268 (100.3% a.i.) was tested up to cytotoxic concentrations with the S. typhimurium strains and the limit of solubility, 250 µg/plate, with E. coli WP2 uvrA-. The positive controls induced the appropriate responses in the corresponding strains. CL 303,268 failed to induce a genotoxic response in any of the tester strains with the exception of a borderline positive result for the TA100 strain at the 1.0 µg/plate dose level. As the result was equivocal and a genotoxic response was not found in any of the other tester strains, **CL 303,268 was determined to not be mutagenic under the conditions of the submitted study.**

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DATA EVALUATION REPORT

PIRATE

Study Type: 85-1; Metabolism - Rat

Work Assignment No. 1-01T (MRID 43492844)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Ann Foster, Ph.D.

Signature: Ann Foster
Date: 3/14/96

Secondary Reviewer:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 3/20/96

Project Manager:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 3/20/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 3/16/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: P. Chin Paul Chin, Date 5/1/96
Review Section II, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, D.V.M. Maryann Copley Date 5/10/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat
OPPTS Number: 870.7485 OPP Guideline Number: §85-1

DP BARCODE: D212558 SUBMISSION CODE: S481410
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): CL 303,630 ([2-pyrrole-^{14}C] Pirate, 97.7%; [phenyl-^{14}C] Pirate; 97.0%)

SYNONYMS: Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)

CITATION: Mallipudi, N.M. (1994) CL 303,630: Metabolism of Carbon-14 Labeled CL 303,630 in the Rat. American Cyanamid Company, Agriculture Research Division, Princeton, NJ, and Hazelton Wisconsin, Inc., Madison, WI. Report No. MET 94-021. October 28, 1994. MRID 43492844. Unpublished.

SPONSOR: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

EXECUTIVE SUMMARY:

In a metabolism study (MRID 43492844), [2-pyrrole-^{14}C] or [phenyl-^{14}C] pirate was administered to 5 HSD:Sprague-Dawley/SD rats/sex/dose by oral gavage at dose levels of 20 mg/kg/day as a single dose or following a 14-day pre-treatment with non-radioactive pirate, or at 200 mg/kg as a single dose.

Low recoveries of the radioactive dose in urine and tissues indicate limited absorption of pirate by rats. The radioactivity in urine from the high dosed rats was about half that from the single and multiple-low dosed rats. More than 80% of the doses were eliminated in the feces. Most of the radioactivity was eliminated in the feces and urine within 48 hours of dosing. After 7 days, 89-121% of the dosed radioactivity was recovered. At sacrifice, female rats had greater (about twice) recovery of radioactivity in the carcass, blood, and fat at all doses than did males. The highest recovery of radioactivity from a single organ was from the liver (0.15-0.48% of dose).

Metabolite extraction and identification accounted for 72-91% of the radioactive doses. The parent was the major radioactive compound found in excreta, accounting for approximately 40-70% of the administered doses. Minor amounts of eight primary and

conjugated metabolites and four unidentified isolated components were detected, each at less than 10% of the dosed radioactivity. Liver and kidney contained several primary and conjugated metabolites and only minor levels of the parent compound (≤8.3% of the radioactivity in the sample). Based on the metabolites identified, the major deposition route of orally administered pirate is fecal excretion of unaltered parent compound. Other pathways include cleavage of the ethoxymethyl side-chain, followed by de-alkylation and ring hydroxylation, and some degree of conjugation of the de-alkylated, ring-hydroxylated metabolite. The two rings of the molecule are not cleaved. Metabolites are excreted primarily in urine; accumulation in tissues is minimal.

This metabolism study in the rat is classified **acceptable** and satisfies the guideline requirement for a metabolism study (85-1) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

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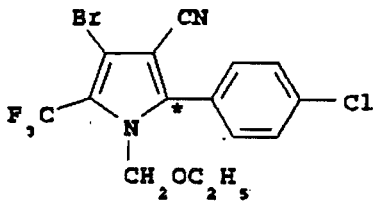
I. MATERIALS AND METHODS

A. MATERIALS:

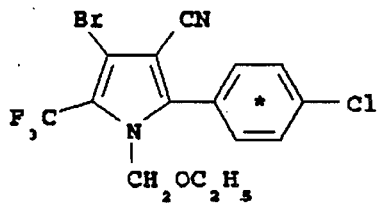
1. Test Compound:

a. [2-pyrrole-^{14}C]Pirate
Radiochemical purity: 100% determined by TLC
Specific activity: low dose 4.04 μCi/mg; high dose 1.33 μCi/mg
Lot/Batch: AC 6918-95A



* indicates position of ^{14}C-label.

b. [phenyl-^{14}C]Pirate
Radiochemical purity: >99% determined by TLC
Specific activity: low dose 4.02 μCi/mg; high dose 1.34 μCi/mg
Lot/Batch: AC 7616-34A



* indicates position of ^{14}C-label.

c. Non radioactive compound pirate,
Purity: 98.8% determined by HPLC
Lot/Batch No.: AC 6937-118
Description: Colorless solid
CAS No.: 122453-73-0

These test substances were diluted with [2-pyrrole-^{13}C]pirate (Lot C13129) as a mass marker, purity 98.9%, ^{13}C-enrichment 99.3%.

2. Vehicle: Sodium salt of carboxymethyl cellulose (CMC, 0.5% aqueous solution).

3. Test animals: Species: Rat
Strain: HSD:Sprague-Dawley/SD
Age and weight at study initiation: Males: 216-245 g, 7 and 9 weeks; females: 165-200 g, 4 and 6.5 weeks
Source: Harlan Sprague Dawley, Inc.
Housing: Nalgene metabolism cages
Diet: Certified Rodent Chow #5002 (Purina Mills) ad libitum
Water: Fresh water ad libitum
Environmental conditions:
Temperature: 19-25°C
Humidity: 50% ± 20%
Air changes: Not applicable
Photoperiod: 12-hour light/12-hour dark
Acclimation period: 7 days

4. Preparation of dosing solutions:

[^{14}C]Pirate was suspended in 0.5% CMC. Dosing solutions were prepared a day before each use.

B. STUDY DESIGN AND METHODS:

The study was designed to determine the absorption, distribution, metabolism, and excretion of labeled [^{14}C]pirate after oral administration to rats. An intravenous study was not conducted owing to limited solubility of the test substance in physiological saline solution or water.

1. Group Arrangements

A preliminary study was conducted using two male and two female rats dosed at 20 mg/kg, each dosed with either [pyrrole-^{14}C] or [phenyl-^{14}C]pirate. A total of 60 rats, randomly assigned, (30 males and 30 females) was used in the definitive study. The test groups and dose levels used in the study are shown below in Table 1. Three male and 3 female untreated rats were maintained as controls.

The study report stated that the high dose (200 mg/kg) was based on data from an oral LD_{50} study, which demonstrated a higher tolerance for pirate in female rats than males. The 200 mg/kg level represented a compromise approximating 2/3 of the oral dose that caused deaths in 3 of 5 males and 1/6 of the oral dose that caused deaths in 3 of 5 females. The low dose (20 mg/kg) approximates the No-Observed-Effect-Level (NOEL) from a rat developmental toxicity study.

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TABLE 1. Dosing groups for pharmacokinetic and metabolism studies for pirate.

Test Group	Dose of labelled material (mg/kg)	Number/sex	Remarks
Preliminary; excretion balance study	20	1	Expired air collected; 7 day sacrifice
Low dose [pyrrole-^{14}C] [phenyl-^{14}C]	 20 20	 5 5	7 day sacrifice
Low dose with pretreatment [pyrrole-^{14}C] [phenyl-^{14}C]	 20 20	 5 5	7 day sacrifice Pretreatment with non-radioactive pirate daily for 14 days
High dose [pyrrole-^{14}C] [phenyl-^{14}C]	 200 200	 5 5	7 day sacrifice

2. Dosing and sample collection:

Each animal received approximately 1.0 mL of dosing solution administered by oral gavage using a syringe equipped with a steel ball-tipped needle.

In the preliminary study, each rat was placed into a glass metabolism cage designed for the collection of expired CO_2 and organic volatiles, urine and feces. Expired volatiles were collected in activated charcoal and CO_2 was trapped in ethanolamine:ethoxyethanol (1:1). Expired air samples were collected every 24 hours. Urine and feces were collected every 24 hours through 7 days after dosing.

In the definitive study, each animal was placed in a metabolism cage designed to separate urine and feces. Urine, feces, and cage rinses were collected at 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours after dosing. After 168 hours, the cages were rinsed with methanol:water (1:1, v/v), which was collected as a cage wash and then wiped with gauze (cage wipe).

Metabolite characterization studies: Animals were sacrificed 168 hours after dosing and the following tissue samples were collected: blood, bone (femur), brain, residual carcass, fat, heart, kidneys, liver,

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lungs, muscle (thigh), ovaries, skin (shaved), spleen, and testes.

Urine was analyzed directly by liquid scintillation counting (LSC). Solid samples were combusted, then analyzed by LSC. For metabolite characterization, urine and feces were each pooled according to ^{14}C-label position, sex, dose group, and time interval (0-12, 12-24, and 24-48 hours).

^{14}C-Residues in urine were digested with protease and sulfatase and the hydrolysates were analyzed by HPLC. Radioactivity in feces was extracted with methanol and soluble residues were analyzed by HPLC. Insoluble residues were extracted with methanol:water:HCl (1:1:0.01, v/v/v) and solubilized residues were reacted with 2,2-dimethoxypropane, then analyzed by HPLC. The remaining insoluble fractions were incubated with protease and hydrolysates were analyzed by HPLC. The solid residue remaining from certain samples after protease treatment were refluxed with 6 N HCl for 4 hours and the released residues were analyzed by HPLC.

