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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of the Comments of Du Pont on the Peer Review of DPX-Y6202 (ASSURE®)

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THROUGH: Marcia van Gemert, Ph.D. 6/11/87
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Recently, the Peer Review Committe evaluated the oncogenicity studies of Assure® on rats and mice, relevant toxicology data, metabolism, and structural activity relationship data of Assure®. The Peer Review Committee classified the oncogenic potential of Assure® as "tentative category B_2" oncogen. This classification was based upon increased incidences of liver tumors in high-dose female rats and male CD-1 mice as well as ovarian tumors in high-dose female mice. The registrant submitted additional historical data on liver tumor incidence of male mice and on ovarian tumor incidence of female mice. The registrant also had the histopathology data of high-dose male rats and of the controls re-evaluated by the pathologists of Experimental Pathology Laboratories, Inc., Durham, NC (EPL).

This reviewer has evaluated the submitted data and comments and has arrived at the following conclusions:

Based upon the currently submitted data, the incidence of hepatocellular tumors observed in high-dose female rats was not compound related. The increased incidences of hepatocellular tumors in high dose CD-1 male mice only and of combined luteomas and granulosa cell tumors in high dose female mice could be considered as marginal and showed some evidence for the oncogenic potential of Assure. Therefore, the Peer Review Committee is requested to reconsidered the classification of the oncogenic potential of Assure.

INTRODUCTION

The Peer Review Committee had evaluated the rat and mouse oncogenicity studies, other relevant toxicology data and metabolism on Assure (NC-302 or DPX-Y2602); the Committee classified the oncogenic potential of Assure as "tentative category B_2". In addition, the Committee requested (1) historical control information on the incidence of hepatocellular adenomas in CD rats from the test laboratory to evaluate the finding related to the combined incidence of hepatocellular adenomas and carcinomas (2) information as to whether liver hyperplasia was looked for in the study but not found and therefore not reported or whether a pathological evaluation for hyperplasia was actually not performed (Attachment A: Memorandum of J. Quest to R. Taylor, Feb 6, 1987).

DISCUSSION

The registrant, Du Pont De Nemours Co., recently has submitted the information requested by the Peer Review Committee and has commented on the classification of the oncogenic potential of Assure as "tentative B_2" (Attachment B; Accession No. 401480-03). The pertinent experimental data from the Data Evaluation Reports of the rat and mouse studies, the findings of the Peer Review Committee, and the comments presented by the registrant are summarized and discussed below.

I. The Incidence of Liver Tumor in Rats (Chronic/Oncogenic Toxicity Study in Rats with NC-302; Attachment C).

TABLE 1
Incidence of Liver Tumors in Assure Treated Female Rats

Dose (ppm):	0	25	100	400
Number examined*:	75	75	75	75
Hepatocellular adenomas	3 (4.0)	1 (1.3)	1 (1.3)	1 (1.3)
Hepatocellular carcinomas	0 (0)	0 (0)	2 (2.6)	4 (5.3)†
Aden. / carci. combined	3 (4.0)	1 (1.3)	3 (4.0)	5 (5.3)

* Includes all rats from 52 weeks on test through terminal sacrifice.
( ) Denote % affected.
† Statistically significant positive dose-related trend.

Table 1 shows the tumor incidence observed in Sprague Dawley derived CD rat oncogenicity study. There was an increase in incidence of hepatocellular carcinomas in mid and high dose treated females relative to the controls, but this increase was within the range of the historical controls for the incidence of hepatocellular carcinomas in SD rats (0-7%).

The Peer Review Committee felt that in high dose females there was a dose-related trend for the incidence of hepatocellular carcinomas (Table 1) and an increased incidence of hepatocellular cytoplasmic eosinophilia. The

Committee felt that hepatocellular eosinophilia may indicate "focal hepatocellilar proliferative lesion which has been reported to progress to adenomas and carcinomas but only in a mildly aggressive manner" (Memorandum of J. Quest to R. Taylor, Feb 6, 1987). Therefore, the Peer Review Committe concluded that the incidences of hepatocellular carcinomas and of hepatocellular eosinophilia provide some evidence for the oncogenic potential of Assure.

