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

NOV 3 - 1993

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: PIRATE® Insecticide-Miticide (AC 303,630): Application for Experimental Use Permit and Petition for Temporary Tolerance for Use in or on Cottonseed

P.C.#: 129093
Submission #s: S442669
Project No. D192279 and 194472
G pet-temp toler
EPA ID#: 3G04223
S446747

From: Guruva B. Reddy, D.V.M., Ph. D.
Section 4
Toxicology Branch I
Health Effects Division (H7509C)

10/25/93

To: Dennis Edwards/Meredith Johnson
Project Manager 19
Registration Division (H7505C)

Thru: Marion P. Copley, D.V.M., D.A.B.T.
Section Head
Section 4, Toxicology Branch I
Health Effects Division (H7509C)

Marion Copley 10/25/93 KB 10/29/93

I. **CONCLUSIONS:**

The data base supports the requested EUP for use in/on cottonseed with a temporary tolerance. All reviewed studies are acceptable, except the Gene mutation study in chinese hamster ovary (CHO) cells and chromosomal aberration assays which were not to or near cytotoxic levels.

A copy of the DERs are attached.

There is no acute toxicity endpoint of concern based on current data. If worker exposure (other than acute) is less than ~~0.0042~~ .04 mg/kg/day, there would be no concern at this time (see 1)

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cc: CCB, OREB (Dorsey)

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II. ACTION REQUESTED:

American Cyanamid Company, has submitted an application for an Experimental Use Permit and a petition for Temporary Tolerance for PIRATE® Insecticide-Miticide. The studies included in this package are listed below and the * against the studies indicate that the DERs are attached.

Guideline #	Study Type	MRID #
81-1*	Acute Oral Toxicity	427702-07/428842-01
81-2*	Acute Dermal Toxicity	427702-08
81-3*	Acute Inhalation Toxicity	427702-09
81-4*	Primary Eye Irritation	427702-10
81-5*	Primary Dermal Irritation	427702-11
81-6*	De....al Sensitization	427702-12
82-1(a)	Subchronic Oral (rodent)	427702-19
82-1(b)*	Subchronic Oral (non-rodent)	427702-20
83-3(a)	Teratology (rodent)	427702-21/428842-02
83-3*	Teratology (non-rodent)	427702-22
84-2*	Gene mutation (Ames)	427702-23
84-2*	Gene mutation (CHO/HGPRT)	427702-24
84-2*	Structural chromosomal aberration	427702-25
84-4*	Other genotoxic effects	427702-26

Formulation: PIRATE® Insecticide-Miticide - EP

Guideline #	Study Type	MRID #
81-1*	Acute Oral Toxicity	427702-13
81-2*	Acute Dermal Toxicity	427702-14
81-3*	Acute Inhalation Toxicity	427702-15
81-4*	Primary Eye Irritation	427702-16
81-5*	Primary Dermal Irritation	427702-17
81-6*	Dermal Sensitization	427702-18

Following is the sponsor furnished product identity:

PIRATE™ insecticide-miticide is referred to by various names in this petition.

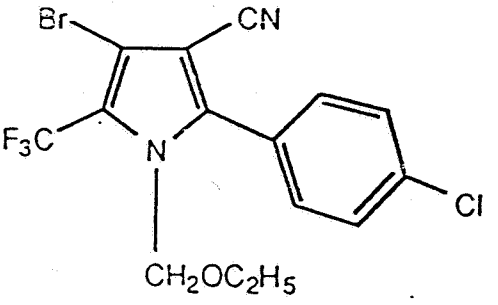
American Cyanamid Company Designations:

Technical	AC 303,630 CL 303,630
Formulated:	PIRATE 3SC Insecticide-Miticide AC 303,630 3SC Insecitcide-Miticide

Chemical Name Designations:

CA Name:	4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile
IUPAC Name:	4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)pyrrole-3-carbonitrile
CAS Registry Number:	122453-73-0
Molecular Formula:	$C_{15}H_{11}BrClF_3N_2O$
Molecular Weight:	407.6

Chemical Structure:



MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

IMPURITY INFORMATION. LOWER CERTIFIED LIMIT.

Composition of Technical:

Minimum	[redacted] AC 303,630
Maximum	[redacted]

The end-use product (formulation) proposed for the experimental use permit is 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile. One gallon of formulation contains 3.0 pounds of active ingredient.

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™ Trademark of American Cyanamid Company

The company supplied the following description: The chemical acts by uncoupling pests' mitochondrial oxidative phosphorylation through an electrochemical gradient. PIRATE® 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. Further, PIRATE® is considered as complementary to the Integrated Pest Management, a much needed direction for pest control, and resistance management programs used by cotton growers.

The submitted experimental program proposes to evaluate product efficacy in control of insects and/or mites on cotton. The petitioner is requesting an authorization for the use of 5,226 lbs of active ingredient during 1994 and 1995 growing seasons based on a base dosage rate of 0.00225 - 0.00233 lb of Pirate® (a.i.)/acre applied by ground or aerial spraying as required but not to exceed 2.0 lbs a.i./acre per year. The label restricts its use to 28 days before harvest. Based on the first year results the sponsor intends to amend the second year request with regard to the application rates and the proposed tolerances. A total of 3990 acres of cotton will be treated in 1994.

The proposed residue tolerance for cottonseed is 0.5 ppm.

III. **DATA REQUIREMENTS:**

For EUP with temp. tolerance. Updated: 10/20/93

Technical: AC 303,630 (Pirate® Insecticide-Miticide, MP)
Use Pattern: Terrestrial food use
Action Type: Experimental Use Permit with Temporary 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
82-1(b)	Subchronic Oral (non-rodent)	Yes	Yes
83-3	Teratology (non-rodent)	Yes	Yes
84-2	Gene mutation (Ames)	Yes	Yes
84-2	Gene mutation (mammalian)	Yes	No
84-2	Structural chromosomal aberration	Yes	No
84-2	Other genotoxic effects	No	Yes

Formulation: PIRATE® Insecticide-Miticide - EP

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

The list of all studies submitted in support of this EUP is attached. Only required studies were reviewed (see Tox. Profile).

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IV. Toxicology Profile Updated: 10/20/93

Guideline #	Study Identification and Classification	Results
Technical		
81-1	Acute Oral Toxicity in Rats MRID 427702-07/428842-01 Study #:T-0417 7/20/1992 **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
81-2	Acute Dermal Toxicity in Rabbits MRID 427702-08 Study #:T-0406 7/20/1992 **Acceptable**	LD_{50} > 2000 mg/kg (Limit Dose) TOXICITY CATEGORY: III
81-3	Acute Inhalation Toxicity in Rats MRID 427702-09 Study (american Cyanamid)#:91-8351 3/25/1993 **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
81-4	Primary Eye Irritation in Rabbits MRID 427702-10 Study #:T-0404 7/20/1992 **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 in Rabbits MRID 427702-11 Study #:T-0405 7/20/1992 **Acceptable**	Non-irritating. TOXICITY CATEGORY: IV
81-6	Dermal Sensitization in Guinea Pigs MRID 427702-12 Study #:T-0439 3/26/1993 **Acceptable**	**Not a skin sensitizer** (Closed-Patch Repeated Insult)

82-1(b)	Subchronic Feeding in Dogs (90-Day) MRID 427702-20 Study (American Cyanamid)#:971-92-118 4/8/1993 **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 (8.1 mg/kg/day)**, based on reduced body weight gain and feed efficiency and emaciation.
83-3(b)	Teratology Study in Rabbits MRID 427702-22 Study (American Cyanamid)#:971-90-179 3/2/1993 **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.**
84-2(a)	Gene Mutation-Ames MRID#: 427702-23 American Cyanamid # 91-02-001; 03/24/93 **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(a)	Gene Mutation - in mammalian cells (CHO/HGPRT) MRID#: 427702-24 American Cyanamid # 91-05-001; 03/25/93 **Not Acceptable**	**Repeatedly negative** at doses up to 250 µg/ml +/- S9, which were not cytotoxic to Guideline levels.
84-2(b)	Structural chromosome aberration - in vivo mouse MRID # 427702-25 American Cyanamid #: 91-18-001; 03/17/93 **Non test**	Although reportedly **negative** for micronucleus induction in mice treated orally up to 20 or 30 mg/kg, the highest dose was lethal without causing cytotoxicity to target tissue.
84-4	Repair in vitro (UDS) MRID #: 427702-26 Microbiological#: T9775.380025 02/23/93 **Acceptable**	**Negative** for inducing unscheduled DNA synthesis in primary rat hepatocyte cultures exposed up to severely toxic concentrations (≥ 30 µg/ml).
PIRATE® Insecticide-Miticide - EP		
81-1	Acute Oral Toxicity in Rats MRID #:427702-14 Study #:T-0515 1/18/93 **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

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81-2	Acute Dermal Toxicity in Rabbits MRID 427702-14 Study #:T-0515 1/18/93 **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 427702-15 Cyanamid #:971-92-109 3/8/93 **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 #: 427702-16 Study #: T-0513 12/4/92 **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 427702-17 Study #T-0514 1/18/93 **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 427702-18 Study #:T-0530 3/5/93 **Acceptable**	Not a sensitizer

V. Data Gaps:

The toxicity data requirements for an **Experimental Use Permit** appear adequate, except for the Gene mutation - mammalian system and chromosomal aberrations using mouse micronucleus assay test. Although these tests will not be required for this EUP (see IX.B.), the registrant will be required to submit an acceptable mammalian Gene mutation and a chromosomal aberration study (other than the micronucleus) for full registration of this chemical.

V. **Data Gaps:**

The toxicity data requirements for an **Experimental Use Permit** appear adequate, except for the Gene mutation - mammalian system and chromosomal aberrations using mouse micronucleus assay test. Although these tests will not be required for this EUP (see IX.B.), the registrant will be required to submit an acceptable mammalian Gene mutation and a chromosomal aberration study (other than the micronucleus) for full registration of this chemical.

VI. **Action Being Taken to Obtain Additional Information or Clarification:**

The sponsor should be notified of the issues discussed under Section V and will be required to rectify it prior to the second year renewal.

VII. **Reference Dose (RfD):**

The recommended PADI (Preliminary Acceptable Daily Intake) is 0.004 mg/kg/day. This value was calculated by using the 90-Day Dog Study NOEL of 4.23 mg/kg/day and a uncertainty factor of 1000, based on extremely limited data base. This has not been presented to the Health Effects Division or Agency RfD Committees.

VIII. **Pending Regulatory Actions:**

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

IX. **Toxicology Issues Pertinent to This Request:**

A. The data indicate no toxicity concerns at this time. It appears that males are more sensitive than females; oral administration resulted in increased absorption over dermal route of administration. Acute toxicity of the technical and the formulation are similar (even though the formulation is only 36% a.i.) which is possibly a result of increased absorption of a.i. due to surfactants and solvents in the formulation. It is not a developmental toxicant in rabbits up to 30 mg/kg/day. The 13-week dog study did not result in any organ pathology identifiable with doses up to 240 ppm. Proposed **EUP** labeling contains common precautionary statements for this type of use.

B. **Mutagenicity** - The current mutagenicity guidelines require 3 studies: Ames and a mammalian Gene mutation assays as well as an acceptable chromosomal aberration assay. The current mammalian test is unacceptable and the chromosomal aberration assay is a non-test due to no

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target organ cytotoxicity at lethal levels. However, there is an acceptable UDS test. Therefore, new studies are not being required for this EUP but will be required for full registration of this chemical.

C. **Risk Concerns:**

Dietary

The sponsor explained that based on the low NOEL, for 90-Day dog of 4.23 mg/kg/day, and rabbit developmental study, of 5 mg/kg/day, supports a preliminary acceptable daily intake (PADI) of 4.2 µg/kg b.w./day or 0.25 mg for a 60 kg person. Also, the maximum daily exposure or theoretical maximum residue contribution (TMRC) of AC 303,630 in cotton seed is 0.001 mg/day (assuming daily diet intake of 1.5 kg and a cotton seed factor of 0.15%) at the proposed temporary tolerance of 0.5 ppm. This would utilize only 0.4% of PADI.

Worker Exposure

There is no endpoint of concern for acute exposure. If worker exposure for non acute exposure is less than 0.042 mg/kg/day, the Margin of Exposure would be at least 100. This is based on NOELs from both the Developmental rabbit study (Maternal NOEL = 5 mg/kg/day) and 90 day dog study (NOEL = 4.2 mg/kg/day).

Note to RD: The remainder of the data submitted with this package has not been reviewed. It has been attached to a new subordinate data package for S442369 with a DP barcode of D196061. These data are currently considered low priority by HED and have been assigned a due date of May 30, 1994.

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Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. concur 8/26/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) Marion Copley 8/26/93

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity/Rat/81-1

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-07/428842-01

GUIDELINE #: 81-1

TEST MATERIAL: AC 303,630

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

STUDY NUMBERS: T-0417

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanmid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Oral LD_{50} Study in Albino Rats with AC 303,630 Technical

AUTHORS: Carolyn A. Lowe

REPORT ISSUED: July 20, 1992

CONCLUSIONS: LD_{50} (95% C.I.) = 626 (274-1085) mg/kg, males and females
LD_{50} (95% C.I.) = 441 (195-832) mg/kg, males
LD_{50} (95% C.I.) = 1152 mg/kg with no range calculable for females

Hyperthermia, prostration and mortality were observed at the 2500, 1250, 625 and 312.5 mg/kg dosage levels. Grossly, congested liver and kidneys, gas filled intestines, tetany of fore- and hind-limbs and stricture of abdominal muscles of deceased animals were observed. Weight gains of the survivors were not effected.

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: II, based on most sensitive sex

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The information presented for this acute oral toxicity study in rats satisfies the criteria set forth in Subdivision F, Series 81-1.

MATERIALS:

1. **Test Compound:** Technical grade AC 303,630, Lot # AC 7504-59A, Purity 94.5% and described as tan powder, was use in this study.

   The test material was mixed with 0.5% carboxymethyl cellulose and distilled water and administered once orally, by gavage, to the overnight fasted rats, at a volume of 10 ml/kg. No untreated controls were used in this study.

2. **Test Animals:** Species: rats, Strain: Crl CD(SD)BR (Sprague-Dawley derived), Age: Young adults, Weight: males - 158-188 and females - 151-174 g, Source: Charles River Labs., Inc.. The animals were housed individually and maintained under atmospheric conditions to a 12-hour dark/light cycles. The rats were acclimated for 7 days to the laboratory environment.

METHODS:

LD_{50} Study: Dosages were selected based on earlier studies. The 156.25 and 312.5 mg/kg groups were added later due to excess mortality in males. The experimental design and mortality for this study is provided in Table 1.

Table 1. Summary of dosages and mortality

Dose (mg/kg)	No. Animals		Mortality	
	♂	♀	♂	♀
156.25	5	not tested	0	not tested
312.5	5	not tested	3/5	not tested
625	5	5	3/5	1/5
1250	5	5	4/5	3/5
2500	5	5	5/5	4/5

The animals were observed four times on the day of dosing and once daily for the remainder of the study for mortality and morbidity. Rectal body temperatures were recorded at pretreatment and 20 minutes, 60 minutes, 2 hours and 24 hours post dosing. Individual body weights were determined on Days 0, 7, and 14 or at death. LD_{50} values and 95% confidence intervals were calculated for males and females using the method of D.J. Finney, Probit Analysis, Ed. 2, Cambridge University Press, Cambridge, 1952.

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QUALITY ASSURANCE:

A statement signed by Kenneth A. Sund, Senior Quality Assurance Auditor, attested that the study was audited thrice and a single report provided.

RESULTS/DISCUSSION:

Mortality is presented in Table 1. Males and females died within 24 hours following administration of compound. Hyperthermia was observed in all animals within 4 hours post administration of compound, except 2, 625 mg/kg group females exhibited hyperthermia up to 24 hours following dosing. Prostration was observed in 5/5 males up to 4 hours and 3/5 up to 8 hours in 625 mg/kg group and 3/5 up to 1 hour in 2500 mg/kg group, following administration of test material. Weight gain of survivors was not effected. Necropsy was unremarkable in the surviving animals. In animals, which died, the most noticeable findings were congested liver and kidneys, gas filled intestines, tetany of fore- and hind-limbs and stricture of abdominal muscles.

The data reporting was thorough and the summary means were supported by individual animal data. The above information supports the following:

LD_{50} (95% C.I.) = 626 (274-1085) mg/kg, males and females
LD_{50} (95% C.I.) = 441 (195-832) mg/kg, males
LD_{50} (95% C.I.) = 1152 mg/kg with no range calculable for females

The study is Core-Acceptable
Toxic Category II, based on mortality at 2000.

As presented the study satisfies the requirements set forth in Subdivision F, Guideline 81-1 for Acute Oral Toxicity in Rats.

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Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 8/26/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T. 8/26/93
Section IV, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity/Rabbit/81-2

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-08

GUIDELINE #: 81-2

TEST MATERIAL: AC 303,630 Technical

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

STUDY NUMBERS: T-0406

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanmid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Dermal LD_{50} Study in Albino Rabbits with AC 303,630 Technical

AUTHORS: Joel E. Fischer

REPORT ISSUED: July 20, 1992

CONCLUSIONS:

Acute dermal LD_{50} was > 2,000 mg/kg. One female rabbit died on day 2, which was unrelated to treatment since the rabbit did not exhibit any clinical signs. **Limit Dose** was reached. No treatment-related clinical signs/necropsy findings was observed in surviving animals.

CLASSIFICATION: Core - Guideline
TOX. CATEGORY: III

The information presented for this acute dermal toxicity study in rabbits satisfies the criteria set forth in Subdivision, F Series 81-2.

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MATERIALS:

1. **Test Compound:** Technical grade AC 303,630, Lot # AC 7504-59A, Purity 94.5% and described as white powder, was use in this study.

   The test material was administered once, moistened with tap water, to the shaved backs of the test animals.

2. **Test Animals:** Species: rabbits, Strain: New Zealand White, Age: Young adults (10 - 16 weeks), Weight: Males - 2233 to 2754, Females - 2127 to 2957 g, Source: Skippack Farms, Skippack, PA.. The animals were housed individually and maintained under atmospheric conditions to a 12-hour dark/light cycles. The rabbits were acclimated for 3 days to the laboratory environment. Purina Laboratory Rabbit Chow and water were provided ad libitum.