Radioactive residues in muscle, liver, and kidney were extracted with methanol and then incubated with pepsin in 0.1 N HCl. The solubilized fractions were analyzed by HPLC. Residues in fat were extracted with methanol and subjected to HPLC analysis.

3. Statistics: No statistical data were reported.

II. RESULTS

A. Pharmacokinetic Studies:

1. Preliminary experiment

In the preliminary experiment, 99.1-101.1% of the dose was recovered from male and female rats administered [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate. Feces accounted for 82.6-86.3% of the dose, 8.7-10.9% was in the urine, and 2.9-9.8% was in the carcass. Radioactivity in expired air, including CO_2 and organic volatiles, was nondetectable (<0.01%).

2. Absorption

A separate pharmacokinetic study was not conducted. As the following sections demonstrate, over 80% of the administered radioactivity was eliminated in the feces. As generally <10% of the radioactivity was excreted in

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the urine and <5% was deposited in tissues, only limited absorption of [2-pyrrole-^{14}C] or [phenyl-^{14}C] occurred in rats.

3. Tissue distribution and excretion

a) Single low dose: Tables 2 and 3 summarize the recovery of radioactivity from excreta and tissues. Seven days after administration of a single dose of [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate, respectively, at 20 mg/kg to rats, 107.3 and 98.5% of the dose was accounted for in males and 104.6 and 96.4% of the dose was accounted for in females. Elimination in the urine accounted for 9.8 and 8.8% of the dose in males and 7.5 and 6.7% in females. Elimination in the feces accounted for 93.7 and 85.9% of the dose in males and 92.0 and 83.9% of the dose in females. Greater than 75% of the dosed radioactivity was eliminated in urine and feces within 48 hours after dosing. At sacrifice, 7 days after dosing with [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate, radioactive residues in tissues plus carcass accounted for, respectively, 2.4 and 2.1% of the dose in males and 4.1 and 4.4% in females (Tables 2 and 3).

As illustrated in Tables 4 and 5, most of the tissue/organ radioactivity was detected in the residual carcass; the percentage of dose recovered in males (1.58 and 1.41%) was lower than that in females (3.06 and 3.37%). The highest percentage of the administered dose for both ^{14}C-labels in specific tissues and organs occurred in the liver (0.37-0.48%). Fat and blood accounted for the next highest tissue levels, with females exhibiting greater accumulation in these tissues than males. Blood radioactivity in females from the [2-pyrrole-^{14}C] and [phenyl-^{14}C] doses, respectively, accounted for 0.21 and 0.22% of the dose, compared to 0.08 and 0.07% in males. Females exhibited approximately twice the percentage of dose in fat (0.26 and 0.35%, respectively, of the [2-pyrrole-^{14}C] and [phenyl-^{14}C] doses) than did males (0.14 and 0.16%). The results were similar for both ^{14}C-label positions.

b) Low dose with pretreatment: Seven days after administration of a single dose of [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate, respectively, at 20 mg/kg to rats following pretreatment with non-radioactive pirate at 20 mg/kg for 14 days, 112.6-102.4% of the dose was accounted for in males and 121.2-106.4% of the dose was accounted for in females (Tables 2 and 3). Values for elimination of the dose in urine and feces were similar to those obtained from the single 20 mg/kg dose,

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without pretreatment. The distribution of radioactivity in organs and tissues following the multiple dose was also similar to the pattern observed following sacrifice of the animals receiving the 20 mg/kg single dose (Tables 4 and 5). Liver accounted for the highest single organ level. Percentages of the doses in blood, fat, and carcass were approximately 2-3 times higher in females than in males.

c) Single high dose: Seven days after administration of a single dose of [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate, respectively, at 200 mg/kg to rats, 93.9 and 88.7% of the dose was accounted for in males and 96.5 and 106.1% of the dose was accounted for in females (Tables 2 and 3). Percentages of dose eliminated in urine were lower than with the single- or repeated-low dose tests, accounting for 4.3 and 5.4% of the dose in males and 4.1 and 4.2% in females. Percentages of the high (200 mg/kg) dose in tissues, including liver and carcass were also about half the level observed from either low dose. As with the two low dose levels, females exhibited roughly twice the proportion of the 200 mg/kg doses in carcass and blood compared with males and approximately 6 times the male dose-percentage in fat.

TABLE 2. Recovery of radioactivity in tissues and excreta of rats after administration of [2-pyrrole-^{14}C]pirate[a].

	Percent of radioactive dose recovered					
	Single low dose		Repeated low dose		Single high dose	
	Male	Female	Male	Female	Male	Female
Tissues[b]	0.8	1.0	0.6	1.0	0.2	0.5
Carcass	1.6	3.1	1.5	3.0	0.6	1.5
Cage wash	1.4	1.0	1.3	1.8	1.0	1.9
Urine	9.8	7.5	9.8	9.4	4.3	4.1
Feces	93.7	92.0	99.4	106.0	87.8	88.5
Total	107.3	104.6	112.6	121.2	93.9	96.5

a = Data extracted from Tables VIII and X, pages 62 and 66 in the study report.
b = Includes blood; to obtain separate tissue and carcass values, the value for carcass on p. 62 was subtracted from the value for tissues (this value includes carcass) on p. 66, then the value for blood (p. 66) was added to the revised tissue value.

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TABLE 3. Recovery of radioactivity in tissues and excreta of rats after administration of [phenyl-^{14}C]pirate[a].

	Percent of radioactive dose recovered					
	Single low dose		Repeated low dose		Single high dose	
	Male	Female	Male	Female	Male	Female
Tissues[b]	0.7	1.0	0.5	0.9	0.2	0.4
Carcass	1.4	3.4	1.1	2.6	0.8	1.4
Cage wash	1.7	1.4	1.5	2.3	2.2	2.6
Urine	8.8	6.7	7.9	7.9	5.4	4.2
Feces	85.9	83.9	91.4	92.7	80.1	97.5
Total	98.5	96.4	102.4	106.4	88.7	106.1

a = Data extracted from Tables VIII and X, pages 62 and 66 in the study report.
b = Includes blood; to obtain separate tissue and carcass values, the value for carcass on p. 62 was subtracted from the value for tissues (this value includes carcass) on p. 66, then the value for blood (p. 66) was added to the revised tissue value.

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TABLE 4. Distribution of radioactivity in rat tissues/organs after administration of [2-pyrrole-^{14}C]pirate[a].

	Percent of radioactive dose administered					
	Single low dose		Multiple low dose		Single high dose	
Tissue/organ	Male	Female	Male	Female	Male	Female
Blood	0.08	0.21	0.10	0.32	0.04	0.10
Bone (femur)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass (residual)	1.58	3.06	1.50	3.02	0.58	1.51
Fat (body)	0.14	0.26	0.07	0.14	0.03	0.17
Heart	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Kidneys	0.02	0.03	0.02	0.03	<0.01	<0.01
Liver	0.48	0.40	0.36	0.38	0.15	0.17
Lungs	<0.01	0.03	0.01	0.03	<0.01	<0.01
Muscle (thigh)	<0.01	0.02	<0.01	0.03	<0.01	<0.01
Ovaries	NA	<0.01	NA	<0.01	NA	<0.01
Skin (shaved)	0.03	0.05	0.03	0.07	0.01	0.04
Spleen	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Testes	0.02	NA	0.02	NA	<0.01	NA

a = Data extracted from Table VIII, p. 62 in the study report

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TABLE 5. Distribution of radioactivity in rat tissues/organs after administration of [phenyl(U)-^{14}C] pirate[a].

	Percent of radioactive dose administered					
	Single low dose		Multiple low dose		Single high dose	
Tissue/organ	Male	Female	Male	Female	Male	Female
Blood	0.07	0.22	0.06	0.22	0.04	0.08
Bone (femur)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass (residual)	1.41	3.37	1.12	2.63	0.76	1.38
Fat (body)	0.16	0.35	0.07	0.20	0.03	0.18
Heart	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Kidneys	0.02	0.03	0.01	0.03	<0.01	<0.01
Liver	0.37	0.38	0.28	0.31	0.17	0.15
Lungs	<0.01	0.02	<0.01	0.02	<0.01	<0.01
Muscle (thigh)	<0.01	0.03	<0.01	0.02	<0.01	<0.01
Ovaries	NA	<0.01	NA	<0.01	NA	<0.01
Skin (shaved)	0.02	0.06	0.02	0.05	0.01	0.03
Spleen	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Testes	0.01	NA	<0.01	NA	<0.01	NA

a = Data extracted from Table VIII, p. 62, in the study report.

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4. Excretion

The data in Tables 6 and 7 indicate that limited absorption of [2-pyrrole-^{14}C] and [phenyl-^{14}C]pirate in rats was observed by 7 days after dosing with [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate at 20 mg/kg (single or repeated dose) or 200 mg/kg (single dose). In males following a single oral dose or the repeated oral dose of 20 mg/kg, 9.4-11.2% of the dose was excreted in the urine by 7 days. In females at these doses, urine excretion was slightly less, 8.1-11.2%. A lower percentage of the single high (200 mg/kg) dose was excreted in the urine, 5.3-7.6%. The majority of the radioactivity (73-84%) excreted during the 7 days was excreted during the first 48 hours of dosing; the collections obtained during the first 48 hours were used for extraction and analysis of metabolites.

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TABLE 6. Cumulative excretion of radioactivity in urine by rats following administration of [^{14}C] pirate[a].

