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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Assure (NC-302): Response to Registrant's Comments and Summaries of Evaluations for the Additional Toxicology Data

Caswell No.: 215D
EPA ID No. : 352-UUR / 5F3252 / 6H5479
Record No. : 203819 / 302820 / 203827
Project No.: 7-1114

TO: Robert Taylor / V. Walters, PM (25)
Registration Division (TS-767c)

FROM: Whang Phang, Ph.D. 12/2/87
Pharmacologist
Toxicology Branch / HED (769c)

THROUGH: Marcia van Gemert, Ph.D. 12/3/87
Head, Section III
and
William Burnam, Deputy Branch Chief
Toxicology Branch / HED (TS-769c) 12/1/87

INTRODUCTION:

The registrant, E.I. du Pont de Nemours & Co., submitted a set of toxicology data in support of permanent tolerances for Assure. After reviewing the data, Toxicology Branch raised concerns about the acceptability of the following studies:

1. Screening Test for Delayed Hypersensitivity (guinea pig)
2. Irritation Effects on Rabbit Skin
3. Irritation Effects on Rabbit Eye Mucosa
4. 21-Day Dermal Toxicity Study (Rabbit)
5. Teratology Study in Rabbits
6. Chronic Feeding Study in Dogs
7. Two-Generation Reproduction Study (Rat)

Peer Review Comments: The Peer Review Committee first evaluated the relevant oncogenicity data of Assure on Nov 5, 1986, at which time the chemical received a tentative Category B_2 classification. The Committee requested additional historical control data on CD-1 mice be provided for further evaluation and for final classification of this chemical. When additional information was available, a second peer review meeting was held on Aug 25, 1987. Assure was classified as a Category C oncogen without risk assessment (see Peer Review Document, Sept 9, 1987).

006529

006529

The registrant has submitted most of the requested data for various studies and has commented on all the concerns raised by the reviewer.

DISCUSSION AND CONCLUSION:

Recently submitted information has been evaluated along with the original studies. The evaluations are presented below.

1). Citation: Ligget, M. et al. Screening test for Delayed Contact Hypersensitivity with NC-302 Technical Product in the Albino Guinea Pig. Huntingdon Research Center, England. Submitted by E.I.du Pont de Nemours. Report No. 82139D / NSA7 / SS. Dated 4/16/82 (EPA Accession No. 073530).

Discussion: This study was previously evaluated by Toxicology Branch (Tox. Document No. 005013). The reviewer concluded that "Assure® is not a skin sensitizer in guinea pigs" under the experimental conditions. The study was classified as supplementary because the report did not present the purity of the test substance and positive control data cited in the submission (HRC Report No. 82138D/ NSA7/SS) with use of formalin.

Currently, the purity of th chemical is reported to be 99.1% Assure. The positive control data are still missing. The findings as stated in the previous DER remain unchanged, and this study is still classified as Core Supplemenatry.

2). Citation: Ligget, M. et al. Irritant Effects of NC-302 Technical Product on Rabbit Skin. Huntingdon Research Center, England. Submitted by E. I. du Pont de Nemours. Study No. 82115/NSA5/SE; dated 3/26/82 (EPA Accession No. 073530).

Discussion: This study was previously reviewed by Toxicology Branch (Tox. Document No. 005013). The results indicated that "Assure® is not a skin irritant in rabbits". The toxicity category of skin irritation for Assure is IV. However, the study was classified as supplementary because the report did not present the information on the purity of the test chemical. The additional data indicate that the purity of the test compound in this study was 99.1% Assure. The study is upgraded from Core Supplementary to Core Minimum.

3). Citation: Ligget, M. et al. Irritant Effects of NC-302 Technical Product on Rabbit Eye Mucosa. Huntingdon Research Center, England. Submitted by E.I. du Pont de Nemours. Study No. 8264D/NSA6/SE; dated 2/26/82; EPA Accession No. 073530.

