02/27/90

PEER REVIEW FILES

007712

007712

CHEMICAL NAME: Bromacil
CASWELL NO.: 111
CAS NO.: 314-40-9
REVIEWER: Taylor

CURRENT AGENCY DECISION

Classification deferred pending receipt and evaluation of repeat mouse study.

TUMOR TYPE / SPECIES

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. / /	2. / /	2. / /	2.
1. 06/23/87	1. 09/09/87	1. 07/18/88	1. Class. deferred

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. / /	1.

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. / /	1. / /
1. 04/07/88	

MISCELLANEOUS:

Stamped 2/2/90; =PR-007712; 130 p ; nha.

Peer Review Documents
(Memo dates)

7/18/88

007712

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

FILE COPY

JUL 18 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Bromacil

FROM: Esther Rinde, Ph.D. E. Rinde 4/13/88
Scientific Mission Support Staff (TS-769c)

TO: Robert Taylor
Product Manager # 25
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on Sept. 9, 1987 to discuss and evaluate the weight-of-the-evidence on Bromacil with particular reference to its oncogenic potential.

A. Individuals ir Attendance:

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

Theodore M. Farber	Theodore M. Farber
Reto Engler	Reto Engler
Richard Hill	—
Richard Levy	Richard Levy
Judith Hauswirth	Judith W. Hauswirth
Jack Quest	Jack A. Quest
Esther Rinde	Esther Rinde

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

Linda L. Taylor

Marcia Van Gemert

Bernice Fisher

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

William L. Burnam

Anne Barton

Robert Beliles

Diane Beal

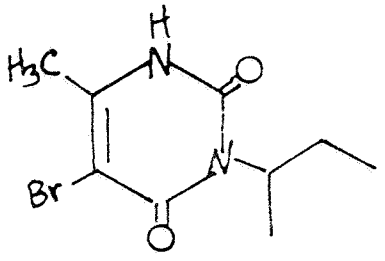
B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. Taylor; tables and statistical analysis by B. Fisher. The material reviewed is attached to the file copy of this report.

C. Background Information:

Bromacil (5-bromo-3-sec-butyl-6-methyluracil) is a non-selective herbicide used to control a wide range of perennial weeds and grasses in industrial and agricultural areas. Bromacil, also called Hyvac X Bromacil Weed Killer, INN 976, BOREA, BROMAX 4G, BROMAX 4L, CYANOGEN, HYVAR X, HYVAR X-L, ROUT, URAGON, UROX "B", UROX "HX", was a Registration Standard (9/30/82), and was issued a tolerance of 0.1 ppm for pesticide residue on citrus fruits.

Structure of Bromacil:



D. Evaluation of Oncogenicity Evidence for Bromacil:

1. Rat Oncogenicity Study
Reference: Two-Year Feeding Study in Charles River CD Rats - Haskell Laboratory; Sherman, H. and Kaplan, A.M. Appl. Pharm. 34:189-196, 1975; Pesticide Petition No. 6F 0499; MRID 00022077

Bromacil was administered in the diet (containing 1% corn oil) to groups of 36/sex/group Charles River CD rats at 0, 0 (replicate controls), 0.005, 0.025, or 0.125% - equivalent to 50, 250, 1250 ppm, respectively, for two years.

NEOPLASTIC LESIONS[1]

Thyroid follicular cell adenomas (found only in the high dose (1250 ppm) females) and thyroid light cell adenomas (in males at all three doses) were not considered to be compound related.

NON-NEOPLASTIC LESIONS[1]

Thyroid follicular cell and light cell hyperplasia were seen in all animals, including controls, but the incidence was slightly higher in the high-dose animals (the degree of hyperplasia was said to be slight in all cases). The increased incidence of follicular cell hyperplasia in high-dose females did not occur in the same 2 females bearing tumors. Hypertrophy and hyperplasia of the parathyroid were also reported in all animals, including controls.

Historical Controls: Data for spontaneous tumors of the thyroid were not available.

This study was considered inadequate and of little use in the weight-of-evidence determination, based on insufficient number of animals tested, and high mortality during study: 58% of Control I, 38% of Control II and 33% of high-dose male animals were found dead. The thyroid lesions (although mainly non-neoplastic) were however noted.

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[1]See Reviewer's Table and comment (page 3a).

Reviewer's Tables

[2]Light cell adenomas, as follows:

FEMALES					MALES				
Control	Control	Low	Mid	High	Control	Control	Low	Mid	High
1	3	1	0	5(1)*	0	0	2	1	1

* () number of adenomas found by LLT

[2]Non-neoplastic lesions of the thyroid and parathyroid were as follows:

	FEMALES					MALES				
	C	C	Low	Mid	High	C	C	Low	Mid	High
FOCAL FOLLICULAR CELL HYPERPLASIA	2	3	0	0	11	1	10	7	8	9
FOCAL LIGHT CELL HYPERPLASIA	8	2	2	0	6	1	0(2)	1	2	13(12)
PARATHYROID HYPERTROPHY	1	3	2	0	2	2	9	10	6	9
PARATHYROID HYPERPLASIA	2	4	3	0	3	2	8	10	6	8

() # found by LLT

COMMENT: The number of animals per group for which no thyroid was available for examination (or were autolyzed) is shown below. There were 36 animals/sex/group at study initiation.

MALES					FEMALES				
Control I	Control IA	Low	Mid	High	Control I	Control IA	Low	Mid	High
21	12	11	11	14	11	10	13	18	14

[2]The denominators (by inference) are presumably as follows: for Males: Control I, Control IA, Low, Mid, and High dose, respectively = 15, 24, 25, 25, 22; and for Females: 25, 26, 23, 18, 22. These data were not confirmed by the Reviewer or analyzed by the Tox Branch Statistics Team, since the study was considered inadequate, as indicated on page 3.

2. Mouse Oncogenicity Study

Reference: Eighteen Month Feeding Study in CD-1 Mice Haskell Laboratory #893-80; EPA Accession # 244069-244071, Dec. 1, 1980.

Bromacil was administered in the diet (containing 1% corn oil) to groups of CD-1 mice, 80/sex/group, at 0, 250, 1250, or 5000 ppm for 18 months.

NEOPLASTIC LESIONS:

There was a statistically significant increase in combined hepatocellular carcinoma/adenoma at the high dose (5000 ppm), compared to concurrent controls and a significant dose-related trend for hepatocellular carcinoma and combined carcinoma/adenoma in male mice. There were no dose-related increases in hepatocellular tumors in female mice. (Tables 3 and 4 in B. Fisher memo[3], 4/7/88 - ATTACHMENT 1).

NON-NEOPLASTIC LESIONS:

Hepatocellular hypertrophy in male mice occurred with the following incidences: 3/69 (4%), 1/69 (1%), 17/68 (25%), 47/68 (69%); the incidences in female mice were: 0/74 (0%), 3/74 (4%), 3/72 (4%), 12/72 (17%) for doses of 0, 250, 1250, 5000 ppm, respectively. In males, there were also dose-related increases in testicular abnormalities.

Historical Controls: Data from control male CD-1 mice sacrificed at 18 months in three 2-year and five 18-month feeding studies run between 1978 and 1985 at Haskell (the testing laboratory) are summarized below. The incidence of hepatocellular carcinoma and carcinoma/adenoma, combined in treated male mice at 5000 ppm exceeded that reported for historical controls at Haskell.

Incidence[4] (%) of Liver Tumors in Control Male CD-1 Mice From 18 Month Studies

	Adenoma	Range(%)	Carcinoma	Range(%)
Males	43/393 (10.9%)	6.8-13.8%	26/393 (6.6%)	5-10%

Combined adenoma/carcinoma (assuming no double-counting) = 69/373 (17.6%)

[3]Based on complete reanalysis of data completed after Peer Review Meeting (results of reanalysis were consistent with data presented at the meeting).

[4]Number of tumors/Number of Animals Examined

D. 2. Mouse Oncogenicity Study (continued)

MTD: The Committee determined that the MTD had been reached at the high dose in males, based on 13% depression in body weight gain; high dose males also had increased atrial thrombosis. In female mice, the MTD was probably exceeded at the high dose, based on an apparently significant increase in mortality: 64% at the high dose vs 46% in controls, with a pairwise significance of $p < 0.05$ (Table 2 in Fisher memo, 4/7/88 - ATTACHMENT 1).

E. Additional Toxicology Data on Bromacil:

1. Metabolism

Bromacil was excreted in the rat urine, principally as 5-bromo-6-hydroxymethyl-3-sec-butyl uracil; trace levels of bromacil and two other metabolites (not identified) were also detected.

2. Mutagenicity

Bromacil gave a positive response in the Drosophila sex-linked recessive lethal test[5] for gene mutations at 2, 3, 5 and 2000 ppm in feeding solution. Positive responses were also seen in two mouse lymphoma L5178Y cell assays[6,7]. Mutagenicity and cytotoxicity of Bromacil are apparently enhanced by metabolic activation[6]. In an Unscheduled DNA Synthesis assay[6], no incorporation of Bromacil (with or without activation) was observed.

Bromacil was negative in the following assays[6]:

Mouse dominant lethal
Salmonella typhimurium (His+ Reversion)
Saccharomyces cerevisiae (Mitotic Recombination)
Escherichia coli WP2 (Try+ Reversion)
Bacillus subtilis (Relative Toxicity)

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[5]J. Environ. Sci. Health B15(6), 867-906 (1980).

[6]Genetic Toxicology-An Agricultural Prospective. R.Flech and A.Hollaender, eds. Plenum Press, New York (1982).

[7]Evaluation of Selected Pesticides as Chemical Mutagens. In Vitro and In Vivo Studies. SRI, PB 268-647 (1977).

E. 3. Developmental and Reproductive Effects:

None of the available studies were adequate.

4. Structure-Activity Correlations

Bromacil is structurally related to 6-methyl-2-thiouracil, which has been shown to produce thyroid tumors in rats[8], in one study; in another study in White rats, no tumors were noted, but the thyroid showed diffuse and focal hyperplasia; in a third study, in albino rats, thyroid adenomas were produced. Another analog, 6-methyluracil produced thyroid adenomas (follicular and solid), hyperplasia, and increased thyroid weight[7] in White rats.

It was noted that although Bromacil and 6-methyluracil structurally resemble thiouracil, neither contains the thionamide structure that is necessary for anti-thyroid activity with that class, which includes thiouracil, propylthiouracil and methimazole.

There is also structural resemblance of Bromacil to another class of thyroid inhibiting compounds, which includes Resorcinol. Resorcinol has demonstrated antithyroid activity in humans and in animals[9], however, 4-Hexyl-resorcinol showed no neoplastic or non-neoplastic lesions of the thyroid in either rats or mice [NTP Peer Review, 3/4/87[10]].

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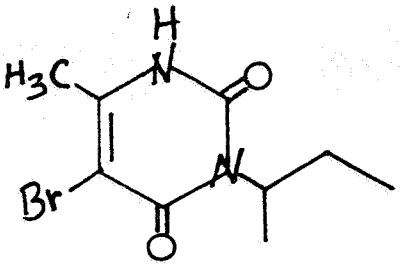
[8]Survey of Compounds Which Have Been Tested for Carcinogenic Activity (1974-75) NCI.

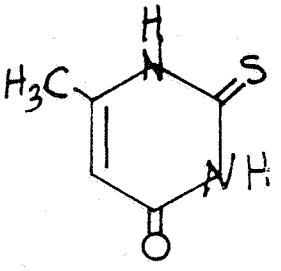
[9] W.L. Green. (1978). Mechanisms of Action of Antithyroid Compounds, In The Thyroid, S.C. Werner and S.H. Ingbar, eds., Chapter 4. New York: Harper and Row. 1978.

[10]Personal Communication from Dr. Richard Hill, 6/8/88.

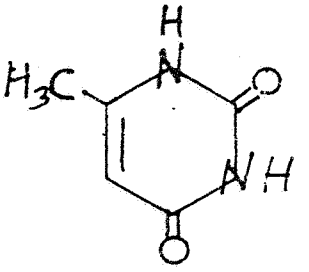
E. 4. Structure-Activity Correlations (Contd.)



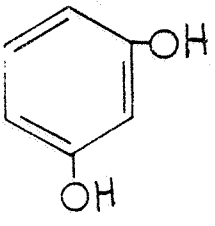
BROMACIL



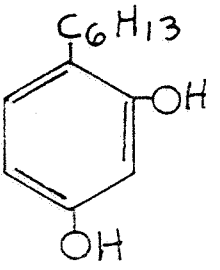
6-METHYL-2-THIOURACIL



6-METHYLURACIL



RESORCINOL



4-HEXYL-RESORCINOL

F. Weight of Evidence Considerations:

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

Bromacil fed in the diet (up to 5000 ppm) to CD-1 mice, produced a statistically significant increase in the incidence of combined carcinoma/adenoma at the HDT in the livers of male mice only ($p<0.01$). There was also a significant dose-related trend for hepatocellular carcinoma ($p<0.05$) and for combined hepatocellular carcinoma/adenoma ($p<0.01$) in these males. The incidence of hepatocellular carcinoma and combined carcinoma/adenoma in treated male mice at 5000 ppm exceeded that reported for historical controls at Haskell (the testing laboratory).

A dietary study (up to 1250 ppm) in Charles River CD rats was considered inadequate by the Peer Review Committee, however thyroid lesions (mainly non-neoplastic), significantly increased in females at the high dose, were noted.

Bromacil is a structural analog of 6-methyl-2-thiouracil and 6-methyluracil, both of which have been shown to produce thyroid tumors in rats (neither Bromacil nor 6-methyluracil contains the thionamide structure, which is associated with anti-thyroid activity with this class). Bromacil is also structurally similar to another class of anti-thyroid compounds, which is includes Resorcinol; 4-Hexyl-Resorcinol was negative for oncogenicity in rats and mice.

Bromacil was mutagenic in the Drosophila Sex-Linked Test, and in 2 mouse lymphoma assays. Bromacil was negative in a number of other assay systems: mouse dominant lethal, S. cerevisiae, E. coli, Bacillus subtilis, Salmonella typhi. It was also reported to be negative in an Unscheduled DNA Synthesis assay.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

Bromacil produced a statistically significant increase in hepatocellular tumors in the male mouse at the HDT. Additional supporting evidence was provided by data from structurally related compounds and some positive mutagenicity studies.

Some members of the Committee considered the above to be "limited evidence" and that therefore Bromacil should be classified as Group C, if the Guidelines were to be rigidly applied. Others thought that the evidence was "inadequate" and thus warranted only a Group D classification, since the tumors were of a common type (mouse liver), seen only in one sex (male), only at the HDT, and only at the end of the study.

Both of the above arguments, and the information that the Registration Standard has asked for the mouse study to be repeated[11], were considered. The Committee agreed to defer classification of Bromacil at this time, pending receipt and evaluation of the repeat mouse study.

NOTE: Although the Registration Standard did not require it, it was strongly recommended that the rat study be repeated, as well (Memo to RD - ATTACHMENT 2).

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[11]The mouse study was to have been a 2-year study; however, it had to be terminated after 18 months due to a high mortality rate at test weeks 52 to 76, especially among male mice.

ATTACHMENT 1

007712

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

APR 7 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Bromacil Mouse Study - Updated Qualitative Risk Assessment

Caswell No.: 111

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Bernice Fisher 4/5/88

TO: Linda L. Taylor, Ph.D.
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Richard Levy 4-7-88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Summary

The analysis of the mouse study for males, indicated no differential survival problems with different dose levels of bromacil for a period of 18 months.

While in female mice there was a significant increasing trend in mortality with increasing doses of bromacil. In the pairwise comparisons with control and the highest (5000 ppm) dose level, mortality rates were significantly different.

1

Male mice had a significantly increasing trend in both liver (adenomas and/or carcinomas) tumors and in liver carcinomas only. The pairwise comparison of controls and the highest (5000 ppm) dose indicated a significantly different liver (adenomas and/or carcinomas) tumor rate.

Female mice showed no significant tumorigenicity.

Background

An 18-month 95% bromacil study in CD-1 strain of mice was conducted by Haskel Laboratory (No. 893-80, Accession No. 244069) for E.I. du Pont and completed in 1981. The study contained 640 mice, stratified by sex and weight and then by randomization, assigned to groups of 80 males and females of equivalent weights. The dose level groups of dietary bromacil were 0, 250, 1250, and 5000 ppm. Evaluation of the toxicological results were to be made at the end of a 2-year period, but because the observed rate of mortality was so large during the test weeks of 52 to 76, especially among male mice, the study was terminated after 18 months.

Survival

No significant increase in male mortality with dose increments of bromacil was found by using the Thomas, Breslow, and Gart program (1977). See table 1 for details.

Female mice did have a significantly ($p = .0076$, Cox's test and $p = .0045$, Gehan-Breslow test) increasing trend in mortality with dose increments of bromacil. Pairwise comparisons with control, indicated significant ($p = .03$, both Cox and the Gehan-Breslow tests) increased mortality in the highest (5000 ppm) dose group. See table 2 for details.

Tumor Analysis

Male mice did have a significant ($p = .012$) increasing trend in liver (adenomas and/or carcinomas) tumor rates with increasing doses of bromacil. Males also had a significant ($p = .020$) trend in liver carcinoma rates. The trend analysis was based upon the Cochran-Armitage Trend test. The pairwise comparisons with controls by means of the Fisher Exact test resulted in a significant ($p = .034$) difference in the high (5000 ppm) dose group for combined liver adenomas and/or carcinomas in the male mice. See table 3 for details.

Since female mice did not have any appreciable liver (adenomas and/or carcinomas) tumor rate differences among the varying dose levels, statistical evaluation was not attempted. See table 4 for details.

Table 1. Bromacil, Mouse Study - Male Mortality Rates[+] and a Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 0-26	27-52	53-80	Total
0	1/80	7/79	43/72	51/80 (64)
250	1/80	3/79	46/76	50/80 (62)
1250	2/80	5/78	39/73	46/80 (58)
5000	0/80	6/80	38/72	44/80 (55)

Table 2. Bromacil, Mouse Study - Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 0-26	27-52	53-80	Total
0	0/80	6/80	31/74	37/80 (46)**
250	3/80	3/77	37/74	43/80 (54)
1250	2/80	6/78	42/72	50/80 (62)
5000	1/80	7/79	43/72	51/80 (64)*

[+]Number of animals that died/number of animals alive at beginning of interval.
()Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.
*$p < .05$, **$p < .01$.

Table 3. Bromacil, Mouse Study - Male Liver Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

Liver Tumor	Dose (ppm) 0	250	1250	5000
Adenoma only	3/69 (4)	7/69 (10)	3/68 (4)[a]	7/68 (10)
Carcinoma	5/69 (7)[b]*	4/69 (6)	4/68 (6)	10/68 (15)
Adenoma and/or carcinoma	8/69 (12)*	11/69 (16)	7/68 (10)	17/68 (25)*

[+]Number of tumor bearing animals/number of animals examined excluding those that died before week 53.
()Percent.
[a]Appearance of first adenoma - week 63.
[b]Appearance of first carcinoma - week 72.
Note: Significance of trend denote at Control.
Significance of pairwise comparison with control denoted at Dose level.
*$p < .05$, **$p < .01$.

Table 4. Bromacil, Mouse Study - Female Liver Tumor Rates[+]

Liver Tumor	Dose (ppm) 0	250	1250	5000
Adenoma only	0/74 (0)	1/74 (1)[a]	0/72 (0)	0/72 (0)
Carcinoma	1/74 (1)	2/74 (3)[b]	0/72 (0)	1/72 (1)
Adenoma and/or carcinoma	1/74 (1)	3/74 (4)	0/72 (0)	1/72 (1)

[+]Number of tumor bearing animals/number of animals examined excluding those that died before week 53.
()Percent.
[a]Appearance of first adenoma - week 81.
[b]Appearance of first carcinoma - week 75.