Dose	Cumulative recovery as percent of administered dose					
	Oral 20 mg/kg		Repeated Oral 20 mg/kg		Oral 200 mg/kg	
Collection interval (hours)	M	F	M	F	M	F
[2-pyrrole-^{14}C]Pirate						
4	0.2	0.2	0.9	1.0	0.1	<0.1
8	1.3	1.3	3.0	2.9	0.2	0.8
12	3.3	2.4	5.0	4.7	0.5	1.4
24	6.3	4.5	7.1	7.4	1.7	2.1
36	8.0	5.7	8.4	9.0	3.3	3.6
48	8.8	6.4	9.1	9.7	3.9	4.4
72	9.7	7.3	9.8	10.3	4.4	4.9
96	10.3	7.7	10.2	10.6	4.6	5.2
120	10.6	8.0	10.5	10.8	4.8	5.3
144	10.9	8.2	10.8	11.0	4.9	5.5
168	11.2	8.5	11.1	11.2	5.3	6.0
[phenyl-^{14}C]Pirate						
4	0.8	0.9	1.2	1.3	0.7	0.7
8	2.3	2.1	2.9	3.1	1.0	1.3
12	4.1	3.1	5.0	4.8	1.5	3.2
24	6.4	4.7	6.6	6.9	3.2	4.2
36	7.7	5.7	7.4	8.0	4.7	5.3
48	8.4	6.4	7.8	8.6	6.1	5.7
72	9.2	7.0	8.4	9.1	6.7	6.1
96	9.6	7.5	8.7	9.5	7.0	6.3
120	9.9	7.7	9.0	9.7	7.2	6.4
144	10.2	7.9	9.1	9.8	7.3	6.5
168	10.4	8.1	9.4	10.2	7.6	6.8

a = Data extracted from Table VI, page 60 in the study report.

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TABLE 7. Cumulative excretion of radioactivity in feces by rats following administration of [^{14}C] pirate[a].

Dose	Cumulative recovery as percent of administered dose					
	Oral 20 mg/kg		Repeated Oral 20 mg/kg		Oral 200 mg/kg	
Collection interval (hours)	M		M	F	M	F
[2-pyrrole-^{14}C]Pirate						
4	NS[b]	NS	NS	ND[c]	<0.1	NS
8	NS	NS	<0.1	NS	<0.1	1.7
12	4.0	9.1	50.5	30.3	26.3	28.2
24	66.7	57.1	77.1	63.2	65.5	57.7
36	78.1	64.0	84.4	77.7	79.2	68.7
48	84.5	74.7	90.9	89.6	83.0	78.6
72	89.0	82.4	94.9	96.8	85.4	84.0
96	91.0	86.5	96.7	101.0	86.5	86.0
120	92.3	89.0	97.9	103.0	87.1	87.1
144	93.1	90.8	98.8	105.0	87.5	87.9
168	93.7	92.0	99.4	106.0	87.8	88.5
[phenyl-^{14}C]Pirate						
4	NS	NS	NS	ND	<0.1	NS
8	NS	NS	NS	NS	<0.1	4.4
12	11.8	14.1	56.7	25.1	24.5	51.5
24	62.3	54.4	79.2	68.0	60.1	72.6
36	70.6	64.6	83.2	75.1	69.4	89.3
48	76.5	70.4	85.7	80.3	74.9	91.4
72	81.0	76.2	88.1	86.0	78.1	94.7
96	82.8	79.2	89.4	88.5	79.0	95.9
120	84.8	81.3	90.3	90.4	79.7	96.7
144	85.6	83.0	90.9	91.8	79.9	97.2
168	86.1	83.9	91.4	92.7	80.1	97.5

a = Data extracted from Table VII, page 61 in the study report.
b NS = No sample.
c ND = Not detectable.

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B. Metabolite characterization studies:

Residues in excreta. The results of HPLC analysis of radioactivity in urine and feces collected 48 hours after dosing with [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate are summarized in Tables 8 and 9. The submission presented separate data sets for the 0- to 12-hour, 12- to 24-hour, and 24- to 48-hour intervals, and the values for 0-48 hours cumulative in Tables 8 and 9 were calculated from the data given for the separate intervals.

The structures of pirate and metabolites are depicted in Attachment 1 (study report pages 11 and 12). A total of 11 compounds (Tables 8 and 9) were isolated and 9 were identified in the excreta of animals dosed with [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate, accounting for 67.1-89.0% of the administered radioactivity. The data indicate that the double-ring structure is not cleaved and metabolite results were similar for the two labels.

The parent compound was the major radioactive compound identified, accounting for 38.5-52.9% of the single and repeated 20 mg/kg doses. The parent was detected at higher levels in excreta from the 200 mg/kg dosed animals, 53.2-72.4% of the dose. Several metabolites in the primary pathway of pirate in rats (depicted in Attachment 2, study report page 207) were identified at minor levels by HPLC, corresponding to known standard compounds (Attachment 1). Metabolites M-8 (0.88-1.87% of the dose) and M-7A (0.70-1.43%) represent, respectively, the removal and acidification of the ethoxymethyl side chain of pirate in the first reactions of two postulated pathways.

In the slightly predominant pathway, M-5 (2.92-10.0% of dose) is a de-brominated, pyrrole-ring hydroxylated product formed from M-8 and M-7 is a phenyl-ring hydroxylated form of M-8. Metabolites M-5 and M-7 were identified by HPLC and the structures were confirmed by GC/MS following derivatization.

In the second pathway, M-6 (1.12-4.03% of the dose) represents the de-bromination of M-7A. Metabolite M-4 (2.41-8.69%) was identified by HPLC, NMR, and MS as a de-fluorinated, pyrrole-ring hydroxylated acid product of M-8. Conjugates M-1 (0.22-1.08%) and M-2 (1.76-5.23%) yielded M-4 following protease and sulfatase hydrolysis. Isolated compounds designated M-1A (0.64-2.75% of the dose), M-3 (2.20-6.63% of the dose), unknown U-2 (0.17-1.81%), and polar unknown U-1 (0.69-

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6.38%) were not identified. Only 8.45-27.50% of the radioactive doses were not accounted for; these proportions include the radioactivity in 48- to 168-hour urine and feces collections, which were not included in the analysis.

Residues in urine. In contrast to the combined-excreta results presented in Tables 8 and 9, reflecting fecal excretion as the main pathway of orally administered pirate in rats, the data on urine *per se* (Table XXVI reproduced from the study report, presented as Attachment 3) show that absorbed pirate is extensively metabolized. The parent compound was not detected in urine. The predominant metabolites in urine were polar metabolites, M-4 (0.34-1.87% of the dose) and M-5 (0.79-3.35%), and polar conjugates M-1 (0.06-0.83%), M-1A (0.19-1.02%), and M-2 (0.62-1.8%).

Residues in tissues. The study also reported metabolite analysis on radioactivity from tissues taken at sacrifice from the single 200 mg/kg dosed rats (Attachment 4, Table XXIX, study report pages 137 and 138). Unchanged parent compound predominated in fat (65.8-93.9% of the total radioactive residue [TRR] in fat) and accounted for 6.5-30.9% of the TRR in muscle. Pirate was extensively metabolized in liver and kidney, where several metabolites were detected at >10% of the TRR and the parent accounted for ≤8%.

The pathway for metabolism of pirate by rats is depicted in Attachment 2. Metabolism of pirate proceeds via oxidation, de-alkylation, and hydroxylation. The pyrrole-ring hydroxylated, de-alkylated metabolite, M-4, is readily conjugated.

TABLE 8. Metabolite profile in excreta of rats dosed with [2-pyrrole-^{14}C]pirate[a].

Dose	Recovery as percent of administered dose					
	Oral 20 mg/kg		Repeated Oral 20 mg/kg		Oral 200 mg/kg	
Compound	M	F	M	F	M	F
Parent	40.16	42.16	43.50	38.53	61.96	68.31
M-1	0.35	0.27	0.22	0.49	0.37	0.23
M-1A	1.38	2.05	2.34	2.75	0.99	0.64
M-2	5.23	2.80	5.06	3.98	2.00	1.76
M-3	6.63	3.11	5.41	4.31	2.83	2.42
M-4	5.90	3.79	7.44	8.69	2.93	2.45
M-5	8.18	6.25	7.20	10.00	3.34	3.79
M-6	2.75	2.13	3.06	4.03	1.41	1.45
M-7	4.25	3.83	4.96	5.34	3.77	2.38
M-7A	1.23	1.07	1.43	1.17	0.78	0.71
M-8	1.18	1.11	1.83	1.87	1.17	1.17
Total identified	**77.24**	**68.57**	**82.45**	**81.16**	**81.55**	**85.31**
U-1 (polar)	5.91	2.30	2.98	5.39	1.00	0.69
U-2	1.41	0.92	1.42	1.49	0.46	0.92
Other unknowns	0.85	0.71	0.88	1.00	0.36	0.48
Total accounted for[b]	**85.41**	**72.50**	**87.73**	**89.04**	**83.37**	**87.40**
Unaccounted[c]	14.59	27.50	12.27	10.96	16.63	12.60
Total	100	100	100	100	100	100

a = Data extracted from Tables XXVI through XXVIII pages 129-135 in the study report.
b = Total accounted for = (Total identified) + (Total unidentified)
c = 100 - (Total accounted for); as only the 0- to 48-hour collections were analyzed, the unaccounted fraction includes radioactivity in excreta collected from 48 to 168 hours.

TABLE 9. Metabolite profile in excreta of rats dosed with [phenyl-^{14}C]pirate[a].