006529

Discussion: This study was previously evaluated by Toxicology Branch (Tox. Document No. 005013). Assure was not shown to be an eye irritant in rabbits. However, the report did not present any information on the purity of the test chemical, and the study was classified as Supplementary. Currently, the test agent has been reported to be 99.1% pure. This study is upgraded as a Minimum study

4). Citation: Loveless, S. E. Subacute dermal toxicity study (21-day) of INY-6202-15 (NC-302) in rabbits. (Unpublished Study No: 411-83; Haskell Lab. for Toxicology and Industrial Medicine, Newark, DE) Submitted by E. I. du Pont de Nemours and Company, Inc.; dated 10/11/83.

Discussion: This study has been re-evaluated along with the new information, and the study is upgraded from Core Supplementary to Core Minimum (for details, please see the attached new DER).

5). Citation: Satoh, R. and Kashima, M. Effect of NC-302 on pregnancy of the New Zealand White Rabbit- Experimental administration of NC-302 to pregnant rabbits during period of organ formation of embryos. (Unpublished study No. NEMRI-H-82-19 by Nippon Experimental Medical Research Institute, Ltd., Japan. for Nissan Chemical Industries, Ltd., Japan; dated 8/30/83) EPA Accession No. 073905.

Discussion: This study was reviewed by Dynamac, Corp., and the DER was approved by Toxicology Branch (Tox. Document No. 005542). Groups of presumed pregnant females (15-18/sex/dose) were gavaged with Assure at dose levels of 7, 15, 30, and 60 mg/kg/day from gestation days 6-18, and the test animals were sacrificed at gestation day 29. The results indicated that no maternal toxicity was observed at 60 mg/kg (HDT). Significant reductions in caudal vertebral ossification were also found in the fetuses of 30 and 60 mg/kg females. However, the report did not present sufficient information for proper evaluation of the both developmental and maternal toxicity; the study was classified as Core Supplementary.

Currently, the registrant has submitted information which include a dose-range finding teratology study, number of female rabbits mated, and the method of determining the status of the uteri which appeared to be non-pregnant. This information was evaluated and found to be insufficient to satisfy the deficiencies listed in the original DER (Tox. Document No. 005542). The reasons are the following:

1). The dose-range finding teratology study was not adequately conducted to show that the highest dose (60 mg/kg) was sufficiently high to cause maternal toxicity. In this dose-range finding study, only 3 pregnant rabbits were used at the highest dose level (60 mg/kg). The number of pregnant rabbits used in the dose-range finding study should be at least 6 to obtain any meaningful information.

2). The requested results of chemical analyses of the test compound were not available.

Therefore, the classification of this study remains Core Supplementary.

6). Citation: Varney, P. (1985) NC-302: 52-Week Oral (Dietary Administration) Toxicity Study in Beagle Dog. (Unpublished study No. 4297-3006/1R) prepared by Hazleton Laboratories Europe Ltd., England. for Nissan Chemical Industries Ltd., Japan; Dated April, 1985. (Submitted by E.I. du Pont de Nemours & Co.,) (EPA Accession No. 073536).

Discussion: This study has been re-evaluated along with the newly submitted information, and the study is upgraded from Core Supplementary to Core Minimum (for details, please see the attached new DER for this study).

7). Citation: Mullin, L. S. Two Generation Reproduction Study in Rats with INY-6202. (Unpublished Study; Du Pont Report No: HLR 633-85; Haskell Lab. for Toxicology and Industrial Medicine, Newark, DE) Submitted by E. I. du Pont de Nemours and Company, Inc.; dated 9/5/85; (EPA Accession No. 074017).

Discussion: This study has been re-evaluated along with the newly submitted data, and the study is upgraded from Core Supplementary to Core Minimum (for details, please see the attached new DER for this study).

006529

Reviewed by: Whang Phang, Ph.D. 11/27/87 006529
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 12/2/87
Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2-Generation Reproduction Study in Rats (Additional Data)

ACCESSION NUMBER: 074017 for the original study

TOX. CHEM. NO. or Caswell No.: 215D

EPA ID No.: 352-UUR/5F3252/6H5479 RECORD NO.: 203819/203820/203827

TEST MATERIAL: Assure, NC-302, or INY-6202 (99.1% pure)

CITATION: Mullin, L. S. Two Generation Reproduction Study in Rats with INY-6202. (Unpublished Study; Du Pont Report No: HLR 633-85; Haskell Lab. for Toxicology and Industrial Medicine, Newark, DE) Submitted by E. I. du Pont de Nemours and Company, Inc.; dated 9/5/85.

