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

EEB 6 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Assure

FROM: John A. Quest, Ph.D., Team Leader
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Robert Taylor, Product Manager #25
Fungicide Herbicide Branch
Registration Division (TS-767)

The Toxicology Branch Peer Review Committee met on November 5, 1986 to discuss and evaluate the data base on Assure. Particular attention was focused on the oncogenic potential of the chemical CD-1 mice and Charles River SD rats.

A. Individuals in Attendance:

1. Peer Review Committee (Signatures indicate concurrence with peer review unless otherwise stated.)

Donald Barnes

Robert Beliles

William Burnam

Reto Engler

Judith Hauswirth

Stephen Johnson

Margaret Jones

Louis Kasza

Herbert Lacayo

John A. Quest

2. Scientific Reviewers: (Non-Committee members responsible for presentation of data; signatures indicate technical accuracy of Committee report.)

Whang Phang

Marica VanGemert

3. Peer Review Committee Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee).

Anne Barton

Diane Beal

Theodore M. Farber

Richard Hill

Bertram Litt

Esther Rinde

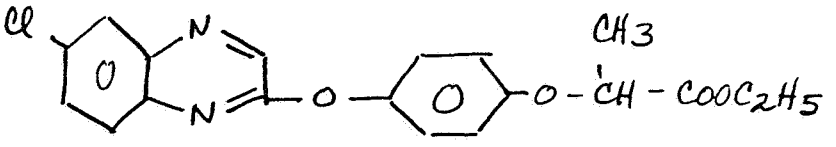
B. Material Reviewed:

The material available for review consisted of a summary of toxicology data on Assure prepared for the Peer Review Committee (Phang/VanGemert memorandum of 10/14/86), DER's of rat and mouse oncogenicity studies of Assure, and information on various reproduction and subchronic studies performed on the chemical.

C. Overview of Toxicology Issues:

Assure (NC-302; DPX-Y 6202) is a selective post emergence herbicide developed for use on broad leaf field crops. The registrant is E.I. duPont de Nemours and Company, Wilmington, Delaware. All required toxicological data has been supplied by the registrant for full tolerance and registration. Assure was brought before the Peer Review Committee to discuss potential oncogenic effects in two rodent species. These include ovarian and liver tumors in CD-1 mice and liver tumors in Charles River SD rats.

Structure:



2-[4-(-chloro-2-quinoxalinyloxy)phenoxy]proprionate

D. Evaluation of Oncogenicity Studies:

1. Mouse Oncogenicity Study:

Assure was administered in the diet to groups of 50 male and 50 female CD-1 mice at concentration of 0, 2, 10, 80 and 320 ppm for 18 months (78 weeks). Additional groups of 10 mice/sex/dose level were subjected to interim sacrifice at study weeks 26 and 52, respectively. The study was conducted by Nissan Chemical Industries, Ltd., Japan, and the study report was prepared by Hazleton Laboratory America. The following incidence patterns of ovarian tumors in female mice and liver tumors in male mice suggestive of a compound related effect were observed. No significant increase in liver tumors occurred in female mice.

Tumor Site and Type	Sex	Dose (ppm) 0	2	10	80	320
Ovary:[e]						
Luteoma	F	0/51(0%)	0/51(0%)	1/46(2%)	0/53(0%)	3/50(6%)[d]
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%)[d]
Luteal hyperpl.	F	0/51(0%)	0/51(0%)	0/46(0%)	0/53(0%)	2/50(4%)
Liver:[f]						
Adenomas	M	3/70(4%)	6/69(9%)	5/69(7%)	7/69(10%)	5/70(7%)
Carcinoma	M	4/70(6%)	4/69(5%)	2/69(3%)	1/69(1%)	10/70(14%)[a]
Combined [c]	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).
c = Number of animals with either or both types of liver neoplasms, not the number of neoplasms.
d = 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%).
e = Histopathological examination of female mice for ovarian tumors was conducted only on the main group of animals in each dose group (i.e., 46 to 53 animals/group were examined; the different denominators reflect the number of animals that remained in each dose group at the end of the study). No ovarian tumors occurred at the 27 and 53 week interim sacrifice periods.
f = Liver tumors were seen in males during the first interim sacrifice (27 weeks); therefore all animals in each dose group that were autolyzed were examined histopathologically.

a) Discussion of Ovary Data:

The Peer Review Committee noted that the ovarian stromal tumors (i.e. luteomas, granulosa cell tumors) produced by Assure in female mice were rare in regard to their site of occurrence. The incidence of the ovarian luteomas, and of the luteomas and granulosa cell tumors combined (i.e. combined stromal tumors) in the treated mice were not statistically significantly different from concurrent control values. With rare tumors, this is often the case, i.e., biological significance is not reflected by statistical significance. However, the incidences of these tumors were statistically significant at the highest dose level tested compared to historical control values from studies conducted at the test laboratory (see above table and accompanying footnote). That is, the larger number of animals evaluated in historical controls allowed for a conclusion on statistical significance. The increased incidence of luteoma at the high dose level was accompanied by a slight increase in luteal hyperplasia (see table). There appeared to be no reduced latency period for the onset of ovarian tumors since the stromal tumors were primary observed at terminal sacrifice and none were seen at the 52-week sacrifice period. On the other hand, the detection of rare tumors in the small number of animals that were sacrificed at 52 weeks (i.e., 10 animals/sex/dose) is very unlikely.