Dose	Recovery as percent of administered dose					
	Oral 20 mg/kg		Repeated Oral 20 mg/kg		Oral 200 mg/kg	
Compound	M	F	M	F	M	F
Parent	40.89	46.30	52.94	42.49	53.19	72.43
M-1	0.92	1.08	0.29	0.52	0.69	0.91
M-1A	1.81	1.15	2.52	2.21	0.93	0.73
M-2	4.97	3.23	2.76	3.59	3.56	2.91
M-3	3.38	3.16	4.60	4.57	2.56	2.20
M-4	3.92	3.25	4.74	6.28	2.42	2.41
M-5	5.04	4.07	5.13	7.58	2.95	2.92
M-6	2.16	1.36	2.42	3.15	1.26	1.12
M-7	2.62	3.01	1.97	3.38	2.27	1.98
M-7A	1.01	1.03	1.38	0.73	1.07	0.70
M-8	0.88	0.88	1.26	1.21	1.00	1.22
Total identified	**67.65**	**68.52**	**79.96**	**75.70**	**71.90**	**89.53**
U-1 (polar)	6.38	2.27	3.16	2.55	2.40	1.20
U-2	1.49	1.15	1.08	1.81	0.17	0.32
Other unknowns	0.49	0.45	0.62	0.85	0.79	0.50
Total accounted[b]	**76.01**	**72.39**	**84.82**	**80.92**	**75.26**	**91.55**
Unaccounted[c]	23.99	27.61	15.18	19.08	24.74	8.45
Total	100	100	100	100	100	100

a = Data extracted from Tables XXVI through XXVIII pages 129-135 in the study report.
b = Total accounted for = (Total identified) + (Total unidentified)
c = 100 - (Total accounted for); as only the 0- to 48-hour collections were analyzed, the unaccounted fraction includes radioactivity in excreta collected from 48 to 168 hours

III. DISCUSSION

A. Investigator's Conclusions. The study author concluded that the majority of [^{14}C]pirate administered to rats is excreted, primarily in the feces and, to a lesser extent, in the urine. The absorption, elimination, and distribution of radioactivity in rats was the same for the [pyrrole-^{14}C] and [phenyl-^{14}C] label. Unaltered pirate accounted for most of the radioactivity in feces. The bond between the pyrrole and phenyl rings remains intact. Polar metabolites predominated in urine. In addition, several polar and non-polar metabolites were detected in feces and urine. Tissues in all groups contained pirate-related residues; tissue radioactivity was substantially higher in females. Pirate was extensively metabolized to several polar and non-polar metabolites in kidney and liver. Unchanged pirate was the predominant radioactive compound in fat and muscle.

B. Reviewer's Discussion. The reviewer concurs with the study author that pirate is only minimally absorbed by rats following oral exposure, and that the metabolism of absorbed pirate results in several non-polar and polar metabolites with intact two-ring structure. Little more than 10% of the single or repeated oral 20 mg/kg dose of either [2-pyrrole-^{14}C] or [phenyl-^{14}C]pirate was absorbed by male or female CD-1 rats. The percent absorption of the single 200 mg/kg dose was less than that of the low dose, with only 4-5% of the dose recovered in the urine. More than 80% was eliminated in the feces. Most of the residue excretion occurred within 48 hours of dosing. Total recovery of the radioactive doses after 7 days was 89-121%.

At sacrifice, total recovery of radioactivity in tissues and carcass was 2-4% of the low doses and 1-2% of the high dose. Most of the radioactivity in the body was in the carcass, 1.12-3.37% of the low doses and 0.6-1.5% of the high dose. The liver had the highest percent of recovered dose of any single tissue, 0.28-0.48% of the low doses and 0.15-0.17% of the high dose. Females accumulated twice as much radioactivity as males in the blood, fat, and carcass.

Eleven radioactive compounds were isolated from excreta of rats dosed with [^{14}C]pirate and nine were identified. Greater than 67% of the radioactive dose was identified and accountability of the radioactive dose was >72%. Unaccountable radioactivity can be attributed to the 48- to 168-hour urine and feces collections, which were not included in the analysis.

The parent compound was the only major residue. Breakdown products of two primary metabolic pathways accounted for minor proportions of the doses (<10%). The de-alkylated, pyrrole-hydroxylated metabolite M-4 (approximately 3-7% of dose) may form amino acid and/or sulfate conjugates, which together accounted for ≤6% of the dose. The two rings of the molecule are not cleaved.

In liver and kidney, the predominant radioactive residues were the primary metabolites and conjugated, and unidentified polar components. The parent was the predominant residue in fat and muscle.

C. Study deficiencies: No significant deficiencies or deviations from Subdivision F were noted in this study.

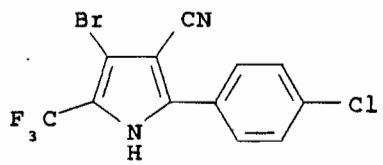
This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: CL 303,268
   Description: Nearly white solid
   Lot/Batch #: AC8979-44B
   Purity: 100.3% a.i.
   Stability of compound: Not specified
   CAS #: Not specified
   Structure:



   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: The test substance was stored at room temperature. Dosing solutions were prepared on the days of testing and samples were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were from 86-106% of the nominal concentrations with coefficients of variation of 4 and 6% for the two replicate trials.

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration: DMSO/0.1 mL per plate

   Positive:
   Nonactivation:

Sodium azide	not used	µg/plate	
2-Nitrofluorene	20	µg/plate	TA98, TA1538
9-Aminoacridine	50	µg/plate	TA1537
Other (list):			
N-methyl-N-nitro-N-nitrosoguanidine	10	µg/plate	E. coli WP2 uvrA-, TA100, TA1535

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Activation:

2-Aminoanthracene	2.5 µg/plate	all S. Typhimurium strains
2-Aminoanthracene	10 µg/plate	E. coli WP2 uvrA-

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

The S9 homogenate was purchased from Microbiological Associates, Inc., Bethesda, MD. The test S9 was purchased frozen (-80 C) and was thawed on the day of testing. The S9:cofactor mix was (1:10) and 0.5 mL of S9 mix was used per plate. The S9 mix composition included NADP, glucose-6-phosphate, potassium chloride, and magnesium chloride-6-hydrate in a sodium phosphate buffer.

4. Test organisms: S. typhimurium strains

___ TA97 x TA98 x TA100 ___ TA102 ___ TA104
x TA1535 x TA1537 x TA1538 ; list any others:

E. coli strain

x WP2 uvrA-

Properly maintained? - **Yes**

Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Preliminary cytotoxicity test: Nine dose levels (1, 5, 10, 25, 50, 100, 250, 500, or 1000 µg/plate) were evaluated with the S. typhimurium strain TA100 or E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and per condition. Vehicle and positive control groups were included.

Mutagenicity assay: S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 303,268 at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, or 5.0 µg/plate. E. coli WP2 uvrA- was tested with CL 303,268 at concentrations of 10, 25, 50, 100, or 250 µg/plate. All evaluated dose levels were assayed with and without S9 activation. Triplicate plates were used for each dose, strain, and condition. Vehicle and positive control groups were included and the assay was run in duplicate.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   _x_ standard plate test
   ___ pre-incubation (___ minutes)
   ___ "Prival" modification *(i.e. azo-reduction method)*
   ___ spot test
   ___ other *(describe)*

2. Protocol: Tester strains were inoculated into nutrient broth culture 1 day prior to dosing and incubated for approximately 11 hours. Test substance and positive control substances were diluted in DMSO to specified concentrations. For the activation conditions, 0.1 mL of the appropriate tester strain culture at a density of $1x10^8$ to $2x10^9$ cells/mL, 0.5 mL of the S9 mix, and 0.1 mL of test material solution, solvent, or positive control were mixed with 2.0 mL of melted top agar supplemented with histidine, tryptophan, and biotin. The mixture was poured over a minimal agar plate. For nonactivation conditions, 0.5 mL of buffer (0.1 M sodium phosphate, 0.008 M magnesium chloride, 0.033 M potassium chloride) was substituted for the S9 mix. After incubation (37 ± 2 C for 48 ± 6 hours), the number of revertant colonies were counted by hand or using an automated colony counter. The background lawn and/or the number of colonies were examined to determine the toxicity of the test compound. Means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

(a) Assay validity: A detailed discussion of the criteria used to determine if the assay was valid was not given. However, the study report states that the assay would be "partially repeated" if there was "excessive toxicity" and that the assay results would be compared with "recent historical control values...to gain perspective on test system performance." An assay was considered valid if the positive control materials demonstrated responses that were consistent with their "published activities".

(b) Positive response: The test material was considered mutagenic if it caused a reproducible dose-related increase in the mean number of revertants over three dose levels with at least one positive dose level. A positive dose level was one where the increase in the mean number of revertants was at least 2-fold higher than the vehicle control for strains TA98

and TA100 and 3-fold for strains TA1535, TA1537, TA1538, and WP2 uvrA-.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: The number of colonies/plate for the preliminary assay are presented in Attachment 1 (study report page 14) of this DER. Nine dose levels (1, 5, 10, 25, 50, 100, 250, 500, and 1000 µg/plate) were evaluated with the S. typhimurium strain TA100 and E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and per condition. The positive controls used were 2-aminoanthracene (activation conditions) and N-methyl-N'-nitro-N-nitrosoguanidine (non-activation conditions). The test material precipitated in the test plates with concentrations ≥250 µg/plate. Toxicity was observed as "greatly reduced or no background lawns, or no bacterial growth" in the TA100 strain at doses ≥10 µg/plate (+S9) and ≥5 µg/plate (-S9). In addition, slight toxicity was observed in the TA100 strain as "reduced background lawns" at doses of 5 µg/plate (+S9) and 1 µg/plate (-S9). For the WP2 uvrA-, no toxicity was observed at any dose level with or without activation.

Based on these results, the mutation assay was performed with a dose range of 0.05-250 µg/plate +/-S9 activation.