In response, the registrant obtained the histology slides of the controls and of the high-dose female rats from the test laboratory, Huntingdon Research Centre (HRC), England, and sent these slides to Experimental Pathology Laboratory (EPL) for re-evaluation. The results are presented in Table 2 (Evaluation of Liver Tissues from Female Rats; Report No. NSA-11/8575; EPA Accession No. 401480-01).

TABLE 2
Incidence of Liver Tumors in NC-302 Treated Female Rats
(Data taken from EPL report)

Dose (ppm):	0	25	100	400
Number examined*:	75	75	75	75
Hepatocellular adenomas	3 (4.0)	2 (2.6)	3 (4.0)	3 (4.0)
Hepatocellular carcinomas	0 (0)	0 (0)	1 (1.3)	2 (2.6)†
Aden. / carci. combined	3 (4.0)	2 (2.6)	4 (5.3)	5 (5.3)

* Includes all rats from 52 weeks on test through terminal sacrifice.
( ) Denote % affected.
† Statistically significant positive dose-related trend (Cochran-Armitage Trend Test) (P = 0.0325) (Statistics performed by the Statistics Team of Toxicology Branch, HED/OPP, USEPA).

Utilizing the NTP nomenclature, the pathologists at EPL found 2 additional hepatocellular adenomas, but 2 hepatocellular carcinomas were judged to be adenomas. Although the re-evaluated histopathology data still show a very marginal increase in hepatocellular carcinomas in high dose female relative to the controls and a significant dose-related trend; this tumor incidence is well within the historical control values, and does not indicate biological significance. The EPL pathologists concluded that this tumor incidence is not compound related. The histopathology data from EPL were presented to Dr. Louis Kasza, and he agreed with their findings as presented in Table 2 and their conclusion.

In the available slides, the EPL pathologists did not find any increased incidence of hyperplasia in the treated females relative to the controls. There was treatment-related increase in centrilobular hypertrophy of hepatocytes in high dose females (control, 0/75; high dose, 63/75). The hypertrophy was characterized by minimal to slightly generalized enlargement of centrilobular hepatocytes, and the enlargement was due to increased amounts of eosinophilic cytoplasm around the central vein. No nuclear alterations were found (EPL report on Evaluation of Liver Tissues From Female Rats; Report No. NSA-11/8575; EPA Accession No. 401480-01).

Based upon the currently available histopathology data, the incidences of cytoplasmic eosinophilia and hepatic enlargement reported by HRC appear

to be representative of the incidnece of centrilobular hypertrophy of hepatocytes without any nuclear alterations. This change may be different from the focal hepatocellular proliferative lesion which was discussed in the Peer Review Summary (Memorandum of J. Quest to R. Taylor, Feb 6, 1987).

Concerning the historical control data on the incidence of hepatocellular adenomas in CD rats, the registrant did not submit additional information except to show that the incidence of carcinomas in high-dose female rats (2.6%) was essentially similar to that of the historical control means (2%) of HRC and was within the range of the historical controls (Attachment B).

II. The Incidence of Liver Tumors in Male Mice (NC-302 Oncogenicity Study in Mice) (Attachment C)

The Peer Review Committee determined that (1) the increased incidence of hepatocellular carcinomas in high-dose male CD-1 mice showed a dose-related trend with Peto Trend Test, and (2) the increase in the incidence of combined hepatocellluar adenomas and carcinomas in high-dose males are significantly increased relative to the concurrent controls (Table 3). (3) In treated male mice, there was also a reduced latency of tumor occurrence. (4) Other non-oncogenic hepatotoxicity was also observed in the treated mice. Based upon these data, the Committee concluded that Assure at 320 ppm produced a compound-related effect on male mice (Memorandum: J. Quest to R. Taylor, Feb 6, 1987).

