US EPA ARCHIVE DOCUMENT

3-25-86

005013

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Toxicology Studies on ASSURE Reviewed by Carolyn Greggorio

FROM: Whang Phang, Pharmacologist 3/25/86
Toxicology Branch
Hazard Evaluation Division (TS-769c)

TO: Robert Taylor, PM #25
Registration Division (TS-767c)

Thru: Alan Katz, Toxicologist 3/25/86
Acting Head, Section III
and
Theodore M. Farber, Ph. D.
Branch Chief
Toxicology Branch / HED (TS-769c)

Chemical: ASSURE (DPX-Y6202)

Petitioner: E. I. Dupont de Nemours and Company, Inc.

Carolyn Gregorio has evaluated and summarized the findings of the following studies on ASSURE:

1.) Acute dermal toxicity study,
2.) Acute oral toxicity study,
3.) Inhalation median lethal concentration (LC_{50}) of INY-6202-15,
4.) Screening test for delayed contact hypersensitivity with NC-302 technical product in the albino guinea pig,
5.) Irritant effects of NC-302 technical product on rabbit skin, and
6.) Irritant effects of NC-302 technical product on rabbit eye mucosa.

In the course of arranging her leave from EPA, she has not had the opportunity to sign her detailed reviews. However, Clint Skinner, who is both a secondary reviewer and section head, has signed these reviews.

1/30

Chemical: ASSURE (DPX-Y6202) 005013

Caswell No.: 215D

Formulation: Technical (99.1% Active Ingredient, Batch No. 800L)

Citation: Acute Dermal Toxicity Study of NC-302 in Rats. Conducted by Nippon Experimental Medical Research Institute. Submitted by E.I. du Pont de Nemours. Study No. not reported. Study Director Ryuicho Sato, M.D.; January 30, 1981.

Petitioner: E.I. du Pont de Nemours

Accession No.: 073530

Reviewed by: Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769)

Secondary Review: Clint Skinner, Ph.D.
Section Head
Toxicology Branch/HED (TS-769)

Clint Skinner 3-20-86 concurs 4/1/86

Material and Methods: Young adult 4-week-old Sprague Dawley rats (10/sex) were clipped free of hair in an area of the skin approximately 4 x 5 cm^2 on the back using electric clippers. The test compound was applied as a single dermal dose of 2500 or 5000 mg/kg without dilution. The test substance was described as "a white crystalline" substance. The site of application was covered for 24 hours with gauze and taped firmly around the trunk of the rat. Animals were observed for clinical signs of toxicity and mortality for 14 days after application. No body weights were reported. Gross necropsy was performed on all animals.

(ack) Reported Results: No signs of clinical toxicity were observed for any animal and no deaths were reported. No dermal irritation was observed for any animal.

Gross necropsy revealed no treatment related pathology.

Conclusion: Under the conditions of this study, the dermal LD_{50} is greater than 5000 mg/kg for males and females.

Toxicity Category: IV

Core Classification: Minimum

005013

Chemical: ASSURE (DPX-Y6202)

Caswell No.: 215D

Formulation: Technical (99.1% Active Ingredient, Batch No. 8002)

Citation: Acute Oral Toxicity Study of NC-302 in Rats. Conducted by Nippon Experimental Medical Research Institute. Submitted by E.I. du Pont de Nemours. Study No. not reported. Study Director Ryuicho Sato, M.D.; January 30, 1981.

Petitioner: E.I. du Pont de Nemours

Accession No.: 073530

Reviewed by: Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769)

Secondary Review: Clint Skinner, Ph.D. Clint Skinner
Section Head 3-22-86
Toxicology Branch/HED (TS-769)

Materials and Methods: Young (4-week-old) Sprague Dawley rats (10/sex/dose) were fasted overnight prior to dosing. The test compound was administered as a single dose of NC-302 as a 30% (weight/volume) solution in corn oil by oral intubation. The doses used are as follows: 833, 1000, 1200, 1440, 1728, 2074, or 2488 mg/kg

The animals were observed for clinical signs for 14 days. Body weights were not reported. Gross necropsy was performed on all animals.

LD_{50} calculations were calculated by Litchfield and Wilcoxon's methods.

