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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Assure[R]: I. Review of eight acute toxicity, two subchronic toxicity, and 3 mutagenicity studies on D+ isomer of Assure[R]

II. Comparison of the toxicology profiles of racemate mixture and D+ isomer of Assure[R]

Caswell No.	215D	HED Proj. No.	9-2183A
EPA ID No.	352-LUR; 352-LUE	EPA Record No.	250157 250158
MRID No.	412061-04 through 412061-16		

TO: Robert Taylor / V. Walters, PM (25)
Registration Division (H7505C)

FROM: Whang Phang, Ph.D. 11/7/90
Pharmacologist
HFAS/Tox. Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head; 11/7/90
James Rowe, Ph.D., Section Head; 11/7/90
and
Marcia van Gemert, Ph.D. 11/8/90
Branch Chief
HFAS/Tox. Branch II/HED (H7509C)

I. Review of the toxicology studies on the D+ isomer of Assure[R]:

The registrant, E.I. du Pont de Nemours and CO., Inc., submitted 8 acute toxicity, 3 mutagenicity, and 2 subchronic toxicity studies on the D+ isomer of Assure[R]. These studies have been reviewed, and the conclusion of each study is summarized below:

1). Fenters, J. D. (1987) Acute oral toxicity of NC-302 (Dextro Plus R compound) in rats. IIT Research Institute; Proj. No. L 8127SN05R; Aug. 5, 1987. EPA MRID No. 412061-04

The combined LD_{50} for NC-301 (D Plus R compound) is estimated to be 1203 mg/kg for male and female rats (Tox. Cat. III). This study meets the data requirements for an acute oral

toxicity study (Guideline No. 81-1) and is classified as core guideline.

2). **Fenters J. D. (1987) Acute oral toxicity study of NC-302 (Levo Plus S compound) in rats. IIT Research Institute; Proj. No. L 8127SN06R; Aug. 5, 1987. EPA MRID No. 412061-05**

The LD_{50} for NC-302 (Levo minus S compound) was estimated to be 952 mg/kg in rats (Tox. Cat. III). The study meets the data requirements for an acute oral toxicity study (Guideline No. 81-1) and is classified as core guideline.

3). **Gerhart, J.M. (1987) 90-day oral (diet) toxicity study of D-NC 302 in rats. IIT Research Institute; Lab. Proj. No. L08138; Aug. 12, 1987. EPA MRID No. 412061-06**

Groups of rats (20/sex/dose) received Assure^R at dietary concentrations of 0, 12, 40, 128, and 1280 ppm for 13 weeks. Ten rats/sex/dose were sacrificed at 13 or 14 weeks of treatment, and the remaining animals (10/sex/dose) were kept on the regular diet for an additional 6 weeks as the recovery groups. Based upon the data presented in the report, the compound-related effects were a decrease in body weight in 1280 ppm males, an increase in liver weights in both males and females of 1280 ppm group, the increases in levels of alkaline phosphatase and albumin in both 1280 ppm males and females, the decreases in the levels of globulin in 1280 ppm males and females, and a decrease in the level of serum cholinesterase and bilirubin in 1280 ppm males. Also, in 1280 ppm males, there was a decrease in levels of triglyceride and cholesterol. Based upon these findings, the no-observed-effect level (NOEL) for subchronic toxicity of this chemical was established as 128 ppm; LEL, 1280 ppm.

The report should contain the calculated actual chemical intake values, but the missing information did not interfere with interpretation of the findings of this study. This study was classified as minimum. This study meets the data requirements for a 90-day toxicity study in rodents (82-1).

4). **Gerhart, J. M., 90-Day oral (diet) toxicity study of D-NC 302 in mice. IIT Research Institute, Lab. Proj. No. L 08137; Aug. 16, 1987. EPA MRID No. 412061-07**

Groups of mice (30/sex/dose) received D-NC 302 at dietary concentrations of 0, 10, 100, 316, and 1000 ppm for 90-days. Subgroups of 10 mice/sex/dose were sacrificed after 4 weeks on the test, and another 10 mice /sex/dose were designated as the recovery groups which were placed on control diet for additional 4 weeks after a 90-day treatment period. The actual

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compound intakes were calculated to be 1.7, 17.4, 55.8, and 175.4 mg/kg/day for males and 2.0, 21.0, 66.8, and 204.9 mg/kg/day for females.

The test compound did not produce changes in survival rate, body weights, food consumption,or hematological parameters. However, clinical chemistry results indicated compound-related effects on the increases in the levels of SGPT, SGOT, alkaline phosphatase, BUN, total protein, albumin, LDH, and serum cholinesterase in 1000 ppm males and females at the interim sacrifice. Essentially similar changes were also seen at the terminal sacrifice. However, at the recovery sacrifice, all the clinical chemistry parameters were comparable between the treated and the control mice.

An increase in liver weights was seen in 100, 316, and 1000 ppm males and females relative to those of the controls at both interim and terminal sacrifices. At the recovery sacrifice, the liver weights of the treated and the control mice were comparable.

The results of the histopathology indicated that D-NC 302 caused unequivocal liver lesions characterized by hypertrophy, necrosis, increased mitosis, pigmentation, and bile duct hyperplasia. The incidence of hypertrophy was seen in all treated males while others were found predominantly in 316 and 1000 ppm males and females. The lesions were found to be more severe in terminal sacrifice animals. No liver hypertrophy or necrosis was found in the recovery animals. The data suggested that the liver toxicity produced by the test compound was reversible.

Based upon the liver toxicity found in D-NC 302 treated male mice, a NOEL could not be established; however, the study was scientifically sound and met the requirements of EPA guidelines for a 90-day rodent study. This study is classified as <u>minimum;</u> however, without a NOEL this study would not be appropriate for regulatory use.

5). Barbera, P.W. (1987) Ames Salmonella mammalian microsomal reverse mutation of D-NC 302. IIT Research Institute; Lab. Proj. No. L 08156. March 11, 1987.
EPA MRID No. 412061-08

Five concentrations of D-NC 302 (0.05 to 5,000 µg/plate) were tested in <u>Salmonella typhimurium</u> microsome mutagenicity assay. Under the testing conditions, the test agent was not mutagenic with or without metabolic activation system (S9). This study is acceptable, and it meets the data requirements for a gene mutation assay (Guideline No 84-2).

6). **Ketels, K. V. (1987) Mouse lymphoma forward mutation assay of D-NC 302. IIT Research Institute; Lab. Proj. No. L 08157. March 19, 1987.**
EPA MRID No. 412061-09

Five nonactivated (5 to 1000 μg/ml) and 5 S9-activated (20 to 500 μg/ml) doses of D-NC 302 were assayed for the potential to induce forward gene mutations in lymphoma L5178Y cells. The report has many serious deficiencies, and it is unacceptable.

7). **Ketels, K. V. (1987) DNA repair assay in primary hepatocytes on D-NC 302. IIT Research Institute; Lab. Proj. No. L 08158. April 21, 1987.**
EPA MRID No. 412061-10

Five doses of D-NC 302 ranging from 10 to 1000 μg/ml were tested on primary rat hepatocytes. The results indicated that D-NC 302 was not genotoxic under the conditions of the study. However, the study was compromised because there was no indication that slides were coded prior to scoring, and the analytical data to support the actual test material concentrations used in the study were not provided. This study was classified as unacceptable, and it did not meet the EPA guidelines (84-4) for other genotoxic effects.

8). **Sarver, J. (1989) Acute oral toxicity study with DPX-Y6202-52 (9.7% EC) in male and female rats. Haskell Lab.; Report No. 101-89. March 1, 1989.**
EPA MRID No. 412061-11

The values of oral LD_{50} for DPX-Y6202-52 were 5900 mg/kg (Tox. Cat. VI) for males and 4100 mg/kg (Tox. Cat. III) for females. The study meets the data requirements for acute oral toxicity study (Guideline No. 81-1), and it is classified as minimum.

9). **Brock, W.J. (1989) Acute dermal toxicity study of DPX-Y6202-52 (9.7%) EC) in rabbits. Haskell Lab.; Lab Report No. 54-89. Feb. 8, 1989.**
EPA MRID No. 412061-12

The data indicated that the acute dermal toxicity for the DPX-Y6202-52 (9.7% EC) was greater than 2000 mg/kg (Tox. Cat. III). This study met the data requirements for an acute dermal toxicity study (81-2), and it was classified as minimum.

10). **Valentine, R. (1989) Acute inhalation toxicity study with DPX-Y6202-52 (9.7%) EC) in rats. Haskell Lab.; Lab Report No. 51-89. Feb. 10, 1989.**
EPA MRID No. 412061-13

The results indicated that the LC_{50} for acute inhalation toxicity of DPX-Y6202-52 (9.7% EC) were 0.36 mg/L for male rats and 0.62 mg/L for female rats (Tox. Cat. III). This study satisfies the data requirements for an acute inhalation study (Guideline No. 81-3) and is classified as minimum.

11). **Brock, W.J. (1988) Primary eye irritation study with DPX-Y6202-45 (D+ isomer) in rabbits. Haskell Lab.; Lab. Report No. 740-88; Dec. 14, 1988.**
EPA MRID No. 412061-14

A 0.1 ml of DPX-Y6202-45 (9.7% of D+ isomer) produced corneal opacity, iritis, chemosis, severe conjunctival redness, and copious blood-tinged discharge in all test animals. Not all of the ocular effects were resolved in one treated rabbit at the termination of the study. This test article is considered as a severe eye irritant (Tox. Cat. I). This study satisfies the data requirements for a primary eye irritation study (Guideline No. 81-4) and is classified as minimum.

12). **Sarver, J.W. (1989) Primary dermal irritation study with DPX-Y6202-52 (9.7% EC) in rabbits. Haskell Lab.; Lab. Report No. 89-89; Feb. 23, 1989.**
EPA MRID No. 412061-15

The results indicated that DPX-Y6202-52 (9.7% EC) was a slight skin irritant (Tox. Cat. IV). The study does not satisfied the data requirements for a primary dermal irritation study (Guideline No. 81-5) and is classified as supplementary.

13). **Brock, W.J. (1989) Closed patch repeated insult dermal sensitization study (Buehler method) with DPX-Y6202-52 (9.7% EC) in guinea pigs. Haskell Lab.; Lab. Report No. 59-89. Feb. 13, 1989).**
EPA MRID No. 412061-16

The results were inconclusive in determining the dermal sensitization potential of DPX-Y6202-52 (9.7% EC). This study does not meet the data requirements for a dermal sensitization study (Guideline No. 81-6) and is classified as supplementary.

II: Comparison of the toxicological profiles of the racemate mixture and the D+ isomer of Assure®

The available toxicological data of the racemate mixture and the D+ isomer are summarized below:

A. Toxicity data of racemate mixture (Technical grade)

Acute oral (rat) : LD_{50} = 1670 mg/kg (M) Tox. Cat. III
1480 mg/kg (F) Tox. Cat. III

Acute dermal (rat): LD_{50} > 5000 mg/kg Tox. Cat. III

Acute inhalation (rat): LC_{50}: 4.8-5.8 mg/L Tox. Cat. III

Primary eye irrit. (rabbit): not an irritant

Primary dermal irrit.(rabbit): negative

Delayed hypersensitivity (g.p.): no dermal reaction

90-Day feeding study (rats): Groups of rats (20/sex/dose) received the test article at dietary concentrations of 0, 40, 120, and 1280 ppm for 90 days. After 90-day treatment period, 15 rats/sex/dose were sacrificed. The remaining test animals (5/sex/dose) were placed on normal diet for an additional 6 weeks as recovery groups. The findings were the following:

a. Statistically significant increases in urea nitrogen, alkaline phosphatase, SGOT, LDH, and plasma cholinesterase levels were seen in 1280 ppm males. Increases in alkaline phosphatase and serum cholesterol levels were also found in 1280 ppm females.

b. An increase in liver weights in 128 and 1280 ppm males and females.

c. Microscopically, slight to minimal centrilobular and/or midzonal enlargements of the liver were seen in 14/15 males or females of 1280 ppm groups at the end of the treatment period. Liver lesions were not seen in the animals of the recovery groups.

d. Decreases in body weights and in testicular weights were seen in 1280 ppm males. Microscopically, an increase in the incidence of testicular atrophy was found in 1280 ppm males.

NOEL = 40 ppm

90-Day feeding study (mice): Groups of mice (20 or 30/sex/dose) received the test article at dietary concentrations of 0, 100, 316, and 1000 ppm for 90 days. At the end of the treatment period, the test animals were sacrificed except that 10/30 mice in the 1000 ppm group were placed on the normal diet for an additional 4 weeks. The results of this studies were summarized as follows:

a. A decrease in body weights was seen in 1000 ppm males.

b. Increases in the levels of total protein, albumin, alkaline phosphatase, blood urea nitrogen, SGPT, and SGOT were found in 1000 ppm males. Alkaline phosphatase, blood urea nitrogen, and SGPT were also increased in 1000 ppm females.

c. Dose-related increases in absolute and relative liver weights were found in all treated males and females. The absolute and relative adrenal weights were also elevated in 316 and 1000 ppm males and females.

d. Microscopically, the dose-related increases in the incidence of degeneration/necrosis of individual hepatocytes and diffuse hepatocytic hypertrophy/hyperplasia were seen in all treated male and female mice. An increase in the incidence of bile duct hyperplasia was found in all treated males and in 316 and 1000 ppm females. In addition, an increase in the incidence of the presence of green-brown pigment was seen in 316 and 1000 ppm males and females. In the kidneys, an increase in the incidence of the tubular cell hypertrophy was seen in 1000 ppm male mice.