B. Mutagenicity assay: The test results for the mutagenicity assays with CL 303,268 are presented in Attachment 1 (study report pages 15-18) of this DER. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 303,268 at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, or 5.0 µg/plate. E. coli WP2 uvrA- was tested with CL 303,268 at concentrations of 10, 25, 50, 100, or 250 µg/plate. All evaluated dose levels were assayed with and without S9 activation. Triplicate plates were used for each dose, strain, and condition. Vehicle and positive control groups were included and the assay was run in duplicate. The test material precipitated in the E. coli WP2 uvrA- 250 µg plates.

There was no significant increase in the mean number of revertant colonies in any of the test strains at any dose level/condition with the exception of the TA100 strain. For the TA100 strain, an increase in the mean number of revertant colonies was demonstrated over the dose range. In addition, at the highest dose tested that did not induce cytotoxicity (1.0 µg/plate), the mean number of revertants was 1.9x (initial trial) and 1.5x (confirmatory trial) the vehicle control. For the TA100 strain, a dose level was

considered positive if the increase in the mean number of revertants was at least 2-fold higher than the vehicle control. Toxicity was observed as "reduced background lawns" with activation and as "greatly reduced or no background lawns" without activation in all S. typhimurium strains at the 5.0 µg dose. All positive controls induced an appropriate increase in the number of revertant colonies. For the E. coli WP2 uvrA- strain, no toxicity was observed at any dose level with or without activation.

Based on the findings, the study author concluded that CL 303,268 was not mutagenic under the conditions of this microbial gene mutation assay.

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

A. The reviewer agrees with the study author's conclusion that CL 303,268 was not mutagenic under the conditions of the submitted microbial gene mutation assay. CL 303,268 was assayed over an appropriate dose range as it was tested to cytotoxic concentrations with the S. typhimurium strains and to the limit of solubility (250 µg/plate) with the E. coli WP2 uvrA- strain. CL 303,268 failed to induce a genotoxic response in any of the tester strains with the exception of the borderline positive result for the TA100 strain at the 1.0 µg/plate dose level. As the result was equivocal and a genotoxic response was not found in any of the other tester strains, we agree with the conclusion that CL 303,268 was not mutagenic under the conditions of the submitted study. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. The study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

CHLORFENAPYR

Page _____ is not included in this copy.

Pages 248 through 252 are not included in this copy.

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.

_____ 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.

DATA EVALUATION REPORT

PIRATE Metabolite/impurity

Study Type: 84-2; *Salmonella typhimurium* and *Escherichia coli*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 101Q (MRID 43492841)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Primary Reviewer:
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Signature: Sandy Daussin by W/A
Date: 1/16/96

Secondary Reviewer:
Steven Brecher, Ph.D.

Signature: Steven Brecher by W/A
Date: 1/16/96

Project Manager:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 1/16/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler by W/A
Date: 1/16/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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[CL 312,094] SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)
(Pirate impurity)

EPA Reviewer: Irving Mauer, Ph.D. , Date 02/16/96
Review Section IO, Toxicology Branch 1 (7509C)
EPA Secondary Reviewer: Marion Copley, DVM, DABT , Date 3/21/96
Review Section 4, Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella typhimurium and Escherichia coli/mammalian activation gene mutation assay
OPPTS Number: 870.5265 and 870.5100 OPP Guideline Number: [§84-2]

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): CL 312,094 (pirate impurity, 96.3% active ingredient)

SYNONYMS: 2-(6-Chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-2-carbonitrile

CITATION: Mulligan, E. (1994) Microbial Mutagenicity Plate Incorporation Assay of CL 312,094. American Cyanamid Company, Genetic Toxicology Laboratory, Princeton, NJ. Study No. 9402002. August 12, 1994. MRID 43492841. Unpublished.

SPONSOR: American Cyanamid Co., Princeton, NJ

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 43492841), strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella typhimurium or Escherichia coli WP2 uvrA- were exposed to CL 312,094 (96.3% a.i.), in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 or E. coli WP2 uvrA- were evaluated with CL 312,094 at concentrations of 25, 50, 100, 250, 500, or 1000 µg/plate (+S9) and at 5, 10, 25, 50, 100, or 250 µg/plate (-S9).

CL 312,094 (96.3% a.i.) was tested up to the limit of solubility. It was not cytotoxic at these concentrations to any of the S. typhimurium strains or the E. coli WP2 uvrA-. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **acceptable**, and satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.

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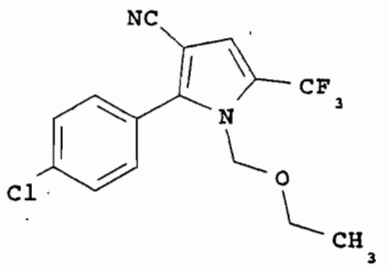
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: CL 312,094
   Description: Tan solid
   Lot/Batch #: AC8978-67A
   Purity: 96.3% a.i.
   Stability of compound: Not specified
   CAS #: Not specified
   Structure:



   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: The test substance was stored at room temperature. Dosing solutions were prepared on the days of testing and samples were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were from 98-107% of the nominal concentrations with coefficients of variation of 2 and 3% for the two replicate trials.

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration: DMSO/0.1 mL per plate

   Positive:
   Nonactivation:

Sodium azide	not used	µg/plate	
2-Nitrofluorene	20	µg/plate	TA98, TA1538
9-Aminoacridine	50	µg/plate	TA1537
Other (list):			
N-methyl-N-nitro-N-nitrosoguanidine	10	µg/plate	E. coli WP2 uvrA-, TA100, TA1535

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Activation:

2-Aminoanthracene	2.5 µg/plate	all S. Typhimurium strains
2-Aminoanthracene	10 µg/plate	E. coli WP2 uvrA-

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

The S9 homogenate was purchased from Microbiological Associates, Inc., Bethesda, MD. The test S9 was purchased frozen (-80 C) and was thawed on the day of testing. The S9:cofactor mix was (1:10) and 0.5 mL of S9 mix was used per plate. The S9 mix composition included NADP, glucose-6-phosphate, potassium chloride, and magnesium chloride-6-hydrate in a sodium phosphate buffer.

4. Test organisms: S. typhimurium strains

___ TA97	x TA98	x TA100	___ TA102	___ TA104
x TA1535	x TA1537	x TA1538 ; list any others:		

E. coli strain

x WP2 uvrA-

Properly maintained? **Yes**

Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Preliminary cytotoxicity test: Eleven dose levels (1, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, or 5000 µg/plate) were evaluated with the S. typhimurium strain TA100 or E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and condition. Vehicle and positive control groups were included.

Mutagenicity assay: S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 or E. coli WP2 uvrA- were evaluated with CL 312,094 at concentrations of 25, 50, 100, 250, 500, or 1000 µg/plate with metabolic activation and at 5, 10, 25, 50, 100, or 250 µg/plate without S9 activation. Triplicate plates were used for each dose, strain, and condition. Vehicle and positive control groups were included and the assay was run in duplicate.

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B. TEST PERFORMANCE

1. Type of Salmonella assay:
   _x_ standard plate test
   ___ pre-incubation (___ minutes)
   ___ "Prival" modification *(i.e. azo-reduction method)*
   ___ spot test
   ___ other *(describe)*

2. Protocol: Tester strains were inoculated into nutrient broth culture 1 day prior to dosing and incubated for approximately 11 hours. Test substance and positive control substances were diluted in DMSO to specified concentrations. For the activation conditions, 0.1 mL of the appropriate tester strain culture at a density of $1x10^8$ to $2x10^9$ cells/mL, 0.5 mL of the S9 mix, and 0.1 mL of test material solution, solvent, or positive control were mixed with 2.0 mL of melted top agar supplemented with histidine, tryptophan, and biotin. The mixture was poured over a minimal agar plate. For nonactivation conditions, 0.5 mL of buffer (0.1 M sodium phosphate, 0.008 M magnesium chloride, 0.033 M potassium chloride) was substituted for the S9 mix. After incubation (37 ± 2 C for 48 ± 6 hours), the number of reverant colonies were counted by hand or using an automated colony counter. The background lawn and/or the number of colonies were examined to determine the toxicity of the test compound. Means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

(a) Assay validity: A detailed discussion of the criteria used to determine if the assay was valid was not given. However, the study report states that the assay would be "partially repeated" if there was "excessive toxicity" and that the assay results would be compared with "recent historical control values...to gain perspective on test system performance." An assay was considered valid if the positive control materials demonstrated responses that were consistent with their "published activities".

(b) Positive response: The test material was considered mutagenic if it caused a reproducible dose-related increase in the mean number of revertants over three dose levels with at least one positive dose level. A positive dose level was one where the increase in the mean number of revertants was at least 2-fold higher than the vehicle control for strains TA98

and TA100 and 3-fold for strains TA1535, TA1537, TA1538, and WP2 uvrA-.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: The number of colonies/plate for the preliminary assay are presented in Attachment 1 (study report page 14) of this DER. Eleven dose levels (1, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, or 5000 µg/plate) were evaluated with the S. typhimurium strain TA100 or E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and per condition. The positive controls used were 2-aminoanthracene (activation conditions)and N-methyl-N'-nitro-N-nitrosoguanidine (non-activation conditions). The test material precipitated in the test plates with concentrations ≥500 µg/plate (+S9) and ≥100 µg/plate (-S9). Toxicity was not observed as at any of the dose level +/-S9 with either tester strain.

Based on these results, the mutation assay was performed to the limit of solubility with a dose range of 5-1000 µg/plate +/-S9 activation.

B. Mutagenicity assay: The test results for the mutagenicity assays with CL 312,094 are presented in Attachment 1 (study report pages 15-18) of this DER. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 or E. coli WP2 uvrA- were evaluated with CL 312,094 at concentrations of 25, 50, 100, 250, 500, or 1000 µg/plate with metabolic activation and at 5, 10, 25, 50, 100, or 250 µg/plate without S9 activation. Triplicate plates were used for each dose, strain, and condition. Vehicle and positive conrtol groups were included and the assay was run in duplicate. The test material precipitated in the test plates with concentrations ≥500 µg/plate (+S9) and ≥250 µg/plate (-S9).