The highest dose of Assure tested in female mice (i.e. 320 ppm) appeared to approach a MTD level, based on hepatotoxic findings of increased liver weight and enzyme activity (i.e. alkaline phosphatase) and liver pathology (i.e. enlarged hepatocytes, hepatocellular pigmentation, sinusoidal cellular pigmentation and focally pigmented macrophages). Similar changes occurred in female mice at the 80 ppm dose level as well.

b) Discussion of Liver Data:

Significant dose-related positive trends for hepatocellular carcinomas; and for adenomas and carcinomas combined, occurred in treated male mice. In addition, hepatocellular adenomas and carcinomas combined were significantly elevated at the high dose level in male mice. In general, there was a reduced latency period for the occurrence of liver tumors in treated male mice compared to the control males. That is, liver tumors were first seen in treated males as early as 27 weeks, but most were observed at 52 to 55 weeks or later as opposed to 68 weeks or later in the control males. No increased incidence of liver hyperplasia occurred in animals

administered Assure. Historical control data on liver tumors were not provided by the test laboratory for comparative purposes.

The highest dose of Assure tested in male mice (i.e. 320 ppm) exceeded a MTD level. This dose level was associated with significantly reduced survival at the end of the study (27/70 survived at the high dose, vs. 41/70 in controls and 34/70 to 40/70 in other treatment groups). An increased incidence of testicular atrophy was seen in high dose males compared to controls. Other signs reflective of hepatotoxicity where observed at both the highest (i.e., 320 ppm) and the next to the highest (i.e. 80 ppm) dose levels tested, and consisted of increases in liver weight and enzyme activity (i.e. alkaline phosphatase) and liver pathology (i.e. enlarged hepatocytes, hepatocellular pigmentation, sinusoidal cellular pigmentation, and focally pigmented macrophages).

The Peer Review Committee requested that additional information regarding historical control data be provided for liver tumors (adenomas, carcinomas) in male mice.

2. Rat Oncogenicity Study:

Assure was administered in the diet to groups of 50 male and 50 female Charles River SD rats at concentrations of 0, 25, 100 and 400 ppm for 104 weeks. Additional groups of rats were subjected to interim sacrifice at study weeks 26 (10 mice/sex/dose level), 52 weeks (10 mice/sex/dose level), and 78 weeks (15 mice/sex/dose level), respectively. The study was conducted by Huntingdon Research Centre, England. The following incidence patterns of liver tumors in female rats suggestive of a compound-related effect were observed. No significant increase in liver tumors occurred in male 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

NOTE: Since the first tumor among all the test animals was observed at week 52 (during the interim sarcifice), the Committee recommended that only those animals sacrificed at or after week 52 (i.e., 75 animals) should serve as the denominator in the above table for tumor tabulation purposes.

a) Discussion of Liver Data:

A significant, dose-related trend for hepatocellular carcinomas was observed in treated female rats. The incidence of carcinomas was also elevated above the concurrent control value at the highest dose level in females, but this change was not statistically significant. The elevation of carcinomas (i.e. 5.3%) seen at the highest dose level, however, did exceed the historical control incidence observed for the tumor type in studies conducted by the test laboratory (mean 2.0%; range 0% - 4.8%). There was no increase observed in adenomas per se. No liver hyperplastic lesions were reported by the test laboratory. It was noted, however, that an increased incidence of hepatocellular cytoplasmic eosinophilia occurred in high dose female rats; the Committee noted that this type of focal hepatocellular proliferative lesion has been reported to progress to adenomas and carcinoma but only in a mildly agressive manner (Hagiwara and Ward, Fundam. Appl. Toxicol. 7: 376-386, 1986). There did not appear to be any reduction in the latency period for the onset of liver carcinomas in treated female rats. That is, all four carcinomas seen at the highest dose level were observed at the 2-year final sacrifice period; one of the two carcinomas seen at the mid dose level also occurred at 2 years whereas the other occurred after 18 months.