In the recovery animals the hepatotoxicity was shown to be reversible.
NOEL < 100 ppm (LDT)

21-Day dermal tox. (rabbit): NOEL = 2000 mg/kg (HDT)

Chronic feeding/onco. (rat): Groups of SD rats (50/sex/dose) received the test article at dietary concentrations of 0, 25, 100, and 400 ppm for 2 years, and a satellite group of 30 rats/sex was attached to each dose level. The results indicated that Assure^{R} elevated the alkaline phosphatase levels in 400 ppm males and females. Histopathologically, an increase in the incidence in liver lesions characterized by generalized hepatocytic enlargement was seen in 400 ppm males, and slight enlargement and cytoplasmic eosinophilia of the centrilobular hepatocytes were also found in 400 ppm females. An increase in the minimal centrilobular enlargement was seen in 100 ppm males and females.

An increase in the liver weights of 400 ppm males and females correlated with the histopathological findings. A treatment-related increase in tumor incidence was not evident.
NOEL = 80 ppm (0.9 mg/kg)

Mouse oncogenicity study: Groups of CD-1 mice (50/sex/dose) received the test article at dietary concentrations of 0, 2, 10, 80, and 320 ppm for 18 months. Satellite groups (20 mice/sex/dose) were also included in this study. The results showed that the test article produced increases in the levels of alkaline phosphatase in 80 and 320 ppm males and 320 ppm females. The absolute and relative liver weights were increased in 80 and 320 ppm males and females. A statistically significant decrease in testicular weights in 320 ppm males and an increase in ovary weights in 320 ppm females were found. The increases in alkaline phosphatase and in liver weight correlated with the histopathological findings of liver lesions characterized by diffuse hepatocytic enlargements, hepatocellular pigmentation, sinusoidal cellular pigmentation, and the presence of focal pigmented macrophages. Testicular atrophy was noted in 320 ppm males. There were increases in the incidence of luteoma in 320 ppm females and in incidence of hepatic tumor in 320 ppm males.

However, the tumor incidence data and other relevant toxicology data were evaluated by the HED Carcinogenicity Peer Review Committee and the EPA Science Advisory Panel (SAP). They concluded that "Assure would probably be best classified as Category D carcinogen (not classifiable as to human carcinogenicity) because limitations in the data from an adequately performed mouse study precluded an accurate interpretation of oncogenic risk". One of the limitations was that highest dose tested appeared to have exceeded MTD, and both luteoma and liver tumor were seen at the highest dose groups. In addition, the incidence of luteoma was within the range of the historical control data, and the increase in the incidence of the mouse liver tumor was marginal.
NOEL = 80 ppm

1-Year feeding (dog)	NOEL = 400 ppm (HDT)
6-Month feeding (dog)	NOEL = 100 ppm
Teratology (rabbit)	Maternal NOEL = 20 mg/kg Develop. NOEL = > 60 mg/kg (HDT)

Teratology (rat)	Maternal NOEL = 30 mg/kg develop. NOEL > 300 mg/kg (HDT)
2-Generation Repro. (rat)	Parental NOEL = 100 ppm Reproductive NOEL = 25 ppm Reproductive LEL = 100 ppm (increase in liver weight and increase in the incidence of eosinophilic changes in the liver)
Mutagenicity Studies	
Unscheduled DNA synthesis	Negative
Chromosomal Aberration (CHO)	Negative
Gene mutation (Ames assay)	Negative
Metabolism studies	Extensively metabolized to the acid form of the test material. Much of the radioactivity was excreted in the feces.

B. <u>The toxicity data of D+ isomer of Assure^{R} (technical grade)</u>

Acute oral toxicity (rat): LD_{50} = 1203 mg/kg (Tox. Cat. III)

90-day oral feeding study (rat): Groups of rats (20/sex/dose) received Assure^{R} at dietary concentrations of 0, 12, 40, 128, and 1280 ppm for 13 weeks. Groups of 10 rats/sex/dose were sacrificed at 13 or 14 weeks of treatment, and the remaining animals (10/sex/dose) were kept on the regular diet for an additional 6 weeks as the recovery groups. Based upon the data presented in the report, the compound-related effects were the following:

a). A decrease in body weight in 1280 ppm males.
b). An increase in liver weights in both males and females of 1280 ppm group.
c). An increase in the levels of alkaline phosphatase and albumin in both 1280 ppm males and females.
d). Decreases in the levels of globulin in 1280 ppm males and females, of serum cholinesterase and bilirubin in 1280 ppm males.
e). A decrease in levels of triglyceride and cholesterol in 1280 ppm males.
f). A slight increase in the incidence of liver necrosis in 1280 ppm males.
g). A slight increase in the incidence of testicular atrophy.

NOEL = 128 ppm

90-day feeding study (mice): Groups of mice (30/sex/dose)

received D-NC 302 at dietary concentrations of 0, 10, 100, 316, and 1000 ppm for 90-days. Subgroups of 10 mice/sex/dose were sacrificed after 4 weeks on the test, and another 10 mice/sex/dose were designated as the recovery groups which were placed on normal diet for additional 4 weeks after a 90-day treatment period. The following treatment-related effects were found:

a). Increases in the levels of SGPT, SGOT, alkaline phosphatase, BUN, total protein, albumin, LDH, serum and cholinesterase were seen in 1000 ppm males and females at the interim sacrifice. Essentially similar changes were also seen at the terminal sacrifice. However, at the recovery sacrifice, all the clinical chemistry parameters were comparable between the treated and the control mice.

b). An increase in liver weights was seen in 100, 316, and 1000 ppm males and females relative to those of the controls at both interim and terminal sacrifices. At the recovery sacrifice, the liver weights of the treated and the control mice were comparable.

c). Unequivocal liver lesions characterized by hypertrophy, necrosis, increased mitosis, pigmentation, and bile duct hyperplasia were found. The incidence of hypertrophy was seen in all treated males while others were found predominantly in 316 and 1000 ppm males and females. The lesions were found to be more severe in terminal sacrifice animals. No liver hypertrophy or necrosis was found in the recovery animals. The data suggested that the liver toxicity produced by the test compound was reversible.

Based upon the liver lesions seen in the treated male mice a NOEL could not be established.

Mutagenicity studies

1). Ames assay (gene mutation)	Negative
2). Unscheduled DNA synthesis assay (primary hepatocytes)	Negative
3). Structural chromosomal aberration	Data not available

Discussion and Conclusion: The available toxicity data (presented above) indicate that the toxicological profiles for both racemic mixture and D+ isomer of Assure® were similar. The acute oral LD_{50} values for the racemic mixture were 1670 mg/kg (males) and 1480 mg/kg (females), and for D+ isomer was 1203 mg/kg. The results of the 90-day feeding studies on rats or mice with racemic mixture offered the best comparisons with those of

90-day feeding studies on the D+ isomer because the dosages used in the corresponding studies were similar (pages 6, 7, 9, & 10). The results of these studies indicated that the target organ of the test agents was the liver, and certain characteristics of the liver lesions were reversible. In the rats, both racemic mixture and the D+ isomer of Assure^{R} induced testicular atrophy at the high dose males, but the racemic mixture appeared to produce higher incidence of testicular atrophy. The available mutagenicity results of the racemic mixture and those of the D+ isomer were also comparable.

The toxicological profiles of the racemic mixture and the D+ isomer were discussed at a Toxicology Branch II meeting attended by Marcia van Gemert, Branch Chief, Clark Swentzel, Section Head, James Rowe, Section head, and this reviewer. It was concluded that the toxicological profiles of the racemic and D+ forms of Assure^{R} were sufficiently similar, and they were considered to be adequate to support a conditional registration for the D+ isomer. However, Tox. Branch II recommends that the deficiencies in mutagenicity studies for the D+ isomer be rectified. In addition, the primary dermal irritation and dermal sensitization studies on the D+ isomer should be upgraded.

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Reviewer: Whang Phang, Ph.D. 8/27/90
HFAS/Tox. Branch II/HED (H7509C)

Secondary Reviewer: K. Clark Swentzel, Section Head
HFAS/Tox. Branch II/HED (H7509C) 8/27/90

DATA EVALUATION REPORT

Chemical: D-NC 302; Assure®; DPX-¥6202

Study Type: 90-Day feeding study (rats)

Caswell No.: 215D **HED Proj. No.:** 9-2183A

MRID No.: 412061-06 **EPA ID No.:** 352-LUE

EPA Record No.: 250158

Sponsor: Nissan Chemical Industries, Ltd., Tokyo, Japan

Testing Laboratory: IIT Research Institute
Life Sciences Research Department
10 West 35th St
Chicago, Illinois 60616

Citation: Gerhart, J. M., 90-Day oral (diet) toxicity study of D-NC 302 in rats. IIT Research Institute, Lab. Proj. No. L 08138; Aug. 12, 1987.

Conclusion

Groups of rats (20/sex/dose) received Assure® at dietary concentrations of 0, 12, 40, 128, and 1280 ppm for 13 weeks. Groups of 10 rats/sex/dose were sacrificed at 13 or 14 weeks of treatment, and the remaining animals (10/sex/dose) were kept on the regular diet for an additional 6 weeks as the recovery groups. Based upon the data presented in the report, the compound-related effects were a decrease in body weight in 1280 ppm males, an increase in liver weights in both males and females of 1280 ppm group, the increases in levels of alkaline phosphatase and albumin in both 1280 ppm males and females, the decreases in the levels of globulin in 1280 ppm males and females, and a decrease in the level of serum cholinesterase and bilirubin in 1280 ppm males. Also, in 1280 ppm males, there was a decrease in levels of triglyceride and cholesterol. Based upon these findings, the no-observed-effect level (NOEL) for subchronic toxicity of this chemical was established as 128 ppm; LEL, 1280 ppm.

The report should contain the calculated actual chemical intake values, but the missing information did not interfere with interpretation of the findings of this study. This study was classified as minimum.

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Materials and Methods

Test article: D-NC 302 (technical) was reported to be 98.1% pure; The test material was stored in the dark at 4°C. The reviewer had a telephone conversation with the study coordinator, and she informed me that the test chemical came in the form of beige powder.

Animals: Male and female Sprague-Dawley rats were obtained from Charles River Breeding Lab., Portage, MI. The rats were approximately 4 weeks old weighing 187-269 gm for males and 166-238 gm for females. Rats were acclimated to the testing environment for two weeks prior to the initiation of the study.

Study Design

Prior to initiation of the studies all rats received a physical examination, and acceptable rats were randomly assigned into 5 dose groups. Each dose group consisted 20 rats/sex, and 10 rats/sex/dose were designated as the recovery group which received the test compound for approximately 90 days and then normal diet for additional 6 weeks. The test groups were summarized as follows:

Conc. (ppm)	90-day		Recovery	
	Male	Female	Male	Female
0	10	10	10	10
12	10	1o	11*	9*
40	10	10	10	10
128	10	10	10	10
1280	10	10	10	10

* One animal was miss-sexed.

All the test animals were housed individually in polypropylene cages throughout the study.

Test diet preparation and administration: For each concentration, appropriate quantity of the test chemical was mixed with the required rat chow. The test diets were prepared weekly, and samples of the test diets were tested for stability and homogeneity. The animals received the test diet for 90 days. At 90 days, the animals in the recovery groups were given normal diet for another 6 weeks.

Clinical observations: The animals were observed daily for signs of illness during the acclimation period. During the treatment period, the test animals were observed twice daily for signs of toxicity and moribundity. Weekly "hand-held physical examination" which included palpation for tissue masses or abnormal distention was performed.

Body weights: From pretest until the termination of the study, body weights of all test animals were measured weekly. For the purposes of calculating the organ-to-body weight ratios, fasted body weights were measured immediately prior to sacrifice.

Food and water consumption: Food consumptions were measured one week prior to the initiation of the study and weekly during the study until termination of the test. During weeks 5 and 10, the volume of water intake for all animals were measured in consecutive 3-day periods.

Eye examination: Eye examinations were conducted on all test animals prior to dosing. At week 13, all of the controls and the high dose animals and 10 rats/sex from the intermediate dose groups were examined. At week 19, all the survivors were also examined.

Hematology: The appended protocol stated that blood samples were to be collected from 5 rats/sex/dose, but the experimental results indicated that the blood samples were obtained from 10 animals/sex/dose in some dose groups. The samples were obtained from fasted animals during pretest, week 4, weeks 12, and week 19, and the following hematologic parameters were examined:

- erythrocyte counts
- hematocrit
- mean corp. volume
- mean corp. hemoglobin
- platelet counts
- prothrombin time
- hemoglobin
- leukocyte counts (total & differential)
- mean corp. hemoglobin conc.
- RBC osmotic fragility
- activated partial thromboplasm time

Clinical chemistry: The following clinical chemistry parameters were examined:

- serum glutamic-pyruvic transaminase (ALT) (SGPT)
- serum glutamic-oxalacetic transaminase (AST) (SGOT)
- alkaline phosphatase
- blood urea nitrogen
- serum creatinine
- albumin
- albumin/globulin ratio
- total bilirubin
- potassium
- calcium
- lactic dehydrogenase
- triglycerides
- glucose
- total protein
- globulin
- cholesterol
- sodium
- chloride
- phosphorus
- cholinesterase
- gama glutamyl transpeptidase

Urinalysis: Urine samples were collected during study weeks 3, 11, and 19. The following parameters were examined:

- volume
- pH
- appearance
- specific gravity

protein	reducing substances
glucose	ketones
bile pigment-urobilinogen	occult blood
sediment	

Gross pathology: Gross examinations were performed on all animals which died on test and those which were sacrificed at weeks 13 and 19. The following tissues were collected and some organs were weighed:

brain *+	pituitary *+
eyes +	esophagus +
stomach +	duodenum +
ileum +	jejunum +
cecum +	colon +
rectum +	pancreas +
adrenals *+	kidneys *+
urinary bladder +	skeletal muscle +
sciatic nerve +	thyroids (including parathyroids) *+
salivary gland +	trachea +
spinal cord +	heart *+
lungs *+	thymus *+
aorta +	spleen *+
lymph nodes +	uterus *+
mammary gland +	epididymides
gonads *+	skin (abdomen) +
prostate	liver *+
bone (femur & sternum) +	
all gross lesions +	

*: organ was weighed. +: Required for subchronic studies.