There was no significant increase in the mean number of revertant colonies in any of the tester strains at any dose level/condition. All positive controls induced an appropriate increase in the number of revertant colonies. No toxicity was observed at any dose level with or without activation for any of the tester strains.

Based on the findings, the study author concluded that CL 312,094 was not mutagenic under the conditions of this microbial gene mutation assay.

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III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

A. The reviewer agrees with the study author's conclusion that CL 312,094 was not mutagenic under the conditions of the submitted microbial gene mutation assay. CL 312,094 was assayed at the limit of solubility as it precipitated at concentrations ≥500 μg/plate (+S9) and ≥250 μg/plate (-S9). CL 312,094 was not cytotoxic or mutagenic at the limit of solubility as it failed to induce a genotoxic response in any of the S. typhimurium strains or in E. coli WP2 uvrA-. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activate positive controls. The study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

CHLORFENAPYR

Page _____ is not included in this copy.

Pages 261 through 265 are not included in this copy.

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.

✓ 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.

DATA EVALUATION REPORT

PIRATE Metabolite

Study Type: 84-2; *Salmonella typhimurium* and *Escherichia coli*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 101R (MRID 43492842)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Sandra Daussin, B.S.

Signature: Sandra Daussin by Jeff
Date: 1/16/96

Secondary Reviewer:
Steven Brecher, Ph.D.

Signature: Steven Brecher by Jeff
Date: 1/16/96

Project Manager:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 1/16/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler by Jeff
Date: 1/16/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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[CL 322,250] **SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)**
(Pirate metabolite)

EPA Reviewer: Irving Mauer, Ph.D. , Date 02/16/96
Review Section IO, Toxicology Branch 1 (7509C)
EPA Secondary Reviewer: Marion Copley, DVM, DABT , Date 2/21/96
Review Section 4, Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella typhimurium and Escherichia coli/mammalian activation gene mutation assay
OPPTS Number: 870.5265 and 870.5100 OPP Guideline Number: [§84-2]

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): CL 322,250 (pirate metabolite, 89% active ingredient)

SYNONYMS: 3-Bromo-5-(p-chlorophenyl)-4-cyano-pyrrole-2-carboxylic acid

CITATION: Mulligan, E. (1994) Microbial Mutagenicity Plate Incorporation Assay of CL 322,250. American Cyanamid Company, Genetic Toxicology Laboratory, Princeton, NJ. Study No. 9402003. August 19, 1994. MRID 43492842. Unpublished.

SPONSOR: American Cyanamid Co., Princeton, NJ

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 43492842), strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella typhimurium or Escherichi coli WP2 uvrA- were exposed to CL 322,250 (89% a.i.), in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. S. typhimurium strains were tested with CL 322,250 at concentrations of 100, 250, 500, 1000, or 2500 µg/plate (+S9) and 50, 100, 250, 500, or 1000 µg/plate (-S9). E. coli WP2 uvrA- was tested with CL 322,250 at concentrations of 250, 500, 1000, 2500 or 5000 µg/plate (+/- S9).

CL 322,250 (89% a.i.) was tested up to cytotoxic concentrations and the limit concentration, 5000 µg/plate. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

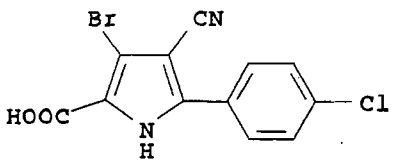
This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: CL 322,250
   Description: Cream, solid, "35 mesh"
   Lot/Batch #: AC9014-97A
   Purity: 89% a.i.
   Stability of compound: Not specified
   CAS #: Not specified
   Structure:



   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: The test substance was stored at room temperature. Dosing solutions were prepared on the days of testing and samples were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were from 82-98% of the nominal concentrations with coefficients of variation of 4 and 6% for the two replicate trials.

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration: DMSO/0.1 mL per plate

   Positive:
   Nonactivation:

Sodium azide	not used	µg/plate	
2-Nitrofluorene	20	µg/plate	TA98, TA1538
9-Aminoacridine	50	µg/plate	TA1537
Other (list): N-methyl-N-nitro-N-nitrosoguanidine	10	µg/plate	E. coli WP2 uvrA-, TA100, TA1535

   Activation:

2-Aminoanthracene	2.5	µg/plate	all S. Typhimurium strains
2-Aminoanthracene	10	µg/plate	E. coli WP2 uvrA-

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3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

The S9 homogenate was purchased from Microbiological Associates, Inc., Bethesda, MD. The test S9 was purchased frozen (-80 C) and was thawed on the day of testing. The S9:cofactor mix was (1:10) and 0.5 mL of S9 mix was used per plate. The S9 mix composition included NADP, glucose-6-phosphate, potassium chloride, and magnesium chloride-6-hydrate in a sodium phosphate buffer.

4. Test organisms: S. typhimurium strains

___ TA97 x TA98 x TA100 ___ TA102 ___ TA104
x TA1535 x TA1537 x TA1538 ; list any others:

E. coli strain

x WP2 uvrA-

Properly maintained? **Yes**

Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Preliminary cytotoxicity test: Eleven dose levels (1, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, or 5000 µg/plate) were evaluated with the S. typhimurium strain TA100 and E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and condition. Vehicle and positive control groups were included.

Mutagenicity assay: S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 322,250 at concentrations of 100, 250, 500, 1000, or 2500 µg/plate (+S9) and 50, 100, 250, 500, or 1000 µg/plate (-S9). E. coli WP2 uvrA- was tested with CL 322,250 at concentrations of 250, 500, 1000, 2500 or 5000 µg/plate (+/- S9). Triplicate plates were used for each dose, strain, and condition. Vehicle and positive control groups were included and the assay was run in duplicate.

B. TEST PERFORMANCE

1. Type of Salmonella assay:

x standard plate test
___ pre-incubation (___ minutes)
___ "Prival" modification *(i.e. azo-reduction method)*
___ spot test
___ other *(describe)*

2. Protocol: Tester strains were inoculated into nutrient broth culture one day prior to dosing and incubated for approximately 11 hours. Test substance and positive control substances were diluted in DMSO to specified concentrations. For the activation conditions, 0.1 mL of the appropriate tester strain culture at a density of $1x10^8$ to $2x10^9$ cells/mL, 0.5 mL of the S9 mix, and 0.1 mL of test material solution, solvent, or positive control were mixed with 2.0 mL of melted top agar supplemented with histidine, tryptophan, and biotin. The mixture is poured over a minimal agar plate. For nonactivation conditions, 0.5 mL of buffer (0.1 M sodium phosphate, 0.008 M magnesium chloride, 0.033 M potassium chloride) was substituted for the S9 mix. After incubation (37 ± 2 C for 48 ± 6 hours), the number of revertant colonies were counted by hand or using an automated colony counter. The background lawn and/or the number of colonies were examined to determine the toxicity of the test compound. Means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

(a) Assay validity: A detailed discussion of the criteria used to determine if the assay was valid was not given. However, the study report states that the assay would be "partially repeated" if there was "excessive toxicity" and that the assay results would be compared with "recent historical control values...to gain perspective on test system performance." An assay was considered valid if the positive control materials demonstrated responses that were consistent with their "published activities".

(b) Positive response: The test material was considered mutagenic if it caused a reproducible dose-related increase in the mean number of revertants over three dose levels with at least one positive dose level. A positive dose level was one where the increase in the mean number of revertants was at least 2-fold higher than the vehicle control for strains TA98 and TA100 and 3-fold for strains TA1535, TA1537, TA1538, and WP2 uvrA-.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay The number of colonies/plate for the preliminary assay are presented in Attachment 1 (study report page 14) of this DER. Eleven dose levels (1, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000 µg/plate)

were evaluated with the strains TA100 or E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and per condition. The test material precipitated in the 5000 µg plates. The positive controls used were 2-aminoanthracene (activation conditions) and N-methyl-N'-nitro-N-nitrosoguanidine (non-activation conditions). Toxicity was observed as "greatly reduced or no background lawns, or no bacterial growth" in the TA100 strain at doses ≥2500 µg/plate (+S9) and ≥1000 µg/plate (-S9). For the WP2 uvrA-, slight toxicity ("reduced background lawns") was evident at 5000 µg/plate without activation. No toxicity was observed at any dose level with activation.

Based on these results, the mutation assay was performed with a dose range of 50-5000 µg/plate +/-S9 activation.

B. Mutagenicity assay: The test results for the mutagenicity assays with CL 322,250 are presented in Attachment 1 (study report pages 15-18) of this DER. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 322,250 at concentrations of 100, 250, 500, 1000, or 2500 µg/plate (+S9) and 50, 100, 250, 500, or 1000 µg/plate (-S9). E. coli WP2 uvrA- was tested with CL 322,250 at concentrations of 250, 500, 1000, 2500 or 5000 µg/plate (+/-S9). Triplicate plates were used for each dose, strain, and condition. Vehicle and positive control groups were included and the assay was run in duplicate.

Toxicity was observed as "greatly reduced background lawns" in all S. typhimurium strains except TA98 at 2500 µg (+S9) and at 1000 µg (-S9). For the TA98 strain, a "reduced background lawn" indicated toxicity at 2500 µg/plate (+S9) and at 1000 µg/plate (-S9). For the E. coli WP2 uvrA- strain, a "greatly reduced background lawn" was observed at 5000 µg/plate without activation and no toxicity was observed at any dose with activation. The test material precipitated in the 5000 µg plates. There was no significant increase in the mean number of revertant colonies in any of the test strains at any dose level/condition. All positive controls induced an appropriate increase in the number of revertant colonies.