METHODS:

A single group of five males and five female rabbits were dosed dermally with 2000 mg/kg of test compound. The backs were clipped (≈ 10%) and the plastic wrap with moistened test material was applied on the clipped area. The test dose was held in place with a cloth bandage. After 24 hours the test site was cleaned and rabbits observed for 14 days.

QUALITY ASSURANCE:

A statement signed by Kenneth A. Sund, Senior Quality Assurance Auditor, attested that the study was audited thrice and a single report provided.

RESULTS:

One female rabbits died on day 2 of the study; exhibited no clinical signs associated with compound administration. Necropsy revealed pale liver and kidneys, mottled lungs and hemorrhages in the ano-genital area. The report indicated that the hemorrhage may be caused by tight bandage. The study authors concluded that the death was unrelated to treatment. Body weight gains were unaffected by treatment. No overt signs of toxic ty were observed in the surviving animals during the study. Necropsy was unremarkable.

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DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data. **Limit Dose** was reached. Based on the data presented the acute dermal LD_{50} was > 2,000 mg/kg.

The study is Core-Acceptable
Toxic Category III.

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-2 for Acute Dermal Toxicity in Rabbits.

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Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 10/20/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) 10/25/93

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation Toxicity/Rat/81-3

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-09

GUIDELINE #: 81-3

TEST MATERIAL: AC 303,630 Technical

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

STUDY/PROJECT NUMBERS: Bio/dynamics Project No. 91-8351
American Cyanamid No. 941-91-109

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: Bio/dynamics Inc.
East Millstone, NJ 08875

TITLE OF REPORT: Acute Inhalation Toxicity Study with AC 303,630 in Rats.

AUTHORS: Gary M. Hoffman

REPORT ISSUED: March 25, 1993

CONCLUSIONS: Doses 0, 0.34, 0.71, 1.8 or 2.7 mg/l in SD rats. Following was the acute inhalation toxicity:

Clinical signs during exposure included labored breathing, gasping and decreased activity and labored breathing for 5 - 6 days post-exposure. Grossly, abnormal tracheal contents and edema of lungs in dead animals; discolored lungs at terminal necropsy.

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

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: III, based on most sensitive sex

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The information presented for this acute inhalation toxicity study in rats satisfies the criteria set forth in Subdivision, F Series 81-3.

MATERIALS:

1. **Test Compound:** Technical grade AC 303,630, Lot # AC 7505-59A, Purity 94.5% and described as tan solid, was used in this study. **Air** was used in controls.

   Sample preparation: For Groups II to IV, the test substance was sieved, desiccated overnight, jar milled for 60 minutes and sieved again prior to exposure. In the case of Group V, in addition to the above procedures, the sample was packed into the Spengler Dust Feeder cup using Carver Laboratory Press prior to exposure.

2. **Test Animals:** Species: rats, Strain: Sprague-Dawley, Age: 8 - 9 weeks, Weight: Males - 253 to 291, and Females - 189 to 224 g, Source: Charles River Breading Labs., Inc., Kingston, NY. The animals were doubly housed during the first week and individually during the second week of acclimatization and all other exposure periods; and maintained at temperature of 18-26°C, relative humidity of 37-79% and 12 hours of light/dark cycles, during 2 weeks of acclimation and all other non-exposure periods. Purina Rodent Laboratory Chow® Brand Animal Diet #5002 and water was provided ad libitum.

   Four groups of five males and five females were exposed to nominal/measured concentrations of 3.9/0.34, 5.8/0.71, 17/1.8 or 80/2.7 mg/l, for 4 hours, respectively. The study design, the achieved concentration and mortality are provided in Table 1.

TABLE 1. SUMMARY OF CHAMBER CONCENTRATION AND MORTALITY

GROUP	CHAMBER CONCENTRATION (MG/L)			MORTALITY		
	NOMINAL	ACHIEVED	GRAVIMETRIC	♂	♀	TOTAL
I	-	0	0	0/5	0/5	0/10
II	3.9	0.34	0.32	1/5	0/5	1/10
III	5.8	0.71	0.71	2/5	0/5	2/10
IV	17.0	1.8	1.8	4/5	1/5	5/10
V	80.0	2.7	2.6	5/5	1/5	6/10

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METHODS:

LC_{50} Study: Exposure by whole body for 4 hours. The plexiglass exposure chamber (100 L) operated dynamically under slight positive pressure at a air flow rate of 20.0 L/min for Groups II, III and V and 40 L/min for Groups I and IV. Air was changed 2.5 to 4.5 times/min. (Groups II and V - 5 times/min., III - 4.5 times/min. and I and IV - 2.5 times/min). Oxygen levels were not measured, however, the above flow rates were considered adequate to maintain 19% level. For Groups II to IV, dust was generated by placing appropriate amount of test substance in a fluiding bed and passing a regulated stream of air (generation flow). The dust generated in the fluidizing bed was mixed with dilution flow and entered the exposure chamber through inlet portal (Appendix A). The Group V rats were exposed to dust generated in Spengler Dust Feeder as shown in Appendix B. Control rats were exposed to air using similar design as Group V rats, except without Drierite® filter and Spengler Dust Feeder.

The nominal concentration of the test substance in the exposure chamber was calculated by dividing consumption of test substance in 4 hours by the total volume of air.

The gravimetric concentration of the AC 303,630 in the breathing zone was determined during the exposure period by drawing appropriate amount of air (L)/time (min) (Groups I to V - 5/1, 5/5, 5/2, 5/1 and 5/1, respectively) once/hour from the normal sampling portal and once/exposure from the distribution sampling portal through Baxter S/P® glass filter paper using the Gelman filter holder and dividing gain in filter wt. by volume of air collected. The actual or analytical test substance concentration was calculated by wt. gain of filter by volume of air.

The particle size range in the test atmosphere was determined using Deltron DCI-6 cascade impactor, about 4 times during the study. The mass median aerodynamic diameter (MMAD), geometric standard deviation (σ_g) and cumulative percent of particles ≤ 1.0, ≤ 3.0, ≤ 5.0, ≤ 7.0 and ≤ 10 μ were calculated based on the amount of material collected on the impactor stages.

The animals were observed immediately prior to exposure and as a group at 15 min. intervals during the first hour and hourly for the remainder of exposure, hourly for 2 hours post-exposure, and once daily for the remainder of the study for general condition and twice daily for mortality.

Rats were weighed Day-1, shortly before exposure and at 7 and 14 days after exposure.

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Detailed gross necropsy was performed on all animals which died or sacrificed at the end of the study.

QUALITY ASSURANCE:

A statement signed by Gary M. Hoffman, Study Director, attested that the data was audited and is in compliance with USEPA's GLPS.

RESULTS:

The nominal, the mean analytical and the mean gravimetric concentration of the test substance ranged from 3.9 to 80, 0.34 to 2.7 and 0.32 to 2.6 mg/l, respectively (see Table 1). Essentially there was no variation between the mean analytical and gravimetric concentrations since same methodology was used in calculating the concentrations. Big difference exists between the nominal concentration and exposure concentrations which may be due to impaction or sedimentation of the dust on the exposure chamber walls.

Table 2 summarizes the analytical concentration, MMAD, σ_g and percent cumulative particle sizes. Percent of MMAD particles with ≤ 1.0 μm ranged from 0.14 - 1.1% (mean = 0.47%), which do not meet the current agency inhalation toxicity guideline of 25%. The mean % of MMAD particles ≤ 3.0, 5.0, 7.0 and 10 μm was 11, 32, 51 and 71, respectively.

TABLE 2. SUMMARY OF PARTICLE SIZE DISTRIBUTION

GROUP	ANALYTICAL CONC. (MG/L)	PARTICLE SIZE DISTRIBUTION*						
		MMAD (μm)	σ_g	CUMULATIVE % OF PARTICLES				
				≤ 1.0μm	≤ 3.0μm	≤ 5.0μm	≤ 7.0μm	≤ 10μm
II	0.34	8.1	2.3	1.1	8.8	25	44	64
III	0.71	7.0	1.9	0.14	9.4	31	51	72
IV	1.8	7.3	2.0	0.24	11	30	48	68
V	2.7	5.9	1.9	0.38	15	40	61	79
MEAN		7.1	2.0	0.47	11	32	51	71

* = MMAD

σ_g = Geometric standard deviation

The particle size distribution of the test material i.e., mean median aerodynamic diameter (MMAD) slightly decreased with increased concentration of the test substance (Table 2). The sponsor indicated that based on preliminary studies using a Wright Dust Feeder, a fluidizing bed and a Spengler Dust Feeder, the maximum attainable chamber concentration that could be achieved was 2.7 mg/l. At 2.7 mg/l, the method clearly provided smallest MMAD of 5.9 μm,

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with highest gravimetric (2.6 mg/l) and analytical concentrations (2.7 mg/l). The calculated LC_{50}, based on the mortality data was 1.9, 0.83 and > 2.7 mg/l for the combined sexes, males and females, respectively. The MMADs for the above LC_{50}s ranged from approximately 5.9 to 7.0 µm, which is outside the range considered acceptable under HED interim guidelines of 1 - 4 µm. Although the study did not meet the HED interim policy for MMADs, the sponsor demonstrated that methodology reached optimal conditions at the highest dose tested.

Clinical signs in males and females during the exposure included labored breathing and/or gasping and decreased activity were observed at all concentrations except in the 0.34 mg/l Group (Table 3). Labored breathing was observed in treated males and females for 5 to 6 days post exposure. Lacrimation and nasal discharge is expected from inhalation of dust but was reported observed in only 0.71 mg/l group. Three of 5 in the 1.8 mg/l and 4/5 males in the 2.7 mg/l Groups died by the end of 4 hour exposure. By Day 1, 1/5, 2/5, 4/5 and 5/5 males died in the 0.34, 0.71, 1.8 and 2.7 mg/l Groups, respectively; one female each died in the 1.8 and 2.7 mg/l Groups (see Table 1).

TABLE 3. INCIDENCE OF CLINICAL SIGNS

CLINICAL SIGNS	ANALYTICAL CONCENTRATION (MG/L)				
	0	0.34	0.71	1.8	2.7
Labored breathing/gasping	None	none	most	some	some
Lacrimation	None	not reported	few	none	not reported
Nasal discharge	none	none	few	some	not reported
Decreased activity	none	none	most/all	most/all	most/all

Few = 10 - 30%
Some = 40 - 60%
Most = 70 - 90%
All = 100%

All surviving males/females gained approximately 43/22, 41/19, 41/15, 49/16 and 0/7%, in the Groups I, II, III, IV and V, respectively, when compared to the initial weights. Females in the 2.7 mg/l Group gained less compared to other Groups because two rats lost about 8% compared to initial weight.

Abnormal tracheal contents and lung edema were seen only in animals which were found dead. In addition, discolored lungs were seen in dead animals and in some males

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that were necropsied at the end of observation period. These changes were considered treatment-related.

DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data. Based on the data presented the acute inhalation toxicity of AC 303,630 is as follows:

Clinical signs during exposure included labored breathing, gasping and decreased activity and labored breathing for 5 - 6 days post-exposure. Grossly, abnormal tracheal contents and edema of lungs in dead animals; discolored lungs at terminal necropsy.

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

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: III, based on most sensitive sex

As presented, the study satisfies the requirements set forth in Subdivision F Guideline, 81-3 for Acute Inhalation Toxicity Study in Rats.

CHLORFENAPYR

Page _____ is not included in this copy.

Pages 23 through 24 are not included in this copy.

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Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 11/26/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) 8/26/93

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation/Rabbit/81-4

TOX. CHEM. NO.: N.A

P. C. NO.: 129093

MRID NO.: 427702-10

GUIDELINE #: 81-4

TEST MATERIAL: AC 303,630

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl) and Pirate®

STUDY NUMBERS: T-0404

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Eye Irritation Study in Albino Rabbits with AC 303,630 Technical

AUTHORS: Joel E. Fischer

REPORT ISSUED: July 20, 1992

CONCLUSIONS:

Corneal opacity (4/6), iritis (2/6) and conjunctivitis (6/6) were observed in rabbits at 48 hours. Iritis was resolved in 72 hours, however, corneal opacity (1/6) and conjunctivitis (6/6) were present. All animals were normal by Day-7.

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: III

The information presented for this primary eye irritation study in rabbits satisfies the criteria set forth in Subdivision, F Series 81-4.

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MATERIALS:

1. **Test Compound:** Technical grade AC 303,630, Lot # AC 7504-59A, Purity 94.5% and described as white powder, was use in this study.

2. **Test Animals:** Species: rabbits, Strain: New Zealand White, Age: Young adults (10 - 16 weeks), Weight: Not given, Source: Skippack Farms, Skippack, PA.. The animals were housed individually and maintained under controlled atmospheric conditions to a 12-hour dark/light cycles. The rabbits were acclimated for 3 days to the laboratory environment. Purina Laboratory Rabbit Chow and tap water were provided ad libitum.

METHODS:

A single group of six males rabbits were instilled with a dosed of 0.1 mg of undiluted test material into conjunctival sac of their right eye. Following dosing, the upper and lower eye lids were held together for 1 second. The contralateral eye of each rabbit acted as the control. At the end of 24 hours exposure period, the treated eyes were rinsed with water and examined with naked eye, ultraviolet light and fluorescein stain. The eyes were examined for irritation at 1, 24, 48, 72 hours, 4 days and 7 days after dosing. Eyes were scored using the Draize scale.

QUALITY ASSURANCE:

A statement signed by Kenneth A. Sund, Senior Quality Assurance Auditor, attested that the data was audited once and a single report provided.

RESULTS:

Corneal opacity was observed.

1 Hour Evaluation

Slight (4/6)-to-moderate (2/6) redness of conjunctivae, slight chemosis (6/6) and a slight (4/6)-to-moderate (1/6) ocular discharge were present. The mean conjunctival (redness + chemosis + discharge; range 2 - 20) score for this evaluation was 6.7.

24 Hour Evaluation

Diffuse areas of corneal opacity (5/6), mild iritis (3/6), slight (2/6)-to-moderate (4/6) conjunctivitis, slight (2/6)-to-moderate (4/6) chemosis and a slight (2/6)-to-

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moderate (3/6) ocular discharge were observed. The mean corneal opacity (degree of density + area of cornea involved; range 5 - 80), iritis (range 5 - 10) and conjunctival scores for this period were 4.2, 2.5 and 9.3, respectively.

48 Hour Evaluation

Diffuse areas of corneal opacity (4/6), mild iritis (2/6), same degree of conjunctivitis observed at 24 hours evaluation and decrease in the conjunctival chemosis and ocular discharge were seen. The mean corneal opacity, iritis and conjunctival scores for this period were 4.2, 1.7 and 4.7, respectively.

72 Hour Evaluation

Corneal opacity (1/6) and conjunctivitis (6/6) were observed. Iritis has resolved. The mean corneal opacity and conjunctival scores were 0.8 and 3.3, respectively.

4 Day Evaluation

Corneal opacity has resolved. Slight (3/6)-to-moderate (1/6) conjunctivitis was present. The mean conjunctival score was 2.0.

7 Day Evaluation

Conjunctivitis has resolved.

Based on the above irritation scores, the chemical is considered moderately irritant (corneal opacity resolved in 7 days) to the rabbit eye.

DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data.

The study is **Core-Acceptable Toxic Category III.**

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-4 for Primary Eye Irritation Study in Rabbits.

010651

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 8/26/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) 8/26/93

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation/Rabbit/81-5

TOX. CHEM. NO.: N.A

P. C. NO.: 129093

MRID NO.: 427702-11

GUIDELINE #: 81-5

TEST MATERIAL: AC 303,630

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl) and Pirate®

STUDY NUMBERS: T-0405

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Skin Irritation Study in Albino Rabbits with AC 303,630 Technical

AUTHORS: Joel E. Fischer

REPORT ISSUED: July 20, 1992

CONCLUSIONS:

Non irritating to rabbit skin.

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: IV

The information presented for this primary dermal irritation study in rabbits satisfies the criteria set forth in Subdivision, F Series 81-5.

MATERIALS:

1. **Test Compound:** Technical grade AC 303,630, Lot # AC 7504-59A, Purity 94.5% and described as white powder, was used in this study.

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2. **Test Animals:** Species: rabbits, Strain: New Zealand White, Age: Young adults (10 - 16 weeks), Weight: Not given, Source: Skippack Farms, Skippack, PA.. The animals were housed individually and maintained under controlled atmospheric conditions to a 12-hour dark/light cycles. The rabbits were acclimated for 3 days to the laboratory environment. Purina Laboratory Rabbit Chow (#5321) and tap water were provided ad libitum.

METHODS:

On the day prior to dosing, six male rabbits were prepared by clipping the trunk free of hair. Two test sites, one control site and one treated site, were selected on opposite sides of the dorsal midline. A 0.5 mg of the test material was applied to 1" square gauze patches, moistened with water and applied to the test site. The patches were held in place with adhesive tape, plastic sheet and filter cloth. The bandages were removed after 4 hours and the site wiped to remove excess material. Observations for dermal irritation were made 1, 24, 48 and 72 hours after patch removal. Draize method was employed for evaluating skin irritation potential of AC 303,603 Technical. Erythema was scored on a scale of 0 to 4, 0 = normal and 4 = severe erythema. Edema formation was scored on a scale of 0 to 4, 0 = no edema and 4 = severe edema.

QUALITY ASSURANCE:

A statement signed by Kenneth A. Sund, Quality Assurance Auditor, attested that the data was audited once and a single report provided.

RESULTS:

No overt signs of toxicity or irritation were observed during the course of the study.

DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data. Based on the primary irritation index the chemical is not irritating to rabbit skin.

The study is **Core-Acceptable**
Toxic Category IV.

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-5 for Primary Dermal Irritation Study in Rabbits.

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Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 12/21/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) 12/27/93

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization- Guinea Pig (Buehler's close patch technique)

TOX. CHEM. NO.: N.A

P. C. NO.: 129093

MRID NO.: 427702-12

GUIDELINE #: 81-6

TEST MATERIAL: AC 303,630 Technical

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl) and Pirate®

STUDY NUMBERS: T-0439

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Dermal Sensitization Study in Albino Guinea Pigs with AC 303,630 Technical using the Buehler Closed Patch Method

AUTHORS: Joel E. Fischer

REPORT ISSUED: March 26, 1993

CONCLUSIONS:

Not a skin sensitizer.