Histopathology: The above organs were processed, and histopathologic examinations were performed. In addition to the above tissues and organs, tongue and seminal vesicles were also collected but not processed.

Statistics: The details of the statistical analyses were excerpted from the report and presented in Appendix A.

Quality assurance: A record of the dates for the quality assurance ininspection and names of the inspector was presented in the report.

Results

Dietary chemical analysis: The samples of the test diets at different dose levels were found to be relatively homogenous. The test chemical in the diet was shown to be stable at 21 days, and the analyzed mean concentrations of the test compound at different

dose levels were reported to be approximately 90% of the nominal concentrations.

Clinical observations:

The report stated that no treatment-related toxic signs were observed, but no clinical observation data were included in the report.

Mortality: No compound-related death was found.

Body weight: The report stated that the values of the body weights of the treated animals were comparable to those of the controls. However, this reviewer analyzed the individual animal data on the body weights of high dose males and those of the controls at weeks 13 and 14 using Student's t-test. The results showed that the body weights of the high dose males were significantly decreased ($p<0.001$) relative to those of the controls (Table 1). The data on the body weight gains showed decreases of approximately 20-30% in high dose male and 10-20% in high dose females relative to those of the corresponding controls (Table 1A). However, these decreases were not statistically significant.

No significant difference was detected in the body weights of the animals in the recovery groups.

Food consumption: The weekly food consumption data were excerpted from the report and presented in Table 2. The results showed no significant difference in food consumption between treated and control rats. The food conversion values were also calculated, but these values did not show a consistent and statistically significant difference between the treated and the control animals.

Hematology: There were no significant changes in the parameters examined in treated animals relative to those of the controls (Table 3).

Clinical chemistry: Table 4 summarizes the clinical chemistry findings at terminal sacrifice for both the treated and control animals. The findings are listed as follows:

a. Statistically significant increases in the level of alkaline phosphatase in both 1280 ppm males and females.

b. An increase in the BUN level in 1280 ppm males. This increase was statistically significant.

c. A statistically significant increase in albumin level in both 1280 ppm males and females.

d. A statistically significant decrease in globulin level in both 1280 ppm males and females.

1280 ppm males and females.

e. An increase in the ratios of albumin to globulin reflects the changes in the levels of albumin and globulin.

f. Statistically significant decreases in serum cholesterol and total bilirubin in 1280 ppm males. A decrease in total bilirubin level in 1280 ppm females was also seen.

g. A statistically significant increase in cholinesterase activity in 1280 ppm males and a decrease in triglyceride levels in 1280 ppm males.

Similar changes in BUN, albumin, globulin, and triglyceride levels in 1280 ppm males were also seen at the interim examination. However, at the recovery period the clinical chemistry parameters between treated and the controls were comparable.

Ophthalmology: No treatment-related eye effects were detected.

Gross pathology: No treatment-related gross lesions were detected at either terminal or recovery sacrifices.

Organ weights: The organ weight data showed that the absolute thyroid/parathyroid weights were decreased in 128 and 1280 ppm males relative to that of the controls (Table 5). Statistically significant increases in absolute and relative liver weights (liver/body weight) were seen in 1280 ppm males and females (Tables 5 & 6). There were slight increases in relative weights of brain, heart, and kidneys in 1280 ppm males, but these changes might be caused by the slight decrease in the body weights of these animals as seen in Table 5. No changes in absolute or relative organ weights were seen in animals of the recovery groups.

Histopathology: A very slight increase in the incidence of testicular atrophy was seen in 1280 ppm animals of the terminal sacrifice group (1/10) and the recovery group (3/10). This lesion was not seen in any other test groups. ~~Slight histological changes were seen in the livers of 1280 ppm males and females; but these did not show a treatment-related effect.~~ A slight increase in the incidence of liver necrosis was seen in 1280 ppm males (control 0%, 1280 ppm 3/10) of the terminal sacrifice group relative to the controls.

Discussion

Groups of rats (20/sex/dose) received Assure® at dietary concentrations of 0, 12, 40, 128, and 1280 ppm for 13 weeks. Groups of 10 rats/sex/dose were sacrificed at or soon after 13 weeks of treatment, and the remaining animals (10/sex/dose) were kept on the regular diet for an additional 4 weeks as the recovery groups. Based upon the data presented in the report, the compound-related effects were a decrease in body weight in 1280 ppm males, an increase in liver weights in both males and females of 1280 ppm group, the increases in levels of alkaline phosphatase and

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albumin in both 1280 ppm males and females, the decreases in the levels of globulin in 1280 ppm males and females, and a decrease in the level of serum cholinesterase and bilirubin in 1280 ppm males. Also, in 1280 pp males, there was a decrease in levels of triglyceride and cholesterol which might be related to the decrease in the body weights. Food consumptions were comparable between treated groups and the controls. Based upon these findings, the no-observed-effect level (NOEL) for subchronic toxicity of this chemical was established as 128 ppm; LEL, 1280 ppm.

The report should contain the calculated actual chemical intake values, but the missing information did not interfere with interpretation of the findings of this study. This study was classified as minimum.

ASSURE TOX R 008154

Page_____ is not included in this copy.

Pages 19 through 26 are not included.

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.

X ____ FIFRA registration data.

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Reviewer: Whang Phang, Ph.D. 10/25/90
HFAS/Tox. Branch II/HED (H7509C)

Secondary Reviewer: James Rowe, Ph.D. James Rowe 10/25/90
Section Head
HFAS/Tox. Branch II/HED (H7509C)

DATA EVALUATION REPORT

Chemical: D-NC 302; Assure^{R}; DPX-46202

Study Type: 90-Day feeding study (mice)

Caswell No.: 215D **HED Proj. No.:** 9-2183A

MRID No.: 412061-07 **EPA ID No.:** 352-LUE

EPA Record No.: 250158

Sponsor: Nissan Chemical Industries, Ltd., Tokyo, Japan

Testing Laboratory: IIT Research Institute
Life Sciences Research Department
10 West 35th St
Chicago, Illinois 60616

Citation: Gerhart, J. M., 90-Day oral (diet) toxicity study of D-NC 302 in mice. IIT Research Institute, Lab. Proj. No. L 08137; Aug. 16, 1987.

Conclusion

Groups of mice (30/sex/dose) received D-NC 302 at dietary concentrations of 0, 10, 100, 316, and 1000 ppm for 90-days. Subgroups of 10 mice/sex/dose were sacrificed after 4 weeks on the test, and another 10 mice /sex/dose were designated as the recovery groups which were placed on control diet for additional 4 weeks after a 90-day treatment period. The actual compound intakes were calculated to be 1.7, 17.4, 55.8, and 175.4 mg/kg/day for males and 2.0, 21.0, 66.8, and 204.9 mg/kg/day for females.

The test compound did not produce changes in survival rate, body weights, food consumption, or hematological parameters. However, clinical chemistry results indicated compound-related effects on the increases in the levels of SGPT, SGOT, alkaline phosphatase, BUN, total protein, albumin, LDH, serum and cholinesterase in 1000 ppm males and females at the interim sacrifice. Essentially similar changes were also seen at the terminal sacrifice. However, at the recovery sacrifice, all the

clinical chemistry parameters were comparable between the treated and the control mice.

An increase in liver weights (absolute & relative) was seen in 100, 316, and 1000 ppm males and females relative to those of the controls at both interim and terminal sacrifices. At the recovery sacrifice, the liver weights of the treated and the control mice were comparable.

The results of the histopathology indicated that D-NC 302 caused unequivocal liver lesions characterized by hypertrophy, necrosis, increased mitosis, pigmentation, and bile duct hyperplasia. The incidence of hypertrophy was increased in all treated males while other liver lesions were found predominantly in 316 and 1000 ppm males and females. The lesions were found to be more severe in terminal sacrifice animals. No liver hypertrophy or necrosis was found in the recovery animals. The data suggested that the liver toxicity produced by the test compound was reversible.

Based upon the liver toxicity found in D-NC 302 treated male mice, a NOEL could not be established. However, the study was scientifically sound and met the requirements of EPA guidelines (82-1) for a 90-day rodent study. This study is classified as minimum; without a NOEL this study would not be appropriate for regulatory use.

Materials and Methods

Test article: D-NC 302 (technical) was reported to be 98.1% pure; Lot No. 302 DT8501. The test material was stored in the dark at 4°C. In a telephone conversation with the study coordinator, this reviewer learned that the test article was a beige powder.

Animals: Male and female CD-1 mice were obtained from Charles River Breeding Lab., Portage, MI. The mice were approximately 4 weeks old weighing 20.9-31.4 gm for males and 17.1-26.1 gm for females. Mice were acclimated to the testing environment for two weeks prior to the initiation of the study. During this time animals were observed daily.

Study Design

Prior to the initiation of the studies all mice received a physical examination, and the acceptable mice were randomly assigned into 5 dose groups. Each dose group consisted 30 mice/sex. Ten mice/sex/dose were designated as the interim sacrifice group which was sacrificed on day 28; 10 mice/sex/dose were designated as the terminal group which were sacrificed on day 90; another 10 mice/sex/dose were designated as the recovery group which received the test article for 90 days and then placed on the control diet

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for an additional 4 weeks. The test groups were summarized as follows:

Conc. (ppm)	Interim SaCc.		90-day		Recovery	
	Male	Female	Male	Female	Male	Female
0	10	10	10	10	10	10
10	10	10	10	10	10	10
100	10	10	10	10	10	10
316	10	10	10	10	10	10
1000	10	10	10	10	10	10

The test animals were housed individually in polypropylene cages throughout the study.

Test article preparation and administration: The test diets were prepared weekly by mixing appropriate amounts of the test chemical with the required quantity of rodent chow. Animal received the test diet and water ad libitum. Samples of the test diets were analyzed for test chemical concentration, stability, and homogeneity.

Clinical observation: During the treatment period, the test animals were observed twice daily for signs of moribundity, mortality, and toxicity. Hand-held physical examinations, which included palpatation for tissue masses or abdominal distention, were performed weekly.

Body weights: All test animals were weighed weekly throughout the test period. The fasted body weights were also measured immediately prior to scheduled sacrifice, and the values were used to calculate the organ-to-body weight ratios.

Food and water consumptions: Food consumption for each animal was measured weekly during the treatment period. The average daily food consumption was calculated. Water consumption was also measured.

Ophthalmic examination: The eye examinations were conducted prior to the initiation of the study on all test animals. At weeks 4, 13, and 17 of the test, 10 mice/sex/dose were subjected to eye examination.

Clinical pathology: The clinical pathologic examinations were performed at the pretest, interim, terminal, and recovery periods. Blood samples were collected from 10 mice/sex/dose at designated periods.

1). Hematology: The following the hematological parameters were measured:

- erythrocyte
- hematocrit
- mean corpuscular volume
- hemoglobin
- leukocyte counts (total & differential)

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mean corpuscular hemoglobin
platelet counts
reticulocyte counts
mean corpuscular hemoglobin concentration

2). Clinical chemistry: All the parameters of clinical chemistry were conducted on the serum.

glutamic-pyruvic transaminase (ALT)
glutamic-oxaloacetic transaminase (AST)
alkaline phosphatase
blood urea nitrogen
glucose
creatinine
total protein
albumin
globulin
albumin/globulin ratios
cholesterol
total bilirubin
sodium
potassium
chloride
calcium
lactic dehydrogenase
cholinesterase

Urinalysis: Urine samples were collected from food-deprived mice during over-night collections. Samples were collected at interim (week 3), interim (week 11), and recovery periods (week 17). The following parameters were evaluated:

Volume
pH
protein
glucose
bile pigments-urobilinogen
appearance
specific gravity
reducing substances
ketones
occult blood
sediment- cells, casts, polymorphonuclear leukocytes, erythrocytes, organisms, & abnormal constituents.

Gross pathology: After 4, 13, and 17 weeks on test, the test animals were sacrificed and gross examinations were performed.

Histopathology examinations: Organs were removed, and certain organs were weighed. The following tissues from all mice were collected, fixed in 10% neutral buffered formalin, and processed for histopathological examination:

brain*
eyes
stomach
ileum
pituitary*
esophagus
duodenum
jejunum

cecum	colon
rectum	pancreas
adrenal*	kidneys*
urinary bladder	muscle
nerve (sciatic)	thyroids-parathyroid*
salivary gland	spinal cord
trachea	lungs*
heart*	aorta
thymus	lymph nodes
spleen*	mammary gland
uterus	gonads*
epididymides	prostate
bulbourethral gland	skin (abdomen)
bone (femur & sternum)	liver*
all gross lesions	

*: These organs were weighed.

Statistics: The details of the statistical methods used in this study are presented in Attachment A.

Quality assurance statement and a GLP statement were included in the report.

Results

Test diet analysis: The chemical homogeneity results indicated that on the average the test diet preparations varied within 10% of the mean. The test diet was shown to be stable for approximately 21 days.

Clinical observations: Clinical signs such as hyperactivity, rough coat,hunched posture, ocular discharge and/or opacity, and lesions at various regions were seen in all treatment groups. These clinical signs were not compound-related.

Mortality: No deaths occurred during the study.

Body weights: The body weights of animals which received 316 ppm or lower were comparable to those of the controls whereas the body weights of 1000 ppm males and females were slightly greater than those of the controls during the treatment period (Table 1). However, this difference was not statistically significant.

During the recovery period, the difference in the mean body weights of 1000 ppm females in comparison to the controls was much less, and that in males was disappeared.

The mean body weight gains were calculated for all the test animals. The mean body weight gains were consistently higher

in 1000 ppm males and females than those of the controls, but the increases were slight and showed no statistical significance.

Food and water consumptions: The mean food consumption values were comparable between treated and control animals (Table 2). The means of the food conversion ratios were calculated for all mice on test. During the duration of the test, there were increases and decreases in food conversion ratios for both treated male and female mice in all dose groups relative to those of the controls, and some of these changes were statistically significant. However, the changes were not consistent; for example, the values varied from 21 gm to 102 gm for 1000 ppm females.