ATTACHMENT 2

007712

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Bromacil Peer Review

TO: Robert J. Taylor
Product Manager (25)
Registration Division (TS-767C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/4/88
Toxicology Branch, Section III
Hazard Evaluation Division (TS-769C)

THRU: Marcia van Gemert, Ph.D. M. van Gemert 2/4/88
Toxicology Branch, Section III
Hazard Evaluation Division (TS-769C)

This is to inform you of the recommendations of the Peer Review Committee regarding Bromacil and additional testing requirements.

At the Peer Review Committee meeting on Bromacil, the Committee agreed to defer classification of Bromacil at this time, pending receipt and evaluation of the repeat mouse study requested in the Registration Standard on Bromacil, and strongly recommended that the rat study be repeated as well.

Qualitative/Quantitative Risk Assessment

4/7/88

007712

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

APR 7 1988

FILE COPY

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PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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Scientific Mission Support Staff
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Richard Levy 4-7-88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Reto Engler

Summary

The analysis of the mouse study for males, indicated no differential survival problems with different dose levels of bromacil for a period of 18 months.

While in female mice there was a significant increasing trend in mortality with increasing doses of bromacil. In the pairwise comparisons with control and the highest (5000 ppm) dose level, mortality rates were significantly different.

Male mice had a significantly increasing trend in both liver (adenomas and/or carcinomas) tumors and in liver carcinomas only. The pairwise comparison of controls and the highest (5000 ppm) dose indicated a significantly different liver (adenomas and/or carcinomas) tumor rate.

Female mice showed no significant tumorigenicity.

Background

An 18-month 95% bromacil study in CD-1 strain of mice was conducted by Haskel Laboratory (No. 893-80, Accession No. 244069) for E.I. du Pont and completed in 1981. The study contained 640 mice, stratified by sex and weight and then by randomization, assigned to groups of 80 males and females of equivalent weights. The dose level groups of dietary bromacil were 0, 250, 1250, and 5000 ppm. Evaluation of the toxicological results were to be made at the end of a 2-year period, but because the observed rate of mortality was so large during the test weeks of 52 to 76, especially among male mice, the study was terminated after 18 months.

Survival

No significant increase in male mortality with dose increments of bromacil was found by using the Thomas, Breslow, and Gart program (1977). See table 1 for details.

Female mice did have a significantly ($p = .0076$, Cox's test and $p = .0045$, Gehan-Breslow test) increasing trend in mortality with dose increments of bromacil. Pairwise comparisons with control, indicated significant ($p = .03$, both Cox and the Gehan-Breslow tests) increased mortality in the highest (5000 ppm) dose group. See table 2 for details.

Tumor Analysis

Male mice did have a significant ($p = .012$) increasing trend in liver (adenomas and/or carcinomas) tumor rates with increasing doses of bromacil. Males also had a significant ($p = .020$) trend in liver carcinoma rates. The trend analysis was based upon the Cochran-Armitage Trend test. The pairwise comparisons with controls by means of the Fisher Exact test resulted in a significant ($p = .034$) difference in the high (5000 ppm) dose group for combined liver adenomas and/or carcinomas in the male mice. See table 3 for details.

Since female mice did not have any appreciable liver (adenomas and/or carcinomas) tumor rate differences among the varying dose levels, statistical evaluation was not attempted. See table 4 for details.

Table 1. Bromacil, Mouse Study - Male Mortality Rates[+] and a Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 0-26	27-52	53-80	Total
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250	1/80	3/79	46/76	50/80 (62)
1250	2/80	5/78	39/73	46/80 (58)
5000	0/80	6/80	38/72	44/80 (55)

Table 2. Bromacil, Mouse Study - Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 0-26	27-52	53-80	Total
0	0/80	6/80	31/74	37/80 (46)**
250	3/80	3/77	37/74	43/80 (54)
1250	2/80	6/78	42/72	50/80 (62)
5000	1/80	7/79	43/72	51/80 (64)*

[+]Number of animals that died/number of animals alive at beginning of interval.
()Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.
*$p < .05$, **$p < .01$.

Table 3. Bromacil, Mouse Study - Male Liver Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

Liver Tumor	Dose (ppm) 0	250	1250	5000
Adenoma only	3/69 (4)	7/69 (10)	3/68 (4)[a]	7/68 (10)
Carcinoma	5/69 (7)[b]*	4/69 (6)	4/68 (6)	10/68 (15)
Adenoma and/or carcinoma	8/69 (12)*	11/69 (16)	7/68 (10)	17/68 (25)*

[+]Number of tumor bearing animals/number of animals examined excluding those that died before week 53.
()Percent.
[a]Appearance of first adenoma - week 63.
[b]Appearance of first carcinoma - week 72.
Note: Significance of trend denote at Control.
Significance of pairwise comparison with control denoted at Dose level.
*p < .05, **p < .01.

Table 4. Bromacil, Mouse Study - Female Liver Tumor Rates[+]

Liver Tumor	Dose (ppm) 0	250	1250	5000
Adenoma only	0/74 (0)	1/74 (1)[a]	0/72 (0)	0/72 (0)
Carcinoma	1/74 (1)	2/74 (3)[b]	0/72 (0)	1/72 (1)
Adenoma and/or carcinoma	1/74 (1)	3/74 (4)	0/72 (0)	1/72 (1)

[+]Number of tumor bearing animals/number of animals examined excluding those that died before week 53.
()Percent.
[a]Appearance of first adenoma - week 81.
[b]Appearance of first carcinoma - week 75.

Reviewer's Peer Review Package for 1st Meeting

14188

007712

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Bromacil Peer Review

TO: Robert J. Taylor
Product Manager (25)
Registration Division (TS-767C)

FROM: Linda L. Taylor, Ph.D. *Linda Lee Taylor 2/4/88*
Toxicology Branch, Section III
Hazard Evaluation Division (TS-769C)

THRU Marcia van Gemert, Ph.D. *M. van Gemert 2/4/88*
Toxicology Branch, Section III
Hazard Evaluation Division (TS-769C)

This is to inform you of the recommendations of the Peer Review Committee regarding Bromacil and additional testing requirements.

At the Peer Review Committee meeting on Bromacil, the Committee agreed to defer classification of Bromacil at this time, pending receipt and evaluation of the repeat mouse study requested in the Registration Standard on Bromacil, and strongly recommended that the rat study be repeated as well.

Per conversation w/ Esther Saito, I've found out that the mouse onco wasn't being repeated. The previous mouse onco should be acceptable.
[illegible]
3/30/88

007712

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

FILE COPY

AUG 13 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Rscheduled Peer Review Meeting on Bromacil

FROM: Jonn A. Quest JA Quest
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

The peer review on Bromacil, originally scheduled for August 12, 1987, has been rescheduled for Wednesday, September 9, 1987 at 11:30 A.M. in Dr. Farber's office (Rm. 821, CM-2). Data packages have already been distributed.

ADDRESSEES

T. Farber
W. Burnam
E. Rinde
J. Hauswirth
R. Engler
L. Kasza
R. Levy
B. Fisner
L. Taylor
M. VanGemert
R. Beliles
D. Beal
A. Barton
D. Barnes
R. Hill

=20 6/12/87. sp

27

007712

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

JUN 23 1987

FILE COPY
OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer-Review of Bromacil

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached for your review is a package on Bromacil prepared by Dr. L. Taylor.

A meeting to discuss the weight-of-the-evidence is scheduled for Wednesday, August 12, 1987, at 11:00 AM in Dr. Farber's office (Rm. 821 CM-2).

Attachment

ADDRESSEES

T. Farber
W. Burnam
E. Rinde
J. Hauswirth
J. Quest
L. Kasza
R. Levy
P. Fisher
L. Taylor
M. Van Gemert
K. Beliles
D. Beal
A. Barton
R. Hill

meeting Cancelled. Will be rescheduled at a later date.

JAC 8/11/87

#19 6/23/87 sp

RE File
007712

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

FILE COPY

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Bromacil, Mouse Study-Males, Re-evaluation of Survival

Caswell No. 111

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Bernice Fisher 5/1/87

TO: Linda L. Taylor, Ph.D., Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Richard A Levy 5-1-87

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

A statistical re-evaluation of the survival component in the 18-month feeding study of 95% Bromacil in CD-1 male mice was needed because previously(see memorandum on Priliminary Risk Assessment for Bromacil-B. Fisher, 12/85) it was evaluated by the Peto Prevalence method. Currently a more relevant way to analyse survival, is to use the Thomas, Breslow, and Gart computer program for Trend analysis and pairwise comparisons.

Data on mortality from the Bromacil male mouse study for dose levels of 0, 250, 1250, and 5000 ppm was used to assess survival. The results indicated as in the above mentioned memorandum, that there was no significant increase in mortality with the given dose increments of Bromacil.

.....

Reference

Thomas, D.G., Breslow, N., and Gart, J.J. (1977)- Trend and Homogenity Analysis of Proportions and Life Table Data, Computers and Biomedical Research 10, pgs 373-381.

007712

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

FILE COPY

JUN 23 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer-Review of Bromacil

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached for your review is a package on Bromacil prepared by Dr. L. Taylor.

A meeting to discuss the weight-of-the-evidence is scheduled for Wednesday, August 12, 1987, at 11:00 AM in Dr. Farber's office (Rm. 821 CM-2).

Attachment

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#19 6/23/87 sp

007712

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review: BROMACIL

TO: The Peer Review Committee for Bromacil
Toxicology Branch (TS-769C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 5/14/87
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Marcia van Gemert, Ph.D. M. van Gemert 5/14/87
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Attached is a report prepared for the Peer Review Committee on Bromacil. One-liners, DER's, and other pertinent information and memoranda are included.

Please contact me if additional data are required prior to the Committee meeting.

3

APPENDICES

A. ONE-LINERS
B. PHASE II TOXICOLOGY PROFILES OF BROMACIL SALTS-11/25/81
C. DER FOR TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS-7/20/81
D. REVIEW OF TERATOLOGY STUDY IN RATS-6/17/83
E. REVIEW OF: REPRODUCTION STUDY IN RATS
   CHRONIC TOXICITY STUDY IN DOGS
   2-YEAR CHRONIC RAT STUDY
   Dated 10/5/66
F. DATA REVIEW OF MOUSE ONCOGENICITY STUDY-10/83
G. PRELIMINARY RISK ASSESSMENT MEMO AND UPDATE-1/4/85 & 5/1/87
H. HISTORICAL CONTROL DATA ON LIVER TUMORS IN CD-1 MICE
I. REFERENCE DOSES (RFD) FOR ORAL EXPOSURE
J. TABLE X
K. TABLES XXIII & XXIV
L. HISTORICAL CONTROL DATA

BROMACIL

I. Background

Bromacil, 5-bromo-3-sec-butyl-6-methyluracil, was a Registration Standard (published 9/30/82) and the subject of a pesticide petition (6F0499) requesting a tolerance of 1 ppm on pineapples and citrus fruits. 40 CFR 180.210 established a tolerance of 0.1 ppm for residues on these fruits. Bromacil is a non-selective herbicide used to control a wide range of perenial weeds and grasses in industrial and agricultural areas. Synonyms: Hyvac X Bromacil Weed Killer, INN 976, BOREA, BROMAX 4G, BROMAX 4L, CYNOGAN, HYVAR X, HYVAR X-L, ROUT, URAGON, UROX "B", UROX "HX".

The acute oral toxicity (LD_{50}) of Bromacil is 3.04 grams/kg. One liners for studies that have been reviewed by the Toxicology Branch (not this (LLT) reviewer) are attached. Only a spot check of the data was performed by LLT.

II. Metabolism

The principal compound isolated from rat urine was 5-bromo-6-hydroxymethyl-3-sec-butyl uracil, which was identified by thin-layer chromotography, infrared spectra, NMR, and mass spectrophotomer. Two unidentified metabolites occurred at trace levels; Bromacil was also present in trace amounts.

5-Bromouracil, a metabolite of Bromacil and a potent mutagen, was not detected in the urine of rats or production plant workers (see Phase II Toxicology Profile of Bromacil and Salts, 11/25/81, attached).

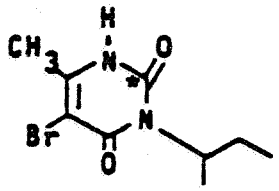
III. Structure-Activity Relationships

Structures of Bromacil and its degradates (Pages 1A-1C), as well as structurally related compounds (uracil, 6-methyl-2-thiouracil, and 6-methyl uracil; page 1D) are shown in the attached pages. The latter 2 compounds are discussed under V.

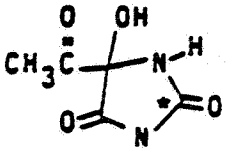
IV. Summary of Short-Term Tests

a. Mutagenicity

Published data from various sources make up the data base on mutagenic potential. Bromacil showed a positive response in the Drosophila Sex-Linked Recessive Lethal Test (2, 3, 5, 2000 ppm in feeding solution).[1] Positive responses were seen in two point/gene mutation in eukaryotes bioassays (mouse lymphoma L5178Y cells and D. melanogaster).[2] Another published assay using L5178Y mouse lymphoma cells heterozygous at the thymidine kinase (TK) locus, with and without metabolic activation, provided evidence that Bromacil increased the mutation frequency in a concentration-related manner, above the spontaneous frequencies observed in the controls. The presence of the metabolic activation system appeared to enhance the mutagenic and cytotoxic effects of Bromacil[3]. In a DNA synthesis test of Bromacil, with and without metabolic activation, no incorporation into DNA was observed.[3] The results from these published studies on mutagenic potential are listed below.



Bromacil



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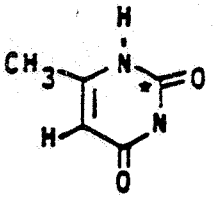

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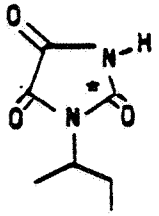

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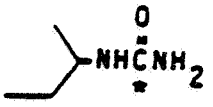

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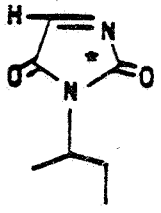

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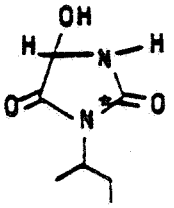
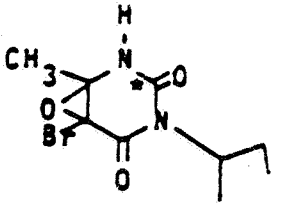
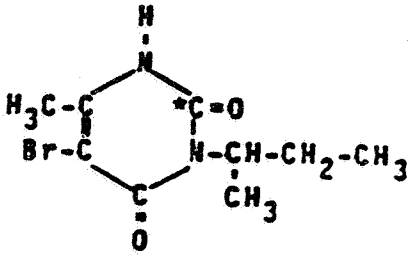


VI

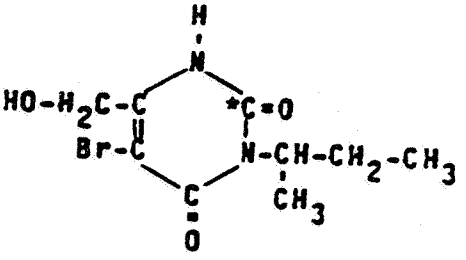


VII

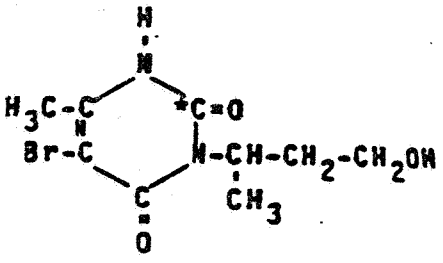
Figure 1. Bromacil and its degradates (* denotes position of the radiolabel): (I) 3-sec-butyl-5-acetyl-5-hydroxyhyantoin; (II) 3-sec-butyl-6-methyl-uracil; (III) 3-sec-butyl-ketohydantoin; (IV) sec-butyl-urea, (V) 3-sec-butyl-3H-imidazole-2,4-dione; (VI) 3-sec-butyl-5-hydroxyhydantoin, (VII) 5-bromo-3-sec-butyl-5,6-epoxy-6-methyl-uracil.
(from photodegradation studies in water)



Bromacil



A



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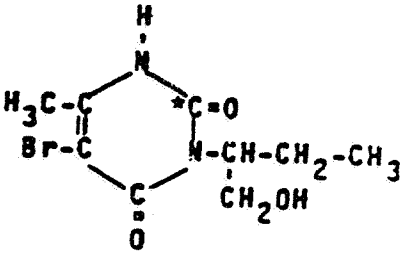
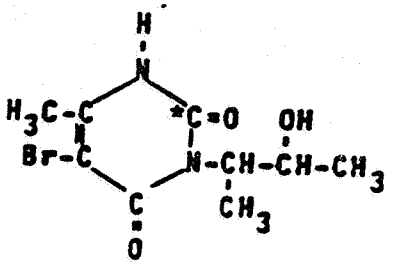


C

Figure 1. Bromacil and its degradates (* denotes position of radiolabel): (A) 5-bromo-3-sec-butyl-6-hydroxymethyluracil; (B) 5-bromo-3-(3-hydroxy-1-methylpropyl)-6-methyluracil; (C) 5-bromo-3-(α-hydroxymethylpropyl)-6-methyluracil.
(degradation studies in soil)



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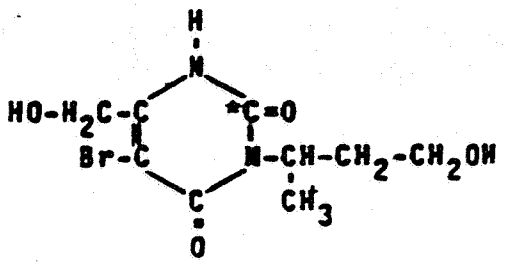

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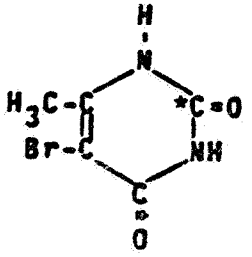
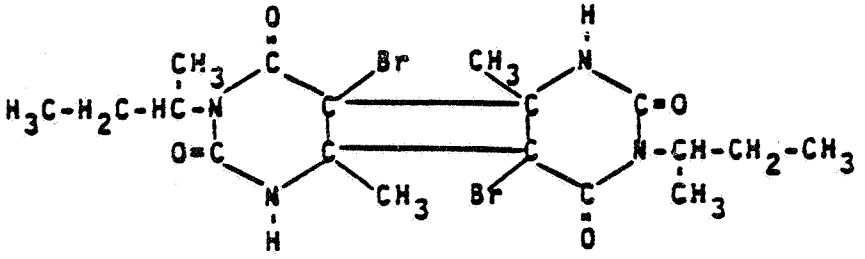
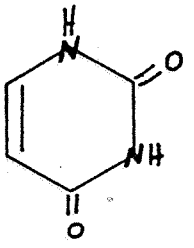


G

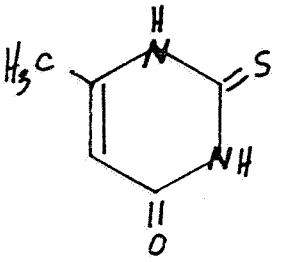


Bromacil dimer

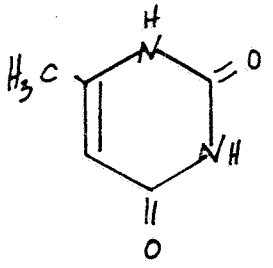
Figure 1. (Continued): (D) 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyl-uracil; (E) 5-bromo-3-(3-hydroxy-1-methylpropyl)-6-hydroxymethyl-uracil; (G) 5-bromo-6-methyluracil; (Dimer) 4A, 10A-dibromo-3,9-di-sec-butyl-4B,10B-dimethyl-cyclobutadi[1,2-D:4-DPR]pyrimidine-2,4,8,10-tetrone.



uracil



6-methyl-2-thiouracil



6-methyluracil

Point/Gene Mutation[2]					Chromosomal Effects[2]		
Prokaryote		Eukaryote					
SAL	WPU	YER	L5T	SRL	SCC	MNM	DLM
–	–	–	+	+	–	–	–

DNA Damage[2]					
Prokaryote			Eukaryote		
REP	REW	SAR	YE3	YEH	UDH
–	–	–	–	–	–

Phase 1[1]								Phase 2[1]			
Ames		WP2		D3		POL A	REC	UDS		DRL	MDL
-MA	+MA	-MA	+MA	-MA	+MA			-MA	+MA		
–	–	–	–	–	–	–	–	–	–	+	–

Bromacil (95.9%) was one of several pesticides tested for genotoxic properties by the use of a battery of in vitro and in vivo methods[3]. Bromacil was negative in all tests (listed below).