CLASSIFICATION: Core - Acceptable

The information presented for this dermal sensitization study in guinea pigs satisfies the criteria set forth in Subdivision, F Series 81-6.

MATERIALS:

1. **Test Compound:** Technical grade AC 303,630, Lot # AC 7504-59A, Purity 94.5% and described as tan powder, was

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use in this study. Positive control: 1-chloro-2,4-dinitrobenzene, Lot # 69F-0232, Purity 99%, was obtained from Sigma Chemical Co., St. Louis, MO..

2. **Test Animals:** Species: Guinea pig, Strain: Dunkin Hartley, Age: Young adults (6 - 8 weeks), Weight: 259 - 323 g, Source: Charles River Laboratories, Inc., Kensington, NY.. The animals were housed individually in a temperature and humidity controlled room and provided free access to Purina Guinea Pig Chow® and water. The animals were acclimated to the lab. conditions for 7 days.

METHODS:

Table 1. Treatment Groups

GROUP	TREATMENT	NO. OF ANIMALS
I	Positive negative control	5
II	Positive control (DNCB)	10
III	AC 303,630 technical negative control	10
IV	AC 303,630 technical treated group (200 mg/animal)	10

Table 1 shows the group distribution of animals for this study. Thirty five acclimated male guinea pigs were divided into four following groups: test, positive (DNCB) and negative control (AC 303,630) groups 10 animals each and positive negative control (DNCB) group 5 animals. Preliminary experiments utilized 25 additional guinea pigs to assess for a nonirritating concentration of the test article. The concentrations tested in the dose-range finding study were undiluted test material of 50, 100, 200 and 400 mg/animal. The test doses were applied to each of 4 sites using a latin square design on 4 test animals. No irritation was observed at 24 hours, however 3/4 animals died by 48 hours. To test toxicity potential of AC 303,630, an additional 2 animals were dosed with 400 mg/animal; all survived and no irritation was observed. Based on the preliminary dose-range finding information, an initial definitive study using 10 animals were tested at 400 mg/animal. After the first induction, 6/10 animals died. Subsequently 9 animals were dosed at 200 mg/animal; no erythema was observed and one animal died without any overt signs of toxicity. A dosage of 200 mg/animal was selected for further testing.

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The induction phase consisted of treating the guinea pigs with 200 mg of undiluted test material or 0.4 ml/ animal DNCB (0.1 % DNCB in 50% ethanol) was applied, using a 25 mm. Hilltop Chamber, to each guinea pig and kept in place with bandage for 6 hours. One application was made each week for 3 weeks. **Negative controls were not treated during this phase.**

The challenge was made 2 weeks after the last induction dose was applied. The challenge dose consisted of 200 mg undiluted test material but only 0.4 ml of 0.05% DNCB applied to each guinea pig and kept in place with bandage for 6 hours. At this time the AC 303,630 technical negative control animals were also treated with a challenge application of test material. The positive control DNCB as well as negative control DNCB guinea pigs were treated. Twenty-four and 48 hours after application of the challenge dose, the guinea pigs were evaluated for dermal reactions. For 24 hour evaluations, the sites were depilated with NEET® Depilatory Cream.

QUALITY ASSURANCE:

A statement signed by Steven M. Boege, Quality Assurance Specialist, attested that the data was inspected once and a single report was provided.

RESULTS:

One test material treated guinea pig (#203) died on day 16, exhibited salivation and tremors prior to death. No overt signs of toxicity were observed in other animals.

The mean body weight gain of the treated animals was (168/211) 20% less than the negative controls. Positive controls gained (196/218) 10% less than the positive control negatives.

No dermal reactions were elicited either by the tested or negative controls groups.

In the positive (DNCB) controls, at the second induction, a mild confluent erythema was observed in 10/10 animals (mean score 2.0) at the 24 and 48 hour evaluations. At the third induction, mild erythema in 10/10 animals with a mean score of 2.0 and a slight non-confluent (2/10)-to-mild confluent (8/10) erythema (score 1.8) was present at 48 hours. At challenge all animals exhibited a mild confluent erythema (mean score 2.0) at both the 24 and 48 hour evaluations. In the negative positive control group, two animals exhibited erythema (one with very slight erythema -

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0.5 and second with slight erythema - 1.0) with a mean score of 0.3 at 24 hours but were normal by 48 hours.

DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data. Based on the dermal sensitization scores the chemical is not a skin sensitizer in guinea pigs.

The study is **Core-Acceptable**

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-6 for Dermal Sensitization Study in Guinea Pigs.

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Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 10/20/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) Marion Copley 10/20/93

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity/Rat

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-13

GUIDELINE #: 81-1

TEST MATERIAL: AC 303,630 3SC Formulation

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

STUDY NUMBERS: T-0516

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanmid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Oral LD_{50} Study in Albino Rats with AC 303,630 3SC Formulation

AUTHORS: Carolyn A. Lowe

REPORT ISSUED: March 19, 1993

CONCLUSIONS: LD_{50} (95% C.I.) = 626 (274-1085) mg/kg, males and females
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.

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: II, based on most sensitive sex

The information presented for this acute oral toxicity study

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in rats satisfies the criteria set forth in Subdivision F, Series 81-1.

MATERIALS:

1. **Test Compound:** AC 303,630 3 SC Formulation (liquid), Lot # AC 8053-87B, Purity 33.25%, Sp. Gravity 1.16 g/ml, was used in this study.

   The test material was administered as received, once orally, by gavage, to the overnight fasted rats. No untreated controls were used in this study.

2. **Test Animals:** Species: rats, Strain: Crl CD(SD)BR (Sprague-Dawley derived), Age: Young adults, Weight: males - 220-250 and females - 161-205 g, Source: Charles River Labs., Inc.. The animals were housed individually and maintained under atmospheric conditions to a 12-hour dark/light cycle. The rats were acclimated for 7 days to the laboratory environment.

METHODS:

LD_{50} Study: Dosages were selected based on earlier studies. The 156.25 and 312.5 mg/kg groups were added later due to excess mortality in males. The experimental design and mortality for this study is provided in Table 1.

Table 1. Summary of dosages and mortality

Dose (mg/kg)	No. Animals		Mortality	
	♂	♀	♂	♀
156.25	5	not tested	1/5	not tested
312.5	5	not tested	3/5	not tested
625	5	5	4/5	1/5
1250	5	5	5/5	3/5
2500	5	5	5/5	5/5

The animals were observed four times on the day of dosing and once daily for the remainder of the study for mortality and morbidity. Individual body weights were determined on Days 0, 7, and 14 or at death. LD_{50} values and 95% confidence intervals were calculated for males and females using the method of C.W. Weil, 1952, "Tables for Convenient Calculation of Median Effective Dose and Instructions in Their Use", Biometrics, volume 8, p 249-263.

QUALITY ASSURANCE:

A statement signed by Steven M Boege, Quality Assurance

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Specialist, attested that the study was audited twice and a single report provided.

RESULTS/DISCUSSION:

Mortality is presented in Table 1. Males and females died within 24 hours following administration of compound. Overt signs of toxicity during the study were decreased activity, salivation, ataxia, hyperthermia, protruded testes and prostration with hind legs extended. Weight gain of survivors was not effected. Necropsy was unremarkable in the surviving animals. In animals, which died, the most noticeable findings were congested and mottled liver and pronounced striations of abdominal muscle wall.

The data reporting was thorough and the summary means were supported by individual animal data. The above information supports the following:

LD_{50} (95% C.I.) = 283 (101-502) mg/kg, males
LD_{50} (95% C.I.) = 999 (431-1821) mg/kg, females

The study is Core-Acceptable
Toxic Category II, based on most sensitive sex.

As presented the study satisfies the requirements set forth in Subdivision F, Guideline 81-1 for Acute Oral Toxicity in Rats.

010651

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Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 10/20/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T. 10/20/93
Section IV, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity/Rabbit

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-14

GUIDELINE #: 81-2

TEST MATERIAL: AC 303,630 3SC Formulation

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

STUDY NUMBERS: T-0515

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanmid Company
Princeton NJ 08543-0400

TITLE OF REPORT: Dermal LD_{50} Study in Albino Rabbits with Ac 303,630 3SC Formulation

AUTHORS: Carolyn A. Lowe

REPORT ISSUED: January 18, 1993

CONCLUSIONS:

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.

CLASSIFICATION: Core - Guideline
TOX. CATEGORY: II, based on most sensitive sex

The information presented for this acute dermal toxicity study in rabbits satisfies the criteria set forth in Subdivision, F Series 81-2.

MATERIALS:

1. **Test Compound:** AC 303,630 3 SC Formulation (liquid), Lot # AC 8053-87B, Purity 33.3%, Sp. Gr. 1.16 g/ml, was used in this study.

   The test material, as received was administered once to the shaved backs of the test animals.

2. **Test Animals:** Species: rabbits, Strain: New Zealand White, Age: Young adults (10 - 16 weeks), Weight: Males - 2436 to 3029, Females - 2291 to 2762 g, Source: Skippack Farms, Skippack, PA.. The animals were housed individually and maintained under atmospheric conditions to a 12-hour dark/light cycle . The rabbits were acclimated for 3 days to the laboratory environment. Purina Laboratory Rabbit Chow and water were provided ad libitum.

METHODS:

Groups of five animals/sex were dosed initially at a rate of 2000 mg/kg of body weight. Mortality in males necessitated additional levels of 1000 and 4000 mg/kg. The backs were clipped (≈ 10%), wrapped with plastic wrap and measured amount of test material was injected under the plastic wrap. The plastic wrap was then covered with cloth bandage. After 24 hours the test site was cleaned and rabbits observed for 14 days.

QUALITY ASSURANCE:

A statement signed by Kevin W. Nolan, Temporary Quality Assurance Auditor, attested that the study was audited twice and a single report provided.

RESULTS:

One male rabbit each died in the 2000 and 4000 mg/kg dose groups within 24 hours; and 2 and 1 rabbits died within 48 hours, respectively. Clinically, excessive salivation (1/5), nasal discharge (1/5) and lacrimation (1/5) were observed in the 1000 and 4000 mg/kg dose groups. Necropsy findings were unrelated to treatment.

DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data. **Limit Dose** was reached in females. Based on the data presented the acute dermal LD_{50} is as follows:

LD_{50} (95% C.I.) = 1782 [1112 - 2856] mg/kg, males
LD_{50} (95% C.I.) > 2000 mg/kg, females

The study is Core-Acceptable
Toxic Category II, based on most sensitive sex.

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-2 for Acute Dermal Toxicity in Rabbits.

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 10/20/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) Marion Copley 10/20/93

010651

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation Toxicity/Rat

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-15

GUIDELINE #: 81-3

TEST MATERIAL: AC 303,630 3SC Formulation

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

STUDY/PROJECT NUMBERS: Bio/dynamics Project No. 92-8396
American Cyanamid No. 971-92-109

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: Bio/dynamics Inc.
East Millstone, NJ 08875

TITLE OF REPORT: Acute Inhalation Toxicity Study with AC 303,630 3SC in Rats.

AUTHORS: Gary M. Hoffman

REPORT ISSUED: March 8, 1993

CONCLUSIONS: Doses 0, 0.84, 1.9 or 2.6 mg/l in SD rats. Following was the acute inhalation toxicity:

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.

LC_{50} (95% C.I.) = 1.3 (0.8 - 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

010651

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: III, based on most sensitive sex

The information presented for this acute inhalation toxicity study in rats satisfies the criteria set forth in Subdivision, F Series 81-3.

MATERIALS:

1. **Test Compound:** AC 303,630 3SC Formulation, Lot # AC 8053-87B, Purity 33.3% and described as tan liquid, was used in this study. Distilled and demineralized water was the vehicle for the test substance. **Air** was used in controls.

   Sample preparation: For Groups II to IV, appropriate amounts of AC 303,630 3SC formulation were combined with demineralized distilled water to obtain a 1:1 (w:w) mixture. Pre-study trials established the optimum conditions to generate 25% of the particles having a mass median aerodynamic diameter (MMAD) of 1.0 μ or less.

2. **Test Animals:** Species: Albino rats, Strain: Sprague-Dawley CD® [Cr]:CD® (SD)BR], Age: 7 - 9 weeks, Weight: Males - 229 to 297, and Females - 204 to 239 g, Source: Charles River Breading Labs., Inc., Kingston, NY. The animals were doubly housed during

TABLE 1. SUMMARY OF CHAMBER CONCENTRATION AND MORTALITY

GROUP	CHAMBER CONCENTRATION (MG/L)			MORTALITY		
	NOMINAL	ACHIEVED	GRAVIMETRIC	♂	♀	TOTAL
I	-	0	0	0/5	0/5	0/10
II	12	0.84	0.89	1/5	0/5	1/10
III	16	1.9	1.9	4/5	0/5	4/10
IV	130	2.6	2.6	4/5	3/5	7/10

the first week and individually during the second week of acclimatization and all other exposure periods; and maintained at temperature of 20-25°C, relative humidity of 34-74% and 12 hour light/dark cycle , during 10 - 17 days of acclimation and all other non-exposure periods. Purina Rodent Laboratory Chow® Brand Animal Diet #5002 and water was provided ad libitum.

Three groups of five males and five females were exposed to nominal/measured concentrations of 12/0.84,

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16/1.9 or 130/2.6 mg/l, for 4 hours, respectively. The study design, the achieved concentration and mortality are provided in Table 1.

METHODS:

LC_{50} Study: Exposure by whole body for 4 hours. The plexiglass exposure chamber (100 L) operated dynamically under slight positive pressure at a air flow rate of 20.0 L/min. Air was changed 5 times/min. Oxygen levels were not measured, however, the above flow rates were considered adequate to maintain 19% level. Aerosol was generated by pumping appropriate concentrations of test substance in distilled water into a Devilbiss glass nebulizer (Groups II and III) or a spray atomizer (Group IV), where it was mixed with air and the resultant mixture was directed into the inlet portal of exposure chamber. The animals remained in the chamber for 30 minutes following exposure to allow chamber to clear.

The nominal concentration of the test substance in the exposure chamber was calculated by dividing consumption of test substance in 4 hours by the total volume of air.

The gravimetric concentration of the AC 303,630 in the breathing zone was determined during the exposure period by drawing appropriate amount of air [(L)/time (min) (Groups II - III and IV - 10/1, 10/1 and 5/1, respectively)] once/hour from the normal sampling portal and once/exposure from the distribution sampling portal through glass filter paper and dividing gain in filter wt. by volume of air collected. Gravimetric concentration of air exposure control was calculated as in the case of test substance treated groups. The actual or analytical test substance concentration was calculated by wt. gain of filter by volume of air.

The particle size range in the test atmosphere was determined, once/hour at a rate of 12.4L/min for 1 min. using a Deltron DCI-6 cascade impactor, during the study. The mass median aerodynamic diameter (MMAD), geometric standard deviation (σ_g) and cumulative percent of particles $\leq$ 1.0, $\leq$ 3.0, $\leq$ 5.0, $\leq$ 7.0 and $\leq$ 10 μ were calculated based on the amount of material collected on the impactor stages using a graphical analysis of an assumed log normal distribution. Particle size distribution of air control samples was determined using a TSI Aerodynamic Particle Sizer, once per hour.

The animals were observed individually immediately prior to exposure and as a group at 15 min. intervals during the first hour and hourly for the remainder of exposure, hourly for 2 hours post-exposure, and once daily for the remainder of the study for general condition and twice daily

for mortality.

Rats were weighed Day-1, shortly before exposure and at 7 and 14 days after exposure.

Detailed gross necropsy was performed on all animals which died or sacrificed at the end of the study.

QUALITY ASSURANCE:

A statement signed by Richard L. Streeton, Jr., Manager, Quality Assurance, attested that the study was audited six times and is a single report is included.

RESULTS:

The nominal, the mean analytical and the mean gravimetric concentration of the test substance ranged from 12 to 130, 0.84 to 2.6 and 0.89 to 2.6 mg/l, respectively (see Table 1). Essentially there was no variation between the mean analytical and gravimetric concentrations since same methodology was used in calculating the concentrations. Big difference exists between the nominal concentration and exposure concentrations which may be due to impaction or sedimentation of the aerosol on the exposure chamber walls.

Table 2 summarizes the analytical concentration, MMAD, σ_g and percent cumulative particle sizes. The MMAD of Group II and III are similar. The Group IV aerosol, which required a different aerosol generator at a much higher nominal level (130 mg/l), resulted in slightly larger (5.0) particle size. Percent of MMAD particles with ≤ 1.0 µm ranged from 0.33 - 3.3% (mean = 1.5%), which decreased with increased analytical/nominal concentration. The percent of particles with MMAD < 1µm do not meet the current agency inhalation toxicity guideline of 25%. The mean % of MMAD particles ≤ 3.0, 5.0, 7.0 and 10 µm was 30, 62, 81 and 92, respectively.