Water intake was comparable between the treated and the control mice.

Test compound intake: From the nominal concentrations of the test chemical in the diet, the food consumptions, and the body weights, the overall average compound intake was calculated and expressed as mg/kg/day. The weekly means of the test compound intake are excerpted from pages 26 and 27 of the report and presented below:

ppm	10	100	316	1000
		(mg/kg/day)		
Male	1.7	17.4	55.8	175.4
Female	2.0	21.0	66.8	204.9

Hematology: Hematological examinations were performed prior to the initiation of the study, at interim sacrifice, terminal sacrifice, and at the end of the recovery period. All the results indicated no compound-related changes in either males or females.

Clinical chemistry: Clinical chemistry parameters were measured before the start of test, at the interim sacrifice, at the terminal sacrifice, and at the recovery period. At the interim sacrifice, statistically significant increases in the SGPT, SGOT, alkaline phosphatase, BUN, total protein, albumin, LDH, cholinesterase, and the ratio of albumin/globulin in 1000 ppm males (Table 3). There were also a significant decrease in the globulin values of 1000 ppm males. Similar changes were also seen in 1000 ppm females, but the magnitude of changes was not as marked as those in males.

At the terminal sacrifice, there were statistically significant increases in SGPT, SGOT, alkaline phosphatase, total protein, albumin, ratio of albumin/globulin, LDH, and cholinesterase in 1000 ppm males (Table 4). In 1000 ppm females, statistically significant increases in SGPT, alkaline phosphatase, total protein, albumin, and LDH were seen. Statistically significant decreases in glucose levels were also found in 100, 316, and 1000 ppm females (Table 4).

At the recovery period, all the parameters examined were comparable between the treated and the control mice (Table 5).

Urinalysis: Urine samples were analyzed at interim and terminal examinations. At the interim examination, there was a decrease in the ketone levels of 316 and 1000 ppm males. An increase in the percentage of the reducing substance in 1000 ppm males was also seen. In 1000 ppm females, increases in the casts, white blood cells, and epithelial cells were detected (Table 6). However, these changes were not found in the urine samples of the treated animals at the terminal examination period.

Ophthalmology: No treatment-related eye effects were seen.

Gross examination: There were small increases in the incidence of liver foci in 1000 ppm females (control, 0/10; 1000 ppm, 3/10) and of enlarged liver in 1000 ppm males (control, 0/10; 1000 ppm, 2/10). No other gross effects could be attributed to the treatment.

Organ weights: The organ weight data were excerpted from the report and presented in tables 7 and 8. At interim sacrifice, the absolute and relative liver weights were increased in 100, 316, and 1000 ppm male and female mice relative to the controls, and the increase in 316 and 1000 ppm animals was statistically significant ($p<0.001$). The adrenal weights in 1000 ppm males were increased. There was a slight decrease in pituitary weights of 10 and 1000 ppm females, but this decrease was not seen in 100 or 316 ppm females (Table 7).

At the terminal sacrifice, the absolute and relative liver weights were increased in 100, 316, and 1000 ppm males and females. The adrenal weights were also increased in the 1000 ppm males and females (Table 8).

At the recovery period, all the absolute organ weights were comparable between the treated and the control mice.

Histopathology: The histopathology results indicated that the liver was the only organ which showed unequivocally compound-related lesions. These lesions were seen in both males and females in similar degree of severity (Table 9). However, the degree of severity varied according to the sacrifice period. In Table 9, the terms "hypertrophy, centrilobular" was used to describe where the "hypertrophy was primarily in the centrilobular and, to some degree, the midzonal regions of the hepatic lobules". The term, "hypertrophy" denoted that the "hypertrophy had progressed to involve the entire lobule more or less uniformly".

At the interim sacrifice, the frequent finding was the increase in the incidence of hypertrophy and hypertrophy, centrilobular in 100, 316 and 1000 ppm males and females. Other findings such as

necrosis and increased mitosis were seen mainly in 316 and 1000 ppm males and females (Table 9).

At the terminal sacrifice, hypertrophy was seen in all 1000 ppm males and females. In addition, 8/10 316 ppm females also showed hypertrophy of the entire hepatic lobule. The incidence of hypertrophy, centrilobular was seen all groups of males whereas, in females, the incidence was seen in 100 ppm or more. The incidence of necrosis was increased in both males and females of 100 ppm or greater groups. The incidence of mitosis, pigmentation, and bile duct hyperplasia was also increased in predominantly 1000 ppm males and females.

At the recovery sacrifice, the occurrence of hepatic hypertrophy increased mitosis, and bile duct hyperplasia were not found. A slight increase in the incidence of necrosis was seen in 316 ppm females, but this finding was not dose-related. The presence of pigment in the liver, was primarily in the 1000 ppm male and female mice. The pigment was not positively identified, but the pathologist thought it was "mostly likely a lipofuscin pigment or bile pigment or a combination thereof. It occurred as a golden to tannish-golden pigment in Kupffer cells and degenerate cells that could not be positively identified". The presence of pigment in the liver persisted in the recovery groups of animals.

Discussion

Groups of mice (30/sex/dose) received D-NC 302 at dietary concentations of 0, 10, 100, 316, and 1000 ppm for 90-days. Subgroups of 10 mice/sex/dose were sacrifice after 4 weeks on the test, and another 10 mice /sex/dose were designated as the recovery groups which were placed on control diet for an additional 4 weeks after a 90-day treatment period.

The treated mice did not show any possible compound-related clinical signs. The mortality rate was not increased in the treated mice relative to that of the controls. The body weights of 1000 ppm males and females were actually slightly increased relative to those of the controls while the food consumption values were comparable between the treated and the control mice.

Hematological examinations did not show any compound related-effects. However, clinical chemistry results indicated compound-related effects of increases in the levels of SGPT, SGOT, alkaline phosphatase, BUN, total protein, albumin, LDH, serum and cholinesterase in 1000 ppm males and females at the interim sacrifice. Essentially similar changes were also seen at the terminal sacrifice. However, at the recovery sacrifice, all parameters examined were comparable between the treated and the control mice.

An increase in liver weights was seen in 100, 316, and 1000 ppm

males and females relative to those of the controls at both interim and terminal sacrifices. At the recovery sacrifice, the liver weights of the treated and the control mice were comparable.

The results of the histopathology indicated that D-NC 302 caused unequivocal liver lesions characterized by hypertrophy, necrosis, increased mitosis, pigmentation, and bile duct hyperplasia. The incidence of hypertrophy was seen in all treated males while other liver lesions were found predominantly in 316 and 1000 ppm males and females. The lesions were found to be more sever in terminal sacrifice animals. No liver hypertrophy or necrosis was found in the recovery animals. The data suggested that the liver toxicity produced by the test compound was reversible.

Based upon the liver toxicity found in D-NC 302 treated male mice, a NOEL could not be established; however, the study was scientifically sound and met the requirements of EPA guidelines for a 90-day rodent study. This study is classified as <u>minimum;</u> without a NOEL this study would not be appropriate for regulatory use.

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Pages 36 through 45 are not included.

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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.

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

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[illegible] (ISO 14663)

EPA No.: 68D80056
DYNAMAC No.: 255-B
TASK No.: 255-B
January 18, 1990

006154

DATA EVALUATION RECORD

ASSURE (D-NC 302)

Mutagenicity--Mouse Lymphoma Forward Mutation Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 1/18/90

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EPA No.: 68D80056
DYNAMAC No.: 255-B
TASK No.: 255-B
January 18, 1990

DATA EVALUATION RECORD

ASSURE (D-NC 302)

Mutagenicity--Mouse Lymphoma Forward Mutation Assay

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 1-18-90

I. Cecil Felkner, Ph.D.
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Signature: Robert Weir for I. Cecil Felkner
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APPROVED BY:

Roman J. Pienta, Ph.D.
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Date: 2/28/90

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 2/26/90

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DATA EVALUATION RECORD

CHEMICAL: Assure (D-NC 302).

STUDY TYPE: Mutagenicity--Mouse lymphoma forward mutation assay.

MRID NUMBER: 412061-09.

TEST MATERIAL: D-NC 302.

SYNONYM(S): Assure.

SPONSOR: E. I. du Pont de Nemours and Co., Inc., Wilmington, DE/Nissan Chemical Industries, Ltd., Tokyo, Japan.

TESTING FACILITY: IIT Research Institute, Chicago, IL.

TITLE OF REPORT: Mouse Lymphoma Forward Mutation Assay of D-NC 302.

AUTHOR: Ketels, K. V.

STUDY NUMBER: L 08157.

REPORT ISSUED: March 19, 1987.

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CONCLUSIONS:

Five nonactivated (5, 100, 400, 800, and 1000 μg/mL) and five S9-activated (20, 200, 350, 400, 500 μg/mL) doses of D-NC 302 were evaluated for the potential to induce forward gene mutations in mouse lymphoma L5178Y ($TK^{+/-}$) cells.

Severe cytotoxicity (≤10% cell survival) was demonstrated at doses of >800 μg/mL -S9 and >450 μg/mL +S9, indicating that the range of test material concentrations selected for the evaluation of mutagenic potential was appropriate. However, the limited data reported for the mutation assay precluded an independent assessment of the results, and these data were insufficient to draw meaningful conclusions. The only reported data were cumulative cell counts, percent relative growth, mutant colony counts, and induced mutation frequencies. The reporting deficiencies include the following:

1. Data Not Reported:

   - Daily cell counts;
   - Number of colonies on nonselective plates;
   - Number of colonies on selective plates;
   - Cloning efficiencies;
   - Mutation frequencies (MF);
   - Methods used to derive these data; and
   - Number of replicate cultures used to determine the means and standard deviations of the mutant colony counts.

2. Study Design Information Not Reported:

   - Cell maintenance and storage conditions;
   - Full composition of the growth medium;
   - Methods used to determine cell counts;
   - Concentration of trifluorothymidine (TFT) in the selection medium; and
   - Number of replicate platings for total population and mutant colony counts.
   - Signed statement of quality assurance.

3. Test Material Information Not Provided:

   - Concentration of the test material that was soluble in growth medium; and
   - Analytical data to support the actual concentrations used in the study.
   - Test material stability both in the solvent and on the shelf.

Study Classification: The study is unacceptable. The study author should furnish a copy of all raw data and a detailed protocol to determine if the study can be upgraded.

A. MATERIALS:

1. Test Compound:
Name: D-NC 302
Description: Fine, light-brown powder
Lot No.: 302. DT 8501
Purity: 98.1%
Contaminants: Not listed
Solvents Used: Dimethylsulfoxide (DMSO).
Other Comments: The test material was stored in the dark at 4°C; test material solutions were prepared on the day of use.

2. Indicator Cells: The mouse lymphoma cell line, L5178Y ($TK^{+/-}$), was obtained from Dr. Donald Clive, Burroughs Wellcome, Research Triangle Park, NC. Cell maintenance and storage conditions were not reported. Cultures were exposed to methotrexate to maintain a low background frequency of TFT-resistant cells and adjusted to 3×10^5 cells/mL in Fischer's medium containing <3% horse serum.

3. S9 Fraction: The rat liver S9-fraction was obtained from Microbiological Associates Bioproducts, Walkersville, MD. The S9 reaction mixture contained the appropriate cofactors and 30% S9 liver homogenate.

4. Positive Controls: Hycanthone at 10 µm/mL and 2-acetylaminofluorene (2AAF) at 100 µg/mL were used as the nonactivated and S9-activated positive controls, respectively.

B. STUDY DESIGN:

1. Preliminary Cytotoxicity Assay: Twelve concentrations of the test material ranging from 2 to 10,000 µg/mL were evaluated in the presence and absence of S9 activation in the preliminary cytotoxicity assay. Following a 4-hour exposure, cells were washed and resuspended in growth medium; cell viability was determined. Based on the results, doses were selected for the mutation assay.

2. Mutation Assay: Cells seeded at 6×10^6 cells/tube were exposed to 13 test material doses, solvent, or positive controls with or without S9 activation for 4 hours. Cells were washed, resuspended in growth medium, and reincubated for 2 days. Daily cell counts were determined, and cells were diluted when appropriate to maintain an optimal growth rate. At the end of the expression period, five doses were chosen for mutant selection.

For mutant selection, 3 x 10^5 cells/mL were mixed with selection medium containing an unspecified concentration of TFT and 4% Noble agar and poured into an unspecified number of petri dishes. Viability was determined by plating 6 cells/mL (the number of replicate plates was not reported) in cloning medium without TFT.

After 11 days of incubation, TFT-resistant colonies and the total number of viable cells were counted; MFs were calculated.

3. Evaluation Criteria:

   a. Assay Acceptability: For the assay to be considered acceptable, the following criteria must be satisfied: (1) the plating efficiency of the solvent control must be ≥70% of the untreated control cultures; (2) relative growth of the solvent control cultures must be ≥70% of the untreated control cultures; (3) the background MF of the untreated and solvent control cultures should range from 15 to 150 x 10^{-6}; (4) the MF for the positive controls should fall between 500 and 1000 x 10^{-6} for hycanthone, and between 150 and 500 x 10^{-6} for 2AAF.

   b. Positive Response: The test material was considered positive if it induced a ≥2-fold increase in the MF as compared with the solvent control over a dose range that included a high dose causing a 90 to 95% reduction in cell survival.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The preliminary cytotoxicity assay was performed with a dose range of 2 to 10,000 µg/mL +/-S9. The study author stated that 10,000 µg/mL of the test material was soluble in DMSO; however, a fine precipitate was observed in cultures exposed to "all but the very low doses." In the nonactivated test, doses ≥2000 µg/mL were severely cytotoxic (<1% survival). Percent survival for the remaining doses ranged from 11% at 1000 µg/mL to 87.4% at the lowest assayed dose (2 µg/mL). In the presence of S9 activation, severe cytotoxicity was apparent at doses ≥500 µg/mL; below these levels, the test material was relatively noncytotoxic. Based on these results, the 13 doses selected for the nonactivated mutation assay covered a concentration range of 1 to 1500 µg/mL; the S9-activated assay was conducted with 13 doses ranging from 1 to 500 µg/mL.