INTRODUCTION: The original study was previouly evaluated by Dynamac Corp., and the data evaluation report of this study was approved by Toxicology Branch (Tox. Document No. 005547). In this study, Assure was tested at dietary concentrations of 0, 25, 100, and 400 ppm. The LOEL of parental toxicity for Assure was 400 ppm; NOEL, 100 ppm. Increased incidence of hematomas was observed in F_{1b} and F_{2a} pups of all treatment groups relative to the controls. Based upon this finding, the LOEL for developmental toxicity was established as 25 ppm (LDT), and NOEL for developmental toxicity could not be determined. The study was classified as supplementary. Currently, the registrant has re-evaluated the study and has also submitted additional information for this sutdy.

DISCUSSION AND CONCLUSION:

The crucial points for this study are (1) there is increased incidence of hematomas in F_{1b} and F_{2a} pups of Assure treated rats and (2) is this finding biologically significant? The incidence of hematomas is presented in Table 1. Statistically significant increase in the incidence of hematomas was observed in F_{1b} pups of 25, 100, and 400 ppm rats and F_{2a} pups of 100 and 400 ppm rats. However, this increase was not dose-related, and was not consistently seen in all generations. No incidence of hematomas was observed in fetuses of Assure treated rats in the teratology study. In addition, in a chronic feeding study with rats, no hematological effects were observed in 25 or 100 ppm animals (EPA Accession No. 073531-5).

Based upon this information, increased incidence of hematomas seen in only F_{2a} and F_{1b} pups of Assure treated rats was not a

reproductive effect, and the biological significance of this finding could not be determined. Therefore, in rats the NOEL of the developmental toxicity for Assure is 25 ppm; LOEL, 100 ppm. At 100 ppm or higher, increased liver weight and increased incidence of eosinophilic changes in the liver of offspring were observed.

With the additionally submitted information, this study is upraded from Core Supplemetary to Core Minimum.

Table 1[a]

Incidence of Hematomas Observed in Offspring of Rats Fed Assure for Two Generations

Dose Level (ppm)	0	25	100	400
F_{1a} No. pups observed	277	188	280	249
No. pups w/hematomas	27(9.7)	21(11.2)	37(13.2)	30(12.0)
F_{1b} No. pups observed	291	257	285	275
No. pups w/hematomas	8(2.7)	32(12,5)*	25(8.8)*	32(11.6)*
F_{2a} No. pups observed	284	275	260	253
No. pups w/hematomas	6(2.1)	15(5.5)	22(8.5)*	18(7.1)*
F_{2b} No. pups observed	241	286	299	238
No. pups w/hematomas	2(0.8)	2(0.7)	3(1.0)	3(1.3)

* : significantly different from control value ($p \leq 0.05$)

† : significant trend across dose groups ($p \leq 0.05$)

( ) : percent

a : Data taken from the submissions.

Reviewed by: Whang Phang, Ph.D. 11/19/87
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 11/19/87
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006529

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding Study in Dogs (Additional data)

ACCESSION NUMBER: None

TOX. CHEM. NO.: 215D

EPA ID No.: 352-UUR/5F3252/6H5479

RECORD NO.: 203819/203820/203827

TEST MATERIAL: Assure, NC-302, or DPX-Y6202 (99.1% pure)

CITATION: Varney, P. (1985) NC-302: 52-Week Oral (Dietary Administration) Toxicity Study in Beagle Dog. (Unpublished study No. 4297-3006/1R) prepared by Hazleton Laboratories Europe Ltd., England. for Nissan Chemical Industries Ltd., Japan; Dated April, 1985. (Submitted by E.I. du Pont de Nemours & Co., 1987)

INTRODUCTION: The original study was previouly evaluated by Dynamac Corp. and approved by Toxicology Branch (Tox. Document No. 005543). In this study, Groups of beagle dogs (6/sex/dose) were fed Assure at dietary concentrations of 0, 25, 100, and 400 ppm for 52 weeks. The data did not indicate any consistent treatment-related effects, and the NOEL for Assure was established at 400 ppm, highest dose tested. However the study was classified as a supplementary study because data on ophthalmology examinations were not reported. In addition, the results of a 26-week dog study was resquested to accertain the proper selection of dose levels for the 1-year study.