No specific discussion of MTD in the Charles River SD rat study was conducted by the Committee. It was noted however, that several changes in liver function and structure occurred in both high dose and/or mid dose female and male rats at the end of the study and at the various interim sacrific periods as well. These included hepatocyte enlargement, increased liver weight, and increased alkaline phosphatase activity. Changes similar to these were also described in a 13-week toxicity study in both sexes of Sprague Dawley rats at the the HDT of 1280 ppm, along with decreases in body weight gain of 25% and 13% in males and females, respectively. These changes were absent or minimal at lower doses of 40 and 128 ppm in the 13-week study. The results of the 13-week study thus suggest that a dose of 1280 ppm would have exceeded a MTD level if it were used in the chronic study. By implication, therefore, the highest dose tested in the chronic study (i.e., 400 ppm) may have approximated, but did not exceed a MTD level.

The Peer Review Committee requested that additional information be obtained for the Assure chronic rat study. The data requested was: 1) historical control information on the incidence of hepatocellular adenomas from the test laboratory to evaluate data related to combined liver tumor incidence; and 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.

E. Additional Toxicology Information:

1. Metabolism:

Several metabolism studies were performed in rats using single oral doses (1.5 or 160 mg/kg) of ^{14}-C Assure (i.e. NC-302). The compound was demonstrated to be absorbed ~~from the~~ from the gastrointestinal tract, and the highest levels of radioactivity (RA) were found in blood, liver, and kidney. Biological T 1/2 values ranged from 18-27 hours in blood and tissues of both males and females. The T 1/2 values in fat were longer, averaging 155 hours in males and 97 hours in females. The major route of excretion was via the feces in males, whereas equal amounts of administered RA were eliminated in the feces and urine in females. In fecal samples collected within 48 hours, unchanged parent compound accounted for about ~~for about~~ 23% of the high oral dose (160 mg/kg) and less than 7% of the low oral dose (1.5 mg/kg). Tne proposed metabolic pathway for the compound is shown in the following diagram; the major metabolite of Assure (NC-302) is the corresponding acid (NC-302 acid) which is further metabolized as indicated.

METABOLIC PATHWAYS OF NC-302 IN RAT

2. Mutagenicity:

Assure was evaluated for mutagenic activity in several in vitro studies using bacteria, yeast, and mammalian cells. The compound was negative in all tests. These included gene mutation assays (E. coli WP-2; S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538), DNA damage assays (B. subtilis M45 and H17; unscheduled DNA synthesis in rat hepatocytes), and a chromosome abberation assay (CHO cells). All tests were acceptable to the Agency.

3. Reproduction and Teratology Studies:

Two studies were considered by the Committee. In a two generation reproduction study in Crl:CD(SD)BR rats, Assure was administered in the diet at dose levels of 0, 25, 100 and 400 ppm. Parenteral toxicity occurred at the 400 ppm dose level in male rats as evidenced by findings of decreased body weights and premating body weight gain in the F_0 and F_1 groups. Developmental toxicity occurred at all dose levels as evidenced by findings of increased incidences of hematomas in pups (F_{1b} and F_{2a}) at all dose levels, increased liver weights and incidences of eosinophilic changes in the livers of offspring (F_{2b}) from the 100 and 400 ppm levels, and reductions in litter size, survival, body weights and spleen weights of offspring from most generations at the 400 ppm level. In a teratology study in rabbits, Assure produced a mild fetotoxic effect manifested by reduced caudal vertebrae ossification at 30 and 60 mg/kg/day (HDT). No maternal toxicity occurred.

4. Structure-Activity Correlations:

The Toxicology Branch conducted a computer search for structural analogues of Assure using Chem Line and Tox Line. Various Congeners of Assure were identified, but no toxicology data was found for these.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding toxicology data on Assure to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Assure, when administered in the diet to female CD-1 mice, was associated with significant increases in ovarian tumors (luteomas, and luteomas plus granulosa tumors combined) at the highest dose level tested (i.e. 320 ppm). The ovarian tumors were considered uncommon in terms of their site of occurrence, and were also accompanied by a slight increase in luteal hyperplasia.

2. Liver tumors (carcinomas, and carcinomas plus adenomas combined) were also seen in male CD-1 mice at the highest dose level tested (320 ppm). Although there was no evidence for the occurrence of hyperplastic changes in the livers of treated males, there was evidence for a reduction in the latency period for time-to-tumor appearance.

3. Assure, when administered in the diet to female Charles River CD rats, was associated with a significant positive dose-related trend for hepatocellular carcinomas. The elevated carcinoma incidence that occurred at the highest dose level tested (400 ppm) exceeded the historical control incidence observed in other studies at the test laboratory, but it was not significantly increased above the concurrent control level. Evidence for a progression in the tumorigenic response to malignancy was not established as no increase in adenomas occurred, and the presence or absence of liver hyperplastic changes was not indicated by the test laboratory. However, cytoplasmic eosinophilia, a mild focal hepatocellular proliferative lesion, was seen at the high dose level. There was no decrease in the latency period for the development of liver carcinomas in treated female rats. No significant increase in liver tumors occurred in male rats.