Reported Results: "Decreased spontaneous movement and crouching were observed at all doses for approximately 10 hours after administration of 833, 1000, 1200, 1440, and 1728 mg/kg doses." Animals dosed at 2074 on 2488 mg/kg displayed "a dose-related increase in severity of clinical manifestation and in mortality." "Before death, they manifested a continuous prone position. A decrease in response to external stimulation (sound and light), the disappearance of righting reflex, ruffled hair, weak or slow respiration, and wasting." Surviving rats in these dose groups showed alleviation of these symptoms by day 3 after dosing.

005013

Gross necrospy or animals that died on study showed congested liver, kidneys, small intestines, and lungs. Gross necropsy of surviving animals revealed lungs with "red flecks accompanied with petechial hemorrhages of various size."

All deaths occurred between day 3 and day 6 after dosing. Mortality data are presented below (Table 1):

Table 1. Mortality Data for Rats given ASSURE NC-302

Males		Females
Dose mg/kg	# Died/Dosed	# Died/Dosed
833	0/10	0/10
1000	1/10	2/10
1200	2/10	2/10
1440	3/10	4/10
1728	5/10	6/10
2074	7/10	8/10
2488	10/10	10/10

Note: This table is reproduced from the Registrant's submission.

Conclusion: This study indicates the following:

Acute Oral LD_{50} (males) = 1670 mg/kg (1438-1939)
Acute Oral LD_{50} (females) = 1480 mg/kg (1310-1672)

Toxicity Category: III

Core Classification: Minimum

Chemical: ASSURE (DPX-Yo202)

Caswell No.: 215D

Formulation: Technical (99.1% Purity; Batch No. not reported)

Citation: Inhalation Median Lethal Concentration (LC_{50}) of INY-6202-15 by EPA Protocol. Conducted by Haskell Lab. Report No. 399-83. MR No. 4581-106. Submitted by E.I. du Pont de Nemours, October 17, 1983.

Petitioner: E.I. du Pont de Nemours

Accession No.: 073530

Reviewed by: Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769)

Secondary Review: Clint Skinner, Ph.D.
Section Head
Toxicology Branch/HED (TS-769)
Clint Skinner 3-20-84

Material and Methods: Young adult albino rats (CRL:CD) were restrained in perforated, stainless steel holders and exposed nose-only for 4 continuous hours followed by a 14-day observation period. The test dust atmosphere was generated by a 3-stage, hour-glass system and dust concentration was monitored by varying the 3 airflows. "At 30-minute intervals, calibrated volumes of chamber atmosphere were drawn through pre-weighed glass-fiber filters. Atmospheric concentration of test material was determined from filter weight gain." Particle size distribution was conducted for each exposure using a Sierra Cascade Impactor. Animals were observed for clinical changes and pathology was performed.

The rats (10/sex/dose) were exposed to the following concentrations of ASSURE: 2.0, 3.4, 4.8 or 5.9 mg/L.

Results: Particle size analysis demonstrated 70 to 90 percent of the particles were less than or equal to 2.7 to 3.1 microns; definitely within the respirable range for rats.

Clinical signs of toxicity noted during the study: "Control rats (male and female) exhibited slight red nasal discharge during exposures and slight weight loss for 1 day postexposure, effects common to rats under restraint. During exposures, rats exposed to 3.4 mg/L exhibited reddish-brown nasal discharge. Observations could not be made at higher concentrations due to the dense cloud in the chamber."

"In a dose-dependent manner, all exposed rats exhibited continuous slight to moderate weight loss for 2-9 days postexposure. Deaths occurred from 5-8 days postexposure. Surviving rats resumed normal weight gain. At all concentrations, exposed rats exhibited hair loss from the head and body. At concentrations greater than 2.0 mg/L, rats exhibited diarrhea and/or wet, stained perineal area, pallor, hunched posture, ruffled fur and dry red nasal discharge for 1-13 days postexposure. In addition, rats felt cold for 2-5 days postexposure. At 4.8 and 5.4 mg/L, a few rats had cloudy eyes for 2-14 days post exposure. At 5.4 mg/L alone, a low incidence of ocular discharge and partially closed eyes was observed throughout the recovery period.