2. Mutation Assay: In the nonactivated mutation assay, <0.1% of the cells survived exposure to 1500 µg/mL; the test

doses selected for cloning were 5, 100, 400, 800, and 1000 μg/mL. Relative suspension growth for the cloned levels was dose related and ranged from 7.1% at 1000 μg/mL to 101.6% at 5 μg/mL.

In the S9-activated assay, relative suspension growth was generally dose related; at the highest assayed level (500 μg/mL), 9.0% of the cells survived. The doses selected for cloning were 20, 200, 350, 400, and 500 μg/mL.

Mutation assay data presented by the study author did not include total population colony counts, cloning efficiency, or MFs; we, therefore, are unable to validate the assay results. Furthermore, the presentation of induced MFs without the actual MFs severely constrained our reviewers' ability to interpret the findings. However, based on the author's criterion for a positive response (i.e., a doubling of the background MF) and the reported MFs required for a positive response (i.e., ≥204 without S9 and ≥132 with S9), we assumed that the background MFs were 102 -S9 and 66 +S9. Using these values, the assumed MFs were calculated for all treatment groups. As shown in Table 1, the assumed MF and the mean mutant colony count for the highest nonactivated dose (1000 μg/mL) showed a slight but <2-fold increase as compared with the solvent control results. The limited data presented for lower nonactivated doses did not suggest a mutagenic response.

In the presence of S9 activation, the highest S9-activated dose induced a ≈2-fold increase in the assumed MF compared with the solvent control and an ≈1.8-fold increase in mutant colonies (Table 1). The significance, if any, of this increase cannot be assessed. Although relative suspension growth at this level indicated severe cytotoxicity (9.0% survival), without total population counts we are unable to determine the possible contribution of severe cytotoxicity to the increased mutant colony counts or the assumed MF. Slight but <2-fold increases in mutant colony counts and the assumed MFs were also noted for the remaining S9-activated doses.

TABLE 1. Representative Results from the Mouse Lymphoma Forward Mutation Assay with D-NC 302

Substance	Dose/mL	S9 Activation	Relative Percent Suspension Growth	Mean Mutant Colonies ± S.D.[a]	Assumed Mutation Frequency[b]	Induced Mutation Frequency[c]
Solvent Control						
Dimethyl-sulfoxide	1%	-	100.0	146.3 ± 11.3	102	0
	1%	+	100.0	103.7 ± 14.0	66	0
Positive Control						
Hycanthone	10 µg	-	23.6	574.3 ± 32.6	1146	1044
2-Acetyl-aminofluorene	100 µg	+	5.3	653.3 ± 24.9	761	695
Test Material						
D-NC 302	1000[d]	-	7.1	213.7 ± 16.3	193	91
	1500	-	<0.1	NC[e]	NC	NC
	400[d]	+	21.4	155.3 ± 11.9	91	25
	500	+	9.0	186.3 ± 9.1	145	79

[a]The number of replicates used to determine the means and standard deviations was not reported.

[b]Mutation frequencies were not reported; based on the author's criteria for a positive effect (i.e., a doubling of the background MF) and the reported values for a positive effect (i.e., ≥204 without S9 and ≥132 with S9), our reviewers assume that the background MFs were 102 -S9 and 66 +S9.

[c]Induced MF = MF of test group - MF of the appropriate solvent control.

[d]Results for lower assayed doses (5, 100, 400, and 800 µg/mL -S9 and 20, 200, and 350 µg/mL +S9) generally showed slight but <2-fold increases in both mean mutant colony counts and the assumed MFs.

[e]NC = Not cloned.

D. STUDY AUTHORS' CONCLUSIONS:

The study authors concluded, "Test article D-NC 302 was non-mutagenic at concentrations of up to 1000 µg/mL without S-9 microsomal activation and up to 500 µg/mL with the S9 microsomal fraction metabolic activation."

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

Owing to numerous reporting deficiencies, we assess that no meaningful conclusion can be reached regarding the potential, if any, of D-NC 302 to induce forward mutations in cultural mouse lymphoma cells. These deficiencies include the following:

1. Study Design Information Not Reported:

- Cell maintenance and storage conditions;
- Full composition of the tissue culture medium;
- Method used to determine cell counts;
- Concentration of TFT in selection medium; and
- Number of replicate platings for total population and mutant colony counts.

2. Data Not Reported:

- Daily cell counts;
- Number of colonies on nonselective plates;
- Number of colonies on selective plates (Note: Although it is acceptable to present means and standard deviations, the number of replicates used to determine these values must be reported);
- Cloning efficiencies;
- MFs; and
- Methods used to derive the above data.

3. Test Material Information Not Provided:

- Concentration at which the test material was soluble in growth medium; and
- Analytical data to support the actual concentrations used in the assay.
- Test material stability both in solution and on the shelf.

We concluded, therefore, that the study is unacceptable. Unless the study author can provide all of the raw data and a detailed protocol, it is doubtful whether the study can be upgraded.

F. QUALITY ASSURANCE MEASURES: An unsigned statement of quality assurance indicated that laboratory operations were inspected on February 10, 12, 23, and 29, 1987, and the report was audited on March 11 and 12, 1987.

G. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 6-9.

APPENDIX A
Materials and Methods
CBI pp. 6-9

ASSURE Dx R 008154

Page_____ is not included in this copy.

Pages 57 through 60 are not included.

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.

X FIFRA registration data.

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

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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.

EPA No.: 68D80056
DYNAMAC No.: 255-C
TASK No.: 2-55C
December 20, 1989

003154

NATIONAL [illegible]

DATA EVALUATION RECORD

ASSURE (D-NC 302)

Mutagenicity--Salmonella typhimurium Mammalian Microsome Mutagenicity Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 12-19-89

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EPA No.: 68D80056
DYNAMAC No.: 255-C
TASK No.: 2-55C
December 20, 1989

DATA EVALUATION RECORD

ASSURE (D-NC 302)

Mutagenicity--Salmonella typhimurium Mammalian Microsome Mutagenicity Assay

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-19-89

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Barbara Lin for I. Cecil Felkner
Date: 12-19-89

APPROVED BY:

Roman Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 12-19-89

Whang Phang, Ph.D.
EPA Reviewer, Section II
Toxicology Branch II
(H-7509C)

Signature: Whang Phang
Date: 12/28/89

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 1/4/90

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DATA EVALUATION RECORD

CHEMICAL: Assure (D-NC 302).

STUDY TYPE: Mutagenicity--Salmonella typhimurium mammalian microsome mutagenicity assay.

ACCESSION NUMBER: 412061-08.

TEST MATERIAL: D-NC 302.

SYNONYM: Assure.

SPONSOR: E.I. du Pont de Nemours and Co., Inc., Wilmington, DE./ Nissan Chemical Industries, Ltd., Tokyo, Japan.

TESTING FACILITY: IIT Research Institute, Chicago, IL.

TITLE OF REPORT: Ames Salmonella Mammalian Microsomal Reverse Mutation Test of D-NC 302.

AUTHOR(S): Barbera, P.W.

STUDY NUMBER: L 08156.

REPORT ISSUED: March 11, 1987.

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CONCLUSION(S) - Executive Summary: Five concentrations of D-NC 302 (0.05 to 5,000 µg/plate) were evaluated in the Salmonella typhimurium microsome mutagenicity assay. The highest assayed dose either with or without S9 activation precipitated; revertant colonies were not scored at this dose level. Results for the remaining four doses indicated that D-NC 302 was neither cytotoxic nor mutagenic in S. typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100 under nonactivated or S9-activated conditions. It was, therefore, concluded that D-NC 302 was not mutagenic in this test system.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material:
Name: D-NC 302
Description: Fine, light-brown powder
Lot No: 302 DT 8501
Purity: 98.1%.
Contaminants: Not listed.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: The test material was stored in the dark at 4°C.

2. Control Materials:
Negative: DMSO
Solvent/final concentration: 100 µL/plate.
Positive: Nonactivation:
Sodium azide 10 µg/plate TA100, TA1535
2-Nitrofluorene 10 µg/plate TA98, TA1538
9-Aminoacridine 100 µg/plate TA1537
Activation: 2-Aminoanthracene (2-anthramine) 10 µg/plate all strains.

3. Activation: S9 derived from

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

If other, describe below. Describe S9 composition: The S9 liver homogenate was purchased from Organon Teknika, Irving, TX. The S9 mix contained ≈10% S9 and the appropriate cofactors.

4. Test Organism Used: S. typhimurium strains

	TA97	x	TA98	x	TA100		TA102		TA104
x	TA1535	x	TA1537	x	TA1538.				

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

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5. Test Compound Concentrations Used:

a. Preliminary Assay: Four concentrations of the test material (5, 50, 500, and 5000 µg/plate) were assayed with strain TA98 in the nonactivated and S9-activated cytotoxicity test. Bacteria either were spread onto nutrient agar plates (nonactivated test) or incorporated into an agar overlay containing S9 mix and poured over minimal glucose agar. Sterile filter discs were placed on the hardened agar and were saturated with the appropriate dilution of the test material. Plates were incubated for 24 hours, and the diameter of the zones of inhibition were measured.

b. Mutation Assay: Based on the findings of the preliminary assay, the concentrations chosen for the mutation assay were 0.5, 5, 50, 500, and 5000 µg/plate with or without S9 activation.

B. TEST PERFORMANCE:

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

2. Preliminary Assay: The test material was not cytotoxic at any nonactivated or S9-activated dose.

3. Mutagenicity Assay: The report stated that precipitation of the highest assayed dose (5000 µg/plate) with and without S9 activation precluded scoring of revertant colonies at this dose level. Representative results from the nonactivated and S9-activated assay are presented in Table 1. These data show that the four assayed doses of the test material, which ranged from 0.5 to 500 µg/plate, were neither cytotoxic nor mutagenic. Although the study author stated that slight "toxicity" was seen at 50 µg/plate in strain TA1537, our reviewers did not consider the reduced revertant counts to be definitive evidence of a cytotoxic effect. By contrast to the negative test material results, all strains responded to the mutagenic action of the appropriate nonactivated or S9-activated positive control. From these data, the study author concluded that D-NC 302 was not mutagenic in this test system.

4. Reviewers' Discussion/Conclusions: We assess that the study was properly conducted and that the author's interpretation of the data was correct. D-NC 302 was assayed up to the limit of solubility and failed to induce a cytotoxic or mutagenic effect in the absence or presence of S9 activation in a well-controlled assay.

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TABLE 1. Representative Results of the Salmonella typhimurium Mutagenicity Assay with D-NC 302

Substance	S9 Activation	Dose (μg/plate)	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	--	11.3±5.9	13.0±8.7	9.7±5.7	21.3±3.2	135±4.0
	+	--	11.0±4.6	6.7±1.2	13.0±2.0	23.0±1.7	177±2.0
Positive Control							
Sodium azide	-	10	968±47.0	--	--	--	911±39.0
2-Nitro-fluorene	-	10	--	--	669±47.0	668±53.0	--
9-Aminoacridine	-	100	--	2100±100.0	--	--	--
2-Anthramine	+	10	273±11.0	142± 6.0	1931±55.0	1601±46.0	1920±102.0
Test Material							
D-NC 302	-	500[b]	13.7±2.5	6.7±1.2	8.0±1.0	14.7±1.5	134.0±7.0
	+	500[b]	10.3±3.1	5.0±2.7	7.0±1.0	23.0±5.0	136.0±7.0

[a]Means and standard deviations of counts from triplicate plates.

[b]Results for lower doses (0.5, 5, and 50 μg/plate +/-S9) were comparable to the appropriate control values; the test material precipitated at the highest assayed dose (5000 μg/plate +/-S9).

5. Quality Assurance Measures: An unsigned quality assurance statement indicating that laboratory operations and the final report were audited was provided.

6. CBI Appendix: Appendix A, Materials and Methods, CBI pp. 6-10.

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APPENDIX A

Materials and Methods

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Page_____ is not included in this copy.

Pages 69 through 73 are not included.

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.

X FIFRA registration data.

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

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

Reviewed by: SanYvette Williams, D.V.M. S Williams 11/29/89
Sec. II, Tox. Branch II (H7509C)
Secondary reviewers: Whang Phang, Ph.D. 11/29/89
Clark Swentzel, Section Head K. Clark Swentzel 11/29/89
Section II, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 008154

CHEMICAL: Propanoic acid, 2-[4-(6-chloroquinoxalin - 2 yloxy)phaenoxy]-, ethyl ester

TOX. CHEM. NO.: 215D **HED NO.:** 9 - 2183A

STUDY TYPE: Acute oral toxicity

ACCESSION NUMBER: 412061-11 **HASKELL NO.:** 17,534

SYNONYMS: DPX-Y6202 D+ Technical

TESTING FACILITY: Agricultural Products Dept., E.I. du Pont de Nemours & Company, Inc.

CITATION: Sarver, John (1989), Acute oral toxic toxicity study with DPX-Y6202-52 (9.7% EC) in male and female rats. Haskell Lab Report No. 101-89; March 1, 1989.

CONCLUSIONS: Based on results of this study, the LD50 for DPX-Y6202-52 in male rats was found to be 5900 mg/kg (Tox. Category IV) which is considered to be low in toxicity, and in female rats the LD50 was 4100 mg/kg (Tox. Category III) which puts it into a slightly toxic category.

CLASSIFICATION: Core - Minimum

A. MATERIALS:

Test compound: Propanoic acid, 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]-, ethyl ester is a reddish-brown liquid composed of 10.2% active ingredients: DPX-Y6202 D+ technical (9.7% D+ isomer of ethyl [the active isomer] and 0.5% L-isomer of ethyl 2-[4-(6-chloroquinoxalin-2-yloxy)-phenoxy]propanoate. 89.8% consisted of inert ingredients.