TABLE 2. SUMMARY OF PARTICLE SIZE DISTRIBUTION

GROUP	ANALYTICAL CONC. (MG/L)	PARTICLE SIZE DISTRIBUTION*						
		MMAD (µm)	σ_g	CUMULATIVE % OF PARTICLES				
				≤ 1.0µm	≤ 3.0µm	≤ 5.0µm	≤ 7.0µm	≤ 10µm
II	0.84	3.6	2.0	3.3	39	70	86	94
III	1.9	3.9	1.8	0.92	33	67	85	95
IV	2.6	5.0	1.8	0.33	18	49	70	87
MEAN		4.2	1.9	1.5	30	62	80	92

* = MMAD

σ_g = Geometric standard deviation

The sponsor indicated that based on preliminary studies using a spray atomizer, Devilbiss nebulizer, Collison nebulizer and a Laskin nebulizer, the achieved sizes (MMAD 2.2 - 4.8) were considered smallest at these targeted exposures. Furthermore, in this study, particle size did not appear related to mortality (Table 1). The calculated LC_{50}, based on the mortality data was 2.1, 1.3 and 2.4 mg/l for the combined sexes, males and females, respectively. The MMADs for the above LC_{50}s ranged from approximately 3.9 to 5.0 μm, which is on the high end of the range considered acceptable under HED'S interim guidelines of 1 - 4 μm. **The study meets the acute inhalation toxicity interim guidelines.**

Clinical signs in males and females during the exposure included labored breathing and excessive salivation (all test groups), eye closure (1.9 and 2.6 mg/l), gasping and decreased activity (2.6 mg/l). In survivors, the most common observations were labored breathing, salivation, decreased activity, rales, dried brown material on face, matted coat, brown material on fur, wet fur and ano-genital staining. Mortality of males/females during the 2 hour post-exposure was 1/0, 4/0 and 3/2 in the 0.84, 1.9 and 2.6 mg/l, respectively; an additional female was reported dead in the 2.6 mg/l group by day 1. **The report failed to account for one male in the 2.6 mg/l group. The report accounts for 3 dead and one terminal sacrifice. It was reported as dead (page 21, mortality table) but the summary of the report (page 23) or the Table 4-5 and 4-6 of the report do not account for this male rat.**

All surviving males/females gained approximately 58/28, 30/13, 45/17 and 41/28%, in the Groups I, II, III and IV, respectively, when compared to the initial weights. Generally, the substance exposed animals gained less, however, not dose-related.

Tan colored fur, due to substance deposition, was observed in some dead animals. Grossly, discolored lungs were seen in some dead animals. These changes were considered treatment-related.

DISCUSSION:

The data reporting was adequate except one male rat in the 2.6 mg/l was not accounted. The rat was considered dead during the experiment and was included in the calculation of LC_{50}. Therefore, the missing information would not alter the conclusions of the study.

Deficiency

The report failed to account for one male in the 2.6 mg/l group. The report accounts for 3 dead and one terminal sacrifice. It was reported as dead (page 21, mortality table) but the summary of the report (page 23) or the Table 4-5 and 4-6 of the report do not account for this male rat.

CONCLUSIONS

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. Based on the data presented the acute inhalation toxicity of AC 303,630 3SC Formulation is as follows:

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

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: III, based on most sensitive sex

As presented, the study satisfies the requirements set forth in Subdivision F Guideline, 81-3 for Acute Inhalation Toxicity Study in Rats.

010651

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 10/20/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) Marion Copley 10/20/93

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation/Rabbit

TOX. CHEM. NO.: N.A

P. C. NO.: 129093

MRID NO.: 427702-16

GUIDELINE #: 81-4

TEST MATERIAL: AC 303,630 3SC Formulation

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl) 3SC Formulation and Pirate®

STUDY NUMBERS: T-0513

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Eye Irritation Study in Albino Rabbits with AC 303,630 3SC Formulation

AUTHORS: Carolyn A. Lowe

REPORT ISSUED: December 4, 1992

CONCLUSIONS:

Slight-to-moderate conjunctivitis (6/6) was observed at one and 24 hours; had resolved by 48 hours.

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: III

The information presented for this primary eye irritation study in rabbits satisfies the criteria set forth in Subdivision, F Series 81-4.

MATERIALS:

1. **Test Compound:** AC 303,630 3 SC Formulation, Lot # AC 8053-87B, Purity 33.2%, Sp. Gr. 1.16 g/l and described

as tan liquid, was use in this study. 010651

2. **Test Animals:** Species: rabbits, Strain: New Zealand White, Age: Young adult males (10 - 16 weeks), Weight: Not given, Scurce: Skippack Farms, Skippack, PA.. The animals were housed individually and maintained under controlled atmospheric conditions to a 12-hour dark/light cycles. The rabbits were acclimated for 3 days to the laboratory environment. Purina Laboratory Rabbit Chow and tap water were provided ad libitum.

METHODS:

A single group of six male rabbits were instilled with a dosed of 0.1 ml of test material into conjunctival sac of their left eye. Following dosing, the upper and lower eye lids were held together for 1 second. The contralateral eye of each rabbit acted as the control. At the end of 24 hours exposure period, the treated eyes were rinsed with tap water and examined under ultraviolet light and fluorescein stain. The eyes were examined at pre-treatment (-3 hours) 1, 24, 48, 72 hours, 4 days and 7 days after dosing for irritation. Eyes were scored using the Draize scale.

QUALITY ASSURANCE:

A statement signed by Barbara C. Fine, Senior Quality Assurance Auditor, attested that the study was audited twice and a single report provided.

RESULTS:

Corneal opacity was not observed.

1 Hour Evaluation

Slight (5/6)-to-moderate (1/6) redness of conjunctivae, slight chemosis (5/6) and slight (4/6) ocular discharge were present. The mean conjunctival (redness + chemosis + discharge; range 2 - 20) score for this evaluation was 5.3.

24 Hour Evaluation

Slight (4/6)-to-moderate (1/6) redness of conjunctivae and slight (1/6) chemosis were observed. The mean conjunctival (redness + chemosis + discharge) score for this luation was 2.3.

48 Hour Evaluation

At this evaluation all signs of irritation in 6 rabbits

had resolved and remained resolved at the termination of study 72 hours post-dosing.

Based on the above irritation scores, the chemical is considered moderately irritant to the rabbit eye.

DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data.

The study is **Core-Acceptable Toxic Category III.**

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-4 for Primary Eye Irritation Study in Rabbits.

010651

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 4/22/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) Marion P Copley 10/20/93

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation/Rabbit

TOX. CHEM. NO.: N.A

P. C. NO.: 129093

MRID NO.: 427702-17

GUIDELINE #: 81-5

TEST MATERIAL: AC 303,630 3SC Formulation

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl) SC Formulation and Pirate®

STUDY NUMBERS: T-0514

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Skin Irritation Study in Albino Rabbits with AC 303,630 3SC Formulation

AUTHORS: Carolyn A. Lowe

REPORT ISSUED: January 18, 1993

CONCLUSIONS:

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.

CLASSIFICATION: Core - Acceptable
TOX. CATEGORY: IV

The information presented for this primary dermal irritation study in rabbits satisfies the criteria set forth in Subdivision, F Series 81-5.

MATERIALS:

1. **Test Compound:** AC 303,630 3SC Formulation, Lot # AC 8053-87B, Purity 33.2%, Sp. Gr. 1.16 g/ml and described as tan liquid, was used in this study.
2. **Test Animals:** Species: rabbits, Strain: New Zealand White, Age: Young adults (12 - 16 weeks), Weight: Not given, Source: Skippack Farms, Skippack, PA.. The animals were housed individually and maintained under controlled atmospheric conditions to a 12-hour dark/light cycle. . The rabbits were acclimated for 3 days to the laboratory environment. Purina Laboratory Rabbit Chow (#5321) and tap water were provided ad libitum.

METHODS:

On day prior to dosing, six male rabbits were prepared by clipping the trunk free of hair. Two test sites, one control site and one treated site, were selected on opposite sides of the dorsal midline. A 0.5 ml of the test material was applied to 1" square gauze patches moistened with water and applied to the test site. The patches were held in place with adhesive tape, plastic sheet and filter cloth. The bandages were removed after 4 hours and the site wiped to remove excess material. Observations for dermal irritation were made 1, 24, 48 and 72 hours after patch removal. Draize method was employed for evaluating skin irritation potential of AC 303,603 3SC Formulation. Erythema was scored on a scale of 0 to 4, 0 = normal and 4 = severe erythema. Edema formation was scored on a scale of 0 to 4, 0 = no edema and 4 = severe edema.

QUALITY ASSURANCE:

A statement signed by Jennifer P. Leonard, Quality Assurance Auditor, attested that the data was audited twice and a single report provided.

RESULTS:

No overt signs of toxicity were observed during the course of the study.

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. The mean erythema/edema 1 hour score was 1.2/0.2; and the 24 hour score was 0.5/0.0, respectively. At 48 hour examination 1/6 exhibited slight erythema which resolved by 72 hours.

DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data. Based on the primary irritation index the chemical is slightly irritating to rabbit skin.

The study is **Core-Acceptable**
Toxic Category IV.

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-5 for Primary Dermal Irritation Study in Rabbits.

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 10/20/93 010651
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) Marion Copley 10/20/93

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization- Guinea Pig (Buehler's close patch technique)

TOX. CHEM. NO.: N.A

P. C. NO.: 129093

MRID NO.: 427702-18

GUIDELINE #: 81-6

TEST MATERIAL: AC 303,630 3SC Formulation

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl) 3 SC Formulation and Pirate®

STUDY NUMBERS: T-0530

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: Dermal Sensitization Study in Albino Guinea Pigs with AC 303,630 3SC Formulation using the Buehler Closed Patch Method

AUTHORS: Lisa M. Boczon

REPORT ISSUED: March 5, 1993

CONCLUSIONS:

Not a skin sensitizer.

CLASSIFICATION: Core - Acceptable

The information presented for this dermal sensitization study in guinea pigs satisfies the criteria set forth in Subdivision, F Series 81-6.

MATERIALS:

1. **Test Compound:** AC 303,630 3SC Formulation, Lot # AC 8053-87B, Purity 33.25%, Sp. Gr. 1.16 g/ml and

described as milky white liquid, was use in this study. Positive control: 1-chloro-2,4-dinitrobenzene, Lot # 69F-0232, Purity 99%, was obtained from Sigma Chemical Co., St. Louis, MO..

2. **Test Animals:** Species: Guinea pig, Strain: Dunkin Hartley, Age: Young adult males, Weight: 309 - 381 g, Source: Charles River Laboratories, Inc., Kensington, NY.. The animals were housed individually in a temperature and humidity controlled room and provided free access to Purina Guinea Pig Chow® and water. The animals were acclimated to the lab. conditions for 7 days.

METHODS:

Table 1. Treatment Groups

GROUP	TREATMENT	NO. OF ANIMALS
	Dose Range Finding Study	4
I	Positive negative control	5
II	Positive control (DNCB)	10
III	AC 303,630 3SC Formulation negative control	10
IV	AC 303,630 3SC Formulation treated group	10

Table 1 shows the group distribution of animals for this study. Thirty five acclimated male guinea pigs were divided into four following groups: test, positive (DNCB) and negative control (AC 303,630) groups 10 animals each and positive negative control (DNCB) group 5 animals. Preliminary experiments utilized 4 additional guinea pigs to assess for a nonirritating concentration of the test article. The concentrations tested in the dose-range finding study were 25, 50, 75 and 100% test material suspension in water % (v/v). The test doses were applied to the anterior right flank region of the animals and were evaluated for irritation at 24 and 48 hours post-dosing. **The method failed to describe number of sites and design used to assign the test sites.** However, the results of the range-finding study indicate that 4 sites were used on each guinea pig to test all 4 concentrations. No irritation was observed at 24 hours, however 2/4 animals had erythema score of 0.5 by 48 hours. **The report did not spell-out the doses selected for the definitive study**, but we assume 100 and 75% was used for the induction and challenge phases, respectively.

The induction phase consisted of treating the guinea pigs with test material (**concentration was not specified**) or 0.4 ml/ animal DNCB (0.1 % DNCB in 50% ethanol) was applied, using a 25 mm. Hilltop Chamber, to each guinea pig and kept in place with bandage for 6 hours. One application was made each week for 3 weeks. **Negative controls were not treated during this phase.**

The challenge was made 2 weeks after the last induction dose was applied. The challenge dose of test material (**concentration not specified**) but only 0.4 ml of 0.05% DNCB applied to each guinea pig (**duration of contact with the test substance not specified**). At this time the AC 303,630 3 SC Formulation negative control animals were also treated with a challenge application of test material. The positive control DNCB as well as negative control DNCB guinea pigs were treated. Twenty-four and 48 hours after application of the challenge dose, the guinea pigs were evaluated for dermal reactions. For 24 hour evaluations, the sites were depilated with NEET® Depilatory Cream.

QUALITY ASSURANCE:

A statement signed by Steven M. Boege, Quality Assurance Specialist, attested that the data was inspected twice and a single report was provided.

RESULTS:

No mortality or overt signs of toxicity were observed during the study.

The mean body weight gain of the treated animals was (197/227) ≈ 14% less than the negative controls. Positive controls gained (174/191) ≈ 9% less than the positive control negatives.

At challenge, 3/10 of test animals exhibited slight-to-slight non-confluent erythema with a mean score of 0.25 at the 24 hours and a very slight barely perceptible erythema (1/10) at the 48 hours observation period. The negative controls exhibited very slight-to-slight non-confluent erythema (4/10) with a mean score of 0.35 at the 24 hours and very slight-to-slight non-confluent erythema (2/10) with a mean score of 0.15 at the 48 observation period. The dermal reaction in the treated group was no higher than the negative controls, therefore, the reaction was considered to be of no toxicological significance.

In the positive (DNCB) controls, at the first induction, a very slight-to-slight non-confluent erythema was observed in 5/10 animals (mean score 0.3) at the 24 and

in 3/10 animals (mean score 0.25) at the 48 hour evaluations. At the second induction, all animals exhibited mild erythema (mean score 2.0) at the 24 and 48 hours evaluation. At the third induction, all animals exhibited mild-to-severe erythema at the 24 (mean score 2.2) and 48 hour (mean score 2.9) evaluations. At challenge, all animals exhibited a mild confluent erythema (mean score 2.0) at both the 24 and 48 hour evaluations. In the negative positive control no erythema was observed at either 24 or 48 hour observation periods.

DISCUSSION:

The report was poorly prepared; however, the data tables were adequately presented to validate the data and to reach conclusions.

Deficiencies

The rangefinding study report is totally inadequate since it lacked the experimental details with regard to number of sites and doses selected for induction and challenge phases of the main study. The report also failed to specify the doses used for the induction phase, challenge phase and duration of contact of the test substance. The sponsor should be made aware of these deficiencies.

CONCLUSIONS:

Based on the dermal sensitization scores the chemical is not a skin sensitizer in guinea pigs.

The study is **Core-Acceptable**

As presented the study satisfies the requirements set forth in Subdivision F Guideline, 81-6 for Dermal Sensitization Study in Guinea Pigs.

010651

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 11/4/93
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T. 9/14/93
Section IV, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90 Day Feeding Study in Dogs

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-20

GUIDELINE #: 82-1 (b)

TEST MATERIAL: AC 303,630

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

STUDY/PROJECT NUMBERS: American Cyanamid NO. 971-92-118

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: Pharmaco LSR, Inc.
Toxicology Services North America
East Millstone, NJ 08875-2360

TITLE OF REPORT: 90-Day Dietary Toxicity Study with AC 303,630 in Beagle Dogs

AUTHORS: Catherine M. Kelly

REPORT ISSUED: April 8, 1993

CONCLUSIONS:

Doses tested in beagles: 0, 60, 120 or 247 ppm (0, 2.16, 4.23 or 6.1 mg/kg/day) in feed. The 247 ppm value 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.

CLASSIFICATION: Core - minimum

This study satisfies the guideline requirements for a 90 day dog feeding study (82-1).

A. MATERIALS:

1. **Test compound:** AC 303,630. Description - Tan solid, Lot # - AC 7504-59A, Purity - 94.5 %.

2. **Test animals:** Species: Canine, Strain: Beagles, Age: 6 months, Weight: Males - 9.9 (9.1 - 11.4) and Females - 9.0 (8.2 - 10.2) kg, Source: Marshall Farms, U.S.A., Inc., North Rose, New York 14516. Animals were identified and housed individually and maintained in an environment with an average temperature of 70°F ± 6°, % R.H. of 57 ± 13 and a 12 hour light/12 hour dark cycles. Animals were acclimated for 1 month, during which time, they were subjected to health assessment (physical exam. and clinical lab. analysis), prophylactic for internal parasites and routine preventive vaccinations. Six dogs were eliminated from the consideration into the study, becuse more than needed number of animals included in pre-clinical screening.

B. STUDY DESIGN:

1. **Animal assignment**

Thirty-two (32) dogs were ranked by body weight and were assigned randomly into 4 blocks of 4 animals per sex. The study groups and the dose levels administered are presented in Table 1.

Table 1. Group Distribution and Summary of Dose Levels During the Study

Test Group	Test Days	Dose in diet (ppm)	Main Study 3 months male	female
1 Cont	1 - 93	0	4	4
2 Low (LDT)	1 - 93	60	4	4
3 Mid (MDT)	1 - 93	120	4	4
4 High (HDT)[a]	1 - 14	300	4	4
	15 - 25	240	4	4
	26 - 93	200	4	4

[a] the same 4 males and 4 females received various dose levels during the study

The doses were based on a range finding study (Pharmaco LSR Study NO. 92-3828), however, no details were provided.

2. **Diet preparation**

Appropriate amounts of test substance was mixed with untreated standard laboratory diet weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at weekly intervals for 4 weeks then monthly thereafter. Pre-study, homogeneity was performed on 60 and 300 ppm levels.

Results - Homogeneity of the test material in the two test diets ranged from 89% to 92% of the nominal concentration. Stability of the test material in the 60 and 300 ppm diets after 7 and 14 days storage at room temperature ranged from 86% to 88% of the nominal concentration. The concentration of the test material in the test diets ranged from 95% to 97% of target.

3. Food consumption was determined by offering 400 grams food for six hours through Week 5 and extended to 22 hours starting at Week 6, because of reduced food consumption in high-dose group. Water was provided ad libitum.

4. **Statistics** - Mean values of body weight, body weight change, food consumption, feed efficiency, hematology and clinical chemistry, organ weights, organ/body and organ/brain weights were analyzed statistically. The means were subjected to one way ANOVA to establish equality and Bartlett's test to determine equality of variance. If the variance were equal and the means were significantly different by ANOVA, Dunnett's test was used to determine which means were significantly different from controls. In the case of unequal variances, the means were analyzed using the Kruskal-Wallis test and a summed rank test (Dunn). Statistical dose trends were analyzed using appropriate methods.

5. A statement of compliance with GLP and a signed statement of quality assurance were included in the submission.

C. **METHODS AND RESULTS:**

1. **Observations:**

Animals were inspected twice daily for signs of

toxicity and mortality.