"No compound-related effects were seen in rats which survived for 14 days postexposure (control and test). Rats which died exhibited lymphoid cell depletion in the spleen and thymus, lung edema, and lipid-like cytoplasmic vacuolation in hepatocytes. All other observations were considered to be artifacts of tissue preparation or to be due to natural causes."

All deaths occurred within 5 to 8 days of exposure. Mortality data are presented below (Table 1):

Table 1. Mortality Data For Rats Exposed to 4-Hour Inhalation

Concentration (mg/L)	Mortality (# deaths/# exposed)	
	Males	Females
2.0	0/10	0/10
3.4	2/10	2/10
4.8	1/10	8/10
5.9	4/10	4/10

Conclusion: Based on the conditions of this study, the inhalation LC_{50} (Probit Analysis) of ASSURE is 4.8 to 5.8 mg/L.

Toxicity Category: III

Core Classification: Minimum

005013

Chemical: ASSURE (DPX-Y6202)

Caswell No.: 215D

Formulation: Technical (Purity and Batch No. not reported)

Citation: Liggat, M. et al. Screening Test For Delayed Contact Hypersensitivity with NC-302 Technical Product In the Albino Guinea Pig. Conducted by Huntingdon Research Center. Submitted by E.I. du Pont de Nemours. Report No. 82139D/NSA7/ss, April 16, 1982.

Petitioner: E.I. du Pont de Nemours

Accession No.: 073530

Reviewed by: Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769)

Secondary Review: Clint Skinner, Ph.D.
Section Head
Toxicology Branch/HED (TS-769)

Clint Skinner
7-22-85

Material and Methods: See Attachment "A".

Results: "No dermal reactions were observed in any of the test or control animals during the 72 hours observation period." See Attachment "B".

Conclusion: Under the conditions of this study, ASSURE is not a skin sensitizer in guinea pigs.

Core Classification: Supplementary (Petitioner must provide (1) purity of test substance; (2) positive control data cited in the submission (HRC Report No. 82138D/NSA7/SS) with use of formalin.)

7

ATTACHMENT "A" : Material and Methods

Assure toxicology review

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___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product 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

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Attachment "B" : Reported Dermal Scores (Registrant's Submission)

Assure toxicology review

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005013

Chemical: ASSURE (DPX-16202)

Caswell No.: 215D

Formulation: Tecnnical (Purity and batch No. Unspecified)

Citation: Ligget, M. et al. Irritant Effects of NC-302 Technical Product on Rabbit Skin. Conducted by Huntingdon Research Center. Submitted by E.I. du Pont de Nemours. Study No. 82115/NSA5/SE, March 26, 1982.

Petitioner: E.I. du Pont de Nemours

Accession No.: 073530

Reviewed by: Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769)

Secondary Review: Clint Skinner, Ph.D.
Section Head
Toxicology Branch/HED (TS-769)

Material and Methods: Young adult New Zealand white rabbits (4/sex) were shaved form the scapular to pelvic region. "A 1 gram amount of NC-302 Technical Product was moistened with 1 ml distilled water and applied under a 2.5 x 2.5 cm lint patch to two intact skin sites on each animal. The treatment sites were occluded with polyethylene covered with 'Elastoplast' elastic adhesive dressing for approximately 6 hours. The animals were not restrained during the exposure period and were returned to their cages. This treatment procedure was repeated on three consecutive days."

The reaction of the skin was appraised 6 1/2 and 24 hours after each application procedure and subsequently at 4 through 7 days following the last application.

The scoring system used is may be found in Appendix "A".

Results: Actual scores are reported in Appendix "B" (Registrant's submission).

No dermal abnormalities were seen in treated and nontreated skin samples.

005013

Conclusion: Under the conditions of this test, ASSURE is not a skin irritant in rabbits.

Toxicity Category: IV

Core Classification: Supplementary (Petitioner must provide purity of test substance).

ATTACHMENT "A": Scoring System Used

APPENDIX 1

GRADES FOR OCULAR LESIONS

005013

(After "Illustrated Guide for Grading Eye Irritation by Hazardous Substances", published by the U.S. Department of Health, Education and Welfare, Food & Drugs Administration, Washington, D.C. 20204).