Test animals: Male and female Crl:CD BR rats, approximately 7 weeks old, were recieved from the Charles River Breeding Laboratories, Raleigh, North Carolina.

B. STUDY DESIGN:

Single oral doses of the test material, as emulsions in Mazola

corn oil, were administered by intragastric intubation to groups of 10 male and 10 female rats. Three groups of 10 male rats each were dosed at 5000, 7000 or 9000 mg/kg and three groups of female rats were dosed at 3000, 5000 or 7000 mg/kg. They were fasted 24 hours prior to dosing, with food being returned to them approximately one hour after dosing. Survivors were weighed and observed daily throughout a 14-day period. All clinical signs and gross pathological changes were noted. The LD50 values were calculated using the Finney method.

C. RESULTS:

The LD50 for male rats was calculated to be 5900 mg/kg with a 95% confidence interval of of 5000-6700 mg/kg. The LD50 for female rats was 4100 mg/kg with a 95% confidence interval of 3300-4800 mg/kg.

Clinical signs of toxicity most often observed in male and female rats included lethargy, ocular, nasal, and oral discharges and wet, stained perineums.

Slight to severe weight losses (up to 24% of the fasted body weight) were observed in all rats up to 4 days post dosing.

D. CONCLUSIONS:

Based on results of this study, the LD50 for DPX-Y6202-52 (9.7% EC) was found to be 5900 mg/kg in male rats and 4100 mg/kg in female rats. It is considered to be very low in toxicity in male rats (LD50 greater than 5000 mg/kg) and slightly toxic in female rats (LD50 500-5000 mg/kg) when administered as a single oral dose to fasted rats.

Reviewer: SanYvette Williams, D.V.M. 1/22/90
Section II, Tox. Branch II (H7509C)
Secondary reviewers: Whang Phang, Ph. D. 1/23/90
Clark Swentzel, Section Head
Section II, Tox. Branch II (H7509C) 1/24/90

DATA EVALUATION REPORT

008154

STUDY TYPE: Acute dermal toxicity in rabbits

TOX. CHEM. NO.: 215D **HED NO.:** 9 - 2183A

ACCESSION NO.: 412061-12 **HASKELL NO.:** 17,534

CHEMICAL: Propanoic acid, 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]-, ethyl ester

SYNONYMS: DPX-Y6202 D+ Technical

CITATION: Brock, William (1989), Acute Dermal Toxicity Study of DPX-Y6202-52 (9.7% EC) in Rabbits. E.I. du Pont de Nemours and Company, Inc. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, De. Haskell Lab Report No. 54-89.

SPONSOR: Agricultural Products Department, E.I. du Pont de Nemours and Company, Inc. Wilmington, DE.

PURITY: 9.7% D+ active isomer

CONCLUSIONS: The results of this study indicate that the skin absorption LD50 for DPX-Y6202 -52 (9.7% EC) is greater than 2000 mg/kg of body weight. (Category III)

CLASSIFICATION: Core - Minimum

A. MATERIALS:

Test compound: Propanoic acid, 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]-, ethyl ester is a reddish-brown liquid composed of 10.2% active ingredients: DPX-Y6202 D+ technical (9.7% D+ isomer of ethyl 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]proponoate (the active isomer). 0.5% L- isomer of ethyl 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propanoate. 89.8% consisted of inert ingredients.

Test animals: Young adult male and female New Zeland White rabbits from Hare Marland, Hewitt, New Jersey were quarantined for approximately two weeks. Their weight ranged between 1974 and 2192 grams on the day of dosing.

B. **STUDY DESIGN:**

On the day before dosing,the hair of each rabbit was closely clipped from the scapular to the lumbar region of the back. Plastic collars were placed around their necks to prevent ingestion of the test material or disruption of the wrappings. The test material (9.7% D+ active isomer) was applied to the skin of 5 male and 5 female rabbits at a dosage of about 2000 mg/kg. Sterile gauze pads were placed on the treated site (approximately 190 square centimeters), followed by wrapping with successive layers of plastic film, stretch gauze bandage and elastic adhesive bandage.

Approximately 24 hours after treatment, the wrappings were removed, and the excess test material was washed off the skin before being dried. Each rabbit was weighed, and observed for clinical signs of toxicity. Observations for clinical signs were made approximately one hour after dosing and then daily thereafter for 14 days. The animals were weighed on days 1, 7, and 14 following treatment. They were also observed daily for mortalities.

C. **RESULTS:**

The dosage regimen of 2000 mg/kg of DPX-Y6202-52 (9.7% EC) produced no mortalities in either the male or female test animals.

Observations at 24 hours post treatment showed that DPX-Y6202 (9.7% EC) produced moderate or severe erythema in both male and female rabbits. By day 2, all had severe erythema with necrosis. There were other dermal effects noted such as blanching and sloughing of the skin, fissuring of the skin, superficial necrosis and epidermal scaling. There was no significant change in the dermal irritation up through day 10, and by day 14, no to moderate erythema and no edema were observed.

In both the male and female rabbits, slight to moderate body weight losses (up to 7% and 4%, respectively, of their initial body weights) were observed one day after treatment. All rabbits recovered from this body weight loss by day 7. One female exhibited slight body weight loss on day 14 post treatment.

D. **CONCLUSION:**

The results of this study indicate that the skin absorption LD50 for DPX-Y6202-52 (9.7% EC) is greater than 2000 mg/kg of body weight.

The author stated that observations for clinical signs were made approximately 1 hour after dosing, but there are no results listed for this time period.

Reviewed by: SanYvette Williams, D.V.M. 6/21/90
Section II, Tox. Br. II (HFAS) (H7509C)

Secondary reviewers: Whang Phang, Ph.D. 6/27/90
Clark Swentzel, Section Head
Section II, Tox. Br. II (HFAS) (H7509C) 6/29/90

DATA EVALUATION REPORT

003154

CHEMICAL: Propanoic acid, 2-[4-(6-chloroquinoxalin- 2-yloxy)phenoxy]-, ethyl ester

TOX. CHEM. NO.: 215D **HED:** 9 -2183A

STUDY TYPE: Acute Inhalation in Rats

ACCESSION NO.: 412061-13 **HASKELL NO:** 17,534

SYNONYMS: DPX-Y6202-52 D+ Technical

AUTHOR: Valentine, Rudolph

SPONSOR: Agricultural Products Department; E.I. du Pont Nemours and Company, Inc. Wilmington, DE.

CITATION: Valentine, Rudolph (1989), Acute Inhalation Toxicity Study with DPX-Y6202-52 (9.7% EC) in Rats. E.I. du Pont de Nemours and Company, Inc.; Haskell Lab for Toxicology and Industrial Medicine, Newark, NJ. Haskell Lab Report No. 51-89.

CONCLUSION(S): Based on the estimated atmospheric concentrations of active ingredient, the 4-hour inhalation LC50 for the active ingredient of the DPX-Y6202-52 (9.7% EC formulation) is 0.36 mg/L for male rats, 0.62 mg/L for female rats, and 0.50 mg/L for male and female rats combined.

Based on the total atmospheric concentrations (active plus inert ingredients), the 4-hour inhalation LC50 for the formulation is 2.6 mg/L in male rats, 4.4 mg/L in female rats and 3.5 mg/L in male and female rats combined.

Under the conditions of this study and based on the LC50 of the DPX-Y6202-52 (9.7% EC) formulation, the test material is considered to have a low inhalation toxicity on an acute inhalation basis with the LC50 exceeding 2 mg/L. [Tox. Category III]

CLASSIFICATION: This study satisfies data requirements for an acute inhalation study according to Guideline #81-3 and is classified as Core-Minimum.

GLP COMPLIANCE: Statement included on page 3.

A. MATERIALS:

Test compound: Propanoic acid, 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]-, ethyl ester is a reddish-brown liquid composed of 10.2% active ingredients: DPX-Y6202 D+ technical (9.7% D+ isomer of ethyl 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propanoate (the active isomer), and 0.5% L-isomer of ethyl 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propanoate. 89.8% consisted of inert ingredients.

Test animals: Young adult male and female Crl:CD BR rats from the Charles River Breeding Laboratories, Raleigh, NC were used in the experiment. They were quarantined one week prior to testing, and were weighed and observed three times during this period. They were housed singly or in pairs (sexes separate) with food and water being available ad libitum except during exposure. At the initiation of the study, the male rats were 8 weeks old and weighed 240-313 g; female rats were 8-9 weeks old and weighed 206-272 g.

B. STUDY DESIGN:

1. Exposure Protocol: Five groups of 5 male and 5 female rats were restrained in perforated, stainless steel cylinders with conical nose pieces. Only the nose of each animal protruded into the chamber and each group was exposed for a single, 4-hour period to an aerosol vapor atmosphere of DPX-Y6202-52 (9.7% EC) in air. The rats were observed for clinical signs during exposure and after exposure. Those survivals were observed and weighed daily for 14 days post-exposure.

2. Atmosphere Generation: Atmospheres of DPX-Y6202-52 (9.7% EC) were generated with a compressed-air nebulizer. The aerosol/vapor mixture was discharged directly into the exposure chamber and was dispersed with a baffle to promote uniform distribution. The concentration of DPX-Y6202-52 was controlled by varying the test material feed rate into the nebulizer.

3. Analytical: Two analytical methods were used to determine the atmospheric concentration of DPX-Y6202-52 (9.7% EC) within the exposure chamber. They were the gravimetric analysis and the gas chromatographic method.

a. The gravimetric analysis was used to determine the atmospheric concentration of aerosol at approximately 30-minute intervals during each exposure. The aerosol concentration was calculated from the difference in the pre- and post-sampling filter weights. For 4 exposures, the filters were weighed immediately after sampling: every other filter was then placed in a desiccator overnight for solvent removal and then reweighed. The remaining filters were placed in 5 ml of acetone immediately after sampling.

Aliquots of the solution were chromatographed. For 1 exposure, each filter was weighed immediately after sampling and then placed in 5 ml of acetone and chromatographed. Because of incomplete overnight drying of the filters, the calculated active ingredient concentrations based on "dry" filter weights from each exposure will not be reported. In a supplemental study estimating the atmospheric concentrations of active ingredient, atmospheres of DPX-Y6202-52 (9.7%) were generated the same as the approximate aerosol concentrations as that measured for each exposure. Triplicate filter samples were collected, weighed and then placed in a desiccator. The filters were dried until a constant filter weight was obtained. The residue was assumed to represent the amount of active ingredient and was used to estimate the atmospheric active ingredient concentration.

b. Gas Chromatographic analysis

The direct measurement of active ingredient on filter samples was attempted using gas chromatography, but this method was found to be unsuitable. Therefore, the gravimetric analysis of dried filter samples was chosen to be the primary method for quantitation of the active ingredient concentrations.

Particle size distributions (mass median aerodynamic diameter (MMAD), geometric standard deviation and percent particles less than 3 and 10 um diameter) were determined with the Sierra series 210 cascade impacter during each exposure.

4. Pathology: All surviving rats were sacrificed and subjected to a gross pathological examination on the 14th day of recovery. Those rats found dead were also necropsied.

C. RESULTS:

1. Exposure Conditions:

Aerosols ranging from 2.6-3.7 um MMAD were generated; all but one exposure had particles with an MMAD less than 2.9 um.

The data of Particle size distributions of the DPX-Y6202-52 (9.7% EC) aerosol were excerpted from the report presented below:

Aerosol Concentration (mg/L)	MMAD	S.D.	PERCENT < 3 um	PERCENT < 10 um
1.2	2.6	2.5	57	94
1.7	2.6	2.6	56	92
2.2	2.9	2.3	52	93
3.9	2.9	2.4	52	92
5.0	3.7	2.4	40	88

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Although the registrant did not report the percentage of particles less than 1 um, the cascade impactor data in Appendix B (pages 25 & 26) of the report show that 10.0 to 27 percent of the particles were 1.1 um or less.

2. Clinical observations:
Because of the dense aerosol which prevented visual observation of the rats, clinical signs of toxicity could not be determined during exposure. However, immediately after release some of the common clinical signs of toxicity included compound-stained fur, nasal and ocular discharges, and hunched posture. At aerosol concentrations of 1.7 mg/L or greater, uncoordinated gait, lethargy, tremors and wet perineum were also observed immediately after exposure.

3. Mortality:
Death occurred within 1-4 days of exposure to DPX-Y6202-52 (9.7% EC) at an aerosol concentration of 2.2 mg/L or greater.

Aerosol	MORTALITY (# deaths/ # exposed)	
Concentration (mg/L)	Males	Females
1.2	0/5	0/5
1.7	0/5	0/5
2.2	2/5	0/5
3.9	5/5	2/5
5.0	5/5	4/5

One day post exposure, most surviving male rats from each group had severe weight losses (avg. 8.5-14% of initial body weight), while most females had moderate to severe weight losses (avg. 3.8-11% of initial body weight). In addition, some female rats exhibited sporadic, slight to severe weight losses during the first and second weeks of the recovery period. The rats began regaining weight about 2-3 days post exposure.

4. LC50 Calculations:
Results of the LC50 calculations, based on the total atmospheric concentrations and the estimated atmospheric concentration of the DPX-Y6202-52 (9.7% EC) formulation for male and female rats have been excerpted from pages 15 and 16 of the report and are listed below:

Summary of Dose-Response Data
Based on Total Atmospheric Concentrations

	LC50 (mg/L)	95% C.I. (mg/L)	Slope
Males	2.6 *	-	37
Females	4.4	0.35 - 8.5	11

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Combined	3.5	3.0 - 4.2	8.0

Summary of Dose-Response Data Based on Estimated Active Ingredient Concentrations

	LC50 (mg/L)	95% C.I. (mg/L)	Slope
Males	0.36*	-	44
Females	0.62	0.42 - 0.94	9.7
Combined	0.50	0.43 - 0.60	7.7

*Value shown is estimated, LC50 value and corresponding confidence limits could not be calculated based on the available data.