Results: Emaciation was the only clinical sign associated with treatment was observed. It was observed in 1 - 2 males and females during the first two months of the study, which was partially due to reduced food consumption associated with palatability of the chemical. This is considered treatment-related. In addition, the author reported emesis in the high-dose group and concluded that they were treatment-related. TB-I disagrees with the author's conclusions since emesis was observed in only one female (#4916) during first week physical and was normal during remainder of the study. This is sporadic and considered to be no toxicological significance.

2. **Body weight**

Animals were weighed pretest, at Day 0, weekly during treatment and terminally (fasting).

TABLE 2. MEAN BODY WEIGHT GAIN (kg)

STUDY INTERVAL (WEEKS)	MALES (GROUP)*				FEMALES (GROUP)			
	I	II	III	IV	I	II	III	IV
-1 - 0	0.3	-0.1	-0.1	0.2	-0.1	0.0	0.0	0.0
0 - 1	0.0	0.4	0.2	-1.1*	0.0	0.4	0.3	-0.2
1 - 2	-0.1	-0.1	-0.1	-0.4*	-0.1	-0.2	-0.3	-0.8*
2 - 3	0.5	0.0	0.2	0.2	0.2	0.1	0.3	-0.1
3 - 4	0.2	0.0	0.0	0.3	0.2	0.3	0.1	0.2
4 - 7	0.1	0.2	0.5	0.5	0.0	0.1	-0.1	0.3
7 - 10	0.5	0.0	0.6	0.7	0.5	0.6	0.3	0.6
10 -13	0.2	0.1	0.4	0.4	0.3	0.5	0.3	0.0

* = $P \leq 0.05$
Groups I = 0, II = 60 ppm, III = 120 ppm and IV = 200 - 300 ppm
* = Group IV males and females received 300 ppm for 2 weeks (14 days), 240 ppm from Days 15 - 25 and 200 ppm till termination

Results: Significant reduction in body weight gain was noticed in high-dose males during the first two weeks and in females during the second week of the study (Table 2). The mean body weight gain was decreased by 30 to 110% in males and 70% in females. The mean body weight gain rebounded and was comparable to the controls, when the dose levels were reduced to 240 ppm

from days 15 to 25 and 200 ppm for remainder of the study. During the first 2 weeks, there was a corresponding decrease in the food consumption. The sponsor concluded that the reduced body weight gain was treatment-related. The reduced body weight gain was only partially due to reduced food consumption which may be associated with palatability of the test material. We concur with the sponsor's conclusions.

3. **Food consumption, compound intake and food efficicency**

Food consumption was determined over a 6 hour period through Week 5 and over 22 hour period starting at Week 6. This was done since the food consumption markedly decreased during the first 2 weeks. Therefore, the dose levels were lowered to 240 ppm from Day 15 - 25 and 200 ppm from Day 26 - termination of the study. Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results: The mean food consumption of high-dose males/females was significantly ($P \leq 0.05$) reduced by 52%/30% and 56%/52%, respectively, when compared to the pre-treatment values. During the corresponding period, the mean food consumption of control males/females increased by 5%/12% and 18%/36%, respectively, when compared to the pre-treatment values. Subsequent to lowering of dose levels from 300 ppm to 240 and 200 ppm, the food consumption in all treated groups was comparable to the controls. The reduced food consumption was considered due to palatability of the compound.

Table 3 provides the group mean compound intake which was calculated from the consumption and dietary concentration.

TABLE 3. MEAN COMPOUND INTAKE (MG/KG/DAY)

DIETARY CONCENTRATION (PPM)	DAYS	MALES	FEMALES	AVERAGE
60	1 - 93	2.1	2.2	2.15
120	1 - 93	3.9	4.5	4.2
300	1 - 15	4.4	6.0	High dose mean =
240	15 - 25	6.0	5.8	6.1
200	25 - 93	7.3	7.1	

Food efficiency of the high dose males (300 ppm) and females (240 ppm) was reduced by ≈ 160 % (121 - 200) and 28% (24 - 31), respectively. It was similar in animals at dose levels of 60, 120 and 200 ppm. The reduced food efficiency was considered treatment-related, since it was associated with reduced body weight gains and emaciation.

4. **Ophthalmological examination**

Performed at termination on all animals. No compound-related effects were observed.

5. **Blood was collected** before treatment, at 1.5 months and termination for hematology and clinical analysis from all animals. 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		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

Results - Hematological parameters evaluated at 1.5 months and at termination were not affected due to treatment.

b. **Clinical Chemistry**

X	Electrolytes:	X	Other:
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Enzymes	X	Total and indirect bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)
	Cholinesterase (ChE)		Triglycerides
X	Creatinine phosphokinase		Serum protein electrophores
X	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		
X	Gamma glutamyl transpeptidase (GGPT)		

Results - No significant treatment-related effects were observed, except for mean serum potassium levels in high-dose males at termination. The mean serum potassium levels were slightly higher (5.3 meq/l; $P \leq 0.05$) in high-dose males at termination, when compared to the controls (4.9 meq/l); however, this increase was not accompanied by any adverse clinical signs and was within the published control ranges of 3.8 to 5.8 meq/l for adult dogs. Therefore, the incidence was considered to be of no toxicological significance.

6. **Urinalysis**

Urine was collected from fasted animals at pre-test, 1.5 months and termination. The CHECKED (X) parameters were examined.

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

Results - Compound-related effects were not observed in all treatment groups.

7. **Sacrifice and Pathology**

All animals were sacrificed under sodium pentobarbital anesthesia on schedule and were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The <u>(XX) organs, in addition, were weighed</u>.

X	Digestive system	X	Cardiovasc./Hemat.	X	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	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic n.)
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	XX	Adrenal gland
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	XX	Parathyroids
XX	Liver	X	Epididymides	XX	Thyroids
X	Gall bladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone
	Respiratory	X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Oviducts	X	All gross lesions and masses
	Nose	X	Vagina		

Organ weight and Gross and/or Microscopic pathology were not affected due to administration of AC 303,630, except for significantly ($P \leq 0.05$) decreased absolute terminal kidney weights in mid and high-dose females. The absolute kidney weight of the females, at the 120 and 200 ppm, decreased 16.5% (39.4) and 13.3% (40.9), respectively, when compared to the controls (47.2). These decreases lacked dose-response and were not accompanied by changes in the relative kidney (brain) weights and renal histopathology. Therefore, the decreased kidney weights were considered to be of no toxicological significance. Other absolute and relative organ weights were similar between controls and treated animals.

D. **DISCUSSION:**

Following administration of AC 303,630 to male and female dogs in feed at dose levels of 0, 60, 120 or ≈ 247 ppm [0, 2.15, 4.2 or ≈ 6.1 mg/kg/day (5.2 from Day 1 - 14, 5.9 from Day 15 - 25 and 7.2 from Day 26 - 93), there were no treatment-related changes in the clinical signs (except emaciation), hematology, clinical chemistry, urinalysis, ophthalmology, absolute/relative organ weights, gross and/or histopathology. Significant reduction in body weight gains, feed efficiency and emaciation were also observed in high-dose males and females. There was a corresponding decrease in food consumption of the high-dose males and females, however, the reduced feed consumption was probably associated with palatability of chemical and therefore of no biological significance. The above information support a NOEL of 120 ppm and LOEL of 247 ppm.

The data reporting was thorough and the summary means were supported by individual animal data.

The study satisfies the requirements set forth in Subdivision F Guideline, 82-1(b) for 90 Day Oral Toxicity Study.

GReddy/AC 303,630/90ddog/9-12-93
Final: 9/14/93

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GUIDELINE: 83-3(b)

Review by: Guruva B. Reddy, DVM, PHD 10/20/93
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, DVM, DABT 10/20/93
Section Head, Review Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rabbit

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 427702-22

TEST MATERIAL: AC 303,630

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

STUDY NUMBER or LAB. PROJECT ID: Argus Project No. 101-016
American Cyanamid No. 971-90-179

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: Argus Research Labs., Inc.
Horsham, PA 19044

TITLE OF REPORT: An Oral Developmental Toxicity (Embryo-Fetal Toxicity Teratogenicity) Definitive Study with AC 303,630 in Rabbits

AUTHOR(S): Alan M. Hoberman

REPORT ISSUED: March 2, 1993

CONCLUSION:

Doses administered: 0, 5, 15 or 30 mg/kg/day, administered by gavage in 0.5% carboxymethylcellulose to pregnant New Zealand White rabbits from Days 7 through 19 of gestation, inclusive.

Maternal NOEL: 5 mg/kg/day. LEL: 15 mg/kg/day, based upon, reduced body weight gain during treatment.

Developmental NOEL > 30 mg/kg/day.

Classification: **Core-Minimum**

The information presented for this developmental toxicity study in rabbits satisfies the criteria set forth in Subdivision F Series, 83-3(b).

A. MATERIALS

1. Test Compound: AC 303,630; Description - Tan solid; Lot # - AC 7504-59A; Purity -94.5%

   Vehicle(s): Carboxymethylcellulose from Sigma Chemical Co.; Lot # - 38F-0529

2. Test Animal(s): Species: Rabbits; Strain: New Zealand White; Age: 5 months old at receipt; Weight: 2.62 - 3.77 kg; Source: Hazleton Research Products, Inc., Denver, PA.; Acclimated for ≈ a month. Human chorionic gonadotropin (HCG; 20 USP units/kg)) was used to induce ovulation.

B. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of AC 303,630 when administered by gavage to timed-pregnant New Zealand White rabbits on gestation days 7 through 19, inclusive. Each pregnant rabbit was offered approximately 180 grams of feed (Certified Rabbit Chow® #5322, Ralston Purina Co., St. Louis, MO) and water was provided ad libitum. Animals were maintained at a temperature of 68 ± 6°F, relative humidity of 52.5 ± 17.5% and a 12 hour light and dark cycle. Air was changed 10/hour. On Day 0, a total of 20 artificially inseminated females each were assigned to the treatment groups, except 19 rabbits to the control group as noted in Table 1 using a weight-stratified randomization procedure:

Group Arrangement:

Table 1

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	19
Low Dose	5	20
Mid Dose	15	20
High Dose	30	20

C. METHODS

1. Mating

   Semen was collected from four proven male breeder rabbits and used to artificially inseminate female rabbits which received human chorionic gonadotropin 20 units/kg 4 hours prior to insemination. Semen from one male was used to inseminate an equal number of females

in each group. The day of insemination was considered day 0 of gestation. Pregnant rabbits were housed individually in suspended stainless steel cages.

2. Dosing

Range Finding Studies: Dose levels were selected based on results of a range finding study in this report (Argus Research Labs., Inc., Study #101-016P). Dose levels included: 0, 12.5, 25, 50, 100 or 200 mg/kg/day. The dosages of 25 mg/kg and higher resulted in reduced body weight gain of ≈ 40% and feed consumption. Deaths occurred in 0/8 (0%), 0/4 (0%), 0/8 (0%), 2/8 (25%), 4/8 (50%), and 4/4 (100%) of the does in the 0, 12.5, 25, 50, 100 and 200 mg/kg/day, respectively. Clinical and necropsy observations in does that died included excess salivation, impaired righting reflex, small red spots in the small intestines, large spleen and extensive discoloration of the liver lobes. None of the doses had any effect on fetal mortality or fetal development.

In the main study, the test substance, diluted in 0.5% carboxymethylcellulose to a constant volume of 10 ml/kg, was administered by gavage. Controls received 0.5% carboxymethylcellulose at a dose equivalent to that used in high dose group. Daily, dosage adjustments were based on the recent body weights.

Test substance analysis: Determination of concentration and homogeneity of AC 303,630 in 0.5% carboxymethylcellulose suspensions was performed using HPLC-UV by the sponsor. Dosage suspensions were prepared every two to three days during the study and were stored refrigerated. Homogeneity of AC 303,630 in 0.5% carboxymethylcellulose aqueous suspension was determined before initiation of the study. Concentration was determined on the first and last day of dosing period.

Results: The purity of undiluted test compound was reported as 94.5%. No impurities were listed. Homogeneity of the samples (1.25 and 20 mg/ml) ranged from 98% to 100% of target concentration. The mean concentrations (2 samples per dose) ranged from 85% to 100% of target concentration.

3. **Observations**

The animals were checked once daily for clinical abnormalities or twice daily for mortality. Post-dosing observations (rectal temp.) were performed

during the treatment period to evaluate overt signs of toxicity. Dams were sacrificed by Beuthanasia®-D Special euthanasia solution on day 29 of gestation and thoracic, abdominal and pelvic cavities and viscera were examined for abnormalities. Uteri and ovaries were removed and live and dead fetuses, and early resorption sites were noted in each uterus. Corpora lutea were counted and recorded for each ovary. Uteri with no implantations were fixed in 10% formalin for determination of early embryo mortality according to Salewski (1964).

All fetuses were counted, weighed, sexed and examined for external and visceral anomalies. Brain was free-hand sectioned according to Staples (Teratology: Detection of Visceral Alterations in Mammalian Fetuses, A37, 1974) and examined for hydrocephaly. All fetuses were eviscerated and stained with alizarin red S according to modified method of Staples for skeletal alterations.

Historical control data were provided from 53 studies on reproductive parameters, maternal necropsy observations and fetal anomalies (visceral and skeletal variations and malformations) to allow comparisons with concurrent controls. The studies covered from 1987 - 1989.

4. **Statistical analysis**

Fetal and maternal body weights, maternal body weight gains, rectal temperatures, food consumption, gravid uterine weights, percent male fetuses, % resorbed conceptus, % fetal implantations, fetal alterations and fetal ossification sites were analyzed using Bartlett's Test of Homogeneity of Variances and one-way analysis of variance (ANOVA), followed by Dunnett's test if significant. Non-homogenous data was analyzed using Kruskal-Wallis, Fisher's Exact or Dunn's Method of Multiple Comparisons Test, as appropriate. All other caesarean sectioning data were analyzed using Kruskal-Wallis Test.

5. **Compliance**

A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided.

D. **RESULTS** 010651

1. **Maternal Toxicity**

a. **Mortality** - Animals were observed twice daily for mortality.

Results - No treatment-related deaths, abortions or premature deliveries occurred during the study.

b. **Clinical Observations** - observed for general appearance several times during the acclimation and Day 0 of pregnancy. Rabbits were examined for clinical signs associated with test substance administration, premature deliveries and/or abortions, immediately before intubation, one-half hour after intubation and once daily during the post-dosage period. In addition, rectal temperatures were recorded prior to dose administration and 30 minutes, 1, 2 and 3 hours post dosage on day 7 of gestation.

Results - Mean rectal temperatures increased significantly ($P \leq 0.05$ to 0.01) in all treated groups 30 minutes post-dosing and in HTD does one hour post-dosing, when compared to the controls. Temperatures in the treated groups ranged from 102.9 to 103.4°F, compared to the control ranges of 102.5 to 102.9°F. There was considerable individual variability observed in all groups (range 101.2 to 104.5°F). The increases lacked dose-relationship or had no evidence of metabolic and/or infectious disease syndrome(s) account for temperature fluctuations. These increases are within the high-end of normal values (101 - 103.2°F) for rabbits of this age and therefore, the fluctuations in rectal temperatures were considered to be of no toxicological significance.

There were no clinical signs indicative of toxicity due to treatment with AC 303,630 were observed.

c. **Body Weight** - Maternal body weights were measured one week before insemination and on gestation Days 0, 6, 9, 12, 15, 19, 24 and 29. Table 2 summarizes body weight gains.

Results - Although mean body weight gains pre-treatment, during the treatment and post-treatment were not significantly different, the body weight gain was biologically significant, when compared to the controls. The mean body weight gain during the treatment period (gestation days 7 - 20) in the 15 and 30 mg/kg/day groups was ≈ 62.5% of the controls (100 gm

G

vs 160 gm). Further, the results of the dose range-finding study suggest that doses of 50 mg/kg/day and above are lethal and 25 and 50 mg/kg/day resulted in marked reduction in body weight gain. At the lethal doses (≥ 50 mg/kg/day), there were signs of failure of righting reflexes, which may be compound-related. In the main study no clinical signs suggestive of clinical toxicity were observed (≤ 30 mg/kg/day). The dose of 30 mg/kg/day is in a narrow range between the maternal toxic dose (25 mg/kg/day) and the lethal dose. Therefore, TB-I concurs with the study author's conclusions that the doses of 15 to 30 mg/kg/day are adequate to test potential developmental toxicity of the chemical.

TABLE 2. BODY WEIGHT GAIN (GRAMS)[a]

	AC 303,630 MG/KG/DAY			
INTERVAL	0	5	15	30
PRETREATMENT: 0 - 7 DAYS	150	150	150	130
TREATMENT:				
7 - 10 DAYS	10	10	-10	0
10 - 13 DAYS	60	70	60	50
13 - 16 DAYS	60	40	40	50
16 - 20 DAYS	40	40	10	0
7 - 20 DAYS	170	160	100	100
POST-TREATMENT: 20 - 29 DAYS	200	180	140	180
GESTATION: 0 - 29 DAYS	520	490	390	420

[a] Data taken from summary Table 5 of study. There are slight differences between the calculated body weight gains using body weights from Table 4 and those values on Table 5.

d. **Food Consumption** - Food consumption was recorded daily on days 0 through 29 of presumed gestation. The data in the study report is given in both g/animal/day and g/kg/day.

Results - Average food consumption, calculated as g/animal/day and g/kg/day was not significantly different during the pre-dose, dosing and post dosing periods, when compared to the controls. The absolute/relative feed consumption in the 15 and 30 mg/kg/day groups, during the dosing period, decreased 6.5%/6% and 9%/9.8%, respectively, when compared to the controls. In the 5 mg/kg/day group consumption increased 4%, when compared to controls. The author

considered reduced feed consumption during treatment was compound-related. TB-I disagrees with the author's conclusions since these differences are so small in magnitude.

e. **Water consumption** - Water consumption was recorded daily throughout the study.