Mouse dominant lethal
Salmonella typhimurium (His^+ Reversion)
Escherichia coli WP2 (Try^+ Reversion)
Saccharomyces cerevisiae (Mitotic Recombination)
Escherichia coli (Relative Toxicity)
Bacillus subtilis (Relative Toxicity)
UDS (DNA Repair)

b. Special Studies

1) Enzyme Induction

No studies on the compound's potential to induce the hepatic mixed function oxidase system were located.

2) Teratology

a) Species/Strain: New Zealand white rabbit
Testing Facility: Hazleton

This study was classified as minimum data, according to the One Liner. However, the DER (attached) states that the study is unsatisfactory and that a new study should be performed. Bromacil was fed in the diet at levels of 0, 50, and 250 ppm on the 8th to the 16th day of gestation. Some of the does were sacrificed and Caesarean sections were performed on day 28 of gestation; the remainder were allowed to deliver normally. One third of the fetuses were prepared for skeletal examination. The maternal and fetal toxic and teratogenic NOELs were stated in the One Liner to be > 250 ppm (HDT). The DER states that this study is without merit for determining the teratogenicity of Bromacil.

b) Species/Strain: Sprague-Dawley Rat
Testing Facility: Stanford Research

This study was classified as minimum (see study review and One Liner, attached). There were 10 animals per group (adult males and females) exposed to Bromacil via inhalation at dose levels of 38+2, 78+6, or 165+6 mg/m^3 for 2 hours per day from the seventh through the fourteenth (13) day of gestation (not clear why males were utilized). The controls (20 animals) were exposed to the solvent, DMSO. The liver and gravid uterus were weighed; live fetuses were weighed and examined; uteri were examined and the number of resorptions recorded; fetuses were prepared for necropsy or fixed for skeletal analysis; pathology was performed on selected tissues (study reviewed in memo dated 6/17/83).

Results

Body weight, food consumption, and litter size were said to be comparable. There were no signs of toxicity. Resorptions were higher at the 165 mg/m^3 (high dose) level compared to the other treated groups, but lower than controls. There was a significant dose-related reduction in fetal weight compared to control, but no teratogenic or pathologic response was reported. It was concluded that the NOEL for terata was 165 mg/m^3, or 7.92 mg/kg.

Comment: It is to be noted that, under the conditions of this study, Bromacil was without significant (maternal) toxicity, indicating that the doses used may not have been adequate. Additionally, justification for the route of exposure was not provided, and too few animals per dose level were studied. This reviewer (LLT) would reclassify this study as supplemental.

3) Reproduction Study

Species/Strain: Charles River CD rats
Testing Facility: Haskell Laboratory for Toxicology & Industrial Medicine

Animals utilized in this study were from the chronic study (reviewed below-MR686). After 12 weeks on the diets, 12 male and 12 female rats were selected randomly from the control (0%) and mid-dose (0.025%) INN 976 groups. This Fo generation produced F1a and F1b generations, and were returned to the chronic study after the F1b generation was weaned. Twelve animals/sex/group of the F1b generation were randomly selected from 5-6 litters at weaning; at approximately 110 days of age, the animals were mated within their respective diet groups to produce the F2a and F2b generations. The same procedure was followed with the F2b generation to produce the F3a and F3b generations. When the pups of the F3b generation were weaned, 2 males and 2 females from each group were selected from each of 5 litters and subjected to histopathological evaluation. The indices reported were listed as fertility, gestation, viability, and lactation. The review (memo dated October 5, 1966) stated that there were no marked differences between control and treated groups (gross or microscopic pathology), and there was no mention of any deformity in the offspring. The One Liner lists this study as core minimum; however, only one dose level was tested, and too few animals were utilized at study initiation to adequately address the reproductive aspects of Bromacil. This reviewer would reclassify this study as core supplemental.

V. Summary of Lifetime Studies

a. Oncogenicity Feeding Study in Mice

Species/Strain: CD-1 mouse
Testing Facility: Haskell Laboratory for Toxicology & Industrial Medicine

Mice (80/sex/group) were administered Bromacil INN-976 (95%) in the diet (1% corn oil suspension) at levels of 250, 1250, and 5000 ppm for 18 months. The study was classified as Core Minimum data. After the first year, mortality was greater in the two highest dose groups compared with controls of both sexes, and the study was terminated at 18 months. Food consumption data were not obtained. No significant difference was observed in the survival rates of male mice using the Peto "Death Rate" method of statistical analysis (see Preliminary Risk Assessment memo dated January 4, 1985). A recent re-evaluation of these data using the Thomas, Breslow, and Gart computer program for trend analysis and pairwise comparisons[4] confirms this. For females, there was a significant ($p<0.05$) increase in the number of animals in the mid- and high-dose groups that died. Throughout the study, body weights of the high-dose animals were significantly lower than controls. There was a significant increase in the mean liver weight of both sexes of the high dose, compared to control at necropsy. Diffuse hepatocellular hypertrophy was observed in both male and female high-dose mice and in the mid-dose males, and centrolobular vacuolization was observed in the low-dose males. Additionally, there was a dose-related increase in testicular abnormalities. There was seminal vesicular distention at the low dose; spermatocyte necrosis, sperm calculi, and interstitial cell hypertrophy/hyperplasia at the mid- and high-dose levels, and focal atrophy of semineferous tubules at all dose levels. The high-dose male group also displayed as increase in atrial thrombosis. No NOEL was established; the LEL was stated to be 250 ppm (memo dated October 1983).

With regard to liver neoplasms, no liver tumors appeared until the second year of the study. The changes in the rate of liver tumors over the 18-month study were analyzed by the "Prevalence and Trend" method of Peto (see Tables III and IV of Risk Assessment memo). It is stated that the dose-related trend for liver carcinoma and/or adenoma was statistically significant ($p<0.02$) for males at the final kill. Additionally, although the trend was significant ($p<0.03$) using the total data, it was mainly affected by the data at the end of the study. Using the X^2 statistic, there was a significant ($p<0.05$) increase in liver tumors, comparing the highest dose males with control males. Further, the combination 0, 250, and 1250 doses compared with the 5000 dose yields a significant difference ($p<0.02$) in the application of Fisher's Exact Test.

MALES	Control	250 ppm	1250 ppm	5000 ppm
hepatocellular adenomas and/or carcinomas	8	11	7	17*
number of mice in group	72	76	73	74

* $p< 0.02$ (see Preliminary Risk Assessment memo dated 1/4/85)

The incidence of hepatocellular hypertrophy in the males was as follows.

	Control	Low	Mid	High
Hepatocellular hypertrophy	3	1	17	47

The control and low-dose hypertrophy was described as diffuse, while the mid- and high-dose hypertrophy was described as midzonal and centrilobular. Females did not exhibit any dose-related effect for liver tumors. However, the incidence of hepatocellular hypertrophy was increased in this group (0 in controls, 3 in both the low- and mid-dose groups, and 12 in the high-dose group).

Historical data from several color studies in CD-1 mice are attached. The combined average incidence of hepatocellular adenomas/carcinomas is reported[5] as 5% for males and 2% for females in studies ranging from 81-105 weeks.

A quantitative risk assessment for Bromacil is described in the Preliminary Risk Assessment memo of 1/4/85. Using the Multistage model, the carcinogenic potency is calculated to be: $Q^*_1 = 3.8 \times 10^{-3}$ E

b. Chronic Oral Toxicity Studies in Dogs

Species/Strain: Beagle dogs
Testing Facility: Haskell Laboratory for Toxicology & Industrial Medicine

Dogs (one to two years of age at start; 3/sex/group) were fed Bromacil for 2 years at dose levels of 0, 50, 250, and 1250 ppm. No deleterious effects were reportedly observed at any dose level. Because of an apparent discrepancy (noted by LLT) in the Reference Doses (RFDs) for Oral Exposure memo, the data for this study were reviewed more thoroughly. In contrast to the original review (memo dated October 5, 1966), two deaths occurred at the high dose level (one of each sex) at week 55, in addition to the one reported in the low dose (female) at 92 weeks. Since these two additional deaths were not reported in the study report, the causes are not known. There was some decline in body weight reported at the high dose level during the first part of the study (both sexes). A spot check of the body weight data by this reviewer (LLT) shows that the decrease at the high dose remained at study termination. The high-dose male weights varied from 74-89% of control from week 4 through week 54, and were 72% of control values at 104 weeks. The high-dose females varied from 85 to 90% of control between weeks 7 and 54. At study termination, the two remaining high-dose females were comparable to the controls. A second discrepancy noted in the RFD memo is that there is only one dog study, not two. The One Liners also list two dog studies. The Toxicology Branch memo dated 6/17/83 is apparently a supplement to the original review. Because of the differences noted in body weight, it would appear that the NOEL should be set at the 250 ppm level. The RFD memo is in error as to the NOEL, which is listed as 12.5 mg/kg. This should read 6.25 mg/kg; the LEL, 31.25 mg/kg. This study was classified as Core Minimum data. This reviewer (LLT) would reclassify this study as Supplemental (too few animals; age of dogs at study initiation).

c. 2-Year Feeding Study in Rats

Species/Strain: Charles River CD rats
Testing Facility: Haskell Laboratory for Toxicology & Industrial Medicine

Rats (36/sex/group) were fed ground Purina Laboratory Chow containing 1% corn oil and 0, 0, 0.005, 0.025, or 0.125% INN 976 for 2 years (two identical control groups were used). The high-dose females showed a consistently inferior body-weight gain throughout the study (75% of Control I, 85% of Control IA at study termination), which was reflected in a lower food consumption for this group. Food efficiency was slightly lower for this group also. Mortality was said to be comparable among the groups (Table X, attached), but the number of animals available at study termination was small (range: 8-17; average: males-10; females-12). At the high dose level, there was said to be a slight effect upon the thyroids (see below). Focal light cell hyperplasia and focal follicular cell hyperplasia were seen in the control animals, but the incidence was slightly higher in the high-dose animals. The degree of hyperplasia was said to be slight in all cases. One follicular cell adenoma was reported in one high-dose female in the original review. A spot check of the data (LLT) indicates the occurrence of a second follicular cell adenoma in another high-dose female and light cell adenomas, as follows.

FEMALES					MALES				
Control	Control	Low	Mid	High	Control	Control	Low	Mid	High
1	3	1	0	5(1)*	0	0	2	1	1

* () number of adenomas found by LLT

Non-neoplastic lesions of the thyroid and parathyroid were as follows:

	FEMALES					MALES				
	C	C	Low	Mid	High	C	C	Low	Mid	High
FOCAL FOLLICULAR CELL HYPERPLASIA	2	3	0	0	11	1	10	7	8	9
FOCAL LIGHT CELL HYPERPLASIA	8	2	2	0	6	1	0(2)	1	2	13(12)
PARATHYROID HYPERTROPHY	1	3	2	0	2	2	9	10	6	9
PARATHYROID HYPERPLASIA	2	4	3	0	3	2	8	10	6	8

() # found by LLT

A list of the various lesions of the thyroid that were observed are presented in Tables A and B, and the tumors and non-neoplastic lesions observed in this study (as provided by the study report) are presented in Tables XXIII and XXIV, attached. The original review memo is dated 10/5/66 (Appendix E).

COMMENT: The number of animals per group for which no thyroid was available for examination (or were autolyzed) is shown below. There were 36 animals/sex group at study initiation.

MALES					FEMALES				
Control I	Control IA	Low	Mid	High	Control I	Control IA	Low	Mid	High
21	12	11	11	14	11	10	13	18	14

Regarding the incidence of thyroid tumors, the thyroid was not among the organs listed in a publication on spontaneous tumors in control CD rats (attached), nor was it listed in the historical data from several color studies in Charles River CD rats (attached). This tumor was displayed only in the high-dose (2) females. The incidence of focal follicular cell hyperplasia (noted above) is increased in the high-dose females, which lends support to the argument of a progressive lesion in this organ. However, neither female displaying the tumor showed hyperplasia.

Note: This study is classified as core minimum in the One Liner; the NOEL was set at 250 ppm (12.5 mg/kg/day); the LEL at 1250 ppm (62.5mg/kg/day), based on slight body weight retardation. However, the number of animals at study initiation was below (36/sex/group) that stated in the criteria. Additionally, the number of animals found dead during the study, and probably lost to analysis, was generally high (39% of Control I, 25% of Control IA, 27% of high-dose males), especially in the males. Therefore, this reviewer (LLT) would reclassify this study as supplemental, although the thyroid effects would appear to be real and this aspect should be further addressed.

Comment: The structurally-related compound, 6-methyl-2-thiouracil, has been shown to produce thyroid tumors in rats[6]. In a study in white rats, no tumors were noted, but the thyroid structure deviated from normal with microfollicular diffuse hyperplasia, irregular diffuse hyperplasia, and focal hyperplasia noted at 10 months (15 mg/100 grams body weight, 6 days/week, I.G., total dose-4.5 grams, 300 days duration). Another study in albino rats given 15 mg/rat, 6 days/week by stomach tube (duration unknown) resulted in thyroid adenomas. Three other studies of limited duration have resulted in thyroid tumors in rats dosed with 6-methyl-2-thiouracil. Another compound, 6-methyluracil, also produced thyroid adenomas (follicular and solid), hyperplasia, and increased thyroid weight[6].

TABLE A

The tables below lists the group and animal number plus the number of days on test and the thyroid lesion(s) observed, by sex.

MALES

Control I	Days	Lesion*
54765	188	F,G
54721	675	B,F
54716	734	A
54894	734	E

Control IA	Days	Lesion*
54845	720	A
54800	735	A
54916	735	A
54734	735	A,B
54780	735	A
54738	735	A
54939	735	A
54943	735	A,B
54909	735	A
54873	735	A

Low Dose	Days	Lesion*
54901	589	A
54865	632	F
54857	709	A
54775	736	A
54747	736	A
54829	736	A,F
54915	736	A,D
54922	736	A
54759	736	B,D

Mid Dose	Days	Lesion
54769	686	A
54758	706	A
54821	736	A
54810	736	A
54938	736	A,B
54819	736	A,B
54863	736	A,D
54967	736	A
54814	736	E

High Dose	Days	Lesion*
54940	370	B
54742	370	B,H
54965	57[illegible]	A,B
54959	640	A
54950	640	A
54963	646	B
54722	703	B
54837	709	A,B
54895	734	A,B

High Dose (cont'd)	Days	Lesion*
54895	734	A,B
54937	734	A
54929	734	D
54729	734	A,B
54868	734	A,B
54850	734	A,B
54951	735	B
54736	735	B

TABLE B

FEMALES

Control I	Days	Lesion*
55096	372	B
54969	589	B
55191	681	B
55095	706	B
55033	737	B
55127	737	A,D
55079	737	A,B
55047	737	B
55152	737	B

Control IA	Days	Lesion*
55217	374	D
55196	374	D
55105	743	A,B
55041	743	B
55215	744	A,D
55148	744	A

Low Dose	Days	Lesion*
55067	512	B
55135	680	F
55021	738	B
55118	743	D,F

Mid Dose	Days	Lesion*
55115	372	E,F
55147	743	E

High Dose	Days	Lesion*
55158	373	A
55073	518	A,B
55055	577	A
54982	737	A,E
55136	738	C
55062	738	A,B
55028	738	A,B
55104	738	A,D
55193	738	C
55138	738	A,B
55172	738	A,B
55170	738	A,B
55042	738	A

*A-focal follicular cell hyperplasia
B-focal light cell hyperplasia
C-follicular cell adenoma
D-light cell adenoma
E-increase in amount of thyroglobulin-filled follicles
F-focal follicular cell necrosis
G-focal interfollicular fibroplasia
H-intracellular colloid depletion

REFERENCES

1. J. Environ. Sci. Health B15(6), 867-906 (1980).
2. Genetic Toxicology-An Agricultural Prospective. R. Flech and A. hollaender, eds. Plenum Press, N.Y. (1982).
3. Evaluation of Selected Pesticides as Chemical Mutagens. In Vitro and In Vivo Studies. SRI, PB 268-647 (1977).
4. Trend and Homogeniety Analysis of Proportions and Life Table Data, Computers and Biomedical Research 10, 373-381 (1977).
5. Spontaneous Tumors in Control F344 and Charles River CD Rats and Charles River CD-1 and B6C3F1 Mice. Toxicology Letters 11, 103-110 (1982).
6. Survey of Compounds Which Have Been Tested for Carcinogenic Activity (1974-75).

