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Chemical:	**Tributyltin benzoate**
PC Code:	**083106**
HED File Code	**13000 Tox Reviews**
Memo Date:	**05/11/93**
File ID:	**TX010245**
Accession Number:	**412-02-0280**

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04/10/2002

CASWELL FILE

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

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MAY 1 1 1993

010245

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Tributyltin Benzoate - Data Evaluation Reports of Toxicity Studies

To: Sue Rathman, PM Team Reviewer
Accelerated Reregistration Branch, SRRD (H7508W)

From: Patricia McLaughlin, Ph.D. P M McLaughlin 5/6/93
Toxicology II Branch, HED (H7509C)

Thru: Elizabeth Doyle, Ph.D., Head
Section IV, Toxicology II Branch, HED (H7509C) E. A. Doyle 5/6/93

Marcia van Gemert, Ph.D., Chief
Toxicology II Branch, HED (H7509C) MvanGemert 5/7/93

Chemical: Tributyltin benzoate
Case/chemical number: 2620/83106
Registrant: Huls America
Data Package: S424289; D181950
MRID Nos. 424158-01, -02, -03; 424125-01, -02, -03.

Attached are the review memoranda for acute oral toxicity, acute dermal toxicity, skin irritation, gene mutatuin, chromosomal aberration, and other genotoxic effects studies in support of reregistration.

Oral LD50--both sexes = 115 mg/kg (97-137 mg/kg); females = 115 (90-148) mg/kg; males = 115 (90-148) mg/kg.
Toxicity category II; Classification: core guideline.

The dermal LD50 of female and male rats was greater than 2.0 g/kg body weight.
Toxicity category III; Classification: core guideline.

A dermal irritation study caused severe dermal reactions with necrosis in all rabbits.
Toxicity category I; Classification: core guideline.

A gene mutation study in Chinese hamster ovary cells (CHO/HGPRT) was not acceptable and should be repeated. Classification: unacceptable.

A mouse micronucleus test for structural chromosomal aberrations did not show effects, but the purity of the test material must be provided. Classification: supplementary.

The UDS study using rat hepatocytes was not acceptable should be repeated. Classification: unacceptable.

Reviewed by: Patricia McLaughlin, Ph. D. P M cLaughlin 11/5/92
Section IV, Toxicology II Branch (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D., Head E A. Doyle 11/13/92
Section IV, Toxicology II Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity (81-1)

TEST MATERIAL: Tributyltin benzoate

P.C. CODE: 83106

MRID NO.: 424158-01

STUDY NO.: 920351D/NDX25/AC

SPONSOR: Huls America Inc.
P.O. Box 365, Turner Place, Piscataway, NJ 08855

TESTING FACILITY: Huntingdon Research Centre Ltd
P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England

TITLE OF REPORT: Acute Oral Toxicity to Rats of Tributyltin Benzoate

AUTHOR: Sarah A. Allan

REPORT ISSUED: July 3, 1992

CONCLUSIONS: Female and male rats were given oral doses of 64, 100, or 160 mg/kg of tributyltin benzoate and observed for 14 days. Some rats in the 100 and 160 mg/kg groups died from day 2 to day 5; these animals had slight weight losses but no abnormalities in gross pathology. Rats displayed pilo-erection, hunched posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of extremities, pink extremities, and diarrhea up to day 6 or 7. Six animals at the low dose and 8 at the mid dose had slightly low weight gains on day 8 but had normal weight gains by day 15.

LD50 -- both sexes = 115 mg/kg (95% confidence limits -- 97-137 mg/kg)
females = 115 (90-148) mg/kg; males = 115 (90-148) mg/kg.

Toxicity category II

Classification: core guideline

This study satisfies the guideline requirements (81-1) for an acute oral toxicity study.

MATERIALS AND METHODS

A. Test Article: Tributyltin benzoate. Purity 97.1 %. Lot 1446-6. Appearance--clear liquid. Expiration date 3/16/94.

B. Animals: Rats. Strain: Hsd/Ola:Sprague-Dawley (CD) from Harlan Olac Ltd., Bicester, Oxon, England. Age: about 4-7 weeks. Weight: 102-131 g.

C. Study design: Animals were randomly assigned to treatment groups. A preliminary study used 2 rats of each sex dosed with 100 mg/kg body weight. The main study used 5 rats/sex/group dosed with 64 or 100 mg/kg on 4/1/92 or with 160 mg/kg on 4/8/92. The test article was prepared at the required concentration in 1% aqueous methylcellulose on the day of dosing. A dose volume of 10 ml/kg was administered by gavage to fasted animals. Animals were observed for mortality and clinical signs at frequent intervals on day 1 and twice a day for fourteen days. Animals were weighed before dosing and on days 8 and 15, or at death. Gross necropsy was conducted on rats that died during the study and on all surviving animals killed on day 15.

D. Statistics: The acute median lethal oral dose and 95 % confidence limits were estimated using the assumption of a fixed slope of 8.2, based on background data.

(This assumption was used because the data did not permit the fitting of a probit line.)

RESULTS

There were no macroscopic abnormalities found in the rats that died or in those killed on day 15. Numbers and times to death are shown in the following table:

GROUPS 5 rats SEX--DOSE mg/kg	DEATH BY DAY day 1	2	3	4	5	6-15
female-- 64						
--100			1			
--160		3	1	1		
male -- 64						
--100				1		
--160		1	2	1	1	

Clinical signs were pilo-erection in all rats starting within five minutes of dosing, hunched posture, and pink extremities for rats at all dose levels. There was a waddling gait in rats dosed at 100 and 160 mg/kg, with ptosis at 160 mg/kg. There were decreased respiratory rate, pallor of extremities, and diarrhea in most rats at all doses. There were slight body weight losses in all rats that died. For surviving rats, slightly low weight gains were recorded on day 8 for 4 males and 2 females at 64 mg/kg and for 4 males and 4 females at 100 mg/kg; these animals had normal weight gains by day 15.
The study was conducted in compliance with GLP regulations.

CONCLUSIONS

Female and male rats were given oral doses of 64, 100, or 160 mg/kg of tributyltin benzoate and observed for 14 days. Some rats in the 100 and 160 mg/kg groups died from day 2 to day 5; these animals had slight weight losses but no abnormalities in gross pathology. Rats displayed pilo-erection, hunched posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of extremities, pink extremities, and diarrhea up to day 6 or 7. Six animals at the low dose and 8 at the mid dose had slightly low weight gains on day 8 but had normal weight gains by day 15.

LD50 for females = 115 mg/kg (95 % confidence limits 90-148 mg/kg).
LD50 for males = 115 mg/kg (95 % confidence limits 90-148 mg/kg).
LD50 for both sexes = 115 mg/kg (95% confidence limits 97-137 mg/kg)

Toxicity category II

Classification: core guideline

This study satisfies the guideline requirements (81-1) for an acute oral toxicity study.