DISCUSSION AND CONCLUSION: The registrant has submitted the ophthalmoscopy data and has also given the EPA Accession No. of the 26-week dog study (250074).

The submitted ophthalmoscopy data did not show any treatment-related ocular changes in the treated animals relative to the controls. These data have substantiated the conclusion presented in the original report (EPA Accession No. 073536).

In the 26-week dog feeding study, the dose levels were 0, 25, 100, and 400 ppm. At 400 ppm, the incidence of testicular atrophy was observed in 2/6 males. No other treatment-related effects were observed. Based upon these data, the dose levels for the 1-year dog study appeared to be properly selected.

The additional data do not change the original conclusions of the 1-year dog study. Based upon the reported data, the NOEL for Assure is 400 ppm, the highest dose tested. This study is upgraded from Core Supplementary to Core Minimum.

Reviewed by: Whang Phang, Ph.D. 4/27/87
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 12/3/87
Section III, Tox. Branch (TS-769C)

006529

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity Study in Rats (Additional Data)

ACCESSION NUMBER: 073530 for the original study

TOX. CHEM. NO. or Caswell No.: 215D

EPA ID No.: 352-UUR/5F3252/6H5479 RECORD NO.: 203819/203820/203827

TEST MATERIAL: Assure, NC-302, or INY-6202 (99.1% pure)

CITATION: Loveless, S. E. Subacute dermal toxicity study (21-day) of INY-6202-15 (NC-302) in rabbits. (Unpublished Study No: 411-83; Haskell Lab. for Toxicology and Industrial Medicine, Newark, DE) Submitted by E. I. du Pont de Nemours and Company, Inc.; dated 10/11/83.

INTRODUCTION:

The original study was previouly evaluated by Dynamac, Corp., and the data evaluation report of this study was approved by Toxicology Branch (Tox. Document No. 005031). In this study, groups of rabbits (5/sex/dose) were dermally administered 0, 125, 500, and 2000 mg/kg for 21 days. No compound-related effects were observed; the NOEL of dermal toxicity for Assure in rabbits was 2000 mg/kg (HDT). The study was classified as supplementary at that time because the data on organ weights, on spicific skin observations, and on food consumption were not included in the report.

DISCUSSION AND CONCLUSION:

Currently the registrant has submitted additional data which were missing in the original report. The mean liver weight of 2000 mg/kg male rabbits showed a slight increase relative to the controls, but the increase was not statistically significant (Table 1). In addition, the liver to body weight ratio was comparable to that of the controls. No other effects were observed in the additionally submitted data.

Although data on food consumption were not available, this set of data might not provide additional information for toxicity of Assure because the body weights of the treated rabbits did not show any significant difference from the control animals. In addition, the acute oral toxicity studies indicated that Assure had relatively low toxicity (Category III).

006529

8

006529

Based upon this information, the NOEL of subacute dermal toxicity for Assure remains as 2000 mg/kg (HDT); the study is upgraded from Core Supplementary to Core Minimum.

006529

TABLE 1

MEAN ABSOLUTE BODY AND ORGAN WEIGHTS (G)
OF MALE RABBITS TREATED FOR 21 DAYS WITH
INY-6202-15 0-DAY RECOVERY

(DATA TAKEN FROM HLC SUBMISSION)

GROUP	CONC.	FINAL WT	LIVER	SPLEEN
CONTROL		2897.7(0.000)	99.163(0.000)	1.143(0.000)
125 MG/KG		3094.0(0.236)	97.510(0.893)	1.307(0.331)
500 MG/KG		2987.7(0.573)	88.683(0.403)	1.273(0.434)
2000 MG/KG		2995.0(0.543)	106.110(0.574)	1.273(0.434)
TEST - HOMOGENEITY		0.662	0.563	0.744
TEST - TREND		0.711	0.757	0.495
BARTLETT'S TEST		0.335	0.384	0.888

GROUP	CONC.	KIDNEYS	TESTES	THYMUS
CONTROL		19.993(0.000)	6.753(0.000)	6.237(0.000)
125 MG/KG		19.613(0.872)	8.123(0.323)	7.233(0.576)
500 MG/KG		19.943(0.983)	6.463(0.829)	6.803(0.749)
2000 MG/KG		21.697(0.477)	6.607(0.913)	5.450(0.058)
TEST - HOMOGENEITY		0.798	0.560	0.753
TEST - TREND		0.473	0.623	0.620
BARTLETT'S TEST		0.120	0.615	0.750

Values in parentheses - P VALUE OF STUDENT T TEST COMPARISON OF TREATMENT MEAN TO CONTROL MEAN.