Results - Administration of AC 303,630 by gavage during the gestation period Day 7 - 19 had no adverse effect on water consumption. The mean water consumption ranged during the treatment period was from 314.5 to 365.2 ml/day. The consumption values pre-dose, post-dose and during the entire gestation were comparable in all dosage groups.

f. **Gross Pathological Observations** - No treatment-related gross pathological observations were noticed in dams at necropsy among any treatment group. **Organ weights or gross pathology was not recorded.**

g. **Cesarean Section Observations**

Cesarian section was performed on a total of 19, 20, 20 and 20 rabbits in the control, 5, 15 and 30 mg/kg/day groups, respectively. No statistically significant differences for the number of live and dead fetuses, early resorptions and late resorptions, fetal weights and sex ratios were observed in dams at necropsy (Table 3). The percent conception in the control, 5, 15 or 30 mg/kg/day groups was 94.1, 95, 80 and 85%, respectively. The litters of one doe each of the control (19209), low (19231) and high dose (19271) groups were all early resorptions. In addition, in the 30/kg/day does, there was a slight reduction in the average litter size (6.9 vs control 8.4))due to increased litter averages for resorptions (0.9) and % resorbed conceptus (41.2) and the number of does (7) with early resorptions. None of these observations were statistically significant and the means were within the historical ranges. These incidences were considered to be of no toxicological significance. There was a non-significant increase in the post-implantation loss, at the 30 mg/kg/day, when compared to the controls. The post-implantation loss (%) in the 5, 15 and 30 mg/kg/day groups was 4.9, 2.3 and 12.7, respectively, compared to 4.7 of the controls. The incidence lacked dose-response and any corroborative evidence suggesting fetal toxicity, therefore, the incidence was not considered real.

TABLE 3. CESARIAN SECTION OBSERVATIONS*

PARAMETER	DOSE (MG/KG/DAY)			
	0	5	15	30
# Animal Mated	19	20	20	20
# Animal Pregnant (% of total)	18 (94.1)	19 (95.0)	16 (80.0)	17 (85.0)
# Non-gravid	1	1	4	3
# Litters Born				
Maternal Wastage				
#Died	0	0	0	0
#Aborted	0	0	0	0
# Animals examined (necropsy)	18	19	16	17
Gravid at Necropsy	17	18	16	16
Total Corpora Lutea	195	193	170	172
Corpora Lutes/Dam	10.8	10.2	10.6	10.1
Total Implantations	150	143	129	125
Implantations/Dam	8.3	7.5	8.1	7.4
Total Live Fetuses	143	136	126	110
Live Fetuses/Dam	8.4	7.6	7.9	6.9
Total Resorptions (Early/Late)	5[c]/2	5[c]/2	2/1	12[c]/4
Resorptions/Dam (Early/Late)	0.3/0.1	0.3/0.1	0.1/0.1	0.7/0.2
Does with resorption N (%)	1 (5.6)	4 (21.0)	3 (18.8)	7 (41.2)
Does with all conceptus resorbed N (%)	1 (5.6)	1 (5.3)	0	1 (5.9)
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)	43.9[b]	45.1	43.3	45.3
Preimplantation Loss (%)	23.1	25.9	24.1	26.7
Postimplantation Loss (%)	4.7	4.9	2.3	12.7
Sex Ratio (% Male)	52.4	50.0	50.8	53.6

* Data extracted from Report Tables 9 and 10. There are slight differences between the calculated cesarian section observations using values from Tables 9 & 10 and those presented in Table 3.

[b] Excludes values from doe 19208, which was accidently sacrificed on day 28 of gestation

[c] One litter had all resorptions

2. **Developmental Toxicity**

A total of 143/17, 136/18, 126/16 and 110/16 fetuses/litter from the control, 5, 15 and 30 mg/kg/day, respectively, were examined for external, visceral and skeletal malformations or variations.

a. **External Examinations** - No treatment-related external

malformations/variations were observed in fetuses at necropsy in any treated groups.

b. **Visceral Examinations** - Treatment with AC 303,630 had no effect on the visceral variations (Appendix I). The total number of various fetal visceral variations/affected litter were 3/2, 2/2, 3/3 and 2/2 in the control, 5, 15 and 30 mg/kg/day groups, respectively. The variations included microphthalmia (30 mg/kg), agenesis of intermediate lung lobe (all groups) and gallbladder (15 mg/kg) and ectopic kidneys (15 mg/kg). The aforementioned incidences lacked dose-relationship, and were within historical ranges for this strain of rabbits. The incidence is considered spontaneous and therefore of no biological significance.

c. **Skeletal Examinations:**

No treatment-related skeletal malformations and/or variations were noted. Sporadic observations observed among all animal groups included malformations of skull, vertebrae/ribs and caudal vertebrae and variations in skull ossifications, hyoid, vertebrae, ribs and sternum (Appendix II). The aforementioned incidences lacked dose-response and were within the historical control ranges, therefore, considered to be of no biological significance.

E. **DISCUSSIONS**

The data reporting was thorough and the summary means were supported by the individual animal data.

F. **CONCLUSIONS**

a. **Maternal NOEL: 5 mg/kg/day. LEL: 15 mg/kg/day**, based upon, reduced body weight gain during treatment.

b. **Developmental NOEL > 30 mg/kg/day.**

As presented, the study satisfies the requirements set forth in Subdivision F Guideline, 83-3 for Developmental Toxicity Study in Rabbits.

Reddy/AC 303,630/dev.der/9-21-93
Final: 9-23-93
Project #: D192279

CHLORFENAPYR

Page _____ is not included in this copy.

Pages 74 through 79 are not included in this copy.

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_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

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_____ 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.

FINAL

010651

DATA EVALUATION REPORT

CL 303,630

Study Type: Mutagenicity: Microbial/
Mammalian Microsome Mutagenicity Assay

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Dean Walton Date 8/13/93
Dean Walton, Ph.D.

Independent Reviewer Nancy E. McCarroll Date 8/13/93
Nancy E. McCarroll, B.S.

QA/QC Manager Sharon Segal Date 8/13/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-132
Clement Number: 425
Project Officer: Caroline Gordon

010651

GUIDELINE §84: MUTAGENICITY
SALMONELLA/E. coli

MUTAGENICITY STUDIES

EPA Reviewer: Irving Mauer, Ph.D.
Immediate Office/HED H7509C
EPA Section Head: Marion Copley, DVM, DABT
Review Section IV,
Toxicology Branch I/HED H7509C

Signature: [signature]
Date: 08/24/93
Signature: [signature]
Date: 8/31/93

DATA EVALUATION REPORT

CHEMICAL: CL 303,630 ($C_{15}H_{11}BrClF_3N_2O$)

Tox Chem Number: None

PC Code: 129093

STUDY TYPE: Mutagenicity: Microbial/mammalian microsome mutagenicity assay

MRID Number: 427702-23

SYNONYM(S)/CAS No.: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl); Pirate™; AC 303,630

SPONSOR: American Cyanamid Company, Princeton, NJ

TESTING FACILITY: American Cyanamid Company, Princeton, NJ

TITLE OF REPORT: Evaluation of CL 303,630 in a Bacterial/Microsome Mutagenicity Assay

AUTHOR: E. Mulligan

STUDY NUMBER: American Cyanamid No. 91-02-001

REPORT ISSUED: March 24, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: Negative in the bacterial (Salmonella typhimurium, Escherichia coli)/mammalian microsome reverse mutation assay with S. typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100 and E. coli strain WP2 uvrA- exposed to doses up to 50 µg/mL CL 303,630 in the presence or absenc. of S9 metabolic activation. Severe cytotoxicity was demonstrated in strains TA1537 and TA1538 at 50 µg/plate +/- S9.

CORE CLASSIFICATION: Acceptable. This study satisfies Guideline data requirements (§84-2a) for genetic effects, Category I, Gene Mutations and is acceptable for regulatory purposes.

A. MATERIALS:

1. Test Material: CL 303,630

Description: Tan powder
Identification number: Batch number: AC 7504-59A
Purity: 94.5%
Receipt date: Not reported
Stability: Stability was evaluated prior to start of the mutagenicity assay but the results were not reported.
Other components: Not reported
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: Dosing solutions were prepared fresh on the day of use and analytical determinations were performed on dosing solutions from Trials 3 and 4 (see Section C, Reported Results).

2. Control Materials:

Solvent/final concentration: DMSO/0.1 mL per plate

Positive:

Nonactivation:		
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	10 µg/plate	TA1535, TA100, WP2 *uvrA*-
2-Nitrofluorene (2-NF)	20 µg/plate	TA1538, TA98
9-Aminoacridine (9-AA)	50 µg/plate	TA1537
Activation:		
2-Aminoanthracene (2-AA)	5 µg/plate	all strains

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

Two batches of the S9 homogenate, identified as lot numbers MBAR417 and MBAR462 were used in the study and were obtained from Microbiological Associates Inc., Bethesda, MD. Prior to use, each batch was characterized for its ability to convert 2-AA and 7,12-dimethylbenz(a)anthracene to mutagenic forms using S. typhimurium strain TA100.

S9 mix composition:

Component:	Concentration
Phosphate buffer	0.1 M
Glucose 6-phosphate (260.14 mw)	5.9 mM
NADP (743.44 mw)	4.2 mM
KCl (74.5 mw)	33.0 mM
$MgCl_2(6H_2O)$ (203.3 mw)	8.1 mM
S9	10%

Note: The concentration of the various components in the S9 mix was calculated by the reviewers based on the provided weight of each component.

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538
list any others: E. coli strain WP2 uvrA-

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

(a) Cytotoxicity assay: Doses ranging from 100 µg/mL to 5000 µg/mL were evaluated.

(b) Mutation assays:

Initial mutation assays:

Trial 1: Five doses (1, 5, 10, 25, and 50 µg/plate) were evaluated in triplicate in the presence and absence of S9 activation; all tester strains were used.

Trial 2: As above

Repeat mutation assays:

Trial 3: In the repeat mutation assay, seven doses (0.5, 1, 5, 10, 15, 20, and 25 µg/mL) were evaluated; only tester strains TA1537 and TA1538 were processed.

Trial 4: As above for Trial 3

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
__x__ Standard plate test
_____ Pre-incubation (_____ minutes)
_____ "Prival" modification
_____ Spot test
_____ Other (_____)

2. Protocol: Similar procedures were used for the preliminary cytotoxicity and the mutation assays. A 0.1-mL aliquot of an 11-hour broth culture of the appropriate tester strain at a density of 0.1-2x10^9 cells/mL and 0.1 mL of the appropriate test material dose, solvent, or positive control were mixed with 0.5-mL volumes of the S9-cofactor mix buffer and 2.0 mL of molten top agar supplemented with

L-histidine, biotin and tryptophan. In the S9-activated tests, 0.5 mL of the S9-cofactor mix replaced the S9-cofactor mix buffer. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E plates and incubated at 37±2°C for ≈48 hours. At the end of incubation, plates were scored. 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: Results were compared to the presented historical background control data to verify assay performance.

(b) Positive response: The test material was considered positive if it caused a dose-related increase in the mean number of revertants per plate over at least three concentrations in at least one strain and the increase was at least 2-fold over the concurrent solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Doses of the test material ranging from 100 to 5000 µg/plate were evaluated in the assay. The data were not provided; however, the study author stated that CL 303,630 was excessively cytotoxic at all doses. Based on this information, a dose of 50 µg/mL was selected as the highest concentration for the mutation assay.

2. Mutation Assays:

a) Initial trials: Representative findings from the first two mutation assay trials are presented in Tables 1 and 2. In general, results from both trials were in good agreement and indicated that the highest dose (50 µg/plate +/- S9) was cytotoxic in S. typhimurium strains TA1537 and TA1538. There was no clear or reproducible evidence of cytotoxicity for the remaining strains. Similarly, no appreciable increase in either histidine (his^+) or tryptophan (trp^+) revertants were observed either in the presence or absence of S9 activation. The 2-fold increase in his^+ colonies of strain TA1538 at 10 and 25 µg/plate +S9 in Trial 2 was not considered by our reviewers to be indicative of a mutagenic effect. This finding was not observed in Trial 1 or subsequent trials and resulted from the low background count for this strain. Our reviewers further noted that background colonies for S. typhimurium strains TA100 and E. coli WP2 *uvrA-* were also relatively low. Nevertheless, all strains responded in the expected manner to the appropriate nonactivated and S9-activated positive controls.

TABLE 1: Representative Results of the Initial Bacterial/Mammalian Microsome Mutation Assay (Trial 1) with CL 303,630

Substance	Dose/ Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]					
			TA1535	TA1537	TA1538	TA98	TA100	WP2 uvrA-
Solvent Control								
Dimethyl sulfoxide	100 µL/mL	-	9±2.5	6±3.0	5±2.5	28±6.1	46±2.1	5±1.2
	100 µL/mL	+	10±1.5	9±1.7	13±3.0	23±4.6	70±15.0	8±3.5
Positive Controls								
N-methyl-N'-nitro-N-nitrosoguanidine	10.0 µg	-	797±221.8	--	--	--	1106±402.5	580±108.2
9-Aminoacridine	50.0 µg	-	--	136±15.5	--	--	--	
2-Nitrofluorene	20.0 µg	-	--	--	1353±123.1	1254±162.2	--	
2-Aminoanthracene	5.0 µg	+	303±15.0	328±15.0	1498±53.8	1614±146.1	2038±35.2	106±13.7
Test Material								
CL 303,630	10 µg[b]	-	5±2.5	3±1.5	5±2.5	29±0.6	62±5.5	6±1.0
	10 µg	+	7±1.5	4±1.0	8±3.5	29±5.0	75±7.8	8±1.7
	25 µg	-	5±3.0	1±0.0	4±1.5	28±7.0	64±8.4	5±4.0
	25 µg	+	7±1.2	3±1.0	6±3.1	18±3.0	66±6.8	7±0.6
	50 µg	-	4±2.0	4±1.0	T	20±1.5	60±12.7	6±2.3
	50 µg	+	4±3.5	T	T	20±5.1	57±3.5	9±1.5

[a]Means and standard deviations of the counts from triplicate plates
[b]Results for lower doses (1 or 3 µg/plate +/- S9) did not suggest a mutagenic effect.

T = Excessive cytotoxicity

Note: Data were extracted from the study report p. 13.

TABLE 2: Representative Results of the Second Bacterial/Mammalian Microsome Mutation Assay (Trial 2) with CL 303,630

Substance	Dose/ Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]					
			TA1535	TA1537	TA1538	TA98	TA100	WP2 uvrA-
Solvent Control								
Dimethyl sulfoxide	100 µL/mL	-	6±4.0	6±2.3	6±2.1	16±3.8	48±5.9	5±2.5
	100 µL/mL	+	5±3.5	4±1.2	2±1.0	16±2.6	56±10.1	13±5.5
Positive Controls								
N methyl-N'-nitro-N-nitrosoguanidine	10.0 µg	-	499±148.5	--	--	--	840±164.5	668±32.8
9-Aminoacridine	50.0 µg	-	--	200±16.1	--	--	--	
2 Nitrofluorene	20.0 µg	-	--	--	1188±176.2	1023±309.7	--	
2-Aminoanthracene	5.0 µg	+	456±10.2	279±4.2	1289±82.7	1675±93.6	2162±16.0	89±3.0
Test Material								
CL 303,630	10 µg[b]	-	6±2.5	3±0.0	5±1.7	16±3.8	53±3.0	6±1.2
	10 µg	+	9±4.5	2±1.0	5±3.8	16±1.0	48±5.7	9±2.1
	25 µg		6±1.7	1±1.5	3±1.0	18±5.0	57±0.0	5±1.0
	25 µg	+	6±3.0	1±0.6	4±3.5	16±5.6	36±5.5	9±6.4
	50 µg	-	6±1.7	T	T	10±4.6	68±2.3	5±1.5
	50 µg	+	5±2.5	T	T	17±7.5	T	6±4.7

[a]Means and standard deviations of the counts from triplicate plates
[b]Results for lower doses (1 or 5 µg/plate +/- S9) did not suggest a mutagenic effect.

T = Excessive cytotoxicity

Note Data were extracted from the study report p. 14

(b) Repeat trials: Owing to severe cytotoxicity for S. typhimurium strains TA1537 and TA1538 at the highest dose evaluated in Trials 1 and 2, repeat experiments were conducted with 0.05, 1, 5, 10, 15, 20, and 25 µg/plate CL 303,630. As the data in Table 3 show, severe cytotoxicity was observed in both strains exposed to the highest dose in the presence of S9 and in strain TA1538 in the absence of S9. No appreciable increases in the numbers of his+ revertants were observed in either strain exposed to the selected doses of the test material. The data from Trial 4 were very similar to, and confirmed. the results obtained in Trial 3. Additionally, the ability of the test system to detect a mutagenic effect was demonstrated with the positive controls in both trials.

3. Analytical Determinations: All dosing solutions from Trials 3 and 4 were analyzed to verify actual concentrations. The second lowest stock solution (0.01 mg/mL) in Trial 4 was 87% of the nominal concentration. All other stock solutions were within ±10% of the target level. Our reviewers assess that the minor deviation from the target concentration of one dose did not affect the outcome of Trial 4.

Based on the overall findings, the study author concluded that CL 303.630 was negative for inducing gene mutations in the presence or absence of S9 activation.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that CL 303,630 was tested at appropriate concentrations including a dose that was clearly cytotoxic in S. typhimurium tester strains TA1537 and TA1538 but failed to induce a mutagenic effect in S. typhimurium or E. coli. We, therefore, conclude that CL 303.630 was not genotoxic in this microbial test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. Quality assurance statements were signed and dated October 29, 1992 (Analytical Determinants. and March 12, 1993 (Microbial Mutation Assay).

CORE CLASSIFICATION: Acceptable. This study satisfies data requirements (§84-2) for genetic effects, Category 1, Gene Mutations and is acceptable for regulatory purposes.

TABLE 3: Representative Results of the Repeat Bacterial/Mammalian Microsome Mutation Assays (Trials 3 and 4) with CL 303,630

Substance	Dose/ Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strains[a] Trial 3 TA1537	Trial 3 TA1538	Trial 4 TA1537	Trial 4 TA1538
Solvent Control						
Dimethyl sulfoxide	100 µL/mL	-	5±3.2	7±2.1	3±2.5	8±5.1
	100 µL/mL	+	6±0.6	9±1.5	6±1.5	13±3.2
Positive Controls						
9-Aminoacridine	50.0 µg	-	388±159.5	--	538±42.1	--
2-Nitrofluorene	20.0 µg	-	--	1587±318.4	--	847±57.1
2-Aminoanthracene	5.0 µg	+	191±53.7	703±152.4	242±17.8	822±127.6
Test Material						
CL 303,630	15.0 µg[b]	-	2±1.5	3±1.2	2±1.0	3±2.5
	15.0 µg	+	1±1.0	2±0.6	3±1.0	0±0.0
	20.0 µg	-	2±0.6	2±1.2	2±1.2	3±2.5
	20.0 µg	+	T	1±0.6	T	0±0.0
	25.0 µg	-	1±1.0	T	2±1.0	T
	25.0 µg	+	T	T	T	T

[a]Means and standard deviations of the counts from duplicate plates
[b]Results for lower doses (0.5, 1, 5, or 10 µg/plate +/- S9) did not suggest a mutagenic effect.