Reviewed by: Patricia McLaughlin, Ph. D. P M Laughlin 11/5/92
Section IV, Toxicology II Branch (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D., Head E A. Doyle 11/13/92
Section IV, Toxicology II Branch

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity (81-2)

TEST MATERIAL: Tributyltin benzoate

P.C. CODE: 83106

MRID NO.: 424158-02

STUDY NO.: 920319D/NDX26/AC

SPONSOR: Huls America Inc.
P.O. Box 365, Turner Place, Piscataway, NJ 08855

TESTING FACILITY: Huntingdon Research Centre Ltd
P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England

TITLE OF REPORT: Acute Dermal Toxicity to Rats of Tributyltin Benzoate

AUTHOR: Sarah A. Allan

REPORT ISSUED: July 3, 1992

CONCLUSIONS: Five female and five male rats were given a single dermal application of tributyltin benzoate at a dose of 2.0 g/kg bodyweight and observed for 15 days. There were no deaths, no signs of systemic reaction to treatment, or macroscopic abnormalities at necropsy. From day 2 to 5, there was slight or well-defined edema at the application sites, and after day 6, scabs and white skin. Slightly low body weights were found in a few rats. The LD50 of females and males was greater than 2.0 g/kg body weight.

Toxicity category III

Classification: core guideline

This study satisfies the guideline requirements (81-2) for an acute dermal toxicity study.

MATERIALS AND METHODS

A. Test Article: Tributyltin benzoate. Purity 97.1 %. Lot 1446-6. Appearance--clear liquid. Expiration date 3/16/94.

B. Animals: Rats. Strain: Hsd/Ola:Sprague-Dawley (CD) from Harlan Olac Ltd., Bicester, Oxon, England. Age: about 7-10 weeks; Weight: 215-239 g.

C. Study Design: Five female and five male rats received a single dermal dose of test compound at 2.0 g/kg bodyweight. About 10 % of the body surface, in the dorso-lumbar region, was shaved one day before treatment. The test substance was spread over an area about 50 mm x 50 mm, which was covered with gauze and a non-irritative dressing around the trunk. After 24 hours, the dressings were removed, the treated area washed with water and blotted dry. Animals were observed frequently on day 1 and twice on each of the following 14 days. Dermal irritation was assessed as erythema, eschar, and edema by a numerical system like that in EPA's subdivision F guideline 81-5. Animals were weighed on days 1, 8, and 15. All animals were killed on day 15 and examined macroscopically.

RESULTS

There were no signs of systemic reaction to treatment, no deaths among the treated rats, and no macroscopic abnormalities at necropsy. Slight or well-defined edema was found at the treated sites on all rats between day 2 and day 5. From day 6, scabs and whitening ot the skin developed in all rats, which prevented assessment of dermal irritation. The dermal reactions resolved at intervals from day 10 to

day 15. Slightly low bodyweight gains were found for four rats (2 f, 2 m) on day 8 and for four rats (1 f, 3 m) on day 15.
The study was conducted in compliance with GLP regulations.

CONCLUSIONS

Five female and five male rats were given a single dermal application of tributyltin benzoate at a dose of 2.0 g/kg bodyweight and observed for 15 days. There were no deaths, no signs of systemic reaction to treatment, or macroscopic abnormalities at necropsy. From day 2 to 5, there was slight or well-defined edema at the application sites, and after day 6, scabs and white skin. Slightly low body weights were found in a few rats.

The LD50 of females and males was greater than 2.0 g/kg body weight.

Toxicity category III

Classification: core guideline

This study satisfies the guideline requirements (81-2) for an acute dermal toxicity study.

Reviewed by: Patricia McLaughlin, Ph. D. P M Laughlin 11/5/92
Section IV, Toxicology II Branch (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D., Head
Section IV, Toxicology II Branch E. A. Doyle 11/13/92

DATA EVALUATION REPORT

STUDY TYPE: Primary skin irritation (81-5)

TEST MATERIAL: Tributyltin benzoate

P.C. CODE: 83106

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MRID NO.: 424158-03

STUDY NO.: 920395D/NDX27/SE

SPONSOR: Huls America Inc.
P.O. Box 365, Turner Place, Piscataway, NJ 08855

TESTING FACILITY: Huntingdon Research Centre Ltd
P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England

TITLE OF REPORT: Skin Irritation to Rabbit of Tributyltin Benzoate

AUTHOR: Michael P. Liggett

REPORT ISSUED: July 1, 1992

CONCLUSIONS: A single dermal dose of 0.5 ml of tributyltin benzoate on intact skin of six rabbits for four hours caused severe dermal reactions with necrosis in all animals.

Toxicity category I

Classification: core guideline

This study satisfies the guideline requirements (81-5) for a primary dermal irritation study.

MATERIALS AND METHODS

A. Test Article: Tributyltin benzoate. Purity 97.1 %. Lot 1446-6. Appearance--clear liquid. Expiration date 3/16/94.

B. Animals: Rabbits. Strain: New Zealand White from Froxfield (U.K.) Ltd., Petersfield, Hampshire, England. Age: about 10-13 weeks. Weight: 2.4 to 2.7 kg.

C. Study Design: Hair was shaved from the dorso-lumbar region of each of six female rabbits. Twenty-four hours later, 0.5 ml of test substance was applied under a 25 mm x 25 mm gauze pad to one intact skin site on each animal. Each site was covered with elastic adhesive dressing for four hours. Then, the dressing and pad were removed; the treatment site was washed with water and blotted dry. The sites were examined about 30 minutes after removal of the test substance, and on each day thereafter. Animals were observed daily for clinical signs. Dermal irritation was assessed for erythema, eschar, and edema by the numerical system in EPA's subdivision F guideline 81-5.

RESULTS

There was slight inflammation of the ears from day 3 to the end of the study, which probably was due to the ears contacting the area of test substance exposure. One female had signs of hyperactivity and brown staining of the muzzle, chest and forepaws from day 3 to 6. After removal of the dressings, there was well-defined erythema with moderate edema. From days 3 through 6, there was well-defined erythema with moderate to severe edema and blanching (scores--erythema 2, edema 4). On day 7, four animals had necrosis, with exudate on one. On day 8, all animals had necrotic lesions extending well beyond the treatment areas (scores--erythema 4, edema 4) and on the back of the ears. The dermal reactions and time of

observation after application of tributyl-tin benzoate are shown for each rabbit in the attached table 1. The dermal responses were scored by the following system:

Erythema	Score	Edema
None	0	None
Very slight	1	Very slight (barely perceptible)
Well-defined	2	Slight (edges raised)
Moderate to severe	3	Moderate (raised about 1 mm)
Severe to slight eschar	4	Severe (raised over 1 mm, extended)

The animals were killed on day 8 due to the severity of the reactions. The study was conducted in compliance with GLP regulations.

CONCLUSIONS

A single dermal dose of 0.5 ml of tributyltin benzoate on intact skin of six rabbits for four hours caused severe dermal reactions with necrosis in all animals.

Toxicity category I

Classification: core guideline

This study satisfies the guideline requirements (81-5) for a primary dermal irritation study.