\+ - SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY LSD

\# - SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY LSD AND DUNNETT'S TEST

HOMOGENEITY - P VALUE OF F TEST OF WHETHER GROUP MEANS ARE EQUAL.

TREND - P VALUE OF F TEST OF WHETHER THERE IS DOSE-RELATED CHANGE IN GROUP MEANS.

BARTLETT'S TEST - P VALUE OF TEST OF HOMOGENEITY OF VARIANCE

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006529

SEP 9 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Second Peer Review Meeting on Assure

FROM: John A. Quest, Ph.D. JA Quest
Team Leader, Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Addressees

This memorandum summarizes the results of a second peer review meeting on Assure. This chemical was first reviewed on November 5, 1986, at which time it received a tentative Category B_2 classification based upon the findings of liver tumors in female rats, ovarian tumors in female mice, and liver tumors in male mice. The Peer Review Committee suggested at the first meeting that additional information, particularly historical control data be provided by the registrant (Dupont) to facilitate a final classification for the chemical. This data, as well as additional pathology information, has recently been received from the Dupont Company. This material is discussed in detail in the attached memorandum (with attachments) of June 11, 1987, from Dr. Whang Phang to Dr. Reto Engler.

The second peer review meeting to discuss available toxicology information on Assure and to provide a final classification was held on August 25, 1987. The attendees were: R. Engler, T. M. Farber, J. W. Hauswirth, R. Levy, W. Phang, J. A. Quest and E. Rinde. The discussion revolved around the aforementioned tumors seen in female rats, female mice, and male mice and the new data received from the registrant.

Discussion of liver Tumors in Female CR-SD Rats:

Assure was initially reported to produce a significant dose-related trend for hepatocellular carcinomas in female CR-SD rats given dietary doses of 0, 25, 100 and 400 ppm for 104 weeks (see Table 1, below, from original peer review memorandum). The study was performed by Huntingdon Research Centre, England, for the Dupont Company.

006529

11

TABLE 1: Liver Tumor Incidence in Assure-Treated Female CR-SD Rats

Liver Tumor Type	Sex	Dose (ppm) 0	25	100	400
Adenomas	F	3/75(4.0%)	1/75(1.3%)	1/75(1.3%)	1/75(1.3%)
Carcinoma	F	0/75(0%)	0/75(0%)	2/75(2.6%)	4/75(5.3%)[a]
Combined	F	3/75(4.0%)	1/75(1.3%)	3/75(4.0%)	5/75(6.7%)

[a] = Statistically significant positive dose-related trend

The elevated incidence of carcinomas (i.e., 5.3%) seen at the highest dose level of Assure (i.e., 400 ppm) exceeded the historical control incidence for similar tumors in studies conduced by the testing laboratory (i.e., mean 2.0%; range 0% to 4.8%). However, no increase in liver hyperplasia or in adenomas per se was seen, no reduction in the latency period for the onset of tumors occurred, and the highest dose level appeared to approximate a MTD level.

The registrant had the above tumor pathology data reevaluated by Environmental Pathology Laboratory (EPL), and the following information was obtained (Table 2).