APPENDIX A

ONE-LINERS

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology-rabbit; Hazleton; #201-163; 1966	Bromacil		Doses 0, 50, and 250 ppm Feto toxic NOEL > 250 ppm (HDT) Maternal toxic NOEL > 250 ppm (HDT) Teratogenic NOEL > 250 ppm (HDT)	I	Minimum 003281
Teratology - rat; Stanford Research; Report ;EPA 600111-78-003; 1/78	Technical	249676	Teratogenic NOEL > 165 mg/m3(HDT) (=7.92 mg/kg) Feto toxic NOEL > 165 mg/m3 Changes in parents were not significant.		Minimum
3 Generation reproduction - rat; EI DuPont	80%		Dose level of 0.025% No difference than controls. (only dose tested)		Minimum 003308
90-Day feeding - rat; EI DuPont	80% WP		NOEL = 500 ppm LEL = 2,500 ppm Dose level used = 0, 50, 500 and 2500 that was raised to 5000 ppm at the 6 week. At 5000 ppm lower growth, low RBC, increase in thyroid activity, enlargment of centrolobular cells of liver		Minimum 003308
2-Week feeding - rat; EI DuPont	80% W.P. (a 15% aqueous suspension)		1/6 death after 10 doses of 1035 mg/kg. Gastrointestinal disturbance CNS incoordination		Supplementary data 003308
2-Year feeding/oncogenic - rat; EL DuPont	80%		Dose levels 0.005, 0.025 & 0.125% NOEL = 250 ppm (0.025%) LEL = 0.125% (weight retardation)		Minimum 003308
2-Year feeding - dog; Additional data E.I. DuPont; 6/14/66	Technical	249676	The thyroid changes were camparable to the controls NOEL = 1,250 ppm.		Minimum

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORL Grade/ Doc. No.
2-Year feeding - dog; EI DuPont	80% Bromacil		NOEL = 0.025% LEL = 0.125% (some decline in body weight) Levels tested: 0.005%, 0.025%, and 0.125%		Acceptable 003308
Metabolism - rat urine; J. Agric Food Chem 17, 967; (1969)	Bromacil	MRID 05002771	Ingestion 1250 mg/kg 20 mg/kg Bromacil, --Excreted 21 mg/kg Free 6-HMU, --Excreted 125 mg/kg Free 6-HMU --Excreted in conjugated form with urine		Acceptable 003280
Metabolism - rat; EI DuPont; J.A. Tardiner	80%		1250 ppm incorporated in diet Principal metabolite found 5-bromo - 6 - hydroxymethyl - 3-sec- butyl-uracil found in rat urine		Acceptable 003308
Mutagenic - Bacteriophages E. Coli K; McGahen et. al.; 1965	Bromacil	MRID 00013309	Bromacil - negative mutagen 5-bromouracil-mutagenic 20 ug/L used (only dose tested)		Not Adequate 003280
Mutagenic incorporation in nucleic acid; EI DuPont; (from publication)	80%		No incorporation into DNA		Not acceptable 003308
Acute oral LD_{50} - rat; Hilltop Res.; #73657; 06/19/80	Heavy aromatic naphtha .83.10% Bromacil (5-bromo-3-sec-butyl-6-methyl-uracil.. 2.47% Trichloroacetic acid 8.62%	243444	LD_{50} > 5 g/kg (male and female, single dose tested)	IV	Guideline 000465

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00771 2

Study/Lab/Study #/Date	Material	No.	LD_{50}, LC_{50}, PIS, NOEL, LEL	Toxicity Category	Source of Info/ Doc. No.
Acute dermal LD_{50} - rabbit; Hilltop Res.; #73657; 06/19/80	Heavy aromatic naphtha..83.10% Bromacil (5-bromo-3-sec-butyl-6-methyl-uracil.. 2.47% Trichloroacetic acid 8.62%	243444	LD_{50} > 2 g/kg (single dose tested) (erythema, edema, atonia, desquamation)	III	Guideline 000465
Primary eye irritation - rabbit; Hilltop Res.; #73657; 06/19/80	Heavy aromatic naphtha..83.10% Bromacil (5-bromo-3-sec-butyl-6-methyl-uracil.. 2.47% Trichloroacetic acid 8.62%	243444	Moderate to severe corneal opacity at 24 hrs in all animals and persisted in majority of animals through day 7.	I	Guideline 000465
Primary dermal irritation - rabbit; Hilltop Res.; #73657; 06/19/80	Heavy aromatic naphtha..83.10% Bromacil (5-bromo-3-sec-butyl-6-methyl-uracil.. 2.47% Trichloroacetic acid 8.62%	243444	At 24 hrs., slight to severe erythema and edema. At 72 hrs., severe erythema and edema. PIS =5.48	II	Guideline 000465
Acute oral LD_{50} - rat; Raltech Scientific;1979	Bromacil	MRID 00022077	LD_{50} = 5.126 g/kg (males) 3.998 g/kg (females)	IV	Minimum 003280
Acute oral LD_{50} - dog; Hazleton Labs; 1966	Hyvar-X	MRID 00013276	LD_{50} = Not determined due to emesis Dose of 5 g/kg		Supplementary 003280
Acute dermal LD_{50} - rabbit; Raltech Scientific; 1979	Bromacil	MRID 00022078	LD_{50} = 2.0 g/kg mild erythema and edema		Minimum 003280

00771 2

Study/Lab/Study #/Date	Material	No.	LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	Core Grade/ Doc. No.
Acute inhalation LC_{50} - rat; Raltech Scientific; 1979	Bromacil	MRID 00022080	1 hour exposure LC_{50} > 57.6 mg/L	IV	Minimum 003280
Primary eye irritation - rabbit; Raltech Scientific; 1979	Bromacil	MRID 00022079	Mild irritant conjunctivitis to 72 hours.	III	Minimum 003280
Priamry dermal irritation - rabbit; Raltech Scientific; 1979	Bromacil	MRID 00022081	P.I.S. = 0.8	IV	Minimum 003280
Acute inhalation LC_{50} - rat; Cavalli, R.D.; 1969	Bromacil (Triox liquid)	MRID 0001322?	1 hour exposure LC_{50} > 16.3 mg/L	III	Minimum 003281
Acute dermal LD_{50} - rabbit; Haskell Lab; #276-69; 10/2/69	Bromacil Technical	MRID 00013272	LD_{50} > 5 g/kg	IV	Minimum 003281
Primary dermal irritation - rabbit; IBT; 8530-0683; 11/9/77	BX-939		Severe irritant PIS 6.2	II	Minimum 003281
Acute inhalation LC_{50} - rat; Raltech Scien; 1979	Bromacil	MRID 00022080	LC_{50} > 57.6 mg/L (in excess of LC_{50} cut off point for initial screening of 5 mg/L	IV	Minimum 003281
Acute oral LD_{50} - rat; EI DuPont	80% W.P. (a 60% aqueous suspension)		LD_{50} = 5,200 mg/kg rapid respiration, postration and weight loss	IV	Minimum 003308
Acute dermal LD_{50} -rabbit; EI DuPont	80% WP		LD_{50} > 5000 mg/kg Only one dose	IV	Minimum 003308
Dermal irritation & dermal sensitization - rabbit & guinea pig; EI DuPont	80% WP a 1% suspension for dermal irritation.		Dermal irritation - mild Negative for sensitization	III	Minimum 003308

Study/Lab/Study #/Date	Material	No.	LD_{50}, LC_{50}, PIS, NOEL, LEL	Category	Doc. No.
Acute inhalation LC_{50} - rat; EI DuPont	4.8 mg/liter 2.1 mg/liter		4 lower exposure LC_{50} > 4.8 mg/L	III	Minimum 003308
Primary irritation - rabbit; EL DuPont	80% Bromacil 10% of 10% suspension in mineral oil		Mild irritation		Supplementary 003308
Acute oral LD_{50}	Granular Herbicide 4% Formula EPA #8123-74		Data requirement waived		003580
Acute dermal LD_{50}	Granular Herbicide 4% Formula EPA #8123-74		Data requirement waived		003580
Primary eye irritation	Granular Herbicide 4% Formula EPA #8123-74		Data requirement waived		003580
Primary dermal irritation	Granular Herbicide 4% Formula EPA #8123-74		Data requirement waived		003580
Risk assessment - mice; Hashel; #893-80			Male mice suriving one year or longer and examined for liver tumors with either carcinoma and /or adenoma yielded a potency estimation $Q^*_1 = 3.8 \times 10^{-3}$		004213
Risk assessment - mice; EPA - Litt			positive mouse liver potency estimation $Q^*_1 = 3.8 \times 10^{-3}$ wt of the evidend to be determined by committee.		004183

0077

0077

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APPENDIX B

PHASE II TOXICOLOGY PROFILE OF BROMACIL SALTS

11/25/81

007712

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: November 25, 1981

SUBJECT: Bromacil and Salts, Input Phase II

FROM: Alex Arce
Toxicology Branch, HED (TS-769)

TO: Ganga Keari, PM
SPRD (TS-791)

THRU: William L. Burnam, Acting Chief
Toxicology Branch, HED (TS-769)

Attached is the Phase II Toxicology Profile of Bromacil and Salts. If you have any questions, please telephone me, X73710.

Attachment

cc:
WButler

VI. TOXICOLOGY

A. TOXICOLOGY PROFILE

B. Human and Domestic Animal Hazard Assessment

C. Summary of Major Data Gaps

A. Toxicology Profile

1. Technical Bromacil (80%)

a. Acute Effects

1) Acute Oral Toxicity

Enough information was available to assess the acute oral toxicity of Bromacil 80% wettable powder. The oral LD_{50} in rats was 5,200 mg/kg with 95% confident limits of 5,024 mg - 5,330 mg/kg. (PP #6G0499) Another study reported a LD_{50} value of 5.126 g/kg in male rats and 3.989 g/kg in females. (Raltech 1979, MRID 0002277)

This is sufficient to assign 80% Bromacil to Toxicity Category III.

The 80% formulation induced an emetic response in dogs. Thus the LD_{50} in dogs was not established. (Paynter, 1966, MRID 000)

2) Acute Dermal Toxicity

An acute dermal toxicity test was conducted in rabbits (Raltech Scientific, 1979, MRID 00022078). The dermal LD_{50} in rabbits was greater than 2 g/kg. In another study (Zapp, 1965), the acute dermal LD_{50} was greater than 5 g/kg which is sufficient to assign Bromacil 80% to Toxicity Category III, indicating that by oral or dermal contact the product has a low hazard potential.

3) Acute Inhalation Toxicity

An acute inhalation test performed by Raltech Labs in 1979 (Biesemeir, 1979, MRID 0002280), reported a LC_{50} greater than 57.6 mg/L during one hour exposure with rats. No mortalities were observed for 14 days.

In another study, rats were exposed for 4 hours to concentrations of 4.8 mg/L and 2.1 mg/L = no deaths. (Zapp, 1965, from PP #6G0499). This is sufficient to assign the product to Toxicity Category IV.

4) Ocular and Dermal Primary Irritation and Sensitization

In a primary dermal irritation study 0.5 mg technical material was applied to the skin of the rabbit (Raltech, 1979, MRID 00022081) and the response was a mild irritation. No sensitization was produced in guinea pigs (Zapp, 1965). The result observed in an eye irritation study was a mild conjunctivitis of temporary nature. Mineral oil was used as a diluent for the material. Despite the fact that the diluent, mineral oil, is not recommended for eye irritation studies, the results indicates a very low hazard by eye contact. (Zapp, 1965) The dermal and ocular irritation studies indicate that the product falls in Toxicity Category IV.

b. Subchronic Effects

A 90-day rat feeding study conducted at 500, 2,500, and 5000 ppm using 80% W.P. produced decreased growth rate among males at the high dose; pathological changes (enlargement of the centrolobullar cells of the liver) at the mid dose, and no observable adverse effects at the low level of 500 ppm. (PP #6G0499, memo from Fitzhugh to Petition Control, Oct 1966).

A 14-day rat intubation study using 80% Bromacil wettable powder in a 15% aqueous suspension produced deaths at 1035 mg/kg; after a 14 day recovery period, the lower level of 600 mg/kg produced no deaths or pathological signs. (Zapp, 1965, PP #6G0499).

c. Chronic Effects

1) Chronic feeding studies

In a chronic oral toxicity study with rats (Sherman, et.al., 1963), 80% Bromacil w.p. was fed to rats for 2 years at dose levels of 0.005%, 0.025% and 0.125%. At the high dose level of 1,250 ppm (0.125%) body weight retardation and thyroid effects were detected. The NOEL was 250 ppm (0.025%). Negative for oncogenicity.

In another study, dogs were fed Bromacil for 2 years (PP #6G0499). at dose levels of 0 control, 0.005, 0.025 and 0.125%. No deleterious effects were observed at any dose level. However, since thyroid effects were observed at the high dose in the 2 year rat feeding oncogenic study, the thyroid of the dog should have had histopathology performed. An analysis of the thyroid tissue in the dogs was not performed, therefore, the dog study can be accepted only as supplementary data.

Due to unanswered questions related to the possibility of the material to induce thyroid changes, we will require an additional dog feeding study.

2) Oncogenicity

The requirements for one of the two species (rat) is satisified by Sherman, et. al. (see 1 above). Data gaps exist for oncogenicity. Testing shall be performed in 2 mammalian species.

The testing in a second species (mouse) was completed but could not be evaluated due to interferring systemic bacterial infection. A new replacement study also using the mouse was begun in 1969 but as of 1981, it has not been received.

3) Teratogenicity

Bromacil 80% w.p. fed to rabbits, (0, 50 and 250 ppm) from the eight to the 16th day of pregnancy, (Paynter, 1966, PP #6F0499), did not produce deformities, gross manifestations or teratogenic effects.

A study using a second specie must be submitted in order to satisfy the current regulatory requirements for teratogenicity testing.

4) Reproduction

In a 2-year chronic toxicity feeding study with rats (Sherman, et. al., 1963, PP #6F0499) 12 male and 12 female rats were allowed to continue to be fed 0.025% Bromacil in the diet for 3 generations. There were no marked differences between the reproductive performance of the control and test animals; no deformed offsprings were observed; no gross or microscopic pathological differences were found; and the fertility, gestation, viability and lactation indices were not significantly different.

This data satisfy the requirements for reproduction studies.

5) Mutagenicity

Bromacil could not be detected in mouse DNA. Bromacil was not inhibitory to E. coli 15T. (McGahen and Hoffman, 1963b). These same authors observed no mutagenic effects of Bromacil on bacteriophage.

Bromacil tested negative to reversion to histidine independence in one test utilizing eight histidine requiring mutants of Salmonella typhimurium, (Anderson, et.al.,1972).

Bromacil was not mutagenic in the dominant lethal assay in mice (Siebert and Lemperle, 1974).

Bromacil was not mutagenic by the bacterial - plate assay method. (Fiscor and Nii Piccolo, 1972).

These data satisfy the requirements for mutagenicity.

6) Metabolism

5-bromouracil, a metabolite of Bromacil, and a potent mutagen, was not detectable in urine of production plant worker (Du Pont, 1966) or in urine of rats (Gardiner et. al., 1969).

Additional metabolites found in lesser quantities were 5-bromo-3-(2-hydroxyl-1-methylpropyl)-6-methyluracil, 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-hydroxy-methyluracil, 3-sec-butyl-6-hydroxymethyluracil, 5-bromo-3-(3-hydroxy-1-methylpropyl)-6-methyluracil, 3-sec-butyl-6-mthyluracil, and an unknown bromine - containing compound of mol. wt 339.

Over 85% of the principal metabolite 5-bromo-3-sec-butyl-6-hydroxymethyluracil was excreted in the urine of rats (Gardiner et. al. - 1966, MRID 00013298).

This data satisfy the requirements for metabolism studies.

B. Human and Domestic Hazard Assessment

The information available to assess potential hazard as a result of chronic exposure is incomplete (see Toxicology Profile for details). A second chronic study; preferable with dogs, with emphasis in possible thyroid tissue changes must be performed, since thyroid changes were reported in the study with rats. The acute oral, dermal, inhalation and eye/skin irritation studies indicate low hazard.

C. Summary of Data Gaps

1. Acute Toxicity

No data gaps

2. Chronic Data

a. Repeat a chronic oral toxicity with dogs, emphasis on thyroid changes.

b. Oncogenicity 1 species

3. Teratogenicity

A second study using another species.

APPENDIX C

DER FOR TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

7/20/81

007712

File No. 003281

DATA EVALUATION REPORT

(1) CHEMICAL:

Bromacil (5-bromo-3-sec-butyl-6-methyl)

(2) FORMULATION:

06 - wettable powder

(3) CITATION:

Paynter O.E. (1966) Reproduction study--Rabbits: Project No. 201-163 (Unpublished study including letter dated May 27, 1966 from O.E. Paynter to J. Wesley Clayton, Jr., received November 22, 1966 under 352-287; prepared by Hazelton Laboratories, Inc., submitted by E.I. duPont de Nemours & Co., Wilmington, Del.; CDL:002921-F)

(4) REVIEWED BY:

Steven G. Oberg
Assistant Professor
Utah State University
Logan, Utah 84322
801-750-2856

Signature Steven G. Oberg

Date 20 JUL 81

(5) APPROVED BY:

Signature _____

Date _____

(6) TEST TYPE:

Teratogenicity Studies
Guideline 40 CFR 163.83-3

(7) CONCLUSIONS:

A. The Bromacil teratogenicity study reviewed is inadequate by guideline standards. See the discussion section for itemized variations from recommended study protocols.

(8) MATERIALS AND METHODS:

A. Twenty-six New Zealand white rabbits were divided into control, low and high Bromacil treatment groups. They were bred by a fertile buck and the rabbits were fed Bromacil in the diet (0, 50 or 250 ppm) from the 8th to the 16th days of gestation.

B. On the 28th or 29th day of gestation 3 controls, 3 low dose and 4 high dose rabbits were sacrificed and Caesarean sections were performed. The remainder of the rabbits delivered normally and were then sacrificed within 24 hours.

61

C. One-third of all the fetuses were prepared for skeletal clearing and staining.

(9) REPORTED RESULTS:

A. All fetuses examined were normal in appearance and behavior.

B. Food consumption was normal during the 9 days of measurement (8th through 16th days of gestation).

C. All skeletal anatomies were found to be normal.

(10) DISCUSSION:

Several deficiencies in the teratogenicity study were noted. Some major variations are listed:

A. Only 1 mammal species was studied and no historical data on the strain was provided.

B. A positive control group was not included in the study.

C. The test compound, Bromacil, was administered only for a selected period during the pregnancies rather than daily.

D. Only control, low and high dose groups were considered--no intermediate dose level was employed. Choice of dose levels was not justified and the doses were not administered according to individual body weights.

E. One-third of the fetuses collected were examined for skeletal abnormalities rather than one-half to two-thirds, and less than 12 pregnant rabbits were included in each dose group.

F. No explanation for administration by diet rather than oral intubation was provided.

G. Maternal and fetal data were brief; expression of data was contrary to guideline protocols.

H. The author's evaluation of the study results was limited since no anomalies were noted that could be related to Bromacil treatments.

The study as presented is unsatisfactory due to inadequate design, execution and reporting. Other than some possible range-finding value, this experiment is without merit for determining the teratogenicity of Bromacil. A new study should be devised and performed after consulting the agency guidelines presented in 40 CFR 163.83-3.

(11) REFERENCES:

None

(12) TECHNICAL REVIEW TIME:

2.25 hours

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APPENDIX D

REVIEW OF TERATOLOGY STUDY IN RATS

6/17/83

007712

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: 6-15-83 JUN 17 1983

SUBJECT: Registration No. ~~352-325~~
Bromacil, Caswell #111
Acc. #249676, Reponse to
Bromacil Registration Standard

TO: Taylor/Remmers PM 21, (TS-767)

FROM: Alex Arce
Tox Branch (TS-769)

THRU: W. Butler,
W. Burnam,
R. Coberly, Tox Branch (TS-769) 6/17/83

Registrant: E.I. duPont de Nemours & Co. of Delaware

Request: Review three studies submitted to satisfy data gaps in the Bromacil Registration Standard.

1. Chronic feeding dog- Data on histopathology - thyroid tissue.

2. Oncogenicity - 18 months feeding mice.

3. Teratogenicity - rat inhalation.

Background Information

This reviewer was in charge of the toxicology phase of the Bromacil Registration Standard published on November 25, 1981 (Copy can be obtained from files at the Tox Branch)..

The data gaps encountered were as follows:

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1. Chronic feeding dog- Data on histopathology - thyroid tissue.

2. Oncogenicity - 18 months feeding mice.

3. Teratogenicity - rat inhalation.

Chronic Data

a. Repeat a chronic oral toxicity with dogs, emphasis on thyroid changes, or submit data in thyroid histopathology.

b. Oncogenicity/specie.

Teratogencity

A second study using another specie.

Recommendation

The duPont response to the data gaps is as follows and the submitted studies has been graded as:.

Chronic Data.

a. The histopathology part of the Chronic Oral Toxicity with dogs has been submitted. The study is acceptable and should be upgraded to core minimum data. Review attached.

b. Oncogenicity

An 18-month mice feeding study has been submitted to EPA but it has not reached this reviewer. Thus, at the time that Acc # 244069-70-71 reaches the Tox Branch it will be reviewed and properly graded.

( )

Teratogenicity

The registrant has submitted a second study in teratology, Report EPA-6001/1-78-003, "Teratology and Acute Toxicology of Selected Chemical Pesticides Administered by Inhalation."

Gordon, W. Newell & James Y. Dilly - January 1978. Stanford Research Institute. Sponsored by EPA. Health Effects Research Laboratory.

This study was conducted in conjunction with other pesticidal products.

The study has been reviewed and found to be acceptable and graded core minimum.

Review of Submitted Data.

a. Chronic Feeding Dog.

The study was previously reviewed and classified as Supplementary data due to the lack of information on the histopathology of the thyroid.

I requested such information because in another chronic study, with rats, thyroid effects were reported.

Results

The major changes observed were:

Thyroid

(a) Chronic inflammation with lymphoid and R.E. cell infiltration of interstitial tissue; hyperplasia of R.E. cells.