TABLE 3.
Incidence of Liver Tumors in NC-302 Treated Male Mice

Dose (ppm):	0	2	10	80	320
Number examined:	70	69	69	69	70
Hepatocellular adenomas	3(4)	6(9)	6(7)	7(10)	5(7)
Hepatocellular carcinomas	4(6)	4(6)	2(3)	1(1)	10(14)*
Aden. / carci. combined	7(10)	10(14)	7(10)	8(12)	15(21)*†

( ) Denote % affected.
* Statistically significant positive dose-related trend.
† Statistically significant relative to the controls ($p < 0.05$)

The registrant has questioned the appropriateness of statistics used in analyzing the trend test for hepatocellular carcinomas. The registrant argues that "the trend test is positive in males only if it is run using the 'actual dose' rather than 'group serial number' or 'dose rank'".

This argument has been evaluated by Mr. Richard Levy, leader of statistics team of Toxicology Branch. The following is his reply (Memorandum of Levy to Phang; June 2, 198; Attachment D).

> The ability to change units of dose for a test of trend depends on the ratios of the gaps between the dose levels. For these data, a test for positive trend with respect to dose would be more sensitive to the increasing ratios between doses, while a test for positive trend with respect to

group serial number, which is equally sensitive to all the differences between successive groups, would be less sensitive to the real effect of Assure. However, it is interesting to note that a Cochran-Armitage positive trend test with respect to serial number is borderline significant (p=0.065) for these data.

Concerning the reduced latency of tumor occurrence, the registrant argued that the finding of liver adenomas or carcinomas in treated animals prior to that found in the controls is considered incidental because the death of the tumor bearing animals was due to another cause which was unrelated to liver tumor. The shortening of time-to-tumor occurrence seen in treated males can not be simply dismissed as a reflection of chance occurrence for there were 5 treated males with adenomas and 3 with carcinomas prior to any tumors seen in the controls.

The registrant provided additional information on the liver tumor incidence of the control CD-1 mice used in Hazleton and Haskell Laboratories. The data are presented in Table 4a, 4b, and 4c.

In Table 4a, the incidence of hepatic tumors in male CD-1 mice from three feeding studies ending in 1983 through 1985 ranges from 4 to 15% for hepatocellular adenomas and 2 to 14% for hepatocellular carcinomas. The period between 1983 and 1985 corresponds approximately to the time when the mouse oncogenicity study with NC-302 was conducted.

In Table 4b, the incidence of liver tumors in CD-1 males from three gavage studies ending in 1979 through 1980 were found to be 0 to 4% for hepatocelluar adenomas and 13 to 17% for hepatocellular carcinomas.

In Table 4c, the incidence of liver tumors in CD-1 mice of four 18 month-feeding studies ranges from 8 to 14% for hepatocellular adenomas and 5 to 10% for hepatocellar carcinomas. These four feeding studies were completed in 1984 through 1986 in Haskell Laboratory.

Based upon the currently submitted hepatic tumor incidence of the historical controls, the incidences of both hepatocelluar adenomas and carcinomas in NC-302 treated CD-1 male mice are within the respective ranges of the historical control values of three feeding studies in Hazleton Laboratory (for example, adenomas: historical control, 4-15%; NC-302 treated, 7%. Carcinomas: historical control, 2-14%; NC-302 treated, 14%). However, the values of the historical controls appear to be selectively chosen because it would be difficult to believe that within 3 years only three feeding experiments were conducted using CD-1 mice for a large laboratory such as Hazleton. The combined incidence of hepatocellular adenomas and/or carcinomas of the mouse historical trols have not been submitted for evaluation (Attachment E).

III. Ovarian Tumors in High-Dose Female CD-1 Mice (NC-302 Oncogenicity Study in Mice; Attachment C)

The Peer Review Committee concluded that the increased incidence of ovarian tumors (combined luteomas and granulosa cell tumor) indicates the oncogenic potential of Assure. Although this incidence was not statistically different from the concurrent controls, it was significantly increased when com-

TABLE 4

A.

Incidence of hepatic tumors in control male CD-1 mice at Hazleton Laboratory from three studies ending in 1983 through 1985:

Group:	Study A	Study B	Study C
# of mice/group:	50	41	50
TUMOR:			
Hepatocellular adenoma	7 (14)	6 (15)	2 (4)
Hepatocellular carcinoma	1 (2)	1 (2)	7 (14)

( ) Denote % affected, rounded to the nearest whole percent.

B.