Sponsor: Hüls America Inc.
920395D/NDX 27/SE

TABLE 1

Dermal reactions observed after application of Tributyltin benzoate

Rabbit number and sex	E = Erythema O = Oedema	Day							
		1*	2	3	4	5	6	7	8
2861♀	E	2	2[A]	2[A]	2[A]	2[A]	2[A]	4[A]	4[C]
	O	3	3	4	4	4	4	4	4
2862♀	E	2	2[A]	2[A]	2[A]	2[A]	2[A]	3[A]	4[C]
	O	3	3	3	3	3	3	3	4
2863♀	E	2	2[A]	2[A]	2[A]	2[A]	2[A]	4[A]	4[C]
	O	3	3	4	4	4	4	4	4
2864♀	E	2	2[A]	2[A]	2[A]	2[A]	2[A]	3[A]	4[C]
	O	3	3	4	4	4	4	4	4
2865♀	E	2	2[A]	2[A]	2[A]	2[A]	2[A]	4[B]	4[C]
	O	3	3	4	4	4	4	4	4
2866♀	E	2	2[A]	2[A]	2[A]	2[A]	2[A]	4[A]	4[C]
	O	3	3	4	4	4	4	4	4

* Approximately 30 minutes after removal of the dressing
A Blanching
B Exudate from dose site
C Not confined to dose area

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FINAL

DATA EVALUATION REPORT

TRIBUTYLTIN BENZOATE

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 Nancy E. McCarroll Date 5/3/93
Nancy E. McCarroll, B.S

Independent Reviewer Lynne Haber Date 5/3/93
Lynne Haber, Ph.D.

QA/QC Manager William L. McLellan for Date 5/3/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-19
Clement Number: 68
Project Officer: Caroline Gordon

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

EPA Reviewer: Patricia McLaughlin, Ph.D.
EPA Review Section IV, Toxicology
Branch II/HED (H-7509C)

Signature: P M Laughlin
Date: 5/5/93

EPA Section Head: Elizabeth Doyle, Ph.D.
EPA Review Section IV, Toxicology
Branch II/HED (H-7509C)

Signature: E A. Doyle
Date: 5/4/93

DATA EVALUATION REPORT

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

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 083106

PC Code: 867AA

MRID Number: 424125-01

TEST MATERIAL: Cotin 310

SYNONYMS/CAS NUMBER: Tributyltin benzoate (TBTB)/4324-30-7

SPONSOR: Hüls America, Inc., Piscataway, N.J.

STUDY NUMBER: 2112-G200-91

TESTING FACILITY: SRI International, Inc., Menlo Park, CA

TITLE OF REPORT: Evaluation of Cotin 310 in the CHO/HGPRT Gene Mutation Assay

AUTHORS: J.P. Bakke and C. J. Rudd

REPORT ISSUED: October 10, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: No definitive conclusions can be reached from the two nonactivated and two S9-activated Chinese hamster ovary (CHO) cell forward gene mutation assays conducted with 0.143-0.35 µg/mL -S9 or 0.33-2.44 µg/mL +S9 Cotin 310. The poor cloning efficiency (CE) at selection for the solvent-treated cells (dimethyl sulfoxide) in the initial nonactivated (32%) and S9-activated (49%) trial as well as the marginally acceptable CEs in the repeat trial (53% -S9; 60% +S9) suggest that assay conditions were not optimal for the detection of a potentially weak mutagen. We are, therefore, unable to determine the relevance, if any of the increased mutation frequencies (MFs) calculated for 0.28 µg/mL Cotin 310 in both nonactivated trials. Purity information on the test material was also not provided.

Based on these considerations, we conclude that the study is unacceptable and recommend that the assay be repeated.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects Category I, Gene Mutations and cannot be upgraded.

A. MATERIALS:

1. Test Material: Cotin 310

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Description: Clear liquid
Identification No.: Not provided
Purity: Not reported
Receipt date: April 5, 1991
Stability: Reported to be stable by the sponsor
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at room temperature, protected from light. The frequency of test material solution preparation was not reported. Analytical determinations to verify actual concentrations used in the study were not performed.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methane sulfonate (EMS) was prepared in an unspecified solvent to yield a final concentration of 200 µg/mL.

Activation (concentrations, solvent): 3-Methylcholanthrene (3-MCA) was prepared in DMSO to yield a final concentration of 5 µg/mL.

3. Activation: S9 derived from adult male Fischer-344

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

The S9 homogenate (lot number 1001) was purchased from Molecular Toxicology, Annapolis, MD. The protein content was determined and found to be 40.7 mg/mL.

S9 mix composition:

Component	Concentration/mL
NADP	4 mM
Glucose 6-phosphate	5 mM
Potassium chloride	30 mM
Magnesium chloride	10 mM
Sodium phosphate buffer	50 mM
Calcium chloride	10 mM
S9 homogenate	5%

One mL of the S9 mix was added to 4 mL of culture medium to yield a final S9 concentration of 1%.

4. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
__x__ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Not reported.

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) 30 µM 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:

- **Trial 1: Thirteen doses (0.4 to 1000 µg/mL) were evaluated with and without S9 activation.**
- **Trial 2: Seven concentration (0.006 to 0.40 µg/mL) were tested with and without S9 activation.**
- **Trial 3: Eleven S9-activated doses (0.64 to 5.96 µg/mL) were assayed.**

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

(1) Nonactivated conditions:

Initial trial: 0.143, 0.179, 0.224, 0.28, and 0.35 µg/mL.

Repeat trial: As above

(2) S9-activated conditions:

Initial trial: 0.33, 0.41, 0.51, 0.64, and 0.80 µg/mL.

Repeat trial: 0.80, 1.00, 1.25, 1.56, 1.95, and 2.44 µg/mL.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, solvent or positive controls for: 4 hours (nonactivated) 4 hours (activated)

(b) After washing, cells were cultured for 7 days (expression period) before cell selection.

(c) After expression, $3x10^6$ cells (~$1x10^6$ cells/plate) were cultured for 14 days in selection medium[a] to determine numbers of mutants and 600 cells (~200 cells/plate) were cultured for 14 days without selection medium[a] to determine cloning efficiency (CE).

Note: Assessment of cytotoxicity after treatment was determined by culturing 500 cells/plate in nonselective cloning medium for 14 days.

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the following criteria were met: (1) the spontaneous mutation frequency (MF) in the negative control should be <60 mutant colonies/10^6 cells; (2) the positive controls induced MFs that were ≥3-fold higher than the solvent control; (3) if the CE for the positive controls was <60%, the induced MFs must be 5-fold higher than the solvent controls.

(b) Positive response: The test material was considered positive if a dose-related increase in the number of mutant colonies and the MFs (at test levels with an initial survival rate of ≥20%) were ≥3-fold higher than the solvent control at one or more concentrations.

4. Protocol: None presented

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[a]Cloning medium contained 0.30% purified agar and 10% fetal bovine serum. The inclusion of agar and serum in the cloning medium allows the plating of a greater number of cells particularly for mutant selection.

C. REPORTED RESULTS:

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1. Preliminary Cytotoxicity Assay: No data were provided from the preliminary cytotoxicity assessment. The study authors stated, however, that the first cytotoxicity assay, which was conducted with 13 nonactivated levels (0.4-1000 µg/mL), was aborted because "most" of the cells detached before the end of treatment. Accordingly, a second cytotoxicity test was performed with a dose range of 0.006 to 0.40 µg/mL +/-S9. The report indicated that the highest nonactivated dose (0.40 µg/mL) reduced the C.E. to ~11% of control; lower nonactivated concentrations were not cytotoxic. There was also no indication of a cytotoxic response in the presence of S9 activation. Based on these data, doses selected for the initial mutation assay ranged from 0.14 to 0.35 µg/mL -S9 and 0.33 to 0.8 µg/mL +S9. However, the failure to achieve an S9-activated dose that resulted in <20% cell survival prompted the performance of a third cytotoxicity test with 11 S9-activated concentrations (0.64-5.96 µg/mL). Results from the third trial indicated that "concentrations of 1.95 µg/mL and higher appeared to increase cell detachment. Therefore, concentrations selected for the second mutagenesis experiment ranged from 0.8 to 2.44 µg/mL."