T = Excessive cytotoxicity

Note Data were extracted from the study report p. 15

FINAL

019651

DATA EVALUATION REPORT

CL 303,630
(PIRATE™)

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date _____
Dean Walton, Ph.D.

Independent Reviewer _____ Date 8/27/93
Nancy McCarroll, B.S.

QA/QC Manager _____ Date 8/27/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-111
Clement Number: 42[illegible]
Project Officer: Caroline Gordon

C10651

GUIDELINE § 84: MUTAGENICITY
MICRONUCLEUS

MUTAGENICITY STUDIES

EPA Reviewer: Irving Mauer, Ph.D.
Immediate Office/HED (H 7509C)
EPA Section Head: Marion Copley, D.V.M., DABT
Review Section IV,
Toxicology Branch I/HED (H-7509C)

Signature: Irving Mauer 8-30-93
Date:
Signature: Marion Copley
Date: 8/31/93

DATA EVALUATION REPORT

CHEMICAL: CL 303,630 ($C_{15}H_{11}BrClF_3N_2O$)

TOX. CHEM. NO.:

PC CODE: 129093

STUDY TYPE: In vivo micronucleus assay in mice

MRID NUMBER: 427702-25

SYNONYM(S)/CAS No.: Pyrrole-3-carbonitrile, 4-bromo-2-[(p-chlorophenyl)-1-ethoxymethyl-5-(trifluoromethyl)] (CA); Pirate™; AC 303,630

SPONSOR: American Cyanamid Company, Princeton, NJ

TESTING FACILITY: American Cyanamid Company, Princeton, NJ

TITLE OF REPORT: Evaluation of CL 303,630 in the In Vivo Micronucleus Assay in Mouse Bone Marrow Cells

AUTHOR: R. K. Sharma

STUDY NUMBER(S): American Cyanamid No. 91-18-001

REPORT ISSUED: March 17, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: Reported to be 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. Although 5 of the 15 males receiving the highest dose died, there were no deaths or clear evidence of test compound-related clinical toxicity among high-dose females, nor any indication of cytotoxicity in the target (bone marrow) cells in either sex at any harvest time. Hence, no evidence has been provided to indicate the transport of a sufficient concentration of test article [or active metabolite(s)] to affect the target tissue. We conclude, therefore, that this in vivo assay, as submitted, may be considered a NON-TEST for mutagenicity (micronucleus induction). The assay with this substance should be repeated using the same route of administration as the positive control, cyclophosphamide (CP), which provided significantly increased micronuclei, namely by intraperitoneal injection.

010651

GUIDELINE § 84: MUTAGENICITY
MICRONUCLEUS

KB 10/25/93
NON-TEST

CLASSIFICATION: ~~Unacceptable~~. The study does not satisfy the requirements for FIFRA Test Guideline 84-2 for Category II, Structural Chromosome Aberrations, and is not acceptable for regulatory purposes. HOWEVER, A REPEAT MICRONUCLEUS TEST IS NOT REQUIRED. RATHER, A CHROMOSOMAL ABERRATION TEST OTHER THAN A MICRONUCLEUS WOULD SATISFY THIS DATA REQUIREMENT. KB 10/25/93

A. MATERIALS:

1. Test Material: CL 303,630

Description: Tan powder
Identification numbers: Lot No. AC 7504-59A
Purity: 94.5%
Receipt date: Not provided
Stability: Found to be stable under the conditions of use. Reported in ARD Chemical Development Report No. T8PL14
Contaminants: Not reported
Vehicle used: Corn oil
Other provided information: The test material was stored at room temperature. Test material solutions were prepared immediately prior to use and analytical determinations were performed on the dosing solutions.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Corn oil was administered once by oral gavage (dosing volume = 10 mL/kg).

Positive/final concentration/route of administration: Cyclophosphamide (CP) was prepared in corn oil and was administered once by intraperitoneal injection at a final dose of 40 mg/kg.

3. Test Compound:

Route of administration: Single oral gavage administration

Dose levels used:

(a) Preliminary toxicity test: 50, 60, 70, and 80 mg/kg were evaluated in males and 30, 40, 50, and 60 mg/kg were evaluated in females (3 animals/sex/dose)

Note: The high dose for either sex was reported to be based on previous toxicology data.

(b) Micronucleus test: 7.5, 15, or 30 mg/kg were evaluated in males and 5, 10 or 20 mg/kg were evaluated in females. Five animals/dose time period were used in the treatment and vehicle groups; and 5 animals/sex were used for the positive control group.

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4. Test Animals:

(a) Species: Mouse Strain: CD-1 Age: 8-10 weeks (at dosing)
Weight range: Not reported Source: Charles River Breeding Laboratories, Inc., Kingston, NY

(b) Number of animals used per test dose: 15/sex

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr

(c) Positive control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr

2. Tissues and Cells Examined:

x bone marrow _____ others (list):

Number of polychromatic erythrocytes (PCEs) examined per animal: 1000

Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Number observed while scoring the first 1000 erythrocytes (PCE + NCE).

3. Details of Slide Preparation: At 24, 48, or 72 hours post-administration of the test material or the vehicle control the appropriate group of animals was sacrificed by CO_2 asphyxiation followed by cervical dislocation. Animals in the positive control group were sacrificed at 24 hours. Bone marrow cells were recovered from the femurs and a drop of cells suspended in fetal calf serum was placed onto slides. Slides were fixed, dried, stained with 10% Giemsa, coded and scored.

4. Statistical Methods: The data for each sex were evaluated for statistical significance ($p \leq 0.05$) using an analysis of variance (ANOVA) and the least square differences (LSD) procedure.

5. Evaluation Criteria: The test was considered valid if the positive control showed a statistically significant ($p \leq 0.05$) response and the vehicle controls were within the range of provided historical control values.

C. REPORTED RESULTS:

1. Toxicity Test: Animals receiving the selected doses of the test material were observed for signs of toxicity after dosing and daily thereafter until sacrifice. At sacrifice, bone marrow smears were made and cells were scored for the ratio of PCEs:NCEs. All males from the three highest dose groups (60, 70, and 80 mg/kg) and 2 of 3 males from the lowest dose group (50 mg/kg) died after exposure to the test material. Two of three females in the highest dose group (60 mg/kg), all females from the 40- and 50-mg/kg dose groups, and one of three females in the low-dose group (30 mg/kg) died. PCE:NCE ratios for survivors did not suggest a cytotoxic effect on the target organ. Based on these findings, doses approximating 60% of the LD_{50} for each sex (30 mg/kg for males or 20 mg/kg for females) were selected as the starting concentrations for the micronucleus assay.

2. Micronucleus Assay: Five males exposed to 30 mg/kg CL 303,630 died (1 at 24 hours, 3 at 48 hours, and 1 at 72 hours). No deaths or clinical signs of toxicity were reported for males in the lower treatment groups. The only sign of compound toxicity observed in females was slight diarrhea in single females of the mid- and high-dose groups. As the data presented in Table 1 indicate, exposure of males to 30 mg/kg or females to 20 mg/kg CL 303,630 did not increase the frequency of micronucleated PCEs in the bone marrow cells harvested at 24, 48, or 72 hours. Similar results were obtained for both sexes in the lower treatment groups. There was also no indication that the test material had a cytotoxic effect on the target organ. By contrast, the positive control (90 mg/kg CP) induced a significant ($p \leq 0.05$) genotoxic response.

3. Analytical Determinations: Analysis of the dosing solutions revealed that one dose in the range-finding study was 121% of the 5.00 mg/mL target and that the majority of doses in the micronucleus assay were within 108%-116% of their respective target concentrations.

From the overall results, the study authors concluded that CL 303,630 did not cause bone marrow cytotoxicity or chromosome damage in this study.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: No conclusion can be drawn from the micronucleus assay conducted with CL 303,630 in male mice at levels up to mg/kg and in female mice at doses up to 20 mg/kg. The high dose was toxic in males, but failed to produce bone marrow cytotoxicity that would

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with CL 303,630

Substance	Dose/kg	Exposure Time[a] (hours)	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Total MPEs per Group	Percent MPEs	Average PCE:NCE Ratio[b]
Vehicle Control							
Corn oil	10 ml						
Males		24	5	5000	11	0.22	0.824
		48	5	5000	5	0.10	0.708
		72	5	5000	7	0.14	0.690
Females		24	5	5000	14	0.28	0.674
		48	5	5000	5	0.10	0.651
		72	5	5000	10	0.20	0.609
Positive Control							
Cyclophosphamide							
Males	90 mg	24	5	5000	261	5.22*	0.667
Females	90 mg	24	5	5000	253	5.06*	0.413
Test Material							
CL 303,630							
Males	30 mg[c,d]	24	4	4000	12	0.30	0.848
		48	2	2000	5	0.25	0.751
		72	4	4000	8	0.20	0.767
Females	20 mg[d]	24	5	5000	9	0.18	0.904
		48	5	5000	10	0.20	0.899
		72	5	5000	9	0.18	0.851

[a]Time after compound or vehicle control administration by oral gavage; the positive control was administered by intraperitoneal injection.
[b]Calculated by the reviewers
[c]Five males died at this dose, 1 each in the 24- and 72-hour groups and 3 in the 48-hour group.
[d]Results from lower doses [7.5 or 15 mg/kg (males) and 5 or 10 mg/kg (females)] did not suggest genotoxic effects.

*Significantly higher ($p<0.05$) than the corresponding vehicle control by ANOVA and LSD analysis

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes

Note: Data were extracted from the study report, pp. 19-21.

indicate that the test material or its metabolite(s) reached the target organ. Additionally, there were no mortalities or definitive evidence of toxicity at any dose in females. The observation of slight diarrhea in single females administered either 10 or 20 mg/kg CL 303,630 is inadequate to determine that a sufficiently high dose was achieved. Therefore, a higher dose of the test material should be examined, using the same route as the positive control, to assure that the test material is systemically available. The sensitivity of the test system to detect a positive response when the test material was delivered intraperitoneally was clearly demonstrated by the significant ($p \leq 0.05$) findings obtained in both sexes with the positive control (90 mg/kg CP).

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (Quality assurance statements, indicating that inspections and audits were conducted, were signed and dated June 30, 1992; September 25, 1992; and March 4, 1993.)

CORE CLASSIFICATION: ~~Unacceptable~~. The study does not satisfy the requirements for FIFRA Test Guideline 84-2 for Category II, Structural Chromosome Aberrations, and is not acceptable for regulatory purposes.

SEE Pg. 3 OF 3.

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FINAL

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DATA EVALUATION REPORT

CL 303,630
(PIRATE™)

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT)

Prepared for:

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

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Dean Walton Date 8/13/93
Dean Walton, Ph.D.

Independent Reviewer Nancy E. McCarroll Date 8/13/93
Nancy E. McCarroll, B.S.

QA/QC Manager Sharon Segal Date 8/13/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-132
Clement Number: 426
Project Officer: Caroline Gordon

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

EPA Reviewer: Irving Mauer, Ph.D.
Immediate Office/HED (H-7509C)

Signature:
Date: 08/24/93

Section Head: Marion Copley, D.V.M., DABT
Review Section IV,
Toxicology Branch I/HED (H-7509C)

Signature:
Date: 9/20/93

DATA EVALUATION REPORT

CHEMICAL: CL 303,630 ($C_{15}H_{11}BrClF_3N_2O$)

TOX CHEM. NUMBER:

PC CODE: 129093

STUDY TYPE: Mutagenicity: Gene mutation in cultured Chinese hamster ovary cells (CHO/HGPRT)

MRID NUMBER: 427702-24

SYNONYM(S)/CAS NUMBER: Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl); Pirate™; AC 303,630

SPONSOR: American Cyanamid Company, Princeton, NJ

TESTING FACILITY: American Cyanamid Company, Princeton, NJ

TITLE OF REPORT: Evaluation of CL 303,630 in the Mammalian Cell CHO/HGPRT Mutagenicity Assay

AUTHOR: R.K. Sharma

STUDY NUMBER: American Cyanamid No. 91-05-001

REPORT ISSUED: March 25, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: Reported to be negative in two independently conducted trials for inducing forward gene mutations at the HGPRT locus in cultured Chinese hamster ovary (CHO) cells at nonactivated doses of 2.5-250 µg/mL or S9-activated doses of 5-250 µg/mL. Relative survival (RS) at the highest dose yielding valid data 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. However, higher levels should have been assayed to insure that the test material was appropriately investigated.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy the requirements for FIFRA Test Guideline 84-2 for genetic effects Category I. Gene Mutations and is not acceptable for regulatory purposes.

A. MATERIALS:

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1. Test Material: CL 303,630

Description: Tan powder
Identification No.: Lot No. AC 7504-59A
Purity: 94.5%
Receipt date: Not reported
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at room temperature. Test material solutions were prepared fresh for each trial and analytical determinations were performed on all dosing solutions.

2. Control Materials:

Negative: Ham's F12 complete medium

Solvent/final concentration: DMSO/1%

Positive:

Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMSO to yield a final concentration of 200 µg/mL.

Activation (concentrations, solvent): 7,12-Dimethylbenz(a)anthracene (DMBA) was prepared in DMSO to yield final concentrations of 3 or 3.5 µg/mL.

3. Activation: S9 derived from a Sprague-Dawley (unspecified sex)

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

S9 liver homogenate (Lot No. R427), purchased from Microbiological Associates Inc., Bethesda, MD, was used in this study and was characterized with 2-aminoanthracene and DMBA.[1,2] The S9-cofactor mix was prepared according to published methods, but the constituents and concentrations were not reported. A volume of 0.02 mL of S9 per mL of treatment medium was used.

4. Test Cells: Mammalian cells in culture

____ mouse lymphoma L5178Y cells
x Chinese hamster ovary (CHO K^1 BH^4) cells
____ V79 cells (Chinese hamster lung fibroblasts)
____ other (list):

[1]O'Neill, J.P., Brimer, P.A., Machanoff, R., Hirsch, G.P., and Hsie, A.W. 1977. A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): Development and definition of the system. Mutat. Res. 45:91-101.

[2]Li, A.P. 1981. Simplification of the CHO/HGPRT mutation assay through growth of the Chinese hamster ovary cells as unattached cultures. Mutat. Res. 85:165-175.

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background?
Maintained in logarithmic growth phase to reduce the spontaneous mutation frequency.

5. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) 1.34×10^{-5}M 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was performed but the data were not presented.

(b) Mutation assay: Two duplicate nonactivated and S9-activated assays were performed; doses tested were as follows:

1 Nonactivated conditions: 2.5, 5, 25, 50, 100, or 250 µg/mL test material (initial and repeat nonactivated trials)

2 S9-activated conditions: 5, 10, 50, 100, 250, or 500 µg/mL test material (initial and repeat activated trials)

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, solvent or positive controls for: __5__ hours (nonactivated) __5__ hours (activated)

(b) After washing, cells were cultured for __7__ days (expression period) before cell selection.

(c) After expression, 2×10^5 cells were added to each of 15 dishes and were cultured for __7-9__ days in selection medium to determine numbers of mutants and 100 cells/dish (3 dishes) were cultured for __7-9__ days without selection medium to determine cloning efficiency (CE).

2. Statistical Methods: The data were evaluated for statistical significance at $p<0.05$ using analysis of variance (ANOVA) and Student's t-test.

3. Evaluation Criteria:

   (a) Assay validity: The assay was considered valid if the following criteria were met: (1) the average absolute CE of the solvent control was between 70% and 115%; (2) the average background mutation frequency was in the range of $0-15x10^{-6}$; (3) at least three dose levels were evaluated; (4) the test material was evaluated at a high dose that resulted in 75%-80% cell mortality or up to a maximum dose of 5000 µg/mL; and (5) the positive controls induced significant ($p\leq0.05$) increases in the mutation frequency (MF) compared to the corresponding solvent control that $>15x10^{-6}$.

   (b) Positive response: The test material was considered positive if it caused a significant ($p\leq0.05$) increase in the MF compared to the solvent control and the average MF was $>15x10^{-6}$.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The study author reported that test material doses ≥500 µg/mL were "markedly" cytotoxic to the CHO cells. Based on this information, high doses of 250 µg/mL -S9 and 500 µg/mL +S9 were selected for the mutation assay.

2. Mutation Assays: Representative results from the nonactivated trials conducted with CL 303,630 are shown in Table 1. RS decreased in a dose-related manner with increasing concentrations of the test material. At the highest evaluated dose (250 µg/mL), RS was 36.7% and 40.1% in the initial and repeat nonactivated trials, respectively. MFs from test-material treated cultures were generally comparable to the solvent controls and, therefore, did not suggest a mutagenic response. The average MFs of CHO cultures exposed to 200 µg/mL EMS for the initial and repeat trials were significantly ($p<0.05$) different from the solvent control.

   Under S9-activated conditions, data from the high-dose group were discarded either because mutant selection plates were contaminated (initial trial) or an insufficient number of cells survived treatment (repeat trial). RS for the highest dose yielding valid data (250 µg/mL) was 23.9% (initial trial) or 38.5% (repeat trial). For the remaining levels in both trials, ≈40% or greater RS was reported. Data presented in Table 2 further indicated that the S9-activated test material did not induce a mutagenic effect.