(b) Focal light cell hyperplasia.

Number of occurrences

a. 2 males - control

1 male - at 50 ppm

~~1~~ 2 male - at 250 ppm

0 at - 1,250 ppm (high dose level)

b. 2 females - control

3 females - at 50 ppm

3 females - at 250 ppm

3 females - at 1,250 ppm

The severity of the changes was similar at all dose levels and the incidence is comparable for controls and dosed groups.

Thus, I concluded that the product does not induce thyroid changes and the study can be upgraded to core minimum data. NOEL = 1,250 ppm.

Teratogenicity

Report # EPA-600/1-78-003. This review includes only the part related to Aromacil. The other products assayed are not

pertinent to the purpose of this Registration Standard of Bromacil.

Product: Bromacil

Animals: Adult male and female Sprague-Dawley rats - 200 to 250 g; healthy. 10 animal per group.

Product Tested: Bromacil

Administration of the Material:

Daily, from the 7 through the 14 day of gestation in an inhalation chamber.

Dose levels:

165 ± 6 mg/m^3, 78 ± 6 mg/m^3 and 38 ± 2 mg/m^3.

Animals exposed for 2 hours daily from day 7 to 13.

Controls (solvent)

Restricted food, air and DMSO. 10 animals/per group for all the above-mentioned levels. 0 controls, 20 animals per group.

Application of the Aerosol

Instrument: ultrasonic or pneumatic generator that regulates the particle size and the concentration of the material. DeVelbiss nebulizer.

Solvent: DMSO

Analysis: By G.C.

Particle size: Analyzed "using a seven-stage cascade impactor." Aerodynamic - 0.44 u

The pesticides were analyzed. The tissue was also analyzed after performing a gross pathological exam. Exam includes weight of liver and gravid uterus.

The live fetuses were weighed and examined.

Uteruses were examined and number of resorptions recorded.

Fetuses were prepared in Bouin's solution for necropsy or fixed for skeletal analysis.

Pathology was performed in the selected tissue.

Results

Particle size: average of 0.5 to 0.65 um diameter.

Weight: comparable to controls

Food consumption: comparable to controls

Signs of Toxicity: none were observed

Litter size: comparable

Resorptions: Higher at the 165 mg/m^3 dose level, than the other treated groups. However, lower than control.

Fetal Weight: Significant dose-related weight reduction in the treated groups as compared to the controls.

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Conclusions: The results indicate that the administration of the material to pregnant rats did not produce a teratogenic or pathological response. The mothers did not exhibit significant changes.

NOEL for terata 165 mg/m^3 = 7.92 mg/kg.

APPENDIX E

REVIEW OF: REPRODUCTION STUDY IN RATS
CHRONIC TOXICITY STUDY IN DOGS
2-YEAR CHRONIC RAT STUDY

DATED: October 5, 1966

007712 6F0499

UNITED STATES GOVERNMENT

Memorandum

TO : Petitions Control Branch

DATE: October 5, 1966

FROM : Dr. O. G. Fitzhugh
Deputy Director
Division of Toxicological Evaluation

SUBJECT: Bromacil (5-bromo-3-sec-butyl-6-methyluracil) on pineapple and citrus fruit.

PESTICIDE PETITION NO. 6F0499

E. I. Dupont de Nemours Company
Wilmington, Delaware
(AF 4-408)

The data in this petition establishes a "no effect" level of at least 50 ppm for the dog and the rat. This is a conservative figure and according to our usual evaluation 250 ppm would be considered a "no effect" level. Therefore, there is sufficient data on acute, subacute, and chronic toxicity and on reproduction to show the safety of the requested tolerance of 1 ppm on pineapples and citrus fruits.

CONCLUSIONS:

The requested tolerance of 1 ppm Bromacil on pineapples and citrus fruits is safe.

cc:

TE
FSA
PP #6F0499

OGFitzhugh:smr 10-5-66

Copy from the Chemistry file

PCB

OCT 19 1966

Evaluation of toxicologic and pharmacologic data for "Hyvac" X Bromacil Weed Killer

Pesticide Petition 6F0499

E. I. Dupont de Nemours Co.
Wilmington, Delaware

The petitioners request that a residue tolerance of 1 ppm for this herbicide be established for pineapples and citrus fruits. To the knowledge of this reviewer no other tolerance has been established for this material.

The active ingredient in this herbicide is 5-bromo-3-sec-butyl-6-methyl-uracil (Bromacil). The weed killer is a wettable powder containing 80% of the active ingredient.

Acute Toxicity (Oral, in Dogs): Vol II of III Haskell

The material tested was the 80% wettable powder. It was not possible to obtain a lethal dose or LD_{50} in dogs because of emesis. The doses ranged from 5.0 g to 100 mg/kg. Besides emesis, the material elicited salivation, mydriasis and incoordination.

Acute Toxicity (Oral, in Rats): Vol II of III Tox Info

The material was administered by intubation as a 60% aqueous suspension of 80% wettable powder. There were 10 animals per dose level and a 14 day test period. The LD_{50} was 5200 mg/kg. Toxic effects observed were rapid respiration, prostration and weight loss.

Subacute Toxicity (Oral in Rats): Vol II of III Tox Info

The material was a 15% aqueous suspension of the 80% wettable powder. It was administered by intubation 5 times a week for 2 weeks. There was 1 mortality; 5 of 6 animals survived 10 daily doses of 1035 mg/kg. There were disturbances to the gastrointestinal tract, CNS and incoordination noted.

90-Day Feeding Study (Rats): Vol II of III Tox Info

There were 10 males and 10 females at each dose level. The levels were 0, 50, 500, 2500 ppm in the diet. After 6 weeks, the upper level was raised to 5000 ppm. After 10 weeks half of these animals were placed on a diet of 6000 ppm for 1 week and then to 7500 ppm for 2 weeks.

Results:

There were no deaths. There was a lower growth rate at the 5000 ppm level and up. Hematology showed low erythrocyte counts for males after

30 days at the highest level. This improved at 90 days. Urinalysis was normal. There was no microscopic pathology or other effects at 50 or 500 ppm. Microscopic changes were observed at 5000 ppm or more for the thyroid and liver (increased thyroid activity; enlargement of centrolobular cells of the liver).

Dermal Toxicity: Vol II of III Tox Info

Skin Absorption (Rabbits):

At 5000 mg/kg there was no indication of toxicity nor gross pathology.

Sensitization: Vol II of III Tox Info

A 50% suspension of the material caused a mild skin irritation for young guinea pigs in 24 hrs.

Inhalation Test: (Rats): Vol II of III Tox Info

At a concentration of 4.8 mg/liter and 2.1 mg/liter of atmosphere, and given 4 hours of exposure, the material caused rapid respiration. There was dried blood around the mouth and nose of 1 out of 4 rats. There were no deaths.

Eye Irritation (Rabbits): Vol II of III Tox Info

Direct application to the eye surface caused a temporary conjunctivitis. There was no corneal injury.

Mutagenic Studies: Section C NATURE PAPER

The possibility of the compound being incorporated into nucleic acids was studied because of the similarity of the compound to certain precursors of DNA. Studies were with the C^{14} labelled material. From the publications presented, in which the suspected DNA is isolated in several instances and examined, the indications are that such incorporation does not occur.

Metabolism and Degradation: Vol I of III Study [illegible]

The principal compound isolated from urine (rat) was 5-bromo-6-hydroxymethyl-3-sec-butyl-uracil. The metabolite was identified by thin-layer chromatography, infrared spectra, NMR and mass spectrophotometer. Traces of 2 other metabolites were not identified. There was also a trace of the administered compound present.

Soil studies with the labelled herbicide showed that after 1 year, 75% of the material is degraded to CO_2. Only 23.5% remained in the soil.

In studies of the uptake and metabolism of the material in orange trees, it was found that the compound was metabolized to the same compound identified in the urine of rats (see above). Less than 4% of the applied radioactive compound was found in orange plants, the root system of which was exposed to 10 ppm of the material. A minor plant metabolite was not identified. Approximately 10% of the applied radioactivity was metabolized to CO_2 by the plant, or otherwise degraded.

No toxicity data are presented for the metabolites.

Two Year Feeding Study in Rats: Vol I of III Haskell

Initially, 259 males and 256 females were housed in pairs (sexes separated). The pre-test period was 14 days. The animals were placed on test in 5 equal weight groups of 36 males and 36 females each (total, 180 males and 180 females). There were 2 control groups and 3 test levels: 0.005, 0.025 and 0.125% of the compound.

Results:

Weight Gain and Food Efficiency:

There was a statistically significant weight retardation for the 0.125% female group at the 1st and 2nd years. The other groups were not affected. The food efficiency for the female 0.125% group was also slightly less than normal.

Clinical Observations and Mortality:

There were no noticable clinical signs of toxicity. Mortalities were not greatly different from the control rats. There were 3 deaths in the control groups and 3 in the treated groups during the 1st year. Mortalities, or animals killed in extremis was greater in the second year. The total mortality, 58% in males and 47% in the females seems quite high.

Hematology:

The following were studied at various times during the 2 year study: differential WBC, RBC, WBC, hemaglobin concentration, hematocrit and cell size. All were in normal range and there was no evidence that their values were altered by the compound at any feeding level.

Urinalysis:

The following were noted at various times during the study: volume, color, appearance, osmolality, blood, sugar, pH and protein of the urine. The values were not markedly different for control or treated rats, except for osmolality in some instances. The difference in osmolality for the 0.125 male group from controls was slightly significant at 2 test periods.

Biochemistry:

No effect of the test material on alk. phosphatase activity was found at any test period. Results for the protein bound iron (PBI) tests, discussed in the Summary, could not be found in the tables presented. It is stated that "there was no difference between control group and test group fed 0.025%" of the compound for 9 months. It is not stated if the protein bound iron tests at higher levels, or after a longer time on the compound were affected. (Reviewer's note: PBI tests are of little value in any case without concurrent determinations of the iron-binding capacity.)

Gross Pathology:

There was no great difference between test and control organ weights.

Histopathology:

There was perhaps, a dose-related effect to the thyroids. Hyperplasia was noticed at the highest level. Also, there was one follicular cell adenoma in a female rat at the highest level which could be compound-related. Examination of rats which died during the study showed nothing related to ingestion of the compound.

Tissue Residue Analysis:

There were detectable amounts of the compound in tissues but there was no evidence of excessive accumulation. The liver and kidneys had the largest amounts (~ at 2 ppm).

Reproduction Studies (Rats): Vol I of III Blue Bill

The animals used for the study were taken from the main feeding (2 yr.) study previously described after approximately 12 weeks of feeding the compound. Animals from only a dietary level group (.025%) and a control group were taken. There were 12 males and 12 females per group. These animals constituted the F_0 generation. The 1st litter was designated F_{1a}; the 2nd litter as F_{1b}. The F_{1b} litter was maintained on the

same diet. At 110 days they were mated to yield F_{2a} and F_{2b} litters. The same procedure was followed yielding F_{3a} and F_{3b} litters.

Results:

Fertility, gestation, viability lactation indices were noted.

There were no marked differences between control and treated groups. There were no pathological changes, gross or microscopic, attributable to the compound. There was no mention of any deformities in the offsprings.

Two Year Study in Dogs: Vol I of III Haskell

There were 3 males and 3 females to each of 4 groups (1 control group; 3 treated). Dietary levels of the compound were 0.005, 0.025 and 0.125%. Vol II of III

Results:

Body weight:

There was some decline in body weight for males and females at the 0.125% level at the start of the experiment. The growth rate stabilized thereafter. Food consumption was not affected by the presence of the compound.

Clinical Observations:

Appearance, rectal temperature, pulse, and respiration were normal. One animal (0.005% level) was sacrificed in extremis. Illness was stated as not dose-related. All other dogs survived the 2 year study.

Hematology:

Erythrocytes, hemoglobin, hematocrit, leucocytes and differential count were not markedly altered or affected over the 2 years by the presence of the compound.

Urinalysis:

It was not affected over the 2 year study.

Biochemistry:

Sugar, urea nitrogen, cholesterol, alkaline phosphatase values were not affected by the compound.

Pathology:

Organ weights were not markedly different from the controls. There were no histopathologic findings to note.

Tissue Residue:

There was no evidence of excessive accumulation of the compound in the tissues.

Comments and Evaluation:

The reproduction study in rats used only 1 dose level. From the data supplied in the petition, this probably was not a toxic dose. The small number of animals initially on test (12 females and 12 males) may be questioned for a study of this kind.

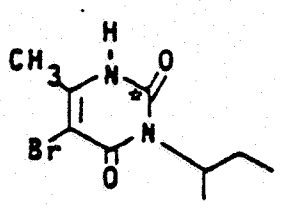
The 2-year dog study had but 6 ~~rats~~ dogs per group. In view of the uneventful findings, however, I would consider the study sufficient.

Metabolite toxicity apparently was not attempted. Acute toxicity of the herbicide is presented only in rats. Short term toxicity was also only in rats. Further studies along these lines in rabbits, perhaps, would be desirable. At least, acute toxicity for the metabolites should be determined.

The data for protein bound iron determinations could not be found, although it is discussed in the rat study Summary. Significance of these tests can not be evaluated.

From the data, it would appear that 0.005% (50 ppm) is a reasonable "no effect" level to consider. USDA figures show per capita consumption in the U. S. of approximately 113.3 lbs of citrus fruit and pineapple per year, or 51200 g. If residue of herbicide of 1 ppm were on these products, per capita intake would be 51.2 mg/capita/year or approximately .0024 mg/kg/day. Considering 50 ppm as a no effect level in dogs, a dog would consume 3.75 mg/kg/day. Thus, there is a safety factor of 1600-fold which suggests no particular hazard. Granting of the tolerance however, should await correction of deficiencies noted in this evaluation.

Junius M. Webb



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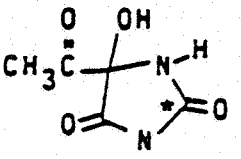

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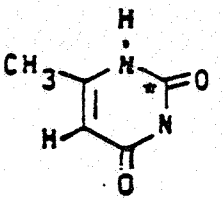

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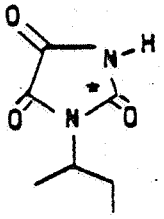

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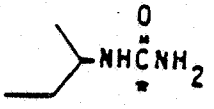

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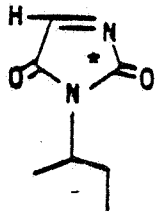

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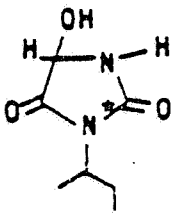

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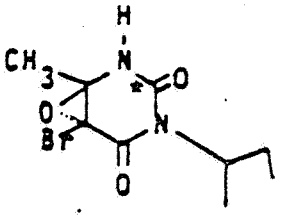
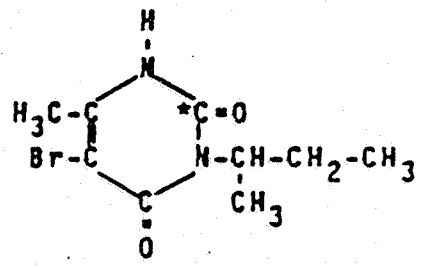
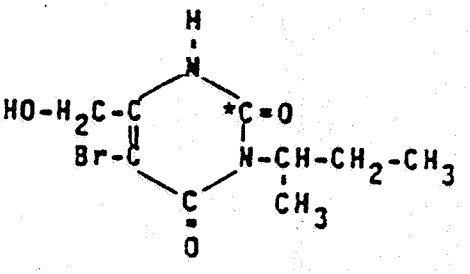


VII

Figure 1. Bromacil and its degradates (* denotes position of the radiolabel): (I) 3-sec-butyl-5-acetyl-5-hydroxyhyantoin; (II) 3-sec-butyl-6-methyl-uracil; (III) 3-sec-butyl-ketohydantoin; (IV) sec-butyl-urea, (V) 3-sec-butyl-3H-imidazole-2,4-dione; (VI) 3-sec-butyl-5-hydroxyhydantoin, (VII) 5-bromo-3-sec-butyl-5,6-epoxy-6-methyl-uracil.
(from photodegradation studies in water)



Bromacil



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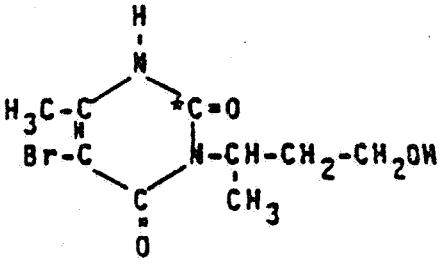

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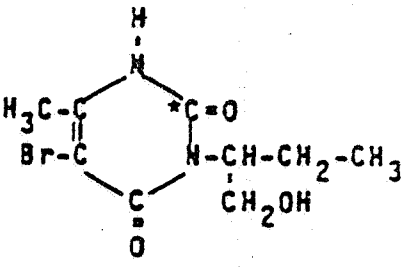

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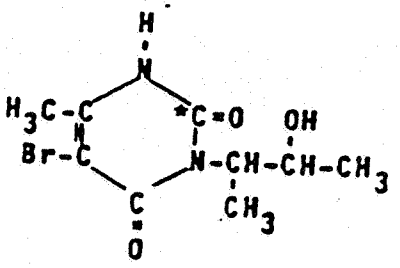
Figure 1. Bromacil and its degradates (* denotes position of radiolabel): (A) 5-bromo-3-sec-butyl-6-hydroxymethyluracil; (B) 5-bromo-3-(3-hydroxy-1-methylpropyl)-6-methyluracil; (C) 5-bromo-3-(∝-hydroxymethylpropyl)-6-methyluracil.

(Degradation Studies in Soil)

33



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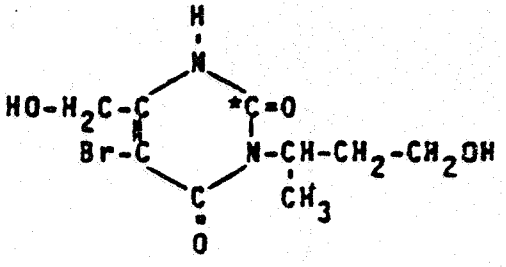

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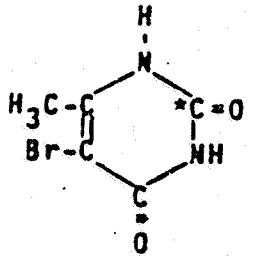
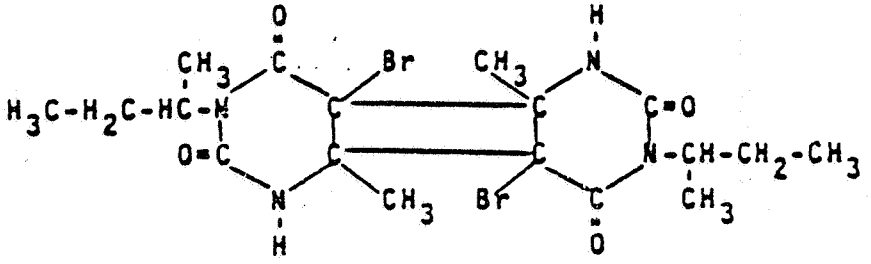


G



Bromacil dimer

Figure 1. (Continued): (D) 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyl-uracil; (E) 5-bromo-3-(3-hydroxy-1-methylpropyl)-6-hydroxymethyl-uracil; (G) 5-bromo-6-methyluracil; (Dimer) 4A, 10A-dibromo-3,9-di-sec-butyl-4B,10B-dimethyl-cyclobutadi[1,2-D:4-DPR]pyrimidine-2,4,8,10-tetrone.