2. Mutation Assays:

(a) Nonactivated conditions: Results from the initial and repeat nonactivated mutation assays with Cotin 310 are presented in Table 1. In the initial trial, relative survival (RS) following exposure to the highest assayed dose (0.35 µg/mL) was 7%; these cultures were not cloned. For the remaining concentrations, survival was dose dependent and ranged from 90% at the lowest level (0.143 µg/mL) to 17% at 0.28 µg/mL. Our reviewers noted the poor absolute CE (at selection) for the solvent control (32%). Since absolute CEs for negative controls that fall below 50% are suggestive of suboptimal culture conditions[1], our reviewers assess that this trial should have been discontinued. Similarly, since the expected CE for CHO cells grown in soft agar is ~70-90% (Li and Shimizu 1983)[2] and the concentration of DMSO used in the solvent-treated cultures (1%) should have been only marginally cytotoxic, the findings tend to point to procedural problems or cells not growing optimally.

In the repeat trial, the absolute CE (at selection) for the solvent control cultures was improved but was only marginally acceptable (53%). RS for cultures exposed to Cotin 310 showed dose dependency and ranged from >100% at 0.143 µg/mL to 18% at 0.35 µg/mL. Owing to the increased survival at the high dose,

[1]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, L.F., 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.

[2]Li, A.P. and Shimizu, R.W. (1983). A modified agar assay for the quantitation of mutation at the hypoxanthine guanine phosphoribosyl transferase gene locus in Chinese hamster ovary cells. Mutat. Res. 111:365-370.

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TABLE 1 Results from the Nonactivated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with Cotin 310

Substance	Dose/mL	Percent Cloning Efficiency (after treatment)[a] Absolute	Relative	Average Total Mutant Colonies[b]	Average Total Viable Colonies[b]	Absolute Cloning Efficiency (at selection)[a,b]	Mutation Frequency x10^{-6}[c]
Solvent Control							
Dimethyl sulfoxide	1%	69[d]	100	17	189	0.32	18
	1%	56[e]	100	15	317	0.53	9
Positive Control							
Ethyl methanesulfonate	200 µg	36[d]	52	204	183	0.31	219
	200 µg	60[e]	107	142	208	0.35	135
Test Material							
Cotin 310	0.224 µg[f]	23[d]	33	15	195	0.33	15
	0.28 µg	12	17	23	160	0.27	28
	0.35 µg	5	7	--	--	--	
	0.224 µg[f]	52[e]	93	10	267	0.45	7
	0.28 µg	15	27	32	260	0.43	25
	0.35 µg	10	18	21	266	0.44	16

[a]Average counts from duplicate cultures for all groups in Trial 1 and from three replicates for the solvent controls and two replicates for test groups and the positive control in Trial 2. Three nonselective and three selective plates were prepared per culture.

[b]Absolute Cloning Efficiency (CE) (at selection) = $\frac{\text{Average Number of Viable Colonies}}{\text{Number of Cells Plated (600)}}$; calculated by our reviewers.

[c]Mutation Frequency (MF) = $\frac{\text{Average Total Number of Mutants}}{\text{No. of Cells Plated for Selection } (3\text{x}10^6) \text{ x CE}}$; calculated by our reviewers.

[d]Results from the initial assay
[e]Results from the repeat assay
[f]Findings for lower doses (0.143 and 0.179 µg/mL) in both trials did not indicate a mutagenic effect.

Note: Data were extracted from the study report; see CBI pp. 18 and 19.

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there were sufficient cells to plate for mutant selection. MFs calculated for groups exposed to 0.179, 0.28, and 0.35 µg/mL were increased compared to the control; however, the increases were not dose related. The relevance, if any of the ~2.8-fold increase over background at 0.28 µg/mL could not be determined. It was, nevertheless, noted that an ~1.6-fold increase in the MF was calculated for a comparable level of Cotin 310 in the trial rejected by our reviewers.

(b) S9-activated conditions: The initial S9-activated trial was also considered to be invalid by our reviewers because of the low absolute CE (at selection) for the solvent control group (49%). Additionally, the highest assayed concentration (0.8 µg/mL) was not cytotoxic (Table 2). For the repeat test, higher Cotin 310 concentrations (0.80, 1.00, 1.25, 1.56, 1.95, and 2.44 µg/mL) were evaluated. As shown in Table 2, the absolute CE (at selection) for the DMSO group was within acceptable limits. The data further indicated that Cotin 310 levels ≥1.56 µg/mL were severely cytotoxic. For the remaining groups, RS ranged from 91% at 0.80 µg/mL to 13% at 1.25 µg/mL. While there was no clear indication of a mutagenic response at any dose, the ~2-fold increase in the MF at 1.00 and 0.80 µg/mL should be interpreted with caution. MFs at these levels (17 at 1.00 µg/mL and 14 at 0.80 µg/mL) fell within the normal background range for CHO cells (0-20 mutants/10^6 survivors)[3].

Based on the overall results, the study authors concluded that Cotin 310 was not mutagenic in the CHO/HGPRT mutagenesis assay.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the inability to draw meaningful conclusions was probably related to the performance of this study under suboptimal conditions. The poor CEs of the solvent controls in both the initial nonactivated and S9-activated trials supports this assessment. Similarly, while background CEs fell within the normal limits in the repeat trial (53% -S9; 60% +S9), CEs for solvent control groups rarely drop below 70% when assay conditions are optimal. Although cultures responded to both the nonactivated and the S9-activated positive controls, these findings provide no assurance that conditions were optimal for the detection of a potentially weak mutagen. Therefore, the relevance, if any, of the increased MF observed at an equivalent dose in both nonactivated trials cannot be evaluated. The study was further compromised because information on the purity of the test material was not provided. Based on these considerations, we conclude that the study is unacceptable and should be repeated.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated October 10, 1991).

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[3]Li, A.P., et al. (1987). Mutat. Res. 189:135-141.

TABLE 2. Results from the S9-Activated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with Cotin 310

Substance	Dose/mL	Percent Cloning Efficiency (after treatment)[a] Absolute	Relative	Average Total Mutant Colonies[a]	Average Total Viable Colonies[a]	Absolute Cloning Efficiency (at selection)[a,b]	Mutation Frequency x10^{-6}[c]
Solvent Control							
Dimethyl sulfoxide	1%	62[d]	100	9	294	0.49	6
	1%	54[e]	100	12	360	0.60	7
Positive Control							
3-Methylcholanthrene	5 µg	57[d]	92	105	218	0.36	97
	5 µg	44[e]	81	334	314	0.52	214
Test Material							
Cotin 310	0.80 µg[f]	61[d]	98	5	309	0.52	3
	0.80 µg	49[e]	91	28	337	0.56	17
	1.00 µg	30	56	25	345	0.58	14
	1.25 µg[g]	7	13	22	416	0.69	11

[a]Average counts from three replicates for the solvent controls and two replicates for test groups and the positive control in both trials. Three nonselective and three selective plates were prepared per culture.