Based on the findings, the study author concluded that CL 322,250 was not mutagenic under the conditions of this microbial gene mutation assay.

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

A. The reviewer agrees with the study author that CL 322,250 was not mutagenic under the conditions of this microbial gene mutation assay. The test material was tested at cytotoxic concentrations and at the limit concentration, and it failed to induce a genotoxic response in any of the S. typhimurium strains or in the E. coli WP2 uvrA-. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. The study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

CHLORFENAPYR

Page _____ is not included in this copy.

Pages 274 through 278 are not included in this copy.

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.

√ 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.

Reviewed by: Irving Mauer, Ph.D. 6/18/96
Immediate Office/HED (7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (7509C) 6/18/96

SUPPLEMENTAL DATA EVALUATION REPORT #2
(HED Doc. # 010986)
010651

STUDY TYPE: Mutagenicity: In Vivo Micronucleus Assay in Mice

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 43187602/42770225

GUIDELINE #: 84-2

TEST MATERIAL: Pirate™; AC 303,630

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl)

STUDY NUMBERS: American Cyanamid Co. 91-18-001

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Evaluation of CL 303,630 in the In Vivo Micronucleus Assay in Mouse Bone marrow Cells

AUTHORS: R.K. Sharma

REPORT ISSUED: March 17, 1993; resubmitted April 7, 1994

EXECUTIVE SUMMARY: **Negative** for micronucleus induction in bone marrow cells of male and female CD-1 mice 24, 48, and 72 hours after the single oral gavage administration of 7.5, 15, or 30 mg/kg (males) or 5, 10, or 20 mg/kg (females) CL 303,630 (MRID No.:43187602/42770225).

This study is upgraded to **Acceptable**, based on the additional information presented and satisfies the requirements for Structural Chromosomal Aberration Assay (84-2).

DISCUSSION: The following additional information justifies upgrading from **UNACCEPTABLE** to **ACCEPTABLE**:

1. Evidence of absorption and circulation to blood and bone marrow reported in a metabolism study in rats

given a single dose of 20 mg/kg (the HDT in female mice in the micronucleus assay).

2. Clinical toxicity (death) at 30 mg/kg, the HDT in male mice of this subject assay, and in high-dose females, at 20 mg/kg (diarrhea), which would be consistent with the language of FIFRA Test Guideline 84-2b for this type of <u>In Vivo</u> mutagenicity assay, which states that acceptable testing should involve administration of the test article at the highest dose that causes clinical toxicity <u>or</u> cytotoxicity at the target tissue. Based upon range-finding testing, animals were dosed at 60% of the LD values, also <u>sufficient</u> for regulatory satisfaction.

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DATA EVALUATION RECORD

PIRATE

Study Type: 84-2; *In vitro* Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells

Work Assignment No. 1-1S (MRID 43492843)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 4/15/96

Secondary Reviewer:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 4/15/96

Project Manager:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 4/15/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 4/15/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Pirate

In vitro cytogenetics (84-2)

EPA Reviewer: I. Mauer, PhD ; Date 05/10/96
Review Section III, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, DVM, DABT , Date 5/10/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: In vitro mammalian chromosome aberrations in Chinese hamster ovary (CHO) cells
OPPTS Number: 870.5375 OPP Guideline Number: §84-2

DP BARCODE: D212558 SUBMISSION CODE: S481410
P.C. CODE: 129093 TOX. CHEM. NO.: NONE

TEST MATERIAL (PURITY): AC 303,630 (Pirate; 94.5% ai)

SYNONYMS: CL 303,630; 4-Bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile

CITATION: Sharma, R.K. (1994) Evaluation of CL 303,630 in the In Vitro chromosome aberration assay in Chinese Hamster ovary (CHO) cells. Genetic Toxicology Laboratory, American Cyanamid Company, Princeton, New Jersey. Laboratory Project ID: Study No. 92-11-001. June 6, 1994. MRID 43492843. Unpublished.

SPONSOR: American Cyanamid Company.

EXECUTIVE SUMMARY:

In a mammalian cell chromosome aberration assay (MRID 43492843), Chinese Hamster ovary (CHO) cell cultures were exposed to AC 303,630 (Pirate; 94.5% ai) in dimethylsulfoxide at concentrations of 6.25, 12.5, 25, or 50 µg/mL with metabolic activation (S-9 mix), or 12.5, 25, 50, or 100 µg/mL without metabolic activation. The high dose was selected so that AC 303,630 was tested to cytotoxic concentrations but sufficient cells remained for evaluation, and the low and intermediate doses were the concentrations corresponding to 12.5, 25, and 50% of the high dose. Cell cultures with metabolic activation were harvested 6, 18, or 42 hours following the termination of exposure (12, 24, or 48 hours following the start of exposure). Cell cultures without activation were harvested approximately 2 hours following the termination of exposure (12, 24, or 48 hours following the start of exposure). Because of cytotoxicity at 50 µg/mL with activation, the activated cultures exposed to AC 303,630 at 6.25, 12.5, and 25 µg/mL were evaluated for aberrant and polyploid cells. The nonactivated cultures exposed at 25, 50, and 100 µg/mL were evaluated.

AC 303,630 had no significant effect on the occurrence of aberrant chromosomes at any harvest time in cultures with or

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without metabolic activation. Analysis of data for polyploidy showed a statistically significant effect at 6.25 µg/mL with activation at the 24-hour harvest only. This effect was not dose-related, since polyploidy values for the 12.5 and 25 µg/mL treatments were similar to the vehicle control, and the data in general exhibited a statistically nonsignificant and negative trend. When statistical analysis of the polyploids was done excluding endoreduplication, no statistical significance was found. **AC 303,630 caused no statistically significant increases in the proportion of aberrant or polyploid chromosomes in Chinese Hamster ovary cells compared to solvent control values.** Positive controls induced the appropriate response.

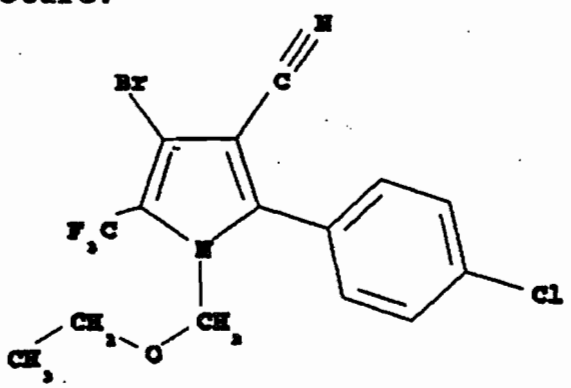
This study is classified as **acceptable** and satisfies the guideline requirement for in vitro cytogenetic mutagenicity studies (84-2).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AC 303,630 (Pirate)
   Description: White solid
   Lot/Batch #: AC 7504-59A
   Purity: 94.5% ai
   Stability of compound: It was reported that "the test material was found to be stable under conditions of the study".
   CAS #: None
   Structure:



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Solvent used: Dimethylsulfoxide
Other comments: Dosing solutions used during the cytotoxicity and cytogenetic assays were analyzed by HPLC to confirm the concentrations of AC 303,630 in solution. Average concentrations of AC 303,630 ranged from 76-100% of nominal for the cytotoxicity tests, and from 100-102% of nominal for the cytogenetic assays.

2. Control Materials:
Negative: Culture medium (nutrient mixture McCoy's 5A)
Solvent/final concentration: Dimethylsulfoxide. The final concentration was not reported; the study protocol stated that the upper limit would not exceed 0.5% (v:v).
Positive:
Activation: Cyclophosphamide (25 µg/mL, solvent not indicated) for clastogenicity
Nonactivation: Mitomycin C (1 µg/mL, solvent not indicated) for clastogenicity

3. Activation
S-9 was derived from:

X	Aroclor 1254	X	Induced	X	Rat	X	Liver
	Phenobarbital		Non-induced		Mouse		Lung
	None				Hamster		Other
	Other				Other		

S-9 and the cofactor mix were purchased from Microbiological Associates Inc., Bethesda, MD, and were mixed at 1 part S-9 to 10 parts cofactor mix. Each flask having metabolic activation was treated with 1 mL of this mixture.

4. Test compound concentrations used
The definitive cytomutagenicity test was conducted at 6.25, 12.5, 25, and 50 µg/mL with S-9 activation, and at 12.5, 25, 50, and 100 µg/mL without activation.

5. Test cells
Chinese hamster ovary (CHO) cells, strain WBL, were grown and subcultured in McCoy's 5A Medium supplemented with 10% fetal bovine serum and 2 mM l-glutamine.

Properly maintained? **Yes**
Cell line or strain periodically checked for Mycoplasma contamination? **Yes**

Cell line or strain periodically checked for karyotype stability? Yes

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay

Three range-finding tests were conducted; each test included both activation and nonactivation conditions. CHO cells suspended in the culture solution were incubated at 37 C and 90% relative humidity in an atmosphere containing 5% carbon dioxide during all tests. Cytotoxicity Test 1 was conducted at 125, 250, 500, 1000, 2000, and 5000 µg/mL. Cytotoxicity Test 2 was conducted at 31.25, 62.5, 125, and 250 µg/mL with activation and 125, 250, 500, and 1000 µg/mL without activation. Cytotoxicity Test 3 was conducted at 50, 100, 150, 200, and 250 µg/mL. All exposures were for 24 hours, with harvest immediately following the termination of exposure. Cytotoxicity was determined by both microscopic evaluation of the cultures prior to harvest, and evaluation of a mitotic index from prepared cells.