APPENDIX F

DATA REVIEW OF MOUSE ONCOGENICITY STUDY

MEMO DATED 10/83

007712

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: October 1983

SUBJECT: Long-Term Feeding Study in Mice with 5-Bromo-3-sec-butyl -6-methyluracil, INN-976, Bromacil ID#352-325
Acc. #244069, 244070 and 244071, Caswell number 111

FROM: Alex Arce, Tox Branch (TS-769)

TO: Taylor, Stavola - PM 25
Registration Division (TS-767)

THRU: W. Butler, Section III W. Butler 12/21/83
W. Burnam, Chief
R. Coberly, Quality Control
Tox Branch (TS-769)

Request: To review a Mouse Long-Term Feeding Study - 18 months duration. Data gap listed in Registration Standard.

Registrant: Dupont

Recommendation

a) The "Long-term feeding study in mice with Bromacil," submitted to fulfill the data gap in the Bromacil Registration Standard, has been reviewed and graded as Core Minimum Data

b)
In the study the NOEL has not been established. A risk assessment evaluation will be required since oncogenic effects are reported at the 5000 ppm dose level.

The product may be a candidate for a Special Review.

Data Review

Haskell Laboratory #893-80
Medical Research Project No. 3155
December 1, 1980

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Product: 5-Bromo-3-sec-butyl-6-methyluracil, INN-976, Bromacil - 95%

Subject: Mice, male & female CD-1

Purpose: "To evaluate the oncogenicity of 5-bromo-3-sec butyl-6-methyluracil (INN-976; Bromacil) in mice"

Dose levels: 0; 250; 1,250; and 5,000 ppm. 18 months study.

Background Information

Reported LD_{50} in rats = 5,175 mg/kg. Six male rats were tested with 10 oral doses of 1500 mg/kg for two weeks. Four died and two survivors showed signs of toxicity. Six rats were dosed with 10 daily doses of 1,035 mg/kg over a 2 week period. One rat died and the five survivors showed signs of toxicity including focal liver cell hypertrophy and hyperplasia.

In a subchronic test, 3-month rat feeding study, no abnormalities up to 500 ppm were detected. At higher dose levels, from 2,500 to 7,500 ppm, signs of toxicity observed were enlarged thyroid gland, increased liver weights and enlarged centrolobular hepatocytes.

Several other studies using rats and dogs exhibited toxic signs at dose levels higher than 250 ppm. Thus, the MTD was established at 5000 ppm.

Product: Bromacil INN-976. 95%

Procedure (18-month feeding study): The product was added to the ground chow as a suspension of 1% in corn oil. Diets were prepared fresh each week and analyzed for Bromacil content at intervals. Weights: each week, each animal, for the first 26 weeks. For the second weighing interval, weeks 26-52, mice were weighed every 2 weeks, and the third weighing interval, weeks 52-76, the mice were weighed every 4 weeks.

Observations: Daily

Food consumption: Determined each week as mean daily food consumption, mean food efficiency, and mean daily intake of Bromacil.

Hematology: At 1, 3, 6, 12, and 18 months, included RBC and WBC, differential hgb and Hct.

Mortality: Observed and recorded.

Sacrifice and necropsy: Started at the 78th week according to prearranged schedule. Major tissues and organs were examined and weighed, and sections were preserved for microscopic examinations. Masses and abnormal tissues were examined in all cases. Urine and feces were also analyzed before sacrifice.

Data from the submitted report: "All mice sacrificed at the terminal sacrifice and mice found dead or sacrificed in extremis during the study were necropsied

and examined grossly. Whenever tissue integrity permitted, the brain, heart, lungs, liver, spleen, kidneys with adrenals attached, testes with epididymides attached, and thymus were weighed and mean organ/body weight ratios (relative organ weights) were calculated. When permitted by tissue integrity, the tissues listed above and other selected tissues listed below were prepared by conventional methods and representative sections were examined microscopically for histopathological nodes (mesenteric, cervical, mandibular, those that were abnormal, and those draining known and suspected tumor sites), aorta, salivary glands (parotid, sublingual and submaxillary), esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, gall bladder, pancreas, bladder, pituitary, thyroid, parathyroid, adrenals, epididymis, prostate, mammary gland, ovaries, uterus, cervix, vagina, spinal cord, peripheral nerve (sciatic), eye, Harderian glands, exorbital lacrimal gland, muscle (thigh, bone (femur), head (3 coronal sections which included nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx and middle ear), all gross lesions with border of normal tissue), and all masses (with adjacent normal tissue)."

Results

Mortality: After the first year, mortality was greater for the treated than for controls at the 1250 and the 5000 ppm dose level, male and female.

Body Weight: The body weight was significantly lower than controls at 5000 ppm for males and females throughout the study.

Food Consumption: No remarkable changes. The daily intake in this type of study is not accurately calculated due to spillage. Clinical observations of alopecia and dermatitis in all animals.

The palpable or absorbable masses observed during the study were not the results of administration of the product.

Serology.

Hematology: A mild increase in HcT, Hgb, was not significant.

Pathology

At 5000 ppm, increase in the mean liver weight for male and female mice was observed. This observation is significant. At the other dose levels, the increase was not significant, 5000 ppm dose level: male control 2.417 - high dose 3.1019, female control 1.9114 - high dose 2.3638.

Oncogenicity (Refer to the attached table extracted from the submitted report)

At 5000 ppm, an increase in neoplasms in the liver of the male mice was observed. Control, 10 hepatocellular adenomas and carcinomas. At the 5000 ppm level, there were 19 adenoma-carcinomas, and this was signficantly different from control ($p < .05$). Also 11 and 8, at 250 and 125 ppm respectively, were reported. Thus the hepatocellular adenomas were present in the male mice and recorded at each dose level, including the 0 control group, but the incidence was almost double at the high-dose level.

Summary Tables extracted from the submitted data are presented as an attachment of this report. Refer to page 5

Non - Neoplastic Abnormalities

Observed at all treatment groups, including the lowest at 250 ppm. Thus, the NOEL is not established in this study.

5000 ppm - diffuse hepatocellular hypertrophy - male and female

1250 ppm - some but in male only

250 ppm - centrolobular vacuolization - males

Other Abnormalities Observed

Testicular abnormalities were observed at all dose levels in a dose-related increment.

250 ppm - seminal vesicular distension.

Focal atrophy of seminoferous tubules at all dose levels. At 1,250 and 5000 ppm - spermatocyte necrosis, sperm calculi and interstitial cell hypertrophy/hyperplasia.

5000 ppm - Atrial trombosis - male.

The NOEL for this study has not been established, since at the lowest dose level of 250 ppm abnormalities are reported.

Conclusion: The submitted study is classified as Core Minimum Data

The dose levels used were 250, 1250 and 5000 ppm. These levels were incorperated into the diet.

The NOEL = Has not been established

The LEL = At 250 ppm

The principal effects observed were:

Oncogenicity - Hepatocellular adenomas and carcinomas at all dose levels including the control, but with a much higher incidence at the 5000 ppm dose level. The increase in combined carcinomas and hepatocellular adenomas was significant to a $p. \leq 0.05$ level of probability. (♂)

NOTE: I have reviewed the submitted study and found it to be acceptable. Any resemblance of my report to the submitted original does not have the intention of plagiarism.

If I have quoted from the original, it is because I believe, to the best of my ability, that the submitted data are thorough, and by adding or changing words I would have only increased the amount of paperwork with no valid or useful purpose.

Table - Summary of combined incidence of neoplasms observed during the Hispathological Examination .

NOTE .- The following table was extracted from the submitted data.

The largest number of neoplasms were found at the high dose level .

Treatment Group (ppm INN-976:)	Male				Female			
	0	250	1,250	5,000	0	250	1,250	5,000
Combined Incidence of Hepatocellular Adenomas and Carcinomas	10	11	8	19*	1	3	0	1
No. Tumor Bearing Mice	8	11	7	17*	1	3	0	1
No. Mice in Treatment Group	80	80	80	80	80	80	79	80

* Different from control at $p \leq 0.05$ level of probability.

Table - The following table, also extracted from the submitted data , shows the number of nodules observed at necropsy .

MALES

GROUP NUMBER	I	III	V	VII
DOSE LEVEL	0 PPM	250 PPM	1250 PPM	5000 PPM
NUMBER NECROPSIED	80	80	80	80
LIVER:				
Cystic lobe-mass/nodule	1	0	2	0
Heavy	0	0	0	1
Pale brown	4	4	2	0
Dark red mottling, dark red cystic nodules	0	0	0	1
Mass, left lobe	1	0	0	0
Cystic lobe, left side-thick irregular	1	0	0	0

MALES

GROUP NUMBER	I	III	V	VII
DOSE LEVEL	0 PPM	250 PPM	1250 PPM	5000 PPM
NUMBER NECROPSIED	80	80	80	80
Heavy with scattered nodules	1	0	0	0
Left lobe-dark	1	0	0	0
Right lobe-raised area	1	0	0	0
Pale brown; right lobe-lacerated	1	0	0	0
Dark	1	0	1	2
Nodules, left and right lobes	0	0	0	1
Pale brown, nodular, swollen, heavy	1	0	0	0
Left lobe-ruptured, filled with clotted blood	1	0	0	0
Left lobe-cystic masses	1	0	0	0
Pale brown, slightly coarse surface	0	0	0	1
Dark red mottling scattered throughout	1	0	0	0
Mass, right and median lobes	1	0	0	0
Lobular markings prominent	2	0	1	3
Cystic and caudate lobes-nodule	0	0	0	1
Cystic lobe-nodular/nodule	1	0	1	2
Heavy; mass, caudate lobe	0	0	0	1
Left lobe-nodule	1	3	0	2
Right lobe-nodule	1	2	0	2
Ventral surface-nodule	0	1	0	0
Cystic structure, cystic lobe	0	1	0	0
Pale brown, lobular markings prominent	0	1	1	0
Nodules throughout	0	1	0	0
Pale brown; red foci, left lobe	0	1	0	0
Caudate lobe-swollen pale brown	0	1	0	0
Cystic structure, caudate lobe	0	1	0	0
Pale brown; right side, adhesions	0	1	0	0
Pale brown; nodules throughout; mass, cystic lobe	0	1	0	0
Pale, nodular throughout; left lobe-nodule	0	1	0	0
Median lobe-cystic structure	0	1	0	0
Heavy; left lobe-mass/nodule	0	0	0	2
Heavy; right side-cystic mass	0	0	1	0
Swollen large, heavy, friable	0	0	0	1
Coarse	0	0	1	2

FEMALES

GROUP NUMBER	III	IV	V	VII
DOSE LEVEL	0 PPM	250 PPM	1250 PPM	5000 PPM
NUMBER NECROPSIED	80	80	79	80

LIVER:

Clear cysts throughout	1	0	0	0
Pale brown, lobular markings prominent	1	0	0	0
Pale brown	7	9	4	2
Yellow-brown nodules throughout	1	0	0	0
Apex-clear cyst	1	0	0	0
Lobular markings prominent	2	0	1	0
Heavy, red focal areas; right lobe-cystic mass	1	0	0	0
Cystic lobe-brown nodule	1	1	0	0
White foci throughout	0	3	0	0
Pale brown areas	0	1	0	0
Cystic lobe-clear cyst	0	2	0	1
Cystic lobe-swollen nodular, mottled brown	0	1	0	0
Left lobe-brown nodules	0	1	0	0
Dull brown	0	1	0	1
Dark red	0	1	0	0
Pale brown, nodular mass; median lobe	0	1	0	0
Heavy	0	0	1	1
Median lobe-clear cyst	0	0	1	0
Cystic lobe-white nodule	0	0	1	0
Large, heavy, granular surface	0	0	1	0
Lobular markings prominent, coarse	0	0	1	1
Coarse surface	0	0	0	3
Cystic lobe-nodule	0	0	0	1
Median lobe-white foci	0	0	0	1
Granular, coarse, irregular on surface	0	0	0	1
Coarse, appeared swollen	0	0	0	1
Right lobe-cyst	0	0	0	1
Left lobe-cyst	0	0	0	1
Dark	0	0	0	1

Tox Chem No. III Bromacil File Last Updated_____ Current Date_____

Study/Lab/Study #/Date	Material	EPA Accession No.	LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2 year feeding, mouse Haskell Laboratory #893-80 Dec. 1, 1980	Bromacil Technical 95%	244069 244070 244071	NOEL - Not established LEL - 250 ppm Testicular abnormalities as focal atrophy of seminiferous tubules. Dose levels: 0, 250, 1250 and 5000 ppm. At 5000, increased liver weight and hepatocellular adenomas and carcinomas.		Core Minimum

93-1

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APPENDIX G

PRELIMINARY RISK ASSESSMENT MEMO AND UPDATE

January 4, 1985 and May 1, 1987

007712

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

MEMORANDUM

JAN 04 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Robert Taylor (PM 25)
Registration Division (TS-767-C2)

FROM: Bernice Fisher, Statistician B Fisher 1/4/85
Toxicology Branch/HED (TS-769)

Bertram Litt, Statistics Team Leader
Toxicology Branch/HED (TS-769)

THRU: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

SUBJECT: Preliminary Risk Assessment for Bromacil
Based on Haskel Study #893-80 in CD-1 Mice, CAS# 111

SUMMARY

The data in the mouse study discussed below indicate that Bromacil is a liver carcinogen in CD-1 mice. The weight of this evidence and its relevance to humans is a determination to be made by the Toxicology Branch Cancer Review Committee.

The number of male mice surviving one year or longer on the study, and examined for liver tumors with either carcinoma and/or adenoma, (see Table 1) yielded a potency estimation $Q^*_1 = 3.8 \times 10^{-3}$.

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Description of the Study

This is an 18-month feeding study of 95% Bromacil IN 976 (MR-3155) in CD-1 strain of mice, Haskel study # 893-80, Accession No. 244069. The reviewer of this study was Alex Arce, TOX Branch (TS-769), 10/83.

The study sample consisted of 640 mice, who were stratified by sex and weight and then randomly assigned to groups of 80 males and females of equivalent weights. Bromacil was mixed into their diets in concentrations of 0, 250, 1250 and 5000 ppm. Evaluation of the toxicological results were to be made at the end of a two-year period but because "the rate of mortality observed during test weeks 52-76, particularly among male mice,...it was terminated 18 months after its initiation." See page 27 of the Haskel Report (Attachment 1).

Food consumption data were not used for evaluation because the initial feeder caused a wide variation in spillage from 0 - 28 weeks and was subsequently replaced by another one, see page 23 of the Haskel Report. (Attachment 2).

Qualitative Evaluation

No significant differences were observed in male mice in the survival rates with the use of Peto's[1] "Death Rate" method of statistical analysis. While in female mice, there was a significant ($P \leq .05$) increase in the number of animals that died on the higher doses of Bromacil. (See Tables I and II).

Weekly weight gains for males were consistently and mostly significantly ($p \leq .05$) lower on the highest dose of Bromacil as compared with the controls. See Table I in the Haskel report. Females also exhibited similar patterns, however, their percent differences were less than the males. See Table III in the Haskel Report. (Attachments 3 and 4.)

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1. Peto et al. - IARC-Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, 1980 - Supplement 2 - Annex pages 311-385.

Table I. Bromacil - Trend Analysis of Mortality; Male Mice

Dose (ppm)	Time (Days)				
	0-189	190-364	365-544	545-569	Total
0	1/80	7/79	35/72	8/37	51/80
250	1/80	3/79	44/76	2/32	50/80
1250	2/80	5/78	33/73	6/40	46/80
5000	0/80	6/80	31/72	7/41	44/80
T	-3750.	2625.79	-22257.7	2673.33	-20708.58
V	159 x10^7	7.97 x10^7	2.92 x10^8	8.21 x10^7	4.70 x10
Z	-0.940	0.294	-1.303	0.295	-0.956
p	0.826	0.384	0.904	0.384	0.830

Table II. Bromacil - Trend Analysis of Mortality, Female Mice

Dose (ppm)	Time (Days)				
	0-189	190-364	365-544	545-569	Total
0	0/80	6/80	27/74	4/47	37/80
250	3/80	3/77	29/74	8/45	43/80
1250	2/80	6/78	35/72	7/37	50/80
5000	1/80	7/79	41/72	2/31	51/80
T	-1500	7394.9	44212.3	-7140.63	42966.57
V	2.37 x10^7	8.29 x10^7	2.90 x10^8	6.35 x10^7	4.60 x10^8
Z	-0.308	0.812	2.598	-0.896	2.004
p	0.621	0.208	0.005	0.815	0.023

T is the sum of the weighted differences between observed and expected frequencies.

V is the variance of the weighted differences between observed and expected frequencies.

$Z = T/\sqrt{V}$

p is the probability associated with the Z Statistic.

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The changes in the rate of liver tumors over the 18 months of the study were analyzed by means of the "Prevalence and Trend" method of Peto[1]. See Tables III and IV.

The dose related trend for liver carcinoma and/or adenoma was statistically significant (P .02) at the final kill. Thus, even though the analysis of the total data, the trend was significant (P .03), it was mainly affected by the data at the end of the study (568 days - see table III).

For the males, with the use of the X^2 statistic, there was a significant ($P \leq .05$) increase in liver tumors, comparing the highest dose of Bromacil with the controls.

In addition, the combination of 0, 250 and 1250 doses and then comparing this total with the 5000 dose group yielded a signifcant difference ($P \_ .02$) in the application of Fisher's Exact Test.

Females did not exhibit any dose related effect for liver tumors.

Quantitative Risk Assessment

The data in table III and IV have shown that no liver tumors appeared until the second year of the study and therefore animals dying during the first year were not considered to be at risk of liver tumors. Accordingly, 8 controls, 4 low, 7 middle and 6 high dosed males and 6 controls, 6 low, 8 middle and 8 high dosed females have been deleted from the total animals used for the low-dose extrapolation procedure. As there was no evidence of increased liver tumor incidence in females, only the males were used for the Bromacil quantitative risk assessment.

Since the study diet of Bromacil was reported in ppm and as food consumption could not be accurately estimated, Lehman's Tables have been used to adjust the ppm of Bromacil in the diet to mg (7 ppm = 1 mg/kg/day for mice). The surface area adjustment described by N. Mantel and M. Schneiderman (Cancer Research, Vol. 35, 1975 June, pages 1379-1386) has been used to estimate exposures and doses in human equivalents expressed in mg/kg/day.

Table III. Bromacil, Trend Analysis of Liver Tumors in Males Examined

Dose (ppm)	Time (days)			
	0-365	366-567	568	Total
0	0/8	4/43	4/29	8/72
250	0/4	2/46	9/30	11/76
1250	0/7	2/39	5/34	7/73
5000	0/6	3/38	14/36	17/74
T	0	1417.17	21445.7	22862.87
V	0	3.96×10^7	1.02×10^8	1.42×10^8
Z	0	0.225	2.119	1.918
p	0	0.411	0.017	0.027

Table IV. Bromacil, Trend Analysis of Liver Tumors in Females Examined

Dose (ppm)	Time (days)			
	0-365	366-567	568	Total
0	0/6	0/31	1/43	1/74
250	0/6	1/37	2/37	3/74
1250	0/8	0/42	0/30	0/72
5000	0/8	0/48	1/24	1/72
T	0	-1659.81	522.39	-1137.42
V	0	4.38×10^6	1.29×10^7	1.73×10
Z	0	-0.793	0.146	-0.274
p	0	0.786	0.442	0.608

The data below were fitted to the Multi-stage, One-hit, Weibull, Probit and Logit models using the assumptions of Independent background (i.e. control rate) effect and separately for the additive background. The independent assumption provided a smaller confidence band and the best fitting results (See Table V).