[b]Absolute Cloning Efficiency (CE) (at selection) = $\frac{\text{Average Number of Viable Colonies}}{\text{Number of Cells Plated (600)}}$; calculated by our reviewers.

[c]Mutation Frequency (MF) = $\frac{\text{Average Total Number of Mutants}}{\text{No. of Cells Plated for Selection } (3\text{x}10^6) \text{ x CE}}$; calculated by our reviewers.

[d]Results from the initial assay
[e]Results from the repeat assay
[f]Findings for lower doses (0.33, 0.41, 0.51, and 0.64 µg/mL in the initial trial) did not indicate a mutagenic effect.
[g]Higher concentrations (1.56, 1.95, and 2.44 µg/mL) were severely cytotoxic and not plated for mutant selection.

Note: Data were extracted from the study report; see CBI pp. 20 and 21.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-15.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-2) for genetic effects Category I, Gene Mutations. The study should be repeated using currently acceptable procedures for the CHO/HGPRT assay.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 8-15

May 11, 1993 Tox Review # 010245

Page _____ is not included in this copy.

Pages 21 through 28 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

FINAL

DATA EVALUATION REPORT

TRIBUTYLTIN BENZOATE

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	Kristin Jacobson Kristin Jacobson, MSPH	Date 4/27/93
Independent Reviewer	Nancy E. McCarroll Nancy E. McCarroll, B.S.	Date 4/27/93
QA/QC Manager	Sharon Segal Sharon Segal, Ph.D.	Date 4/27/93

Contract Number: 68D10075
Work Assignment Number: 2-19
Clement Number: 69
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY
MICRONUCLEUS

MUTAGENICITY STUDIES

EPA Reviewer: Patricia McLaughlin, Ph.D.
Review Section IV, Toxicology Branch II
Health Effects Division (H-7509C)
Signature: P M Laughlin
Date: 5/5/93

EPA Section Head: Elizabeth Doyle, Ph.D.
Review Section IV, Toxicology Branch II
Health Effects Division (H-7509C)
Signature: E.A. Doyle
Date: 5/4/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

EPA IDENTIFICATION NUMBERS:

Tox Chem. Number: 867AA

PC Code: 083106

MRID Number: 424125-02

TEST MATERIAL: Cotin 310

SYNONYM/CAS NUMBER: Tributyltin benzoate (TBTB)/4324-30-7

SPONSOR: Huls America, Inc., Piscataway, NJ

STUDY NUMBER: 2112-C100-91

TESTING FACILITY: SRI International, Menlo Park, CA

TITLE OF REPORT: Bone Marrow Erythrocyte Micronucleus Assay of Cotin 310 in Swiss-Webster Mice

AUTHOR: K.G. O'Loughlin

REPORT ISSUED: June 24, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: The oral gavage administration of 50, 100 or 200 mg/kg/day Cotin 310 to male or female mice for 2 consecutive days did not cause a significant increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) in bone marrow cells harvested 24 or 48 hours after the final dosing. Mortality (1 of 6 animals) occurred in mice exposed to 200 mg/kg/day in the range-finding study; no deaths occurred in the micronucleus test. Clinical signs of toxicity, including rough fur and humped backs, were observed in both the range-finding and definitive micronucleus assays at levels ≥100 mg/kg/day. There was also suggestive evidence of a dose-dependent suppression of the ratio of polychromatic erythrocytes (PCE) to total erythrocytes (RBC) (i.e., PCE/RBC) in the bone marrow of treated animals. It was concluded, therefore, that Cotin 310 was tested over an adequate range of doses approaching the maximum tolerated dose (MTD) and was

found to be nongenotoxic in the mouse micronucleus assay. Although the assay was performed in a technically acceptable manner, the study is incomplete because information on the purity of the test material was not provided.

STUDY CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations, but can be upgraded if the missing test material purity information is provided.

A. MATERIALS:

1. Test Material: Cotin 310

Description: Clear, colorless liquid
Identification numbers: Range-finding study: Lot number 1446-06
Micronucleus assay: Lot number not reported
Purity: Not reported
Receipt dates: Range-finding study: August 22, 1990
Micronucleus assay: April 25, 1991
Stability: Reported by the sponsor to be stable in absence of heat or open flame
Contaminants: Not reported
Vehicle used: Corn oil
Other provided information: The test material was stored at room temperature, protected from light. Dosing solutions were prepared immediately prior to testing. Analytical determinations were not performed on test solutions.

2. Control Materials:

(a) Acute dose range-finding study:

Vehicle/route of administration: Mice received corn oil via oral gavage at a dosing volume of 10 ml/kg/day for 2 consecutive days.

(b) Cytogenetic assay:

Vehicle/route of administration: Corn oil was administered by oral gavage in 10 mL/kg dosing volumes in two doses, 24 hours apart.

Positive/final dose/route of administration: Benzene, at a final dose of 500 mg/kg/day, was prepared in corn oil and administered via oral gavage.

3. Test Compound:

Route of administration: Oral gavage (2 administrations separated by 24 hours)

Volume of test substance administered: 10 mL/kg

Dose levels used:

(a) Acute dose range-finding study: 25, 50, 100, 200 and 400 mg/kg/day

(b) Micronucleus assay: 50, 100 and 200 mg/kg/day

4. Test Animals:

(a) Species: Mouse Strain: Swiss-Webster Age: ≈15-20 weeks (at dosing) Weight Range (means): Acute study: 28.7-33.0 g (males); 24.6-30.3 g (females); Micronucleus assay: 33.1-38.8 g (males); 26.3-28.1 g (females) Sex: Males and females Source: Charles River Laboratories, Inc., Portage, MI.

(b) Number of animals used per dose:
Acute study: 3 (males); 3 (females) /treatment group

Micronucleus assay (primary dose groups/sacrifice time):

- Treatment groups: 5 males 5 females
- Positive control: 5 males 0 females
- Vehicle control: 5 males 5 females

Note: A secondary high-dose group (5 males and 5 females exposed to 200 mg/kg) was included in the study, for use in the event of mortality in the primary high-dose groups. None of these secondary animals were needed and they were sacrificed at study completion.

(c) Were test animals properly maintained? Yes. Although humidity in the animal rooms (ranging from 10% to 65%) was outside the range recommended by NIH guidelines (40-70%), and temperature in the animal rooms (62.6-77.0°F) was briefly outside of the recommended range (64.4-78.8°F), these deviations were judged to have not adversely affected the outcome of the study.

B. TEST PERFORMANCE:

1. Acute Dose Range-finding Study: The selected doses of Cotin 310 were administered once a day for 2 days via oral gavage. Animals were observed for signs of toxicity and/or mortality for 48 hours after the second exposure, after which time all animals were sacrificed and weighed. Peripheral blood (from tail prick samples) and bone marrow (one femur per animal) were evaluated for cytotoxicity by determining the ratio of polychromatic erythrocytes (PCE) to the total number of erythrocytes (RBC).

