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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 3 1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Third Peer Review of Baygon (Propoxur)

FROM: Esther Rinde, Ph.D. E. Rinde 7/5/91
Science Analysis and
Coordination Branch
Health Effects Division (H7509c)

TO: Dennis Edwards
Product Manager #12
Registration Division (TS-767c)

The Health Effects Division Peer Review Committee (PRC) met on April 10, 1991 to discuss and evaluate the weight-of-the-evidence on Baygon with particular reference to its carcinogenic potential. The PRC concluded that additional mechanistic information would be needed if the classification and method of quantitative RA is to be affected. The classification of Baygon remains unchanged: Group B2.

A. Individuals in Attendance:

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

Penelope A. Fenner-Crisp	Penelope A. Fenner-Crisp
William L. Burnam	
Reto Engler	
Marcia Van Gemert	
Karl Baetcke	
Marion Copley	
Julie Du	
Richard Hill	
Hugh Pettigrew	

A. 1. Peer Review Committee (contd.)

Jean Parker

Esther Rinde

Yin-Tak Woo

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

Byron Backus

Clark Swentzel

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.)

Robert Beliles

Kerry Dearfield

George Ghali

William Sette

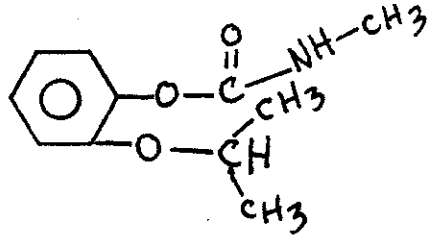
4. Other Attendees: (Observers)

Ed Budd, Larry Dorsey, Gary Burin, Lori Brunsman, Jess Rowland (HED) and Dr. Carmine Pellosie (Uniformed Services) were present.

B. Material Reviewed:

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

Structure of Baygon:



C. Background Information:

1. Previous classification based on the 1984 Rat Study

Baygon was first evaluated by the Toxicology Branch Peer Review Committee as a Group B2 Carcinogen on June 26, 1986 [Memo: Rinde to Ellenberger, 9/4/86]. This classification was based on evidence of neoplasia in one species (SPF fed Wistar Rat) in a 1984 study. These tumors (bladder papillomas and carcinomas) considered to be relatively rare in rodents (especially, as in this case, in the absence of crystalline deposits in the bladder), occurred in both sexes with an unusually high incidence (67-75% at the HDT vs 0% in control), with an early onset of bladder epithelial hyperplasia.

Additionally, in female rats, there was a borderline statistically significant increase in the incidence of uterine carcinoma, associated with early dose-related deaths; there was a definite tendency for this tumor to develop earlier and/or grow more rapidly in the high-dose group, compared to the controls.

In this study, Baygon was administered in Altromin^{R} 1321 diet.

2. Negative 1984 Mouse Study

Baygon in Altromin^{R} pulverized feed was also tested in the SPF mouse, in which it appeared to be negative for carcinogenicity; however, concern was expressed by the Committee as to the validity of the study (classified as core minimum). This study was subsequently rejected by the California Department of Food and Agriculture for "no justification of dose selection, tissue autolyis, and no analyses of diet" and the Registrant agreed to redo the study.

3. Mutagenicity Data Available in 1985

The Peer Review Committee concluded that adequate mutagenicity studies were not available and that the Registrant should be asked to provide a complete battery of tests. Some concern was also expressed that some of the mutagenicity tests may not be sensitive to this class of chemicals (Baygon has some structural similarity to Urethane, a carcinogen for rodents, which is generally negative in the Ames Assay).

4. Current Office of Research and Development Classification

The ORD Carcinogen Assessment Group of the Office of Health and Environmental Assessment classified Baygon as a Group C carcinogen based on the available information in 1986. This classification then was undertaken before the more recent additional carcinogenicity study (see below) became available.

D. Additional (More Recent) Carcinogenicity Study

The Registrant conducted a subsequent 2-year dietary chronic study using female Wistar rats.

Reference: Chronic Feeding Test on Female Wistar Rats Over 2 years. Testing Facility: Bayer AG Institute of Toxicology, August 15, 1988 (study completion date). MRID No: 408811-01.

Baygon was administered in the diet to groups of 70 female Wistar rats at 0 (control) 50, 250, 1000, 3000, 5000 or 8000 ppm (with satellite groups of 10 animals at each dose and one additional group of 50 animals at 8000 ppm) for 2 years. The test material was administered in the food (Altromin® 1321). There were interim sacrifices at 4, 7, 12, 26, 53, 78 weeks with final autopsy at 104 weeks. This study, as did the previous one, used SPF-bred Wistar rats, Bor strain WISW (SPF Cpb) from the laboratory animal breeding firm Winkelmann, Borchen.