Cornea	
No ulceration or opacity	0
Dulling of normal lustre, or fine stippling of cornea, positive staining with fluorescein	1
Scattered or diffuse areas of opacity, details of iris clearly visible	(2)*
Easily discernible translucent areas, details of iris slightly obscured	3
Nacreous areas, no details of iris visible, size of pupil barely discernible	4
Complete corneal opacity, iris not discernible pannus formation (= abnormal membrane on cornea), ulceration	5
Iris	
Normal	0
Slight congestion, deepening of folds, or circumcorneal injection	1
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or any combination of these) iris still reacting to light (sluggish reaction is still positive)	(2)*
No reaction to light, haemorrhage, gross destruction (any or all of these)	3
Conjunctivae	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	(2)*
Diffuse beefy red	3
Chemosis	
No swelling	0
Slight swelling above normal (including nictitating membrane)	1
Obvious swelling with eversion of lids	(2)*
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

* A reaction is interpreted as being positive when the grades of reaction shown in brackets, or higher grades, are present in the rabbit eye from 24 hours after instillation of the test material.

ATTACHMENT "B": Reported Dermal Scores

Assure toxicology review

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005013

Chemical: ASSURE (DPX-Y6202)

Caswell No.: 2150

Formulation: Technical (Purity not specified; Batch No. not specified).

Citation: Ligget, M. et al. Irritant Effects of NC-302 Technical Product on Rabbit Eye Mucosa. Conducted by Huntingdon Research Lab. Submitted by E.I. du Pont de Nemours. Study No. 8264D/NSA6/SE, February 26, 1982.

Petitioner: E.I. du Pont de Nemours

Accession No.: 073530

Reviewed by: Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769)

Secondary Review: Clint Skinner, Ph.D.
Section Head
Toxicology Branch/HED (TS-769)

3-22-82

Material and Methods: Young adult 16 to 19-week-old New Zealand white rabbits (6 animals) were tested by introducing 0.1 ml (approximately 50 mg of test substance) of ASSURE into the conjunctival sac of the eye; the other eye served as controls. After application, the eyelids were held closed for one second and released. The treated eyes of 3 animals remained unrinsed; the treated eyes of 3 animals were rinsed with 200 ml of tap water approximately 30 seconds after treatment.

The eyes were graded for irritation potential using the "Illustrated Guide for Grading Eye Irritation by Hazardous Substances," published by the U.S. Department of Health, Education and Welfare, Food and Drug Administration (See Appendix "A") method at 1 and 6 hours and on days 1 through 7.

Results: The scores for the unrinsed and rinsed eyes are attached in Appendix "B".

Conclusion: Under the conditions of this test, ASSURE is not considered to be an eye irritant in rabbits.

Toxicity Category: IV

Core Classification: Supplementary (Petitioner must provide purity of test substance).

ATTACHMENT "A": Scoring System Used

005013

3.4. Observations and scoring

3.4.1. Examination of the treated skin sites was made approximately 6.5 hours and 24 hours after each application of the bandage and lint patches.

The sites were also assessed daily for a further four days.

3.4.2. The dermal reactions were assessed using the following numerical scoring system:

Erythema and eschar formation:

No erythema	0
Pale pink (slight erythema)	1
Redness (well-defined erythema)	2
Severe redness (moderate erythema)	3
Beet redness (severe erythema to eschar formation)	4

Oedema formation:

No oedema	0
Soft skin (slight oedema)	1
Oedema (well-defined oedema)	2
More definite oedema (moderate oedema)	3
Severe oedema	4

A note was also made of any lesion not covered by this scoring system.

3.5. Histological examination

3.5.1. On completion of the observation period, all the rabbits were killed with an intravenous overdose of pentobarbitone sodium*.

3.5.2. A sample of skin was taken from the centre of each treated area for histological examination. A sample of control (untreated) skin was also taken from each animal.

3.5.3. The samples were fixed in buffered formalin, processed and embedded in paraffin wax (M.P. 56°C). Sections were cut at 5 µm and were stained with haematoxylin and eosin.

* "Expiral" pentobarbitone sodium (200 mg/ml), Abbott Agricultural and Veterinary Supplies, Kent, England.

ATTACHMENT "B": Reported Eye Scores

Assure toxicology review

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