2. Micronucleus Assay:

Treatment and sampling times:

1. Test compound:
Dosing: ____ once __x__ twice (24 hours apart)
Sampling (after last dose): _____ 6 hours _____ 12 hours
__x__ 24 hours __x__ 48 hours _____ 72 hours

2. Vehicle control:
Dosing: ____ once __x__ twice (24 hours apart)
Sampling (after last dose): __x__ 24 hours __x__ 48 hours
_____ 72 hours

3. Positive control:
Dosing: ____ once __x__ twice (24 hours apart)
Sampling (after last dose): __x__ 24 hours __x__ 48 hours
_____ 72 hours

3. Tissues and Cells Examined:

__x__ bone marrow __x__ other (list): peripheral blood
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Number observed while scoring 200 erythrocytes (PCEs+NCEs)

4. Details of Cell Harvest and Slide Preparation: At 24 and 48 hours after the final administration of the test material, vehicle or positive control, the appropriate groups of animals were sacrificed by cervical dislocation. Peripheral blood smears were made from animals used in the range-finding study, and bone marrow cells were harvested from the animals in the preliminary study and the micronucleus test. The bone marrow from one femur of each animal was aspirated, mixed with fetal bovine serum, centrifuged, resuspended and spread onto slides. Slides were air-dried, fixed with methanol and coded. One slide per animal was stained with acridine orange, coded and scored for micronucleated PCEs (MPEs), total PCEs, and total erythrocytes.

Note: When stained with acridine orange, PCEs will uniformly fluoresce bright orange and are referred to as RNA^+ erythrocytes; NCEs will not take up the acridine orange and are referred to as RNA^- erythrocytes; MPEs are RNA^+ erythrocytes with a visible chromatid structure.

5. Statistical Methods: The micronucleus frequency data were evaluated for statistical significance ($p<0.05$) using a conditional binomial test, as tabulated by Kastenbaum and Bowman (1970)[1]. The Cochran-

[1]Kastenbaum, M.A. and Bowman, K.O. (1970) Tables for determining the statistical significance of mutation frequencies. Mutat Res 9:527-549.

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MICRONUCLEUS

Armitage trend test was used to evaluate MPE frequency dose-response relationships. The ratio of PCEs to total RBCs was evaluated using the Kruskall-Wallis non-parametric analysis of variance. Mean body weights were analyzed using Student's t-test. Males and females were analyzed separately.

6. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if (1) the frequency of MPEs for the vehicle control group was within an unspecified normal historical control range; (2) the positive control induced a significant increase in MPEs; and (3) there were at least three surviving animals per sex with a ratio of RNA^+ to total erythrocytes ≥0.1 in two or more treatment groups.

(b) Positive response: The test material was considered positive if the frequency of MPEs was significantly ($p<.05$) increased in two dose groups, or if a positive, dose-related increase in MPEs was seen.

7. Protocol: Not provided

C. REPORTED RESULTS:

1. Acute Dose Range-finding Study: Mortality following the two oral gavage administrations of the selected doses of Cotin 310 in the range-finding study was as follows:

	Number Dead/Number Tested		
Dose (mg/kg/day)	Males	Females	Combined
25	0/3	0/3	0/6
50	0/3	0/3	0/6
100	0/3	0/3	0/6
200	1/3	0/3	1/6
400	1/3	3/3	4/6

As the above data show, all females and one of three males in the 400-mg/kg/day group, and one of three males at 200 mg/kg/day died prior to sacrifice. Clinical signs of toxicity in moribund animals included rough fur, greasy fur near the tail, humped back, slow movement, and labored breathing. No deaths occurred in animals receiving ≤100 mg/kg/day. All animals surviving treatment with ≥100 mg/kg/day exhibited rough fur, greasy fur near tail, and humped backs; several animals from the lower dose groups also exhibited greasy fur near the tail or rough fur. Reductions in final body weight of animals surviving treatment was ≥10% in males at 400 mg/kg/day (82% of initial weight) and females at 200 mg/kg/day (89% of initial weight).

The study author reported that cytotoxicity, as indicated by a suppression in the mean PCE:RBC ratio, occurred in both peripheral blood and bone marrow samples of surviving males and females at dose levels ≥200 mg/kg/day. However, suppression of the PCE:RBC ratio in peripheral blood samples, which occurred only in males in the 200-mg/kg/day group, was not considered by our reviewers to be biologically significant because it was not dose dependent. Similarly, the bone marrow data suggest a cytotoxic response in surviving males of the 200- and 400-mg/kg/day dose groups; however, the effect was not dose related. In females administered 200 mg/kg/day, the slightly reduced PCE:NCE ratio did not provide definitive evidence of an adverse effect on erythropoiesis.

Based on the observed mortality at 400 mg/kg/day, the study author determined that the maximum tolerated dose (MTD) was approximately 200 mg/kg/day; accordingly, the doses selected for the micronucleus assay were 50, 100, and 200 mg/kg/day.

2. Micronucleus Assay: No deaths were reported following administration of the three selected doses of Cotin 310. Clinical signs similar to those seen in the range-finding test (i.e., rough fur and humped backs) were observed in all animals exposed to levels ≥100 mg/kg/day; also, approximately 50% of the animals in the high-dose group had greasy fur near the tail region. There were no appreciable changes in body weight of any dose groups.

   Representative results from the micronucleus assay are presented in Table 1. Reduced PCE:RBC ratios, suggestive of cytotoxicity in the target cells, was noted in both males and females of all treatment groups; the effect also appeared to be dose related. However, these PCE/RBC ratios were within the normal range for bone marrow cells (i.e. 40-50%)[2]. There were no significant increases in the frequency of MPEs in either sex at any dose level. By contrast, the frequency of MPEs in mice exposed to the positive control (500 mg/kg benzene) was significantly increased ($p<0.05$).

Based on the overall results, the study author concluded that Cotin 310 was not genotoxic in this in vivo mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: The range of Cotin 310 doses that were investigated (50, 100, and 200 mg/kg/day) produced signs of slight toxicity and possible cytotoxicity but did not result in an increase in the frequency of micronuclei in bone marrow erythrocytes. Although our reviewers disagree with the study author's statement that the highest dose tested, 200 mg/kg/day, represented the MTD, we believe that the selected high dose approached the MTD. Therefore the data provide sufficient

[2]Mavournin, K.H., Blakey, D.H., Cimino, M.C., Salamone, M.F. and Heddle, J.A. (1990) The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 239:29-80.

Table 1. Representative Results of the Micronucleus Assay in Mice with Cotin 310

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean Percent MPEs ± S.E.	Mean Percent PCE/RBC ± S.E.
Vehicle Control								
Corn oil	10 mL	24	M	5	5090	11	0.22 ± 0.06	52.30 ± 1.64
		48	M	5	5076	10	0.20 ± 0.04	50.79 ± 1.43
	10 mL	24	F	5	5065	7	0.14 ± 0.02	53.15 ± 1.69
		48	F	5	5070	10	0.20 ± 0.03	52.32 ± 1.23
Positive Control[b]								
Benzene	500 mg	24	M	5	5099	115	2.25 ± 0.28*	48.21 ± 1.20
		48	M	5	5084	63	1.24 ± 0.23*	51.02 ± 1.73
Test Material								
Cotin 310	200 mg[c]	24	M	5	5080	9	0.18 ± 0.06	47.14 ± 2.05
		48	M	5	5091	10	0.20 ± 0.07	44.94 ± 1.04
	200 mg[c]	24	F	5	5064	6	0.12 ± 0.04	47.74 ± 2.36
		48	F	5	5062	11	0.22 ± 0.02	48.65 ± 2.01

[a]Time after final compound administration by oral gavage for two consecutive days
[b]Positive control groups consisted of males only
[c]Signs of toxicity in males and females exposed to ≥100 mg/kg/day included rough fur and humped backs. No clastogenic effects were seen in the low- (50 mg/kg) or mid- (100 mg/kg) dose groups.