TABLE 1. Representative Results from the Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with CL 333,630 in the Absence of S9 Metabolic Activation

Substance	Dose/mL	Average Percent Relative Survival (posttreatment)[a]	Average Total Mutant Colonies	Average Absolute Cloning Efficiency[a]	Average Mutation Frequency/ 10^6 survivors[b]
Negative Control	--	103.3[c]	1.0	79.0	0.5
	--	102.2[d]	14.0	83.5	5.6
Solvent Control					
Dimethyl sulfoxide	0.1	100.0[c]	1.0	77.0	0.5
	0.1	100.0[d]	12.0	77.5	5.3
Positive Control					
Ethyl methane-sulfonate	200 µg	59.4[c]	345.0	70.0	210.6[e,*]
	200 µg	76.3[d]	504.5	72.5	232.1[*]
Test Material					
CL 333,630	100 µg[f]	63.9[c]	4.5	94.5	1.60
	250 µg	36.7	0.5	111.5	0.20
	100 µg[f]	53.1[d]	1.5	83.5	0.6
	250 µg	40.1	11.5	78.0	4.5

[a]Average values from duplicate cultures for all groups; calculated by our reviewers.

[b]Mutation Frequency = $\frac{\text{Average Total Mutant Colonies}}{\text{No. of Dishes (15) X No. of Cells Plated } (2X10^5) \text{ X Absolute Cloning Efficiency}}$

[c]Results from the initial trial
[d]Results from the repeat trial
[e]Based on an average total of 12.5 plates (15 plates for one culture and 10 plates for the other culture)
[f]Results for lower levels (2.5, 5, 25, or 50 µg/mL in both trials) did not suggest a mutagenic response.

[*]Significantly ($p<0.05$) greater than the corresponding vehicle control

Note: Data were extracted from the study report, pp. 16, 21-24, and 26

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TABLE 2. Representative Results from the Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with CL 303,630 in the Presence of S9 Metabolic Activation

Substance	Dose/mL	Average Percent Relative Survival (posttreatment)[a]	Average Total Mutant Colonies	Average Absolute Cloning Efficiency[a]	Average Mutation Frequency/ 10^6 survivors[b]
Negative Control	--	93.6[c]	19.5	85.5[c]	7.6
	--	98.4[d]	27.5	91.5[d]	10.0
Solvent Control					
Dimethyl sulfoxide	0.1 mL	100.0[c]	7.5	99.5[c]	2.6
	0.1 mL	100.0[d]	22.0	95.0[d]	7.8
Positive Control					
7,12-Dimethylbenz-(a)anthracene	3.5 µg	15.6[c]	1077.0	67.0[c]	621.9[e*]
	3.0 µg	18.2[d]	786.5	68.5[d]	387.1[*]
Test Material					
CL 303,630	100 µg[f]	42.2[c]	4.5	106.5[c]	1.4
	250 µg	23.9	0.0	107.0	0.0
	500 µg	18.3	--[g]	103.0	--[g]
	100 µg[f]	44.9[d]	24.0	89.0[d]	9.0
	250 µg	38.5	1.0	89.5	0.4
	500 µg[h]	--	--	--	--

[a]Average values from duplicate cultures for all groups; calculated by our reviewers.

[b]Mutation Frequency = $\frac{\text{Average Total Mutant Colonies}}{\text{No. of Dishes (15) X No. of Cells Plated (2X10}^5\text{) X Absolute Cloning Efficiency}}$

[c]Results from the initial trial
[d]Results from the repeat trial
[e]Based on an average total of 12 plates (15 plates for one culture and 9 plates for the other culture)
[f]Results for lower levels (5, 10, or 50 µg/mL +S9) did not suggest a mutagenic response.
[g]Mutant colony selection plates were contaminated and, therefore, discarded.
[h]Reported to be severely cytotoxic

-- = Data were not reported.

[*]Significantly ($p<0.05$) greater than the corresponding vehicle control

Note: Data were extracted from the study report, pp. 15, 17-20, and 25.

3. Analytical Determinations: The analysis of dosing solutions used in both or the nonactivated and S9-activated trials indicated that the majority of dosing solutions contained >100% of the target dose. In some instances, the difference between nominal and actual concentrations was excessive (i.e., ≥270%).

Based on the overall findings, the study author concluded that CL 303,630 was not mutagenic in this mammalian cell gene mutation assay.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the study does not provide definitive evidence that CL 303,630 is not mutagenic in the CHO/HGPRT assay because the recommended level of cytotoxicity (i.e., 10%-30% survival)[3] was either not achieved (nonactivated conditions) or was not reproduced (S9-activated conditions) at the highest assayed level (250 µg/mL -S9 or 500 µg/mL +S9). The study is, therefore, considered unacceptable and should be repeated.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. Two quality assurance statements indicating that the study was audited by the sponsor laboratory's Quality Assurance Unit were signed and dated August 10, 1992 and March 16, 1993).

CORE CLASSIFICATION: Unacceptable. The study does not satisfy the requirements for FIFRA Test Guideline 84-2 for genetic effects Category I. Gene Mutations and is not acceptable for regulatory purposes.

[3]Li, A.P., Carver, J.H., Choy, W.N. Hsie, A.W., Gupta, R.S., Loveday, K.S., O'Neill, J.P., Riddle, J.C., Stankowski Jr., and Yang, L.L. 1987. A guide for the performance of the Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl transferase gene mutation assay. Mutat. Res. 189:135-141.

FINAL

010651

DATA EVALUATION REPORT

CL 303,630
(PIRATE™)

Study Type: Mutagenicity: Unscheduled DNA Synthesis (UDS) Assay in Primary Rat Hepatocytes

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Dean Walton Date 8/13/93
Dean Walton, Ph.D.

Independent Reviewer Nancy E. McCarroll Date 8/13/93
Nancy E. McCarroll, B.S.

QA/QC Manager Sharon Segal Date 8/13/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-132
Clement Number: 428
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY
UDS

MUTAGENICITY STUDIES

EPA Reviewer: Irving Mauer, Ph.D. Signature: [signature] 8/24/93
Immediate Office/HED H7509C Date:
EPA Section Head: Marion Copley, D.V.M., DABT Signature: [signature]
Review Section I.
Toxicology Branch IV/HED H7509C Date: 8/31/93

DATA EVALUATION REPORT

CHEMICAL: AC 303,630 ($C_{15}H_{11}BrClF_3N_2O$)

TOX CHEM NUMBER:

P.C. CODE: 129093

STUDY TYPE: Mutagenicity: Unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes.

MRID Number: 427702-26

SYNONYMS/CAS No.: Pyrrole-3-carbonitrile,4-bromo-2-[(p-chlorophenyl)-1-ethoxymethyl)-5-(trifluoromethyl)] (CA); Pirate™; CL 303,630

SPONSOR: American Cyanamid Company, Princeton, NJ

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: Unscheduled DNA Synthesis in Rat Primary Hepatocytes with AC 303,630

AUTHOR: R.H.C. San

STUDY NUMBERS: Microbiological Associates Project No. T9775.380025; American Cyanamid No. 971-91-101

REPORT ISSUED: February 23, 1993

CONCLUSIONS-EXECUTIVE SUMMARY: Negative for inducing unscheduled DNA synthesis (UDS) in primary rat hepatocytes treated with doses of the test material up to 0.15 µg/mL (actual dose, based on analytical determinants was 0.118 µg/mL). Higher concentrations (≥0.30 µg/mL) were severely cytotoxic.

STUDY CLASSIFICATION: Acceptable. The study satisfies the requirements for FIFRA Test Guideline 84-4 for genetic effects Category III, Other Mutagenic Effects, and is acceptable for regulatory purposes.

A. MATERIALS:

1. Test Material: AF 303,630

Description: Tan powder
Lot number: AC 3504-59A
Purity: 94.5%
Receipt date: February 14 1991
Stability: Not reported
Contaminants: Not reported
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored in the dark at room temperature. Dosing solutions of the test material were verified by analysis (see Section D--Experimental Results).

2. Indicator Cells: Rat hepatocytes, harvested from adult male rats (Strain: Fischer 344) purchased from Harlan Sprague Dawley, Frederick, MD

3. Control Substances:

- The negative control was William's Medium E (WME) containing 2 mM L-glutamine, 1.01 M HEPES, and antibiotics.
- The solvent control was DMSO at a final concentration in the media of 10 µL/mL.
- The positive control was 7,12-dimethylbenz(a)anthracene (DMBA) dissolved in DMSO at final concentrations in the media of 3 and 10 µg/mL.

4. Medium: WME containing 10% fetal bovine serum (WME+)

5. Test Compound Concentrations Used:

(a) Cytotoxicity assays:

Initial assay: An independent cytotoxicity assay was performed using ten doses of the test material ranging from 0.15 to 5000 µg/mL.

Repeat assay: Owing to severe cytotoxicity at all levels in the initial cytotoxicity test, a repeat assay was performed with 10 doses ranging from 0.00005 to 50 µg/mL.

(b) UDS assays:

Initial assay: Ten doses ranging from 0.0015 to 0.50 µg/mL were evaluated. Due to a technical error in media preparation cells were not scored for UDS. However, data from a parallel cytotoxicity assay were used to set doses for the repeat study.

Repeat assay: Seven doses were evaluated and cells exposed to 0.050, 0.075, 0.1, 0.125, and 0.15 µg/mL AC 303,630 were scored.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion technique: Each rat was anesthetized by inhalation of metofane and the livers were perfused with Hank's balanced salts containing 0.5 mM EGTA and 0.01 M HEPES buffer solution (pH 7.3) and with WME containing 80-100 U/mL collagenase (Type I). Each liver was excised, cleaned of extraneous tissue, shaken in the collagenase perfusion solution, and either combed to release the hepatocytes or passed through a stainless-steel sieve.

(b) Hepatocyte harvest/culture preparation: Recovered cells were collected, counted and seeded at a density of $5x10^5$ cells, either into preconditioned 35-mm tissue culture dishes for the cytotoxicity assay or onto coverslips in 35-mm tissue culture plates for the UDS assay. Cultures were placed in a humidified 5% CO_2 incubator for 90-180 minutes, washed and refed prior to use.

(c) Preliminary Cytotoxicity Assay: Duplicate cultures of prepared cells were exposed to the selected doses of the test material, the negative control (WME) or the solvent control (DMSO) for 18-20 hours. Following exposure, aliquots of the treatment medium were removed, centrifuged and measured for lactate dehydrogenase (LDH) activity. Relative cytotoxicity was assessed by subtracting the LDH activity of the media control from the LDH activity in the treated cultures and comparing the values to the amount of LDH released by exposure of high-dose cultures or solvent control cells to 1% Triton.

2. UDS Assay:

(a) Treatment: Five replicate monolayer cultures were exposed to the selected test material doses, the solvent or positive control for 18-20 hours in media containing 10 µCi/mL [3H]thymidine. Duplicate cultures from each treatment group were grown directly on dishes and were used to assess LDH activity as described for the cytotoxicity assay. The remaining cultures, seeded into plates containing coverslips, were used for the assessment of UDS activity.

(b) UDS slide preparation: Treated hepatocytes attached to coverslips were washed, swollen with 1% sodium citrate, fixed in ethanol/glacial acetic acid, dried, and mounted.

(c) Preparation of autoradiographs/grain development: Slides were dipped into Kodak NTB2 emulsion, dried for 1.5 hours and stored in a refrigerator for 7 days in slide boxes containing desiccant. Slides were developed in Kodak D-19, fixed, stained with hematoxylin-sodium acetate-eosin, coded and counted.

(d) Grain counting: The nuclear grains of 150 randomly selected cells with appropriate background counts and normal morphology (50/slides from each [illegible] material dose, negative and positive control group were scored for the incorporation of tritiated thymidine into DNA. Net nuclear grain counts were determined by subtracting the nuclear grain count of each cell from the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus. Means and standard deviations were calculated for each treatment group. The percentage of cells undergoing replicate DNA synthesis was determined by scoring 300 nuclei per slide; the number of nuclei completely blackened with grains was recorded.

3. Evaluation Criteria:

(a) Assay validity: For the assay to be considered valid, the following criteria must be satisfied: (1) the proportion of cells in repair in the solvent control must be <15% and the net nuclear grain count of the solvent control must be <1, and (2) the positive control compound must induce a "significant" increase in the net nuclear grain count (≥5 grains/nucleus over the negative control).

(b) Positive response: The assay was considered positive if the test material induces a dose-related increase in mean net nuclear grains and one or more of the doses had an increase in the mean net nuclear grain count that was ≥5 grains/nucleus over the negative control. In the absence of a dose-related effect, a compound that showed significant increases in mean nuclear grain counts over two successive doses was also considered positive.

4. Statistical Methods: The data were not analyzed for statistical significance.

C. REPORTED RESULTS:

1. Cytotoxicity Assays:

(a) Initial cytotoxicity assay: Ten doses (0.15-5000 µg/mL) of the test material were examined in the initial cytotoxicity assay. The study author stated that droplets of the test material were visible in the media at the initiation of treatment with 500, 1500, or 5000 µg/mL. At the end of the treatment, droplets were still apparent at 1500 and 5000 µg/mL. Owing to the increased levels of LDH with accompanying relative percent cytotoxicity

≥64%, a second cytotoxicity assay was performed with lower doses of AC 303,630.

(b) Repeat cytotoxicity assay: In the repeat trial, LDH levels were elevated at the three highest doses (0.5, 5, and 50 µg/mL) and the relative percent cytotoxicity ranged from 70% to 77%. At 0.05 µg/mL AC 303,603 and lower, LDH levels were similar to the solvent control. Visual examination of the hepatocytes indicated high levels of cytotoxicity at concentrations of ≥0.5 µg/mL, low-level cytotoxic effects at 0.05 and 0.015 µg/mL, and normal cell morphology at lower doses. Based on these results, 0.5 µg/mL was chosen by the study author as the highest concentration for the first UDS assay.

2. UDS Assays:

(a) Initial UDS Assay: The initial UDS assay was aborted due to a technical error in the preparation of the media. However, data from the cytotoxicity phase of the assay were used to set doses for the repeat UDS assay.

(b) Repeat UDS Assay: Six doses (0.05, 0.075, 0.1, 0.125, 0.15, and 0.3 µg/mL AC 303,603) were evaluated. Results presented in Table 1 show that the highest test level was severely cytotoxic and doses ≤0.15 µg/mL had no adverse effect on the monolayers. Accordingly, cells exposed 0.05, 0.075, 0.1, 0.125, or 0.15 µg/mL were scored for UDS. None of the doses selected for evaluation of UDS induced an appreciable increase in net nuclear grains. Similarly, data from the evaluation of nuclei undergoing replicative DNA synthesis indicated that the test material did not have an inhibitory effect on DNA. By contrast, the positive control, DMBA at 3 and 10 µg/mL, showed marked increases in UDS activity.

3. Analytical Determinations: Analysis of the dosing solutions used in the initial cytotoxicity assay revealed that the percent recovery for the majority of levels was <90%. Only one dose in the repeat cytotoxicity assay was outside of the acceptable range (±10% of the nominal value). Analytical determinations performed on all dosing solutions evaluated in the UDS assay indicated that percent recovery for the majority of levels was <90%. For the three highest dosing solutions (30.0, 15.0, and 12.5 µg/mL) the percentage of the nominal value was 88%, 79%, and 89%, respectively. Based on this information, the actual concentrations for the three highest doses were 0.265, 0.118, or 0.112 µg/mL AC 303,630.

Based on the overall results, the study author concluded, therefore, that AC 303,603 was negative for inducing UDS in this test system.

TABLE 1. Representative Results of the Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assay with AC 303,630

Treatment	Dose/mL	Cytotoxicity: Average Lactate Dehydrogenase (LDH) Activity (Units/L)[a]	Cytotoxicity: Corrected LDH Activity (Units/L)[b]	Cytotoxicity: Percent Relative Cytotoxicity[c]	UDS Activity: Number of Cells Scored/ Group	UDS Activity: Net Nuclear Grains[d]	UDS Activity: Percent of Cells with >5 Net Nuclear Grains
Negative Control							
William's Medium E	--	77.0	-24.5	-6%	150	-1.7±2.4	0%
Solvent Control							
Dimethyl sulfoxide	10 µL	101.5	0.0	0%	150	-2.0±2.4	0%
Dimethyl sulfoxide + 1% Triton	10 µL	521.5	420.0	100%	--	--	--
Positive Control							
7,12-Dimethyl-bens[a]anthracene	3 µg	141.5	40.0	10%	150	14.5±5.0[e]	99%
Test Material							
AC 303,603	0.125 µg[f] (0.112 µg)[g]	113.0[h]	11.5	3%	150	-2.1±2.5	0%
	0.150 µg (0.118 µg)	136.0	34.5	8%	150	-1.8±2.3	1%
	0.300 µg[i] (0.265 µg)	484.5	383.0	91%	--	--	--

[a]Average LDH activity of two cultures per group
[b]Corrected LDH = Average of Test Group - Solvent Control LDH

[c]Percent Relative Cytotoxicity = $\frac{\text{Corrected LDH of Test Group}}{\text{Corrected LDH of Solvent Control Cultures Exposed to 1\% Triton}}$

[d]Mean and standard deviations of the count from 3 slides (50 cells/slide)
[e]Conforms to the reporting laboratory's criteria for a positive response
[f]Target concentration
[g]Actual concentration, based on analytical determinations (see Study Report p. 36)
[h]Results from lower doses (0.05, 0.075, or 0.1 µg/mL) did not suggest a genotoxic effect.
[i]Cells at this dose were not scored for UDS because of severe cytotoxicity.

Note: Data were extracted from the study report pp. 15 and 17.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We agree with the study author's conclusions that AC 303,603 did not induce genotoxic effects at the concentrations tested in this rat hepatocyte DNA repair assay. Although the analytical test values showed that a majority of the prepared solutions contained <90% of the nominal values, observation of cytotoxicity confirmed that the material entered the hepatocytes. Therefore, the lack of genotoxic effect was not related to inaccurate dose preparation or the inability of the test material to interact with the target cells. In addition, the sensitivity of the test system to detect UDS was adequately demonstrated by the results obtained with the positive control (3 and 10 µg/mL DMBA). We conclude, therefore, that the assay provided valid evidence of a negative response in this test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. Quality assurance statements were signed and dated July 23, 1991 (Sponsor's inspection) and February 24, 1993 (Performing laboratory's inspection).

CORE CLASSIFICATION: Acceptable. The study satisfies the requirements for FIFRA Test Guideline 84-4 for genetic effects Category III, Other Mutagenic Effects, and is acceptable for regulatory purposes.