TABLE 2: Reevaluation of Liver Tumor Incidences in Assure-Treated Female Rats by EPL

Liver Tumor Type	Sex	Dose (ppm) 0	25	100	400
Adenomas	F	3/75(4.0%)	2/75(2.6%)	3/75(4.0%)	3/75(4.0%)
Carcinoma	F	0/75(0%)	0/75(0%)	1/75(1.3%)	2/75(2.6%)[a]
Combined	F	3/75(4.0%)	2/75(2.6%)	4/75(5.3%)	5/75(6.7%)

[a] = Statistically significant positive dose-related trend

The revised data from EPL indicated (a) an increased number of adenomas in all groups and (b) a reduction in the number of carcinomas in the mid (100 ppm) and high (400 ppm) dose groups. The Peer Review Committee considered this information and concluded: 1) Assure still produced a statistically significant positive trend for carcinomas in female rats (Table 2), but the observed low increases in carcinomas were not biologically significant; and 2) the revised lower incidences of carcinomas reported by EPL (Table 2) did not exceed the historical control incidences for liver carcinomas reported by the test laboratory (i.e., 0% to 4.8%). Based on this new data provided by the Dupont Company, the Peer Review Committee concluded that Assure did not appear to be associated with an increase in liver carcinomas in female CR-SD rats.

Discussion of Ovarian Stromal Tumors in Female CD-1 Mice:

Assure produced a significant increase in ovarian stromal tumors (luteomas, and luteomas and granulosa cell tumors combined) in female CD-1 mice given dietary doses of 0, 2, 10, 80 and 320 ppm for 78 weeks (see Table 3, below, from original peer review memorandum). The ovarian cell tumors were also considered to be uncommon in terms of their site of occurrence. The study was performed by Nissan Chemical Industries, Ltd., Japan, for the Dupont Company.

TABLE 3: Ovarian Stromal Tumor Incidence in Assure-Treated Female CD-1 Mice

Tumor Site and Type	Sex	Dose (ppm) 0	2	10	80	320
Ovary:						
Luteoma	F	0/51(0%)	0/51(0%)	1/46(2%)	0/53(0%)	3/50(6%)[a]
Granulosa Cell	F	0/51(0%)	0/51(0%)	0/46(0%)	0/53(0%)	1/50(2%)
Combined	F	0/51(0%)	0/51(0%)	1/46(2%)	0/53(0%)	4/50(8%)[a]
Luteal hyperpl.	F	0/51(0%)	0/51(0%)	0/46(0%)	0/53(0%)	2/50(4%)

a = Statistically significant ($p < 0.05$) when compared to historical control values for ovarian luteoma (0/196 or 0%) or ovarian luteomas/granulosa cell tumors combined (1/196 or 0.5%).

The Committee reevaluated the findings of ovarian stromal tumors in female mice, and noted several points which appeared to diminish the weight of the evidence for a positive oncogenic effect of Assure. First, additional consideration was given to the fact that ovarian tumors were determined to be significantly elevated when compared to historical control values, but not when compared to concurrent control values (see footnote a in Table 3, and also original peer review memorandum for details). Since the source of this historical control data could not be identified, the committee decided to omit it from the data base used for statistical comparisons and therefore to designate the female mouse ovarian stromal tumor incidences listed in Table 3 as being not significantly elevated. Second, the registrant provided historical control data from 4 studies conducted at Haskell Lab indicating that ovarian stromal tumors (luteomas and granulosa cell tumors) in female CD-1 mice range from 0% to 6.6%; the incidence of ovarian stromal tumors (8%) produced by Assure fell slightly outside of this range (Table 3). However, in some of the studies conducted at Haskell, the numbers of luteomas observed

were similar to (i.e., 3/80 in one study) or exceeded (i.e., 4/76 in another study) those seen in the Assure study (i.e., 3/50; see Table 3). Furthermore, data on file with the Agency relating to FD&C dye studies at several different test laboratories also show incidences of ovarian stromal tumors greater than those seen in the Assure study. Third, the ovarian stromal tumors in the Assure study were not accompanied by hyperplasia of the ovary, by signs of endocrine activity related to ovarian function, or by a dose-response relationship with respect to the incidences observed. On the basis of this information, the Committee believed that the ovarian stromal tumors observed in the Assure female mouse study were probably not compound-related. There was some minor reservation about this conclusion, however, which was related to the question of the rarity of the observed tumors. Although historical data provided by the registrant and that available in Agency files suggest these tumors may not be as uncommon as was once believed, the Committee felt that this question might be better resolved if historical data from the laboratory that acutally performed the Assure study (Nissan Chemical Industries, Ltd.) were available for evaluation.