*Significantly higher ($p<0.05$) than the corresponding vehicle control by conditional binomial test.

Abbreviations used: PCE = polychromatic erythocyte; MPE = micronucleated polychromatic erythrocyte; RBC = total red blood cells (polychromatic + normochromatic erythrocytes)

Note: Data were extracted from study report, pp. 29-30.

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evidence that Cotin 310 was adequately tested and found to be nonclastogenic in this test system. In addition, the sensitivity of the assay to detect a genotoxic response was demonstrated by the significant ($p<0.05$) results obtained in male mice exposed to the positive control (500 mg/kg/day benzene). We conclude, therefore, that the study was technically sound. However, the study is incomplete because information on the purity of the test material was missing.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated June 24, 1991.)

F. STUDY APPENDIX: Appendix A, Materials and Methods, Study pp. 10-20.

CORE CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations but can be upgraded if the missing test material purity information is provided.

APPENDIX A

MATERIALS AND METHODS
Study pp. 10-20

May 11, 1993 Tox Review # 010245

Page _____ is not included in this copy.

Pages 39 through 49 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

FINAL

DATA EVALUATION REPORT

TRIBUTYLTIN BENZOATE

Study Type: Mutagenicity: Unscheduled DNA Synthesis 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 Lynne T. Haber, Ph.D. Date 4/29/93

Independent Reviewer Nancy E. McCarroll, B.S. Date 4/27/93

QA/QC Manager Sharon Segal, Ph.D. Date 4/29/93

Contract Number: 68D10075
Work Assignment Number: 2-19
Clement Numbers: 70 and 71
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY
UDS

MUTAGENICITY STUDIES

EPA Reviewer: Patricia McLaughlin, Ph.D. Signature: P. McLaughlin
Review Section IV,
Toxicology Branch II/HED (H7509C) Date: 5/5/93
EPA Section Head: Elizabeth Doyle, Ph.D. Signature: E. A. Doyle
Review Section IV,
Toxicology Branch II/HED (H7509C) Date: 5/4/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION Numbers:

PC Code: 083106

Tox Chem. Number: 867AA

MRID Numbers: 424125-03 and 424125-04

TEST MATERIAL: Cotin 310

SYNONYMS/CAS NUMBER: Tributyltin benzoate (TBTB)/4324-30-7

SPONSOR: Hüls America, Inc., Piscataway, NJ

STUDY NUMBER: 1482-V01-90

TESTING FACILITY: SRI International, Menlo Park, CA

TITLES OF REPORTS: **Evaluation of the Potential of Cotin 310 to Induce Unscheduled DNA Synthesis in the In Vitro Hepatocyte DNA Repair Assay Using the Male F-344 Rat, and Evaluation of the Potential of Cotin 310 to Induce Unscheduled DNA Synthesis in the In Vitro Hepatocyte DNA Repair Assay Using the Male F-344 Rat, Amendment One.**

AUTHORS: **Bakke, J. P., Mirsalis, J.C., and Sutherland, R.M.**

REPORTS ISSUED: **November 30, 1990 and July 14, 1992**

CONCLUSIONS-EXECUTIVE SUMMARY: No conclusions can be reached from the parallel primary rat hepatocyte unscheduled DNA synthesis (UDS) assays conducted with Cotin 310 at doses ranging from 0.005 µg/mL to 25 µg/mL. Levels ≥10 µg/mL were cytotoxic. Net nuclear grain counts for the medium and solvent controls were unusually low in both trials and ranged from -4.2 to -6.8 (medium) and from -8.8 to -10.2 (DMSO). Although the study author stated that the results for the solvent controls fell within historical ranges, no data were provided to support this statement. Since net nuclear grain counts

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UDS

for negative controls typically range from -2 to 1[1], our reviewers assess that technical problems with the staining procedure were the probable cause of the net nuclear grain counts being lower than expected from the literature. Therefore, the relevance, if any, of the decreasing negative values, particularly in the repeat trial, for cultures exposed to 1, 2.5, or 5 µg/mL could not be determined. Similarly, the significance of the increased percentage of cells in repair at 2.5 and 5 µg/mL (initial trial) and at 1 and 5 µg/mL (repeat trial) cannot be evaluated. The decreasing negative net nuclear grains calculated for these levels might be attributable to genotoxicity, but it is equally probable that cytotoxicity accounts for these results. Without the primary data, our reviewers are unable to determine whether the results are indicative of a genotoxic or cytotoxic response. In addition, the study was compromised by the absence of purity information on the test material.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-4) for genetic effects Category III, Other Mutagenic Mechanisms.

A. MATERIALS:

1. Test Material: Cotin 310

   Description: Liquid at room temperature
   Identification number: Lot number: 1446-06
   Purity: Not reported
   Receipt date: August 22, 1990
   Stability: Reported by the sponsor to be stable.
   Contaminants: None listed
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: Stored at room temperature protected from light. Solutions of the test material were prepared immediately prior to use. Analytical determinations were not performed to verify actual concentrations used in the study.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the livers of male Fischer-344 rats purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN.

3. Control Substances: WME (Williams' Medium E with 2 mM L-glutamine and gentamycin) was the negative control and DMSO (1%) was the solvent control. The positive control was 2-acetylaminofluorene (2-AAF) prepared at 3 µg/mL in DMSO. A second, unspecified concentration of 2-AAF was also tested but not scored.

4. Medium: WME; WME+: WME with 10% fetal bovine serum.

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[1]Probst, G.S., McMahon, R.E., Hill, L.E., Thompson, C.Z., Epp, J.K., and Neal, S.B. (1981). "Chemically-Induced DNA Synthesis in Primary Rat Hepatocyte Cultures: A Comparison With Bacterial Mutagenicity Using 218 Compounds." Environ. Mutagen. 3:11-32.

5. Test Compound Concentrations Used:

(a) First UDS assay: The ten doses evaluated initially were 1, 5, 10, 25, 50, 100, 250, 500, 750, and 1000 µg/mL; slides were not scored due to cytotoxicity at all but the two lowest dosage levels.

(b) Second and third UDS assays: Ten doses of the test material (0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, and 25 µg/mL) were tested. Mechanical failure of the incubator prevented completion of these experiments.

(c) Fourth and fifth UDS assays: Ten doses of the test material (0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, and 25 µg/mL) were evaluated; cells treated with 0.05, 0.1, 0.5, 1, 2.5 and 5 µg/mL were scored.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion techniques: Rats (one/experiment) were anesthetized with sodium pentobarbital and the livers were perfused with an unspecified collagenase solution. Livers were combed to release hepatocytes.