Bromacil - Males

Human Equivalent Doses (Mg/kg/day)	0	3	15	60
Number at risk	72	76	73	74
Number of Tumor bearing animals	8	11	7	17

Table V. Bromacil - Male Mice, Liver Tumors
Estimation of Dose Associated in mg/kg/day with Risk
(via Independent Assumption)

Risk	Models					
	Multi-Stage		Weibull		Probit	
	MLE	Lower 95% Bound	MLE	Lower 95% Bound	MLE	Lower 95% Bound
10^{-4}	1.6	2.6×10^{-4}	46.	4.2×10^{-4}	38.	1.6×10^{-5}
10^{-6}	1.6×10^{-1}	2.6×10^{-4}	38.	2.1×10^{-4}	31.	9.5×10^{-6}

As there are no metabolic or other data indicating that there is a basis for the use of a particular extrapolation model, the Multistage model was used as recommended by the Agency for estimating human risks. The Multistage model when fitted to the above data estimated the carcinogenic potency as $Q_1^* = 3.8 \times 10^{-3}$ for mg/kg/day.

Characterization of Risks

The only exposure data available are the published tolerances CFR 180.210 (Code of Federal Regulations 40, Parts 150-189, July 1, 1983 page 359) for citrus fruits and pineapples. These tolerances have been adjusted by their contribution to the human diet, 3.81 percent and 0.3 percent of 1.5 kg intake. The dietary intake is then divided by 60 kg (average human weight - See Table VI) in order to obtain the exposure in mg/kg as shown in Table VII.

Table VI

Food	Tolerances (ppm)	% of Diet	Amount of Exposure (1.5 kg/day) x % of Diet
Citrus fruits	0.1	3.81	5.7×10^{-3}
Pineapples	0.1	0.30	4.5×10^{-4}
Total			6.2×10^{-3}

Table VII. Bromacil - Male Mice - Estimation of Human Exposure and Risk

Food	Amount of Human Exposure[1] mg/kg/day	Upper 95% Bound on Risk[2]
Citrus fruit	9.5×10^{-5}	10^{-7} to 10^{-6}
Pineapples	7.5×10^{-6}	10^{-8}
Total	1.0×10^{-4}	10^{-7} to 10^{-6}

1 Amount of Exposure divided by 60 kg (avg. human wt.)

2 Q^*_1 (3.8×10^{-3}) x Amount of Exposure

Bromacil RIN: 1607-93

Page_____ is not included in this copy.

Pages 102 through 107 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.

✓ 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.

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Bromacil, Mouse Study-Males, Re-evaluation of Survival

Caswell No. 111

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Bernice Fisher 5/1/87

TO: Linda L. Taylor, Ph.D., Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Richard A Levy 5-1-87

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

A statistical re-evaluation of the survival component in the 18-month feeding study of 95% Bromacil in CD-1 male mice was needed because previously(see memorandum on Priliminary Risk Assessment for Bromacil-B. Fisher, 12/85) it was evaluated by the Peto Prevalence method. Currently a more relevant way to analyse survival, is to use the Thomas, Breslow; and Gart computer program for Trend analysis and pairwise comparisons.

Data on mortality from the Bromacil male mouse study for dose levels of 0, 250, 1250, and 5000 ppm was used to assess survival. The results indicated as in the above mentioned memorandum, that there was no significant increase in mortality with the given dose increments of Bromacil.

Reference

Thomas, D.G., Breslow, N., and Gart, J.J. (1977)- Trend and Homogenity Analysis of Proportions and Life Table Data, Computers and Biomedical Research 10, pgs 373-381.

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APPENDIX H

HISTORICAL CONTROL DATA ON LIVER TUMORS IN CD-1 MICE
(from color studies)

COLOR ADDITIVE	Hepatocellular Adenoma M	Hepatocellular Adenoma F	Hepatocellular Carcinoma M	Hepatocellular Carcinoma F
D&C Green No.5	0/59	2/60	5/59	1/60
(Hazleton)	0/59	0/59	2/59	0/59
D&C Red No.36	2/60	0/60	4/60	0/6
(Hazleton)	2/60	0/60	4/60	0/6
FD&C Blue No.1	0/60	0/60	5/60	0/6
(IRDC)	0/60	0/60	3/60	0/6
FD&C Blue No.2	0/47	2/34	0/47	4/34
(Bio-dynamics)	3/48	3/48	2/48	0/48
D&C Orange No.5	2/60	0/60	5/60	2/6
(Bio-dynamics)	1/60	0/60	9/60	1/6
D&C Red No.27	4/58	0/59	2/58	0/59
(Litton-Bionetics)	1/59	1/59	5/59	0/59
FD&C Green No.3	3/45	1/44	7/45	0/4
(Bio-dynamics)	2/49	0/47	5/49	0/4
D&C Red No.21	5/60(7/59)*	0/60	1/60(0/59)*	0/6
(IRDC)	5/60(6/59)*	0/60	2/60(1/59)*	0/6
D&C Red No.6	6/60	1/60	4/60	1/60
(IRDC)	5/60	0/58	3/60	0/58
D&C Red No.19	4/59	2/56	5/59	0/56
(Bio-dynamics)	4/60	2/59	4/60	0/59
D&C Orange No.17	5/60(3/55)*	1/60	6/60(5/55)*	0/60
(Bio-dynamics)	5/60(4/59)*	1/59	4/60(4/59)*	1/59
D&C Red No.33	2/60	1/60	1/60	1/60
(IRDC)	0/60	0/60	10/60	1/60
D&C Yellow No.10	6/60	1/60	6/60	1/60
(Bio-dynamics)	5/60	1/60	2/60	0/60
D&C Red No.9	13/59	1/60	4/59	0/60
(Litton-Bionetics)	6/60	0/60	6/60	0/60
FD&C Red No.3	2/60	0/60	6/60	0/60
(IRDC)	2/60	0/60	1/60	1/60
FD&C Yellow No.5	4/60	0/60	2/60	3/60
(IRDC)	3/60	1/60	7/60	0/60

+ FDA DP/3F re-evaluation of liver slides.

APPENDIX I

REFERENCE DOSES (RFD) FOR ORAL EXPOSURE

REFERENCE DOSES (RfDs) FOR ORAL EXPOSURE

Chemical: Bromacil

CAS #: 116-06-3
Caswell #: 111

Carcinogenicity: Hepatocellular adenomas and carcinomas in mice

Systemic Toxicity: See below.

Preparation Date: 2/18/86

Endpoint	Experimental Doses	UF	MF	RfD
Sherman et al. (1963); E I Dupont 2-Year Dog Feeding Study	~~12.5~~ mg/kg/day NOEL	100	—	0.13 mg/kg/day
Decline in body weight	~~62.5~~ mg/kg/day LEL			
2-Year Feeding/ Oncogenic Rat Study	250 ppm (12.50 mg/kg/day) NOEL			
weight retardation	1250 ppm (62.5 mg/kg/day) LEL			

Endpoint and Experimental Doses:

Sherman et al. 1963.
2-Year Dog Feeding Study.
E.I. Dupont; Report No. PP 6G0499

Dogs were fed Bromacil for 2 years at dose levels of 0, 0.005, 0.025, and 0.125%. No deleterious effects were observed at any dose levels. The thyroid of the dog should have had histopathology performed since thyroid effects were observed at 1250. ppm (62.50 mg/kg) in the 2 year rat feeding oncogenic study.

112

Uncertainty Factors (UFs):

An uncertainty factor of 100 was used to account for the inter- and intraspecies differences.

Modifying Factors (MFs):

None

Additional Comments:

Bromacil in a 2 year mice feeding study, at 5000 ppm, produced hepatocellular adenomas and carcinomas.

Data Considered for Establishing the RfD

1) 2-Year Feeding - Dog NOEL = 0.025%, LEL = 0.125% (some decline in body weight); Levels tested: 0.005%, 0.025%, 0.125%; core grade minimum Supplementary

2) 2-Year Feeding - Dog NOEL = 1250 ppm (31.25 mg/kg/day)(the thyroid changes were comparable to the controls); core grade minimum → what about WT changes?

3) 2-Year Feeding/Oncogenic - Rat NOEL=250 ppm (12.50 mg/kg/day)(0.025%), LEL = 1250 ppm (62.5 mg/kg/day)(0.125%) (weight retardation); core grade minimum

4) 3-Generation Reproduction - Rat Dose level of 0.025%; No difference than controls (only dose tested); core grade minimum

5) Teratology - Rat Teratogenic NOEL > 165 mg/m^3 (7.92 mg/kg)(HDT); Fetotoxic NOEL > 165 mg/m^3 (changes in parents not significant); core grade minimum

6) Teratology - Rabbit Maternal toxic NOEL > 250 ppm (HDT); Fetotoxic NOEL > 250 ppm (HDT); Teratogenic NOEL > 250 ppm (HDT); core grade minimum

Data Gap(s)

None

Other Data Considered

1) 2-Year Feeding - Mice NOEL < 250 ppm (37.5 mg/kg/day)(testicular abnormalities as focal atrophy of seminiferous tubules) At 5000 ppm increased liver weight and hepatocellular adenomas and carcinomas were observed; core grade minimum

113

Other Data Considered (cont.)

2) 90-Day Feeding-Rat NOEL=500 ppm (25 mg/kg/day), LEL=2500 ppm (125 mg/kg/day)(at 5000 ppm lower growth, low RBC, increase in thyroid activity, enlargment of centolobular cells of liver); core grade minimum

.....

Confidence in the RfD:

Study: High (which study) Data Base: High RfD: High

The critical study appears to be of sufficient quality and is given high confidence rating. Since the data base on chronic toxicity is complete, the RfD is given a high confidence.

.....

Documentation of RfD and Review:

Registration Files

.....

Agency RfD Review:

First Review: 11/25/85
Second Review: 12/16/86
Verification Date: 12/16/86

U.S. EPA Contact:

Primary: George Ghali FTS 557-7490

Secondary: Reto Engler FTS 557-7491

APPENDIX J

TABLE X

ICK 1-13-82

Two Year Rat Study

IC.

TABLE X

MORTALITY TABLE OF RATS FED VARIOUS LEVELS OF INN-976
(36 Males and 36 Females Per Group)

Group	Dietary % INN-976	Males: Killed by Design*	Males: Killed in Extremis	Males: Found Dead	Males: Survived 2 Years	Females: Killed by Design*	Females: Killed in Extremis	Females: Found Dead	Females: Survived 2 Years
I	0	11	2	14	9	12	8	3	13
Ia	0	10	6	9	11	12	8	6	10
II	0.005	12	8	8	8	12	6	9	9
III	0.025	12	3	9	12	11	8	5	12
IV	0.125	10	8	8	10	12	3	4	17

* A maximum of 12 (4 at 3 months, 2 at 6 months, and 6 at 12 months).

00167

APPENDIX K

TABLES XXIII AND XXIV.

TABLE XXIII (1)

0110

NUMBER OF RATS WITH TUMORS (3 MONTHS TO 2 YEARS)

INN-976

Tumor	FEMALES Group I	IA	II	III	IV	MALES Group I	IA	II	III	IV
Reticulum cell sarcoma, liver	1	1	0	0	0	1	0	0	0	0
Adenoma, adrenal cortex	2	0	0	1	1	0	0	0	0	0
Adenoma, adrenal medulla	2	0	0	0	1	0	0	3	1	0
Adenoma, anterior pituitary	5	13	16	4	7	2	2	5	5	5
Adenoma, islet cell	0	0	0	0	0	0	0	0	1	0
Thymoma	1	0	0	1	0	0	0	1	0	1
Lymphosarcoma, lymph node	1	0	0	0	0	0	0	0	0	0
Reticulum cell sarcoma, lymph node	0	0	0	0	0	0	1	0	2	0
~~Fibroma, mammary gland~~	~~8~~	~~8~~	~~11~~	~~11~~	~~1~~	~~0~~	~~0~~	~~1~~	~~1~~	~~0~~
Fibroadenoma, mammary gland	6	4	1	2	8	0	0	0	0	0
Adenocarcinoma, mammary gland	2	1	2	3	1	0	0	0	0	0
Fibrosarcoma, mammary gland	1	0	1	1	0	0	0	0	0	0
Reticulum cell sarcoma, spleen	0	2	5	4	0	0	2	3	1	3
Polyp, uterus	3	0	0	2	0	-	-	-	-	-
Fibroma, ovary	0	0	0	1	0	-	-	-	-	-
Adenoma, ovary	0	1	0	0	0	-	-	-	-	-
Follicular cell adenoma, thyroid	0	0	0	0	1	0	0	0	0	0
Light cell adenoma, thyroid	1	3	1	0	5	0	0	2	1	1
Trichoepithelioma, skin	0	0	0	0	0	0	0	0	0	1
Squamous cell carcinoma, skin	2	0	0	0	0	0	0	0	0	1
Lymphoma, lymph node	0	0	0	0	0	0	1	0	2	0

~~093-4~~

RAVEN Systems
D.D. LUCK 1-13-82

00771

007712

011

TABLE XXIII (2)

Tumor	FEMALES Group I	IA	II	III	IV	MALES Group I	IA	II	III	IV
Lipoma, subcutis	0	0	0	1	1	0	0	0	0	3
Fibroma, skin	1	1	0	1	1	2	1	3	3	3
Fibrosarcoma, skin	0	0	1	0	0	1	0	0	0	0
Hyperu[illegible]uroma, kidney.	0	1	0	0	0	0	0	0	0	0
Mesothelioma, peritoneal cavity	0	0	1	0	0	0	0	0	0	0
Hibernoma, thymus	0	0	0	0	1	0	0	0	0	0
Ganglioneuroma, posterior pituitary	1	0	0	0	0	0	0	0	0	0
Adenoma, parathyroid	0	0	0	0	1	0	0	0	0	0
Adenocarcinoma, parathyroid	0	0	0	1	0	0	0	0	0	0
Reticulum cell sarcoma, lungs	0	0	0	0	0	0	0	0	1	0
Fibrosarcoma, uterus	3	2	0	0	1	-	-	-	-	-
Osteogenic sarcoma	0	1	0	0	0	0	0	0	0	0
Sebaceous gland adenoma	0	0	0	0	0	0	0	1	0	0
Total tumors	40	38	39	33	30	6	7	19	16	18

~~09930~~

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007712

TABLE XXIV (2)

011.

Lesion	FEMALES Group I	IA	II	III	IV	MALES Group I	IA	II	III	IV
Arteriosclerosis, aorta	2	0	1	0	1	1	6	2	0	0
Prosoplasia, exorbital lacrimal gland	1	1	0	0	1	5	10	9	7	9
Focal lymphoid cells, exorbital lacrimal gland	0	1	6	3	0	5	3	4	2	3
Hyperplasia, spleen	0	4	0	1	1	0	0	2	1	0
Depletion of lymphoid follicles, spleen	17	17	11	24	19	13	10	8	9	12
Focal hematopoiesis, spleen	7	11	14	19	0	9	15	11	16	13
Bone marrow hyperplasia	6	7	3	5	12	3	8	5	7	5
Glandular hyperplasia, uterus	5	1	0	2	5	-	-	-	-	-
Neutrophilic endometritis	4	4	5	7	1	-	-	-	-	-
Cystic graffian follicle	5	1	4	3	7	-	-	-	-	-
Parathyroid hypertrophy	1	3	2	0	2	2	9	10	6	9
Parathyroid hyperplasia	2	4	3	0	3	2	8	10	6	8
Focal follicular cell hyperplasia, thyroid	2	3	0	0	11	1	10	7	8	9
Focal light cell hyperplasia, thyroid	8	2	2	0	6	1	0	1	2	13
Transitional cell hyperplasia, kidney	0	1	4	5	4	6	6	5	1	2
Leg muscle atrophy	1	0	1	4	6	3	5	2	2	7
Fatty change, exorbital lacrimal gland	0	1	2	6	0	0	0	0	0	0
Focal fibrosis, exorbital lacrimal gland	0	0	0	0	0	4	7	2	3	4
Focal atrophy, exorbital lacrimal gland	0	0	0	0	0	0	1	1	0	3
Fibroplasia of islets of Langerhans	0	0	0	0	0	4	4	0	0	3

* At least - cases

~~09992~~

RAVEN Systems
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007712

TABLE XXIV (1)

0112

NUMBER OF RATS WITH COMMON* HISTOLOGIC
NON-NEOPLASTIC LESIONS - 3 MONTHS TO 2 YEARS

IMN-976

Lesion	FEMALES Group I	IA	II	III	IV	MALES Group I	IA	II	III	IV
Chronic nephritis	16	10	10	15	10	22	25	23	26	24
Bile duct hyperplasia	12	11	7	9	15	8	11	10	9	15
Fatty change, liver	6	9	8	7	4	1	4	4	5	7
Microgranuloma, liver	14	10	10	13	12	6	4	10	5	5
Hepatocyte hypotrophy	2	8	6	3	1	1	4	5	4	6
Adrenal, cortical focal fatty change	1	3	0	3	1	1	1	4	3	0
Dilated adrenal sinusoids	16	16	22	14	12	0	1	2	1	3
Focal hyperplasia, anterior pituitary	8	6	6	13	9	4	9	3	8	6
Chronic murine pneumonitis	18	9	9	10	13	16	7	14	8	10
Upper trachea	15	4	9	9	6	4	2	4	8	6
Focal testicular atrophy	-	-	-	-	-	7	5	11	6	2
Chronic arteritis, testis	-	-	-	-	-	6	8	10	6	3
Prostatic hyperplasia	-	-	-	-	-	2	4	0	2	2
Prostatic fibrosis	-	-	-	-	-	4	1	2	0	1
Duct hyperplasia, pancreas	1	0	1	0	0	1	4	4	3	0
Chronic arteritis, pancreas	1	0	2	4	0	2	0	4	5	2
Dilated gastric glands	1	8	3	0	1	4	14	20	18	17
Intestinal nematodes	4	0	3	0	5	3	5	4	2	5
Focal fibrosis, heart	1	2	1	0	0	9	12	6	12	3
Histiocytic foci, heart	0	1	0	0	0	4	0	1	3	1

~~C922~~

APPENDIX L

HISTORICAL CONTROL DATA

Thyroid Gland TUMOR INCIDENCES IN CONTROL Charles River CD Rats

(By C.S. Lin)

COLOR ADDITIVE	Follicular Cell Tumor				Parafollicular (C-) Cell Tumor			
	Adenoma		Carcinoma		Adenoma		Carcinoma	
	M	F	M	F	M	F	M	F
D&C Green No.5	1/65	0/69	0/65	0/69	5/65	3/69	3/65	3/69
(Haglatom)	0/66	0/69	0/66	0/69	4/66	1/69	5/66	2/69
D&C Red No.30	0/69	1/70	1/69	0/70	0/69	1/70	5/69	3/70
(Haglatom)	1/68	1/70	0/68	0/70	1/68	0/70	4/68	5/70
FD&C Blue No.1	0/67	1/65	0/67	0/65	0/67	2/65	0/67	0/65
(IRPC)	1/68	2/70	0/68	0/70	0/68	2/70	0/68	1/70
FD&C Blue No.2	10/65[a]	6/64[a]	0/65	2/66[d]	0/65	0/66	0/65	0/66
(Bio-dynamics)	4/64[b]	10/66[c]	0/64	0/66	0/64	1/66[e]	0/64	0/66
D&C Orange No.5	1/57	2/58	2/57	1/58	4/57	2/58	1/57	0/58
(Bio-dynamics)	5/59	0/55	0/59	0/55	4/59	0/55	0/59	0/55
D&C Red No.27	0/57	0/58	1/57	0/58	5/57	6/58	4/57	3/58
(Litton-Bionetics)	1/54	0/54	1/54	0/54	3/54	4/54	3/54	3/54
FD&C Green No.3	3/62*	2/63*	2/62**	8/63**	a) Listed as "Adenoma (solid/follicular/papillary/thyroid)			
(Bio-dynamics)	3/61*	1/66*	11/61**	2/66**	**) Listed as "carcinoma (solid/medullary/papillary/follicular)			
D&C Red No.21	3/59	1/59	0/59	1/59	only reported as "adenoma" & "carcinoma".			
(IRDC)	3/52	2/58	0/52	0/58				
D&C Red No.6	0/70	0/68	0/70	0/68	0/70	1/68	0/70	0/68
(IRPC)	2/70	0/69	0/70	0/69	1/70	2/69	0/70	0/69
D&C Red No.19	3/59 (2/57)	1/58 (2/57)	0/59 (1/57)	0/58 (0/57)	7/59 (5/57)	10/58 (6/57)	0/59 (0/57)	0/58 (2/57)
(Bio-dynamics)	4/58	4/59	0/58	0/59	7/58	6/59	4/58	1/59

a) Reported as "medullary adenoma". b) Included 2 rats with "medullary adenoma" and 1 rat with "follicular carcinoma"