2. Cytogenetic Assay

None of the doses tested in the range-finding cytotoxicity tests resulted in a "clear" 50% depression in the mitotic index compared to the solvent control. Therefore, the high dose in the cytogenetic assay was selected so that AC 303,630 was tested to cytotoxic concentrations, but sufficient cells remained for evaluation. The low and intermediate doses were the concentrations corresponding to 12.5, 25, and 50% of the high dose.

a. Cell treatment

CHO cells suspended in the culture solution were incubated at 37 C and 90% relative humidity in an atmosphere containing 5% carbon dioxide throughout the study. Cell cultures with S-9 activation were exposed to AC 303,630 at 6.25, 12,5, 25, or 50 µg/mL for 6 hours. Cell cultures without activation were exposed to AC 303,630 at 12.5, 25, 50, or 100 µg/mL for 12, 24, or 48 hours.

For each set, two cell cultures were treated with each dose level, and two were treated with the solvent control. For the 24-hour harvest only, two activated cultures were treated with cyclophosphamide at concentration of 25 µg/mL, and two nonactivated cultures were treated with mitomycin C at 1 µg/mL.

"Because of scattered incidences of polyploidy in the initial assay...and to examine the reproducibility of

the results", additional cell cultures with activation were exposed to AC 303,630 at 6.25, 12.5, or 25 μg/mL for 6 hours. Cell cultures without activation were exposed to AC 303,630 at 25, 50, or 100 μg/mL for 24 or 48 hours. No positive controls were conducted in conjunction with the repeat study.

b. Spindle inhibition
Inhibitor used/concentration: Colcemid at 1μM
Administration time: 2 hours before cell harvest

c. Cell harvest
Cell cultures with metabolic activation were harvested 6, 18, or 42 hours following the termination of exposure (12, 24, or 48 hours following the start of exposure). Cell cultures without activation were harvested approximately 2 hours following the termination of exposure (12, 24, or 48 hours following the start of exposure). The cells were detached from the flasks by mitotic shake-off, swollen in a 0.075 M KCl solution, then fixed with methanol:glacial acetic acid (3:1).

The retest cell cultures with and without activation were harvested as described 24 or 48 hours after the start of exposure.

d. Details of slide preparation
Cell suspensions were placed onto microscope slides and air-dried. The cells were stained with 3% Giemsa; and the slides were air-dried, cleared in xylene, and mounted with coverslips.

e. Metaphase analysis
No. of cells examined per dose: 200
No. of cells examined in the solvent control: 200
No. of cells examined in the cyclophosphamide positive control: 100
No. of cells examined in the mitomycin C positive control: 100
Scored for structural aberrations: Yes
Scored for numerical polyploidy: Yes
Coded prior to analysis: Yes

f. Evaluation criteria
A positive response was claimed if a treatment-related and statistically significant ($p \leq 0.05$ by ANOVA) increase was observed in the number of cells containing chromosome aberrations, or in the number of polyploid cells. "Biological significance of the results was also taken into consideration in determining the response of the test article." The

study was considered valid if the positive controls exhibited a statistically significant response, and the vehicle controls fell within expected range of historical values.

g. Statistical analysis

Data on the proportion of aberrant and polyploid cells were evaluated for statistical significance using ANOVA at $p < 0.05$. If significance was determined, the test for linear trend in dose-response was performed using the least significant difference. Fisher's exact test was used to compare the data from the positive and vehicle controls.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assays

Cytotoxicity, determined by both microscopic examination of the cell cultures prior to harvest and the mitotic index, was determined in three range-finding tests encompassing AC 303,630 concentrations between 31.25 and 5000 µg/mL with activation (rat S-9 mix), and between 50 and 5000 µg/mL without activation. All exposures were for 24 hours, with harvest by trypsinization immediately following the termination of exposure. Toxicity and a depression of the mitotic indices were observed at all concentrations tested, both with and without metabolic activation. None of the doses resulted in a clear 50% reduction in the mitotic indices. In cell cultures with metabolic activation that were treated at 50-250 µg/mL, the mitotic index (number of mitotic cells ÷ number of cells evaluated) was 0.5-0.7% for the 50 and 100 µg/mL treatments, 0.0-0.1% for the 150-250 µg/mL treatments, and 7.9% for the solvent control. In cell cultures without activation that were treated at 50-250 µg/mL, the mitotic index was 5.3% for the 50 µg/mL treatment, 1.7-2.8% for the 100-250 µg/mL treatments, and 8.5% for the solvent control. (Data were presented as study Tables 1-3, study pages 17-19).

Therefore, in order to test AC 303,630 to cytotoxic doses, but still allow sufficient cells for evaluation, 50 µg/mL with activation and 100 µg/mL without activation were selected as the high doses for the cytogenetic assay. Three lower doses (12.5, 25, and 50% of the high dose) were included to provide at least three doses for evaluation.

B. Cytogenetic assay

Cell cultures with S-9 activation were exposed to AC 303,630 at 6.25, 12,5, 25, or 50 µg/mL for 6 hours and harvested 12, 24, or 48 hours after exposure; the 6.25, 12.5, and 25 µg/mL treatments were evaluated for aberrations and polyploidy

because of cytotoxicity at 50 µg/mL. Cell cultures without activation were exposed to AC 303,630 at 12.5, 25, 50, or 100 µg/mL for 12, 24, or 48 hours, and harvested at approximately the same intervals; the 25, 50, and 100 µg/mL treatments were evaluated.

AC 303,630 had no significant effect on the occurrence of aberrant chromosomes at any harvest time in cultures with or without metabolic activation. The positive controls induced significant increases in percent aberrant cells. The vehicle control values were within the expected range. Data are presented in Attachment 1 (study report pages 26-28).

Analysis of data for polyploidy showed a statistically significant effect at 6.25 µg/mL with activation at the 24-hour harvest only; this observation was repeated in a subsequent assay conducted under identical conditions. In both cases, the effect at 6.25 µg/mL was not dose-related, since polyploidy values for the 12.5 and 25 µg/mL treatments were similar to the vehicle control, and the data in general exhibited a statistically nonsignificant and negative trend. When statistical analysis of the polyploids was done excluding endoreduplication, no statistical significance was found. Data are presented in Attachment 2 (study report pages 29-33).

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study author concluded that AC 303,630 caused no statistically significant increases in the proportion of aberrant or polyploid cells, in either the presence of S-9 activation mix or in its absence at any treatment time.

B. Reviewer's Discussion

AC 303,630 was neither clastogenic nor polyploid-inducing in cultured CHO cells over the dose ranges tested (6.25-50 µg/mL with activation and 12.5-100 µg/mL without activation) and exposure times up to 48 hours.

The solvent (dimethylsulfoxide) and untreated controls had comparable low frequencies of chromosome aberrations and polyploidy. However, the final concentration of solvent in the culture flasks was not indicated. The sensitivity of the assay system to detect damage to chromosomes was adequately demonstrated at the 24-hour harvest by the results obtained with the positive controls. Positive controls were not used at the 12 or 48 hour harvests and a positive control for polyploidy was not used at any harvest time. Since the

normal cell cycle for CHO cells is 12-14 hours, a harvest at 12 hours may not provide the time necessary for enough cells to pass through a complete cycle, especially in the presence of a compound that could potentially damage cells and delay the cycle.

In the definitive cytogenetic assay, the report states that the 50 µg/mL dose (with activation) produced cytotoxicity and was not used for cytogenetic evaluation. It further states that the lower dose levels 6.25, 12.5, and 25 µg/mL (with activation) and the 25, 50, and 100 µg/mL (without activation) provided sufficient cells for analysis at all harvest times. However, the level of cytotoxicity at these doses is not indicated.

Although the test chemical did not produce a statistically significant increase in chromosome aberrations or polyploidy, Attachment 2 indicates that there was a definite increase in the number of chromatid gaps (TG) at all dose levels of the test chemical in the absence of metabolic activation at the 24-hour harvest [100 µg/mL (67), 50 µg/mL (60), 25 µg/mL (29), positive control (5), solvent control (1)]. The laboratory did not consider these gaps true aberrations and did not include them in their statistical evaluation. (The OPPTS Guidelines state that gaps should be recorded separately and not included in the total aberration frequency.) No increase in gaps was seen in the presence of metabolic activation; however, these cultures were exposed to the test chemical for only 6 hours. Some gaps have been shown to be true discontinuities in DNA[1,2] and, if the aberration frequencies in this study were on the borderline of statistical significance above controls, further investigation would be required. Still, the registrant should provide the rationale for not considering gaps in the evaluation of the results, particularly in this case, where the number of gaps is rather high in comparison to controls.

The preceding deficiencies would not be expected to compromise the study as a whole. We conclude, therefore, that the results of this study provide sufficient evidence to consider AC 303,630 negative in this <u>in vitro</u> test system.

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[1] Heddle, J.A. and D.J. Bodycote. 1970. On the formation of chromosomal aberrations. <u>Mutation Research</u> 9:117-26.

[2] Satya-Prakash, K.L., T.C. Hsu, and S. Pathak. 1981. Chromosome lesions and chromosome core morphology. <u>Cytogenetics and Cell Genetics</u> 30:248-52.

IV. STUDY DEFICIENCIES

No deficiencies that would be expected to alter the conclusions of the study were identified. Minor deficiencies that were identified were:

The level of cytotoxicity for each dose level evaluated in the definitive cytogenetics assay was not reported.

The high frequency of chromatid gaps at all dose levels without metabolic activation at the 24-hour harvest was not addressed.

There was no positive control for polyploidy.

The harvest at 12 hours may not represent one complete cell cycle.

Analysis of the test chemical was not included in the study report.

ATTACHMENT 1

CHLORFENAPYR

Page _____ is not included in this copy.

Pages 292 through 314 are not included in this copy.

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.

__✓__ 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.