5. Pathology:
There was no evidence of organ-specific toxicity detected in those rats found dead or killed by design 14 days after exposure to DPX-Y6202-52 (9.7%EC).

D. CONCLUSIONS:

Based on the estimated atmospheric concentrations of active ingredient, the 4-hour inhalation LC50 for the active ingredient of the DPX-Y6202-52 (9.7% EC formulation) is 0.36 mg/L for male rats, 0.62 mg/L for female rats, and 0.50 mg/L for male and female rats combined.

Based on the total atmospheric concentrations (active plus inactive ingredients), the 4-hour inhalation LC50 for the formulation is 2.6 mg/L in male rats, 4.4 mg/L in female rats and 3.5 mg/L in male and female rats combined.

There was no organ-specific evidence of toxicity observed based upon pathological examination of rats found dead or sacrificed at 14 days post exposure.

Under the conditions of this study and based on the LC50 of the DPX-Y6202-52 (9.7% EC) formulation, the test material is considered to have a very low inhalation toxicity on an acute inhalation basis with the LC50 exceeding 2 mg/L.

This study satisfies data requirements for an acute inhalation study according to Guideline #81-3 and is classified as Core-Minimum, Tox. Category III.

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Reviewed by: SanYvette Williams, DVM SWW 2/28/90
Section II, Tox. Br. II (HFAS) (H7509C)
Secondary reviewers: Whang Phang, Ph. D. WP 3/1/90
Clark Swentzel, Section Head
Section II, Tox. Branch II (HFAS) (H7509C) CS 3/5/90

DATA EVALUATION REPORT 008154

CHEMICAL: Propanoic acid, 2-[4-(6-chloroquinoxalin - 2 yloxy)phenoxy]-, ethyl ester

TOX. CHEM. NO.: 215D **HED NO.:** 9-2183A

STUDY TYPE: Eye Irritation in Rabbits

ACCESSION NO.: 412061-14

SYNONYMS: DPX-Y6202 D+ Technical

SPONSOR: Agricultural Products Dept., E.I. du Pont de Nemours and Company, Inc.

CITATION: Brock, William (1988), Primary eye irritation study with DPX-Y6202-45 (D+ isomer) in rabbits. Haskell Laboratory for Toxicology and Industrial Medicine; Haskell Lab. report #740-88; Dec. 1988.

CONCLUSIONS: Because there was not complete reversibility of the ocular effects of DPX-Y6202-45 (D+ isomer), under conditions of this study, it is considered a severe eye irritant.

CLASSIFICATION: Core - minimum. (Tox. Category I)

A. MATERIALS:

Test compound: Propanoic acid, 2-[4-6-chloroquinoxalin-2-yloxy)phenoxy] -, ethyl ester is a reddish-brown liquid composed of 10.2% active ingredients: DPX-Y6202 D+ technical (9.7% D+ isomer of ethyl [the active isomer] and 0.5% L-isomer of ethyl. 89.8% consisted of inert ingredients.

Test animal: Young adult male New Zeland White rabbits were used in the experiment. Initial body weights ranged from 2829 to 3226 grams on the day of treatment. They were supplied by Hare Marland in Hewitt, New Jersey.

B. STUDY DESIGN:

On the day of study initiation, the eyes of 6 male rabbits were examined using flourescein dye. Those animal showing preexisting corneal or conjunctival injury or irritation were not

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used. A 0.1 ml aliquot of DPX-Y6202-45 (D+-isomer) was introduced into the lower conjunctival sac of the left eyes of the six rabbits. The right eyes were used as controls. The treated and control eyes of all animals remained unwashed. They were examined for evidence of eye irritation at approximately 1, 24, 48, and 72 hours, and 7, 14, and 21 days post treatment.

Summary of Ocular Responses to DPX-Y6202-45 (D+ isomer)

Response	1hr	24hr	48hr	72hr	7 days	14 days	21 days
Corneal opacity	6/6	6/6	6/6	6/6	6/6	0/6*	1/6
Iritis	6/6	6/6	6/6	6/6	2/6	0/6	0/6
Conjunctiva							
Redness	6/6	6/6	6/6	6/6	6/6	6/6	1/6
Chemosis	6/6	6/6	6/6	6/6	2/6	0/6	0/6
Discharge	6/6	6/6	6/6	6/6	0/6	0/6	0/6

* The corneal opacity observed at day 21 in Animal #23258 was considered to be present at day 14. But, because the opacity was obscured by the nicitating membrane in this rabbit on day 14, the opacity was not noted.

At each examination both the treated and control eyes were examined using illumination and magnification and scored for ocular reactions using the Draize scale. Any other unusual effects indicative of corrosion were also noted. Biomicroscopic examinations for corneal injury were conducted at the 24-hour observation and at each subsequent observation. The treated eyes were scored according to the biomicroscopic classification.

C. **RESULTS:**

DPX-Y6202-45 (D+ isomer) produced several ocular signs including corneal opacity, iritis and chemosis, severe conjunctival reddness and copious blood-tinged discharge in all rabbits that were treated. Moderate to severe or severe corneal damage was noted using the biomicroscopic evaluation. Other ocular effects noted were aqueous flare in all treated eyes and vascularization of the cornea in the eyes of two treated rabbits. There were also areas of alopecia and epithelial sloughing noted around the eye.

At study termination (21 days post treatment), all ocular irritation had resolved in 5 out of 6 treated rabbits with the 6th one exhibiting moderate corneal opacity at study termination. Because of this, complete reversibility of the ocular effects of DPX-Y6202-45 (D+ isomer) was not demonstrated in this study.

D. **CONCLUSION(S):**

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Because there was not complete reversibility of the ocular effects of DPX-Y6202-45 (D+ isomer), under the conditions of this study, it is a severe eye irritant (Tox. Category I).

This study satisfies the data requirements for guideline #81-4. It is classified as core - minimum.

Reviewed by: SanYvette Williams, DVM SWilliams 10/24/89
Section II, Tox Branch II (HFAS) (H509C)
Secondary reviewers: Whang Phang Ph.D. 10/27/89
K. Clark Swentzel, Section Head
Section II, Tox Branch II (HFAS) (H7509C) 10/27/89

DATA EVALUATION REPORT

002154

CHEMICAL: Propanoic acid, 2-[4-(6-chloroquinoxalin - 2 yloxy)phenoxy]-, ethyl ester

TOX. CHEM. NO.: 215D **HED NO.:** 9-2183A

STUDY TYPE: Dermal Irritation in Rabbit

ACCESSION NUMBER: 4 12061-15

SYNONYMS: DPX-Y6202 D+ Technical

SPONSOR: Agricultural Products Dept., E.I. du Pont de Nemours & Company, Inc.

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine

CITATION: Sarver, J.W. (1989), Primary dermal irritation study with DPX-Y6202-52 (9.7% EC) in rabbits. Haskell Laboratory for for Toxicology and Industrial Medicine; Haskell Lab. Report #89-89; Feb. 23, 1989.

CONCLUSION(S): Based on results of this study, DPX-Y6202-52 (97% EC) with a primary irritation index of 1.4 was shown to be a slight skin irritant (Category IV).

CLASSIFICATION: Supplementary- The report should contain the results of the dermal examination within 30-60 minutes of removal of the test material.

A. MATERIALS:

Test compound: Propanoic acid, 2-[4-6-chloroquinoxalin-2-yloxy)phenoxy] -, ethyl ester is a reddish-brown liquid composed of 10.2% active ingredients: DPX-Y6202 D+ technical (9.7% D+ isomer of ethyl [the active isomer] and 0.5% L-isomer of ethyl. 89.8% consisted of inert ingredients.

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Test animals: Young adult New Zeland White male and female rabbits were used in the experiment. Initial body weights ranged from 1937-2802 grams. They were supplied by Hare Marland in Hewitt, New Jersey

B. STUDY DESIGN:

On the day before the initiation of the study, the hair of 3 male and 3 female rabbits was clipped to expose the skin from the scapular to the lumbar region of the back. Each singly housed rabbit was placed into a stock fitted with an 8"x18" piece of rubber sheeting. Approximately 0.5ml of DPX-Y6202-52 (9.7%) was applied to 2"x2" gauze pads, placed on the test sites, secured in place and left for approximately 4 hours. After this time, the rubber sheeting was loosened, the test area marked and the gauze removed from the test site. The skin was washed with warm water and gently wiped dry on each animal. Then, the test sites were evaluated for signs of erythema, edema and any other evidence of dermal effects. In addition, the skin effects were examined at 4, 24, 48, and 72 hours, and 4 days post exposure. Primary irritation indices were calculated for each animal by adding the numerical erythema and edema scores recorded at 4, 24, 48, and 72 hours (a total of 2 values obtained from the test site of each animal at 4 time intervals) and dividing that sum by 4(1).

RESULTS:

DPX-Y6202-52(9.7% EC) produced severe erythema in 2 rabbits and moderate erythema in 4 rabbits by 4 hours post exposure. Slight edema was observed in 2 rabbits at this time. At 24 hours post treatment, 2 rabbits exhibited moderate erythema and 4 exhibited mild erythema. There was no edema observed. After 4 days, no dermal irritation was seen in any of the rabbits.

All rabbits exhibited red, swollen nictitating membranes, and had a milky-white ocular discharge during the study. Yet, these clinical signs can not be specifically attributed to DPX-Y6202-52 (9.7% EC) because 3 other test materials were being evaluated concurrently on other test sites.

A summary of responses of individual skin irritation scores and individual dermal irritation scores is presented in Tables I and II. According to the report, the average primary irritation index was calculated to be 2. Scores in the range between 2-5 place the chemical in the moderate range for an irritant.

CONCLUSION:

Based on the results of this study, the calculation of the primary irritation index as 2 is incorrect. The index should have been calculated by taking the average of the results at 24 and 72 hours.

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The calculated index then would be 1.4 which would cause the chemical to be classified as a slight skin irritant. Also, the report should contain the results of the skin examinations which were conducted within 30-60 minutes of removal of the test compound from the application sites. In addition, further information is needed in regard to the cause of the eye irritation observed in all the rabbits because there were three other test materials applied on different areas of skin along with DPX-Y6202 (9.7%EC). This leaves a question as to the significance of the eye irritation results.

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TABLE I *

RABBIT SKIN IRRITATION TEST

SKIN RESPONSES OBSERVED IN TEST RABBITS FOLLOWING TOPICAL EXPOSURE TO DPX-Y6202-52 (9.7% EC)

Rabbit Number	Erythema				
	4 hr	24 hr	48 hr	72 hr	4 days
23426	4	3	2	0	0
23427	3	3	2	2	0
23456	3	2	0	0	0
23458	4	2	1	0	0
23459	3	2	2	0	0
23466	3	2	1	1	0

Rabbit Number	Edema				
	4 hr	24 hr	48 hr	72 hr	4 days
23426	0	0	0	0	0
23427	0	0	0	0	0
23456	1	0	0	0	0
23458	0	0	0	0	0
23459	0	0	0	0	0
23466	1	0	0	0	0

*Primary Dermal Irritation Study with DPX-Y6202-52 (9.7% EC) in Rabbits Haskell laboratory Report No. 89-89. page 12.

TABLE II**

PRIMARY DERMAL IRRITATION
SCORES OF RABBITS TOPICALLY EXPOSED TO
DPX-Y6202-52 (9.7% EC)

Rabbit Number	Primary Dermal Irritation Scores
23426	2.3
23427	2.5
23456	1.5
23458	1.8
23459	1.8
23466	2.0

**Primary Dermal Irritation Study with DPX-Y6202-52 (9.7% EC) in Rabbits
Haskell Laboratory Report No. 89-89, page 12.

Table III ***

DRAIZE[2] SCALE FOR SCORING PRIMARY SKIN IRRITATION

Evaluation of Skin Reactions	Value
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1 (Slight)
Well-defined erythema	2 (Mild)
Moderate to severe erythema	3 (Moderate)
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4 (Severe)
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1 (Slight)
Slight edema (edges of area well defined by definite raising)	2 (Mild)
Moderate edema (raised approximately 1.0 mm)	3 (Moderate)
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4 (Severe)

A = Abraded	F = Fissuring	L = Sloughing
I = Intact	N = Necrosis	R = Raw Areas
T = Thickening	G = Fissuring with Bleeding	X = Compound Adhered to Skin
C = Eschar	S = Epidermal Scaling	SN = Superficial Necrosis
- = No Effect		
B = Blanching		

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[2] Draize, J. H., "Dermal Toxicity." Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Editorial Committee of the Association of Food and Drug Officials of the United States, Austin, Texas, 1959, pp. 46-59.

***Primary Dermal Irritation Study with DPX-Y6202-52 (9.7% EC) in Rabbits Haskell Laboratory Report No. 89-89, page 11.

Review by: SanYvette Williams, D.V.M.
Section II, Tox. Branch II (HFAS)(H7509C)
Secondary Reviewers: Whang Phang, Ph.D.
Clark Swentzel, Section Head
Section II, Tox. Branch II (HFAS) (H7509C)

5/30/90
5/31/90

DATA EVALUATION REPORT

006154

CHEMICAL: Propanoic acid, 2-[4-(6-chloroquinozalin-2 -yloxy)phenocy]-, ethyl ester

TOX. CHEM.NO. : 215D **HED NO.**: 9-2183-a

STUDY TYPE: Dermal Sensitization in Guinea Pigs

ACCESSION NUMBER: 412061-16 **HASKELL NO.**: 17,534

SYNONYMS: DPX-Y6202 D+ Technical

CITATION: Brock, William (1989), Closed-Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with DPX-Y6202-52 (9.7% EC) in Guinea Pigs. Haskell Laboratory for Toxicology and Industrial Medicine; Haskell Lab. Report No. 59-89; Feb. 13, 1989.

CONCLUSIONS: There is inconclusive evidence in determining the dermal sensitization potential of DPX-Y6202. Therefore, this study should be repeated.