(b) Hepatocyte harvest/culture preparation: Recovered cells were seeded at an unspecified cell density onto coverslips in 6-well culture dishes containing WME+, and allowed to attach for 1.5-2 hours in a 37°C, CO_2 incubator. Unattached cells were removed; viable cells were fed WME containing 10 µCi/mL [^{3}H] thymidine.

2. UDS Assay:

(a) Treatment: Triplicate cultures were exposed to each of the selected test material doses, the negative control (medium), the solvent control (DMSO), or the positive control (2-AAF), for ≈19 hours. Treated hepatocytes, attached to coverslips, were washed, exposed to 1% sodium citrate, fixed in acetic acid:ethanol (1:3) and mounted.

(b) Preparation of autoradiographs/grain development: Slides were coated with Kodak NTB-2 emulsion, exposed for 7 days at -20°C, developed and stained with 1% methyl-green Pyronin Y.

(c) Grain counting: Slides were visually evaluated for cytotoxicity (dead or morphologically altered cells) before being coded and counted. The nuclear grains of at least 90 morphologically normal cells (30/coverslip) from a randomly selected area of each slide were counted. Cytoplasmic background counts were determined by counting two nuclear-sized areas adjacent to the nucleus. Net nuclear grain counts were determined by subtracting the higher

cytoplasmic background count from the nuclear grain count. The percentage of cells in repair (cells with ≥5 net nuclear grains) was also determined.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the solvent control data were within unreported historical ranges, and if both mean net nuclear grain counts and the percent of cells with ≥5 net nuclear grains (percent in repair) were significantly elevated for the positive control. The definition of significance was not provided; no statistical methods were used.

(b) Positive response: The test material was considered positive if it induced a mean net nuclear grain count of >5 grains/nucleus at any dose.

4. Protocol: A protocol was not included with the study report.

C. REPORTED RESULTS: Three unsuccessful assays were conducted; no data were furnished from these three aborted trials. The first unsuccessful assay evaluated ten levels ranging from 1 µg/mL to 1000 µg/mL. The study authors stated that since cytotoxicity was observed in the initial assay at concentrations ≥10 µg/mL and only two doses were available for evaluation, slides were not prepared. Based on these findings, two parallel experiments were performed with ten doses ranging from 0.005 µg/mL to 25 µg/mL. Trials 2 and 3 were also aborted, because of mechanical difficulties with the incubator. Accordingly, two additional parallel trials were undertaken with a concentration range similar to that attempted in Trials 2 and 3.

In the first successful assay, doses ≥10 µg/mL were severely cytotoxic and not scored. One of three cultures in the 2.5- and 5-µg/mL groups was reported to be unscorable because of a high proportion of pyknotic nuclei and sparse cells. Therefore, our reviewers assumed that 60 cells/group were scored from cultures treated with 2.5 or 5 µg/mL Cotin 310. However, we are unable to verify this assumption since the number of scored cells was not indicated on the summary table and primary data were not submitted. In the repeat trial, levels ≥10 µg/mL were severely cytotoxic. All cultures exposed to 5 µg/mL appeared normal, but "some" pyknotic nuclei were observed in one culture at 2.5 µg/mL. Summarized results from the two completed UDS assays with Cotin 310 are presented in Table 1.

As shown, the number of net grains in the negative and solvent controls for both trials was very low. Net nuclear grain counts for negative control hepatocyte cultures typically range from -2 to 1[2], but the values for these assays ranged from -4.2 to -6.8 (medium) and from -8.8 to -10.2 (DMSO). Owing to these low values, the relevance, if any, of the decreasing negative values for the highest scored level in the initial

[2]Probst, G.S. et al. (1981). Environ. Mutagen. 3:11-32.

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trial (5 µg/mL) and the three highest scored doses in the confirmatory trial (1, 2.5, and 5 µg/mL) was not clear. Our reviewers further noted the increased percentage of cells in repair at 2.5 and 5 µg/mL (initial trial) and at 1 and 5 µg/mL (repeat trial). However, the increase was not dose related.

Based on the overall results, the study authors concluded that Cotin 310 was negative in the primary rat hepatocyte UDS assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that no conclusions can be reached from the two UDS assays conducted with Cotin 310. The low net nuclear grain counts for the medium and solvent controls in both trials renders the findings with the test material uninterpretable. Although the study author stated that the results for the solvent controls fell within historical ranges, no data were provided to support this statement. We assess, however, that technical problems with the staining procedure were the probable cause of the net nuclear grain counts being lower than expected from the literature[3]. Therefore, the relevance, if any, of the decreasing negative values, particularly in the repeat trial, for cultures exposed to 1, 2.5, or 5 µg/mL could not be determined. It is conceivable that the decreasing negative net nuclear grains calculated for these levels can be attributed to genotoxicity, but it is equally probable that cytotoxicity accounts for these results. However, without the primary data, our reviewers are unable to determine whether the data are indicative of a genotoxic or cytotoxic response. The study was further compromised by the absence of purity information on the test material.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (Quality assurance statements for the two reports were signed and dated November 28, 1990, Study Report Number 1482-V01-90 and July 24, 1991, Study Amendment 1.)

F. APPENDICES: Appendix A, Materials and Methods, Study Report Number 1482-V01-90, pp. 8-18, and Study Amendment One, p. 5.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-4) for genetic effects Category III, Other Mutagenic Mechanisms.

[3]Probst, G.S. et al. (1981). Environ. Mutagen. 3:11-32.

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TABLE 1. Results of the Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assays with Cotin 310

Treatment	Dose/mL	Initial Trial		Confirmatory Trial	
		Net Nuclear Grains±S.E.[a]	Percent cells with ≥5 Net Nuclear Grains	Net Nuclear Grains±S.E.[a]	Percent cells with ≥5 Net Nuclear Grains
Negative Control					
Culture medium	--	-6.8±0.5	0	-4.2±1.0	4
Solvent Control					
Dimethyl sulfoxide	1%	-8.8±2.0	1	-10.2±1.5	4
Positive Control					
2-Acetylaminofluorene	3 µg	19.7±4.3	83	22.6±2.4	89
Test Material					
Cotin 310	0.05 µg[b]	-13.5±0.7	0	-8.5±1.1	3
	0.1 µg	-9.3±0.7	0	-10.1±0.4	3
	0.5 µg	-9.7±2.0	1	-10.8±1.5	1
	1 µg	-8.9±2.4	1	-7.0±2.5	7
	2.5 µg	-9.1±1.9[c]	9	-8.6±1.5	2
	5 µg[d]	-5.2±0.8[c]	8	-5.0±1.2	6

[a]Means and standard errors of the count of 90 cells; thirty cells from triplicate slides were analyzed.

[b]Lower doses (0.005 and 0.01 µg/mL in both assays) were not scored.

[c]Severe cytotoxicity was observed in one of three cultures; only two cultures (presumably 30 cells/culture) were scored.

[d]Higher doses (10 and 25 µg/mL in both trials) were cytotoxic.

Note: The data were extracted from the study report, p. 20.

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APPENDIX A

MATERIALS AND METHODS
Study Report Number 1482-V01-90, pp. 8-18,
Study Amendment One, p. 5

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