4. The primary target organ of toxicity of Assure was the liver, as evidenced by similar changes in liver function and structure (e.g., increased organ weight and alkaline phosphatase activities, enlarged and pigmented hepatocytes, and cytoplasmic eosinophilic changes) in both the mouse and rat chronic oncogenicity studies, in a 13-week rat toxicity study, and in a two generation rat reproduction study.

5. Metabolism studies were not performed in mice. However, studies conducted in rats demonstrated a preferential concentration of the compound in the liver. This finding is consistent with the liver being a target organ for Assure's toxicity.

6. No evidence for mutagenic activity of Assure was obtained in several in vitro studies, using bacteria, yeast and mammalian cells, which were acceptable to the Agency.

7. Several structural analogues of Assure were identified following a computerized data base search, but toxicity data was not available for these analogues.

8. Assure produced adverse effects in a two-generation rat reproduction study in parenteral males (i.e. decreased body weights and premating body weight gain) and in offspring of both sexes (e.g., increased incidence of hematomas, increased liver weight and eosinophilic changes, and reductions in survival and body weights), but reproductive performance was not altered. No teratogenic effects were seen in rabbits.

G. Classification of Oncogenic Potential:

The Committee concluded that the data available for Assure provided sufficient evidence of oncogenicity for the chemical in animals. The conclusion was based on the findings: 1) that ovarian tumors were produced in female CD-1 mice at the highest dose tested. The tumors were considered to be uncommon in regard to site of occurrence and were also accompanied by hyperplasia suggesting a progression in the oncogenic response; 2) that liver carcinomas and carcinomas plus adenomas in combination occurred in male CD-1 mice with positive dose-related trends. The tumors were accompanied by a reduction in the latency period to tumor appearance; and 3) that liver carcinomas occurred in female Charles River CD rats with a positive dose related trend. The tumors were accompanied by a mild focal hepatocellular degenerative lesion (cytoplasmic eosinophilia) but no other signs of progression in the oncogenic response (i.e. no hyperplasia or adenomas). Although the elevated carcinoma incidence at the highest dose tested in female rats was not significantly increased compared to concurrent controls, it did exceed the historical range for this tumor in other studies at the test laboratory. Assure was not mutagenic in several in vitro systems, and no adequate toxicology data was available for several analogues of the chemical identified in a computerized structure-activity search.

Based on the above information and the criteria in EPA Guidelines for Carcinogen Risk Assessment (CFR, September 24, 1986), the Peer Review Committee classified Assure as a tentative Category B_2 (probable human) carcinogen. That is, Assure produced increased incidences of malignant tumors or combined malignant and benign tumors in mice (i.e., ovarian luteomas or luteomas and granulosa cell tumors combined in female mice, and liver carcinomas or adenomas and carcinomas combined in male mice). In addition, the ovarian tumors in female mice were uncommon with regard to site of occurrence, and the liver tumors in male mice occurred earlier in treated animals than in control animals. Assure also produced a marginal increase in liver carcinomas in female rats (statistically significant trend). The Committee also considered criteria for classifying a carcinogen in the C category, but Assure appeared to exceed the criteria specified for this classification. That is, Assure produced a malignant tumor response in mice which was supported by a marginal increase in same tumor type in female rats. It also produced increases in tumors that were more than just marginally statistically significant, and this also occurred in studies that were generally adequate in design and reporting. Finally, more than an increase in benign tumors alone was seen for this agent that showed no response in short-term tests for mutagenicity (i.e., carcinomas were also seen).

In summary, the opinion of the Committee was that the occurrence of an uncommon tumor type (ovarian luteomas, and luteomas and granulosa cell tumors combined) in female mice alone provided some rationale for the B_2 category. Furthermore, the B_2 classification was considered to receive support from evidence indicating tnat the liver was a target organ of toxicity in both mice and rats, and that similar hepatocellular tumors (carcinomas) occurred in both male mice (with a reduced latency period) and in female rats treated with Assure. It was also, recognized, however that additional information pertaining to the oncogenicity studies of Assure might be provided by the registrant for consideration by the Committee. This information includes historical control data for liver tumors (adenomas, carcinomas, and adenomas/carcinomas combined) in male CD-1 mice, historical control data for liver tumors (adenomas, and adenomas/carcinomas combined) in female Charles River CD rats, and data on the incidence of liver hyperplastic changes (if available) in female Charles River CD rats. Pending the potential receipt of this information, the Committee agreed to defer a final classification of oncogenic potential and to assign the tentative B_2 classification to Assure.

#16 11/21/86 sb