CLASSIFICATION: Core - Supplementary. This study does not meet the data requirements for a dermal sensitization study (Guideline 81-2), and it should be repeated.

GLP COMPLIANCE: Statement is included on page 3.

A. MATERIALS:

Test compound: Propanoic acid, 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]-, ethyl ester is a reddish-brown liquid composed of 10.2% active ingredients: DPX-Y6202 D+ technical (9;7% D+ isomer of ethyl [the active isomer] and 0.5% L-isomer of ethyl. 89.8% consisted of inert ingredients.

Test animals: Young adult male and female Duncan Hartley albino guinea pigs were received from Charles River Breeding Laboratories, Stone Ridge, New York. The singly housed guinea pigs were weighed and quarantined one week prior to study initiation.

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B. **STUDY DESIGN:**

A 25% concentration of emulsion of the test material in distilled water was used for the main study based on results of a preliminary rangefinding test. The sensitization study consisted of 2 phases: an induction phase and a challenge phase. The induction phase (dermal application) was conducted in 5 male and 5 female guinea pigs, weighing from 392 to 468 grams, by applying 0.4 ml of 25% (v/v) emulsion of the test material in distilled water onto a 25 mm Hill Top Chamber Delivery System (patch). The patch was placed onto the shaved, intact skin of each animals back. A piece of plastic wrap was placed over the patch followed by an adhesive bandage. This induction procedure was done once a week for 3 consecutive weeks. In addition, 5 of the guinea pigs were being treated with a 0.4 ml suspension of DNCB. After a 6-hour exposure period the bandages and patches were removed from the test sites. Irritation responses were scored approximately 24 and 48 hours after treatment.

Two weeks after the last induction period, the test guinea pigs were challenged for sensitization by applying 0.4ml of a 25% (v/v) emulsion of the test material in distilled water to an unexposed test site. In addition, 5 naive positive control guinea pigs were treated by applying 0.4 ml of a 0.1% suspension of DNCB in 80% ethanol. Also, 5 guinea pigs were treated with 0.4 mL of a 25% (v/v) emulsion of the test material and 0.1% DNCB and served as negative controls. The bandages were removed from the test sites approximately 6 hours after exposure and washed with warm water. Approximately 22 hours after treatment, the test site were depilated for 30 minutes. Irritation responses were scored 2 hours after depilation and again 48 hours after treatment.

Approximately one week after challenge, the test guinea pigs were rechallenged for sensitization in the same manner as described for the challenge phase. In addition, a naive group of 5 guinea pigs served as another negative control group. Responses were scored 24 and 48 hours after test material application.

The incidence of sensitization is reported and defined as the number of animals in each group sensitized to the test material divided by the total number of animals tested in that group. The severity of the irritation response is the sum of the test scores in each group divided by the total number of animals tested in that group for both 24 and 48 hour evaluations.

C. **RESULTS:**

During the induction phase, one animal exhibited slight erythema following the first induction treatment at both the 24 hour and 48 hour observations. Following the second induction, 7/10 and 4/10 animals exhibited slight erythema at 24 and 48 hours respectively. After the third induction phase, 7/10 animals exhibited slight

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erythema and 2/10 exhibited mild erythema at 24 hours, while at 48 hours 8/10 displayed slight erythema and 2/10 displayed mild erythema.

Summary of Skin Responses

Challenge Phase

Response	24 hr	48 hr	Negative Control DPX-Y6202-52 24 hr	DPX-Y6202-52 48 hr	DNCB 24 hr	DNCB 48 hr
No reaction	6/10	6/10	2/5	2/5	5/5	5/5
Slight erythema	4/10	4/10	2/5	1/5	0/5	0/5
Mild erythema	0/10	0/10	0/5	1/5	0/5	0/5
Mod. erythema	0/10	0/10	0/5	0/5	0/5	0/5
Severe erythema	0/10	0/10	1/5	1/5	0/5	0/5

Response	Positive Control 24 hour	48 hour
Mild erythema	4/5	3/5
Moderate erythema	1/5	2/5

Following challenge, 5 test animals exhibited slight erythema while severe irritation with necrosis was observed in one negative control animal. The response prompted a rechallenge of the test group because the overall dermal responses in the negative control animals was greater than that observed in the test animals.

Summary of Skin Responses
Rechallenge Phase

Response	24 hr	48 hr	Negative Control 24 hr	48 hr
No reaction	0/10	0/10	3/5	3/5
Severe erythema	10/10	10/10	2/5	2/5

Following rechallenge, severe erythema with necrosis or superficial necrosis was observed in all of the test animals (10/10) at both 24 and 48 hours. Severe erythema with necrosis or superficial necrosis was also observed in 2/5 negative control animals. Previous studies with DPX-Y6202 formulations have demonstrated that it is not a dermal sensitizer but is a moderate to severe skin irritant in rabbits and guinea pigs. The irritation responses observed in the present study are considered to be related to an increased sensitivity of the skin because of repeated application of the test material and has been termed "skin fatigue". The response observed to DPX-Y6202-52 (9.7% EC) may be related to this

physiological change and unrelated to sensitization.

D. CONCLUSIONS:

This study has produced more questions than answers for the following reasons:

1. The irritation response during the induction phase shows that the investigator did not do an adequate job of finding the primary irritation threshold.

2. The negative control should not produce irritation.

3. "Skin fatigue" can not be used to explain irritation observed during the rechallenge phase with the negative control.

4. One would expect the positive control to induce a more definitive response.

5. In regard to the incidence responses at challenge on page 23 of the report, there are several miscalculations noted. In the test group animals, the incidence of sensitization should be 0.4 and 0.4 at 24 and 48 hours, respectively. The incidence values for the negative control animals should have been 0.6 and 0.6 at 24 and 48 hours, respectively.

Considering these questionable results, this study is classified as Core-supplementary and should be repeated.

EPA No.: 68D80056
DYNAMAC No.: 255-A
TASK No.: 2-55A
December 20, 1989

008154

DATA EVALUATION RECORD

ASSURE (D-NC 302)

Mutagenicity--Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 12/19/89

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EPA No.: 68D80056
DYNAMAC No.: 255-A
TASK No.: 2-55A
December 20, 1989

DATA EVALUATION RECORD

ASSURE (D-NC 302)

Mutagenicity--Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-19-89

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Robert Julian for I. Cecil Felkner
Date: 12/19/89

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 12-19-89

Whang Phang, Ph.D.
EPA Reviewer, Section II
Toxicology Branch II
(H-7509C)

Signature: Whang Phang
Date: 12/29/89

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 1/4/90

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DATA EVALUATION RECORD

CHEMICAL: Assure (D-NC 302).

STUDY TYPE: Mutagenicity--Rat primary hepatocyte unscheduled DNA synthesis assay.

MRID NUMBER: 412061-10.

TEST MATERIAL: D-NC 302.

SYNONYM(S): Assure.

SPONSOR: E. I. du Pont de Nemours and Co., Inc., Wilmington, DE/ Nissan Chemical Industries, Ltd., Tokyo, Japan.

TESTING FACILITY: IIT Research Institute, Chicago, IL.

TITLE OF REPORT: DNA Repair Assay in Primary Rat Hepatocytes on D-NC 302.

AUTHOR: Ketels, K. V.

STUDY NUMBER: L 08158.

REPORT ISSUED: April 21, 1987.

CONCLUSIONS(S)/Executive Summary:

Under the conditions of the assay, five doses of D-NC 302 ranging from 10 to 1000 µg/mL did not induce an appreciable increase in the net nuclear grain counts of treated rat hepatocytes. Cytotoxicity was clearly demonstrated at concentrations ≥5000 µg/mL, and compound precipitation was observed at doses ≥100 µg/mL. It is concluded, therefore, that D-NC-302 is not genotoxic in the primary rat hepatocyte unscheduled DNA synthesis (UDS) assay.

However, the overall study was compromised because there was no indication that slides were coded prior to scoring, and analytical data to support the actual test material concentrations used in the study were not provided.

Study Classification: The study is currently unacceptable but can be upgraded if the identified missing information can be furnished by the study author.

A. MATERIALS:

1. Test Material
   Name: D-NC 302
   Description: Fine, light-brown powder
   Lot No.: 302 DT 8501
   Purity: 98.1%
   Contaminants: Not listed
   Solvent Used: Dimethylsulfoxide (DMSO)
   Other Comments: The test material was stored in the dark at 4°C, and all solutions were prepared on the day of use.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the liver of a male Fisher F-344 rat; the source of the animal was not reported.

B. STUDY DESIGN:

1. Perfusion Technique: The liver was perfused with 0.5 M EGTA and 100 U/mL collagenase, excised, and removed to a culture dish; hepatocytes were collected in Williams' Medium E (WME) containing 10% serum, and assayed for viability.

2. Preliminary Cytotoxicity Assay: Recovered hepatocytes were seeded into triplicate tissue culture flasks at a cell density of 1 x 10^6, allowed to attach for 2 hours, and exposed to six test material doses ranging from 100 to 50,000 µg/mL. Following the 18- to 20-hour exposure, trypan blue (0.4%) was added to each flask; cells were

washed, fixed in 5% formalin, and counted. Based on the findings of the cytotoxicity test, six doses were selected for the UDS assay.

3. UDS Assay:

   a. Treatment: Approximately 1 x 10^6 cells were seeded into tissue culture flasks containing plastic coverslips, allowed to attach for 2 hours, and rinsed with WME. Prepared slides were exposed in triplicate to the selected test material doses, the solvent (DMSO), and the negative (100 nM/mL biphenyl) or positive (200 nM/mL 2-acetylaminofluorene, 2AA) controls for 18 to 20 hours in the presence of 10 µCi/mL [^{3}H]thymidine.

   b. UDS Slide Preparation: Following exposure, treated hepatocytes attached to the coverslips were washed, fixed in ethanol:acetic acid (3:1), dried, and mounted.

   c. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak NTB emulsion, dried for 10 days at 4°C in light-tight boxes, developed in Kodak D-19, stained with Harris' alum hematoxylin and eosin, and counted. The report did not indicate whether slides were coded prior to scoring.

   d. Grain Counting: The nuclear grains on 150 morphologically normal cells (50 cells per slide) for each test dose and the untreated, solvent, negative, and positive control groups were counted microscopically. Net nuclear grain counts were determined by subtracting the nuclear grain count of each cell from the highest cytoplasmic grain count adjacent to the nucleus.

4. Evaluation Criteria: The assay was considered positive if an increase in the net nuclear grain count of ≥5 grains/nucleus on triplicate coverslips occurred within a nontoxic range (>10% survival).

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The six doses evaluated in the preliminary cytotoxicity assay were 100, 500, 1000, 5000, 10,000, and 50,000 µg/mL of the test material. The author stated that a precipitate was formed in all assayed levels. Percent viability at 5,000, 10,000, and 50,000 µg/mL was 18.0, 3.4, and 25.8%, respectively. These results suggested to our reviewers that the lack of a dose-dependent response may have been associated with compound precipitation. Below 5000 µg/mL, survival proceeded in a dose-related manner and ranged from 32.9% at 1000 µg/mL to

64.2% at the lowest assayed dose (100 µg/mL). Based on these results, the study author selected 10, 50, 100, 500, 1000, and 5000 µg/mL of the test material for the UDS assay.

2. UDS Assay: The report indicated that the highest assayed dose (5000 µg/mL) was cytotoxic; this concentration was, therefore, not scored for UDS.

As shown in Table 1, the net nuclear grain count for the highest scored dose (1000 µg/mL) was slightly higher than the solvent control value, but was well below the minimum count of ≥5 grains/nucleus to conclude a positive effect. Results for the remaining doses did not suggest a genotoxic response. By contrast, the positive control, 2-AAF, induced a marked increase in the net nuclear grain count, whereas the negative control compound, biphenyl, was clearly inactive. These results indicate that the test system had an appropriate level of sensitivity to distinguish between a known genotoxicant and a known nongenotoxic substance. Based on these findings, the study author concluded that D-NC 302 was negative in this test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We assess that the study design was adequate to conclude that none of the assayed doses of D-NC 302 induced an appreciable increase in UDS. The cytotoxic effect demonstrated at doses ≥5000 µg/mL indicated that the test substance entered the hepatocytes, and that the lack of response was not due to the inability of the test material to penetrate the cell membrane. We further assess that the inclusion of a known nongenotoxic substance as an additional negative control is commendable. However, it was not reported whether slides were coded to eliminate bias, and no analytical data were provided to support actual test concentrations used in the study; these reporting deficiencies cannot be overlooked. We conclude, therefore, that the study is unacceptable (incomplete) but can be upgraded if the missing information can be supplied.

E. QUALITY ASSURANCE MEASURES: An unsigned quality assurance statement indicating that laboratory operations and the final report were audited was provided.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 6-8.

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TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with D-NC 302

Treatment	Dose/mL	Cells Scored	Average Net Nuclear Grain Counts ± Average S.D.[a]
Cell Control			
Medium only	--	150	0.35 ± 1.07
Solvent Control			
Dimethylsulfoxide	1%	150	0.53 ± 2.18
Negative Control			
Biphenyl	100 nM	150	0.46 ± 1.49
Positive Control			
2-Acetylaminofluorene	200 nM	150	24.87 ± 12.84*
Test Material			
D-NC 302	1000μg[b]	100	0.68 ± 2.13

[a]Individual means and standard deviations from the count of 50 cells per slide were presented by the study author; average counts and average standard deviations for replicate slides were calculated by our reviewers.

[b]Highest dose scored; higher concentrations were cytotoxic. Results for lower doses (10, 50, 100, and 500 μg/mL) did not indicate a genotoxic effect.

*Fulfills reporting laboratory's criteria for a positive effect (i.e., $\geq$5 grains/nucleus).

Appendix A

Materials and Methods

ASSURE TOX R 008154

Page_____ is not included in this copy.

Pages 104 through 106 are not included.

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.

X 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.