Thyroid Gland TUMOR INCIDENCES IN CONTROL Charles River CD Rats (Cont'd)

(By C.S. Lin)

COLOR ADDITIVE	Follicular Cell Tumor				Parafollicular (C-) Cell Tumor			
	Adenoma		Carcinoma		Adenoma		Carcinoma	
	M	F	M	F	M	F	M	F
D&C Orang. No.17.	2/59 (1/56)	0/57 (1/52)	0/59 (4/56)	1/57 (0/52)	2/59 (4/56)	6/57 (9/52)	2/59 (3/56)	3/57 (3/52)
(Bio-dynamics)	1/58	0/55	0/58	2/55	3/58	5/35	1/58	1/55
D&C Red No.33	0/57 (1/57)	2/59 (0/56)	0/57 (1/57)	2/59 (0/56)	9/57 (1/57)	7/59 (4/56)	0/57 (3/57)	1/59 (1/56)
(IRDC)	1/58	0/58	1/58	1/58	0/58	5/58	4/58	3/58
D&C Yellow No.10	1/58 (2/60)	0/59 (1/59)	1/58 (0/60)	0/59 (0/59)	3/58 (8/60)	5/59 (7/59)	2/58 (4/60)	0/59 0/58 (0/59)
(Bio-dynamics)	3/58	0/58	1/58	0/58	3/58	6/58	2/58	
D&C Red No.9	1/56 (0/68)	0/52 (0/67)	0/56 (0/68)	0/52 (0/67)	0/56 (1/68)	3/52 (3/67)	0/56 (0/68)	0/52 (0/67)
(Hilltop Biomatics)	0/57	0/54	0/57	0/54	1/57	0/54	0/57	0/54
FD&C Red No.3	0/59 (0/59)	0/60 (0/56)	0/59 (1/59)	1/60 (0/56)	5/59* (3/59)	2/60* (2/56)	0/59 (1/59)	0/60 (0/56)
(IRDC)	1/58	0/57	0/58	0/57	4/58**	1/57**	0/58	0/57
FD&C Yellow No.5	2/56 (3/57)	0/59 (0/60)	0/56 (0/57)	0/59 (0/60)	1/56 (3/57)	8/59 (9/60)	0/56 (1/57)	0/59 (0/60)
(IRRC)	1/57	0/56	1/57	0/56	3/57	4/56	0/57	0/56

* One was listed as "clear cell adenoma" and the other one as "light cell adenoma".

** Listed as "clear cell adenoma".

Among them, 3 were listed as "light cell adenoma" and 2 as "para-follicular adenoma"; but among them, 3 were listed as "light cell

Toxicology Letters, 11 (1982) 103–110
Elsevier Biomedical Press

SPONTANEOUS TUMORS IN CONTROL F344 AND CHARLES RIVER-CD RATS AND CHARLES RIVER CD-1 AND B6C3HF1 MICE

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(Received October 23rd, 1981)
(Accepted November 10th, 1981)

SUMMARY

The incidence of spontaneous neoplasms in outbred, inbred and F1 hybrid strains was compared using the Charles River-CD rat and mouse, the F344 rat, and B6C3HF1 mouse. These strains are commonly used in carcinogenic studies.

Each strain has a consistent pattern of tumor occurrence; testicular, pituitary and lymphoreticular neoplasms are common in F344 rats, mammary and pituitary neoplasms are common in Charles River-CD rats, liver neoplasms are uncommon in CD-1 mice, while hepatic tumors are frequent in male B6C3HF1 mice. There is considerable variation in tumor incidence in individual studies regardless of strain and there appeared to be greater variation in incidence between laboratories using the same strain than in different laboratories using unlike strains.

Therefore, the choice between these strains may be fortuitous or recommended by governmental agencies. Regardless of the strain selected, it is vital to develop sufficient historical tumor data on the strain used at the particular test laboratory.

INTRODUCTION

Chronic studies in mice and rats have been used to evaluate the carcinogenic potential of drugs, food additives, and chemicals. There have been differences in opinions expressed concerning the use of inbred and outbred strains in such studies. The Canadian Food and Drug Directorate [1] has suggested that animals with heterogeneous genetic constitution (outbred strains) be used to 'determine the potential carcinogenicity of a hitherto untested compound.' When basic mechanisms in carcinogenesis are studied, an 'inbred strain that is known to respond to a particular test compound or group should be selected.' The guidelines for carci-

Abbreviation: MSDRL, Merck, Sharp and Dohme Research Laboratories.

0378-4274/82/0000–0000/$02.75

nogenic testing for the United Kingdom [2] recommend the use of outbred strains of rats and hamsters or an F1 hybrid mouse. Other investigators [3] have recommended the use of inbred mouse strains because of 'genetic stability and stable, reproducible background noise'.

To demonstrate the variability in spontaneous tumor incidence in commonly used strains, tumor incidence in an inbred rat strain (F344), an outbred rat strain (Charles River-CD), and F1 hybrid mouse (B6C3HF1) and an outbred strain of mouse (Charles River CD-1) were compared.

As a survey of 14 pharmaceutical companies has shown, these strains are commonly used (see below):

Strain	Number of companies
Charles River-CD rat	7
F344 rat	2
CD-1 mouse	6
B6C3HF1 mouse	5

The National Cancer Institute had used the F344 strain of rat and B6C3HF1 mouse exclusively since 1972.

METHODS AND MATERIALS

Reports of carcinogenic studies issued by the National Cancer Institute* were scanned for studies using the B6C3HF1 mouse or F344 (Fischer) rats. The tumors in control mice and rats from 22 and 23 studies, respectively, performed by Laboratory A were compiled. 20 male and 20 female controls were started on each study although the final number autopsied varied. The animals were usually 6 weeks old at initiation and were obtained principally from Charles River Breeding Laboratories or the Frederick Cancer Research Center. Data from nine control groups from similar studies performed by Laboratory B were also compiled.

Absorb Dri® hardwood chip bedding from two principal suppliers, (Wilner Wood Products-Norway, Maine and Northeast Products Warrensburg, N.Y.) was used for both rats and mice in studies sponsored by the National Cancer Institute. In three of the studies, hardwood chip bedding (Sanichips®) was supplied by Shurfire Products, Beltsville, MD, or Pinewood Sawdust Co., Moonachie, NJ. Contact bedding in MSDRL studies was either Absorb Dri® or Betta Chips® hardwood bedding supplied by Lab Products, Secaucus, NJ.

Wayne Lab Blox or Wayne sterilizable lab meal (Allied Mills Inc., Chicago, IL)

*National Cancer Institute Bioassay of compounds for possible carcinogenicity Washington, DC. U.S. Dept. of Health Education and Welfare, 1978–1980.

was used for b
supplied by Bu
Certified rodent
below:

Crude Pro	
Crude Fat	
Crude Fib	
Ash max	
Added Mi	

Tumor data f
the Departmen
groups of cont
tabulated. Both
tories and were
studies were of

RESULTS

B6C3HF1 mou

Overall tumo
10% to 70% in
80% for males
Neoplasms o
Lymphoreticula
to be more freq
were considerab
glands, adrenal
incidence not e

Study durati

Duration (weeks)	
90–100	
101–108	

was used for both rats and mice in all NCI contract studies. Purina Lab diets supplied by Buckshire Corp., Perkasie, PA, was used in all MSDRL studies. Certified rodent diets were introduced in June, 1979. Analysis of the diets is shown below:

	Non-certified No. 5001	Certified No. 5002
Crude Protein min	23%	20.0%
Crude Fat min	4.5%	4.5%
Crude Fibre max	6.0%	5.5%
Ash max	–	7.0%
Added Minerals max	–	2.5%

Tumor data from carcinogenic studies of new human health drugs performed in the Department of Safety Assessment, MSDRL were compiled. Data from 24 groups of control CD-1 mice and 23 groups of Charles River-CD rats were tabulated. Both mice and rats were obtained from Charles River Breeding Laboratories and were 4 to 6 weeks of age when the studies were initiated. Almost all studies were of 81 weeks duration in mice and 100 to 105 weeks in rats.

RESULTS

B6C3HF1 mouse (Table I)

Overall tumor incidence in Laboratory A varied from 20% to 89% in males and 10% to 70% in females. In Laboratory B, the range of tumor incidence was 13% to 80% for males and 20% to 60% for females.

Neoplasms of the lung were much more frequent in males than in females. Lymphoreticular neoplasms were one of the most commonly observed and appeared to be more frequent in Laboratory B than Laboratory A studies. Liver neoplasms were considerably more frequent in males than in females. Tumors of the mammary glands, adrenals and thyroid were quite rare occurring in only a few studies at an incidence not exceeding 10%.

Study duration varied as shown below.

Duration (weeks)	Number of studies	
	Lab A	Lab B
90–100	10	4
101–108	12	5

TABLE I

PERCENT (%) INDICENCE OF NEOPLASMS IN CONTROL B6C3HF1 AND CD-1 MICE

Duration	Lab A B6C3HF1 90–108 weeks		Lab B B6C3HF1 91–105 weeks		MSD Studies CD-1 81–105 weeks	
	Male	Female	Male	Female	Male	Female
Number necropsied:	425	426	321	324	1232	1240
Total tumors range:	20–89	10–70	13–80	20–60	24–56	21–60
Average:	49	29	49	40	38	40
Number of groups:	22		9		24	
Lung						
Range – adenomas:	0–30	0–12	0–16	0–10	0–38	0–41
adenocarcinomas:	0–21	0–6	0–5	2	0–16	0–12
Average – adenomas:	8	1	6	3	17	14
adenocarcinomas:	5	1	2	1	5	3
Combined average:	13	2	8	4	22	17
Liver						
Range – adenomas:	0–42	0–6	0–6	0–5	0–12	0–14
adenocarcinomas:	0–37	0–5	0–35	0–10	0–8	0–6
Average – adenomas:	11	2	2	1	3	2
adenocarcinomas:	13	1	20	2	2	1
Combined average:	24	3	22	3	5	2
Lymphoreticular						
Range:	0–35	0–45	4–30	5–40	0–16	3–22
Average:	9	16	15	27	6	11

Overall tumor incidence, as well as tumors at sites of high incidence (liver, lymphoreticular) increased with study duration.

CD-1 mouse (Table I)

Lung tumors occurred at the highest incidence in both males and females. Lymphoreticular neoplasms were frequent, and at a somewhat higher incidence in females than males. Liver neoplasms were infrequent in both males and females. Overall tumor incidence was 38% in males and 40% in females.

F344 rat

The distribution of neoplasms for selected tumor sites is shown in Table II. Overall tumor incidence was quite high, 96% in males and 62% or 78% in females.

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As is readily apparent, the most common tumors in males were benign testicular interstitial cell tumors and in females, pituitary tumors. Mammary tumors, principally adenomas, were frequent in females. Lymphoreticular neoplasms occurred in both males and females and at a higher incidence in Laboratory B.

Charles River-CD (Table II)

Charles River-CD rats also had a high incidence of tumors averaging 71% in males and 88% in females. Pituitary tumors, both adenomas and carcinomas, occurred commonly in both males and females. Mammary tumors were frequent in females. Liver and lymphoreticular tumors were infrequent.

DISCUSSION

Ward et al. [4] compiled spontaneous tumors in over 2500 control B6C3F1 mice of both sexes from National Cancer Institute carcinogenic studies. Laboratory variability was not analyzed. As seen below, the most common tumors were also lung, liver and lymphoreticular.

	Male (%)	Female (%)
Pulmonary	13	4
Lymphoreticular	8	17
Liver	22	4

Goodman et al. [5] also compiled tumor incidence in about 1800 control F344 rats from National Cancer Institute studies. The most common tumors observed were also interstitial cell tumors of the testis in males, mammary and pituitary tumors in females, and lymphoreticular tumors in both sexes.

	Male (%)	Female (%)
Testis	81	–
Mammary	1	18
Lymphoreticular	12	10
Pituitary	11	30

Compilation of published results in Charles River strains have shown good agreement with MSDRL results [6].

The average incidence of selected tumor types was compared in studies done at Laboratories A and B and MSDRL (Tables I and II).

There frequently was a greater variation in incidence between laboratories using the same strain than between different laboratories using unlike strains. For

example, lymphoreticular neoplasms in female B6C3HF1 mice were almost twice as frequent in Laboratory B as in Laboratory A studies (27% vs. 16%). In MSDRL studies of CD-1 mice, the overall incidence of lymphoreticular neoplasms was 11%. The same pattern held for lymphoreticular tumors in male B6C3HF1 and CD-1 mice (9%, 15% and 6%) in Laboratory A, B, and Merck studies, respectively.

Overall tumor incidence was higher in female B6C3HF1 mice in Laboratory B studies than in Laboratory A studies (40% vs. 29%). Overall tumor incidence in female CD-1 MSDRL studies was 40%.

Adrenal medullary tumors on the average were twice as frequent in male Laboratory B F344 rats as in Laboratory A F344 rats (17% vs. 8%) compared to 9% in Merck CRCD rats. Lymphoreticular neoplasms were more frequent in both males and females in Laboratory B than Laboratory A studies (26% vs. 11% and 16% vs. 9%).

Tarone et al. [7] have recently reported on the variability in spontaneous tumor rates in two strains, F344 rats and B6C3HF1 mice. Data from 72 control F344 rat groups from six laboratories and 54 control B6C3HF1 mice from five laboratories were analyzed. The data were obtained from the NCI Carcinogenesis Bioassay Program. This group also found significant intralaboratory variation for certain tumor types for both the rat and mouse. Significant interlaboratory variability occurred in 2 of 6 laboratories for the F344 rat and 1 of 5 laboratories for the B6C3HF1 mouse.

The data presented in this report show that the outbred strains of Charles River-CD rat and Charles River CD1 mouse, as well as the F1 hybrid mouse (B6C3HF1), are commonly used in carcinogenic studies. Each strain has a relative pattern of tumor occurrence; testicular, pituitary and lymphoreticular neoplasms are common in the F344 rat, mammary and pituitary neoplasms are common in the Charles River-CD rat, and liver neoplasms are relatively uncommon in the CD-1 mouse. There is considerable variation in tumor incidence in individual studies regardless of strain and there frequently was greater variation in incidence between laboratories using the same strain that different laboratories using unlike strains. In recent years understanding of the relationship of spontaneous tumors to certain environmental factors including the type of bedding used, the type of cage, the presence of aflatoxin in the diet, etc. has improved [8]. The variation in spontaneous tumor incidence observed may be related to other environmental factors not clearly identified including wild viruses, stress, etc. [8, 9].

Whichever strain is selected, it is vital to develop sufficient historical tumor data on the strain used at the particular test laboratory. Gart et al. [10] and Ward et al. [4] have commented on the value of historic controls. Historic control information may call attention to tumor incidences that are unusually low or high, e.g., as a result of inadvertant environmental contamination or randomization error. Historic data may also 'indicate the degree of expected variability of spontaneous tumor types from study to study and allow more critical evaluation of the incidences in test animals.'

Tarone et al. [7] have recently pointed out that 'the most appropriate and important comparison of a treated group is with its matched control...when the comparison...leads to equivocal results, however, the historical control rats can sometimes provide data needed to make a clear interpretation of the results.'

REFERENCES

1 Draft of Preclinical Toxicologic Guidelines, Bureau of Drugs Health Protection Branch – Health and Welfare, Canada October, 1979.
2 Notes for Guidelines on Carcinogenicity Testing on Medicinal Products, MAIL 24 (Medicines Act Information Letter), June, 1979.
3 M.B. Shimkin, in L. Goldberg (Ed.), Carcinogenic Testing of Chemicals, CRC Press, Cleveland, OH, 1974, p. 15.
4 J.M. Ward, D.G. Goodman, R.A. Squire, K.C. Chu and M.S. Linhart, Neoplastic and nonneoplastic lesions in aging C57BL/6N X C3H/HeN)F1 (B6C3F1) Mice, J. Natl. Cancer Inst., 63 (1979) 849–854.
5 D.G. Goodman, J.M. Ward, R.A. Squire, K.C. Chu and M.S. Linhart, Neoplastic and non-neoplastic lesions in aging F344 rats, Toxicol. Appl. Pharmacol., 48 (1979) 237–248.
6 S.P. Sher, Tumors in control hamsters, rats, and mice: literature tabulation, Crit. Rev. Toxicol., (1981) in press.
7 R.E. Tarone, K.C. Chu and J.M. Ward, Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N × C3H/HeN)F1 (B6C3F1) Mice, J. Nat. Cancer Inst., 66 (1981) 1175–1181.
8 F.J.C. Roe, Spontaneous tumors in rats and mice, Food Cosmet. Toxicol., 3 (1965) 707–720.
9 V. Riley, Psychoendocrine influences on immunocompetence and neoplasia, Science, 212 (1981) 1100–1109.
10 J.J. Gart, K.C. Chu and R.E. Tarone, Statistical issues in interpretation of chronic bioassay tests for carcinogenicity, J. Natl. Cancer Inst., 62 (1979) 957–974.

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EFFECT OF SILY

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SUMMARY

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INTRODUCTION

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TABLE II

PERCENT (%) INCIDENCE OF NEOPLASMS IN CONTROL F344 AND CHARLES RIVER-CD RATS

Duration	Lab A F344 102–107 weeks		Lab B F344 104–106 weeks		MSD Studies Charles River-CD 98–128 weeks	
	Male	Female	Male	Female	Male	Female
Number of necropsied:	459	459	448	450	1211	1204
Total tumors range:	85–100	35–95	90–100	72–90	35–90	57–100
Average:	96	62	96	78	71	88
Number of groups:	23		6		23	
Liver						
Range – adenomas:	0–5	0–5	0–10	0–6	0–6	0–2
adenocarcinomas:	0–10	0	0–4	0–4	0–16	0–12
Average – adenomas:	1	1	3	1	1	1
adenocarcinomas:	2	0	1	1	5	2
Combined average:	3	1	4	2	6	2
Mammary gland						
Range – adenomas:	0–5	0–6	0–2	14–38	0–10	27–72
adenocarcinomas:	0–5	0–5	0–4	0–4	0–4	6–40
Average – adenomas:	1	13	1	24	3	49
adenocarcinomas:	1	1	1	2	1	20
Combined average:	1	14	1	26	4	69
Pituitary						
Range – adenomas:	0–65	5–80	2–14	28–48	16–62	32–90
adenocarcinomas:	0	0–10	0–2	0–2	0–10	0–16
Average – adenomas:	14	34	7	39	36	65
adenocarcinomas:	0	1	1	1	2	5
Combined average:	14	35	8	39	38	70
Testis						
Range – benign:	0–100	–	78–92	–	> 0–20	–
malignant:	0–90	–	0–2	–		–
Average – benign:	80	–	86	–	> 7	–
malignant:	8	–	0.2	–		–
Combined average:	88	–	86.2	–		–
Lymphoreticular						
Range:	0–30	0–20	14–46	6–32	0–12	0–6
Average:	11	9	26	16	4	3
Adrenal medulla						
Range:	0–15	0–10	6–26	0–8	0–20	0–7
Average:	8	2	17	3	9	2

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