Incidence of hepatic tumors in control male CD-1 mice from Haskell Laboratory:

Group:	Study Aa	Study Bb	Study Cc	Study Dd
Study Completed:	1984	1984	1985	1986
# of mice/group:	80	80	80	80
TUMOR:				
Hepatocellular adenoma	9 (11)	11 (14)	9 (11)	6 (8)
Hepatocellular carcinoma	8 (10)	4 (5)	4 (5)	4 (5)

( ) Denote % affected, rounded to nearest whole percent.

C.

Incidence of hepatic tumors in control male CD-1 mice at Hazleton Laboratory from three studies ending in 1979-1980:

Group:	Study D	Study E	Study F
# of mice/group:	55	61	24
TUMOR:			
Hepatocellular adenoma	2 (4)	0 (0)	1 (4)
Hepatocellular carcinoma	7 (13)	8 (13)	4 (17)

( ) Denote % affected, rounded to the nearest whole percent.

pared to the historical data (Table 5). In addition, this tumor type in CD-1 mice is also considered as a rare tumor. There were two high dose females with luteal hyperplasia which lend some supports to the Committee's conclusion.

TABLE 5
Incidence of Liver Tumors in NC-302 Treated Male Mice

Dose (ppm):	0	2	10	80	320	Historical
Number examined:	51	51	46	53	50	Control
Luteoma	0(0)	0(0)	1(2)	0(0)	3(6)†	0/196
Granulosa cell tumor	0(0)	0(0)	0(0)	0(0)	1(2)	1/196
Combined	0(0)	0(0)	1(2)	0(0)	4(8)†	1/196
Luteal cell hyperplasia	0(0)	0(0)	1(2)	0(0)	2(4)	a

( ) Denote % affected.
† Statistically significant relative to the controls ($p < 0.05$).
a Data not available.

The registrant argues that the increased incidence of luteomas seen in CD-1 mice is an equivocal result, and no true relationship to NC-302 treatment can be established because in recent article, Haseman et al. showed that statistical differences can occur with rare tumors in dual control Groups (Attachment F).

In the article of Haseman et al., the authors analyzed the tumor incidence from 18 carcinogenicity studies which used male and female CD-1 mice and CD rats. The purpose was to determine "if the frequency of significant ($p < 0.5$) pairwise differences between the 2 concurrent control groups used in these studies exceeded chance expectant". The authors found that there were 23 observed statistically significant ($p < 0.05$) paired control differences in tumor incidence in these 18 studies which were listed in Table 4, page 580 of Attachment F, but the ovarian tumor was not one of them.

Additional control data on the incidence of ovarian tumors in CD-1 mice from Haskell Laboratory have been submitted to show the incidence of luteomas was found in the controls of three studies (Table 5). The registrant stated that the average incidence at Haskell Laboratory was 2.6% for luteoma. The incidence of 2.6% is much higher than that reported in the Haseman article (1.7%) for ovarian stroma cell tumors which may include several type of tumors, such as luteoma, granulosa cell tumors, theca cell tumors, and arrhenoblastomas.

TABLE 5
Incidence of Ovarian Tumors in Control CD-1 Female Mice (Haskell Laboratory)

Group:	Study As	Study Bb	Study Cc	Study Dd
Study completed:	1984	1984	1985	1986
No. of mice/group:	73	80	76	80
Luteoma	0	3	4	1
Granulosa/theca cell tumor	0	0	1	0
Adenoma/cystadenoma	0	0	1	1

The registrant also argues that the incidence of luteal cell hyperplasia was found merely in the same slide, same section, and the same mice as did luteoma. This reviewer disagrees with this argument because, for example, one of the hyperplasia was observed in a mouse which had granulosa cell tumors instead of luteomas.

CONCLUSION:

Based upon the currently submitted data, the incidence of hepatocellular tumors observed in high-dose female rats could not be considered as compound related. The increased incidences of hepatocellular tumors in high dose CD-1 male mice only and of combined luteomas and granulosa cell tumors in high dose female mice would be considered as marginal and showed some evidence for the oncogenic potential of Assure.

In the presence of additional information, the Peer Review Committee is requested to reconsider the classification of the oncogenic potential of Assure.