Discussion of Liver Tumors in Male CD-1 Mice:

Assure produced significant dose-related trends for hepatocellular adenomas, and for adenomas and carcinomas combined, in male CD-1 mice given dietary doses of 0, 2, 10, 80 and 320 ppm for 78 weeks (see Table 4, below, from original peer review memorandum). In addition, hepatocellular adenomas and carcinomas combined were significantly elevated at the highest dose level tested in male mice (Table 4). This study was performed by Nissan Chemical Industries, Ltd., for the Dupont Company.

TABLE 4: Liver Tumor Incidences in Assure-Treated Male CD-1 Mice

Tumor Site and Type	Sex	Dose (ppm) 0	2	10	80	320
Liver:						
Adenomas	M	3/70(4%)	6/69(9%)	5/69(7%)	7/69(10%)	11/70(7%)[a]
Carcinomas	M	4/70(6%)	4/69(5%)	2/69(3%)	1/69(1%)	10/70(14%)
Combined	M	7/70(10%)	10/69(14%)	7/69(10%)	8/69(11%)	15/70(21%)[a,b]

a = Statistically significant positive dose-related trend (liver tumors).
b = $p < 0.05$ compared to controls (liver tumors).

Upon reevaluation of the male mouse study, the Committee noted several points which appeared to diminish the weight of the evidence for an effect of assure to produce liver tumors. First, the registrant provided historical control data from both Hazleton Labs and Haskell Lab indicating that liver carcinomas in male CD-1 mice ranged from 2% to 17%; the incidence of carcinomas (14%) produced by Assure fell within this range (Table 4). Second, an elevated incidence of carcinomas appeared to be primarily responsible for the oncogenic effect of Assure, but these tumors were not accompanied by increases in liver adenomas or by liver hyperplasia. Furthermore, Assure was not mutagenic. Third, the tumors did not occur in a strictly dose-related manner: they were seen only at the high dose level. Finally, the high dose level exceeded a MTD level. On the other hand, the Committee observed that the historical data provided by the registrant was not from the laboratory that performed the study (Nissan Chemical Industries, Ltd.), that the combined incidence of liver adenomas/carcinomas was significantly elevated at the high dose level of Assure compared to the concurrent controls, and that there was evidence for a reduction in the latency period for the time-to-tumor appearance. On the basis of all of the above information, the Committee concluded that the possibility that Assure acted to produce liver tumors in male mice could not be ruled out.

Conclusions:

The Committee reconsidered the toxicology data base on Assure in the light of new historical control data and pathology information provided by the registrant and reached the following conclusions:

1. Assure does not appear to be associated with an increased incidence of liver carcinomas in female CR-SD rats.

2. Assure does not appear to be associated with an increased incidence of ovarian stromal cell tumors in female CD-1 mice. This conclusion, however, is encumbered by a question relating to the rarity of these tumors with possible resolution of the issue attendant upon the receipt of historical control data from the test laboratory.

3. Assure was associated with an increased incidence of hepatocellular tumors (adenomas plus carcinomas combined) in male CD-1 mice, but only at the highest dose level tested which was in excess of a MTD level. The tumors appeared to occur with a reduced latency period for development.

The above information led the Peer Review Committee to revise the classification of Assure, as derived at the initial peer review meeting held on November 5, 1986, from that of a tentative Category b_2 carcinogen (producing liver tumors in female rats and male mice, and ovarian tumors in female mice) to a Category C carcinogen (producing liver tumors in male mice, and possibly uncommonly occurring ovarian tumors in female mice). Because the overall evidence for the oncogencity of Assure was considered to be weak, it was further recommended that no quantitative risk assessment be performed for the chemical. Finally, the Committee concluded its deliberations on Assure by recommending that the registrant attempt to obtain historical control information on both liver (adenomas/carcinomas combined) and ovarian stromal tumors in male and female CD-1 mice, respectively, from the test laboratory. Receipt of this data would prompt a further effort by the Committee to more accurately classify the chemical.

ADDRESSEES

T. Farber
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R. Engler
E. Rinde
J. Hauswirtn
R. Levy
M. Van Gemert
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M. Jones
R. Beliles
D. Beal
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#20 9/4/87 sp