The emphasis in this more recent study was on findings associated with the urinary system Other organs (including the uterus) were examined for histopathology only if macroscopic changes were observed. Uterine carcinoma occurred in two female rats in the highest dose group (a total of 6 uteri were examined at this dose level following final sacrifice). **(A borderline statistically significant increase in uterine carcinoma was also reported in female rats in the 1984 study.)**

The neoplastic findings are given in Table 1. There was a significant increase in urinary bladder carcinomas ($p<0.05$), papillomas ($p<0.01$) and in combined carcinoma/papilloma ($p<<.01$) at the HDT (8000 ppm). There were also significant increases in papillomas and in combined carcinoma/papilloma at 3000 ($p<0.05$) and at 5000 ($p<<0.01$) ppm. The trend for carcinoma, papilloma and combined carcinoma/papilloma was also highly significant ($p<<0.01$). **(statistically significant increases and trends for urinary bladder carcinoma, papilloma and combined carcinoma/papilloma were also reported in the previous 1984 study)**[1]. The incidence of these tumors in Control animals was zero. **(This again bears out the rarity of this tumor type, as it did in the 1984 study, in which the same zero incidence was reported for control animals.)**

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[1]**The dose-related occurrence of papillary and/or nodular hyperplasia of the bladder was also observed in female SPF-bred Sprague Dawley rats administered 3000 and 8000 ppm dietary Baygon for one year in another study (conducted by Bayer AG Toxicology Division; review in Caswell File 8191).**

Table 1. Baygon - Wistar Female Rats, Bladder Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)						
Tumors	0	50	250	1000	3000	5000	8000
Carcinomas	0/38	0/35	0/39	0/37	0/40	2/39	5[a]/33
(%)	(0)	(0)	(0)	(0)	(0)	(5)	(15)
p=	0.000**	1.000	1.000	1.000	1.000	0.253	0.018*
Papillomas	0/38	0/35	0/39	0/37	6/40	11/39	7[b]/33
(%)	(0)	(0)	(0)	(0)	(15)	(28)	(21)
p=	0.000**	1.000	1.000	1.000	0.015*	0.000**	0.003**
Both	0/38	0/35	0/39	0/37	6/40	13/39	12/33
(%)	(0)	(0)	(0)	(0)	(15)	(33)	(36)
p=	0.000**	1.000	1.000	1.000	0.015*	0.000**	0.000**

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a] First carcinoma observed at week 68, dose 8000 ppm.
[b] First papilloma observed at week 79, dose 8000 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then p<.05 and if ** then p<.01.

D. Additional Carcinogenicity Study (contd.)

Although there was a numerical difference in survival between controls and treated animals, it was not statistically significant (Table 2).

The findings of this study, particularly those relating to the bladder are consistent with those observed in the previously reviewed 1984 study, except that in that study the NOEL was 200 ppm. In this more recent study, macroscopic bladder changes (increased consistency and/or decreased transparency) were dose-related, and occurred at even the lowest exposure level (50 ppm), indicating that the NOEL for Baygon is substantially below 200 ppm.

E. Additional Information

1. Mutagenicity Data

In a Registrant-submitted UDS study utilizing rat bladder epithelium cells from animals that had received a one-week dietary exposure to Baygon (in Altromin®) at 40, 200, 1000 or 8000 ppm, there was a reported dose-related increase in the proportion of cells in S-phase. The Registrant maintains that a number of "flaws" (including lack of homogeneous cell type preparations, poor presentation of data) in this study call into question the reporting of a positive mutagenic response. The increase in proportion of cells in S-phase at 8000 ppm was, however, apparently real.

There is also a report in the open literature[2] of micronuclei induction and increased number of sister-chromatid exchanges in human lymphocytes following exposure to Baygon (propoxur). Finally, a study has been received from the Registrant in which Chinese hamster ovary cells exposed for two hours to Baygon at 2500 and 5000 ug/ml with exogenous metabolic activation, with a delayed (20 hour) cell harvest time, showed significant increases in chromosomal aberrations. However, this was ascribed to precipitation of the Baygon.

Additional comments on the mutagenicity of Baygon, not available at the time of the meeting, are provided in Appendix I.

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[2]Cid, M.C, Lori, D. and Matos, E. (1990) Genotoxicity of the pesticide propoxur and its nitroso derivative, NO-propoxur, on human lymphocytes in vitro. Mutation Research 232:45-48.

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

	Weeks								
Dose(ppm)	4&7&12[a]	12-26	26[a]	27-52	53[a]	53-78	79[a]	79-104[b]	Total
0	15/15	0/55	5/5	0/50	10/10	1/40	10/10	4/29	5/30(17)
50	15/15	1/55	5/5	2/49	10/10	3/37	10/10	4/24	10/30(33)
250	15/15	1/55	5/5	0/49	10/10	0/39	10/10	5/29	6/30(20)
1000	15/15	0/55	5/5	1/50	10/10	3/39	10/10	3/26	7/30(23)
3000	15/15	0/55	5/5	0/50	10/10	1/40	10/10	9/29	10/30(33)
5000	15/15	1/55	5/5	0/49	10/10	0/39	10/10	6/29	7/30(23)
8000	15/15	0/49	5/5	0/44	10/10	3/34	10/10	5/21	8/24(33)

[+] Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[++] Thomas, D.G., Breslow, N., and Gart, J.J. - Trend and Homogeneity Analysis of Proportions and Life Table Data, version 2.0.

( ) percent

a Interim Sacrifices at weeks 4(5 animals), 7(5 animals), 12(5 animals), 26, 53 and 79 weeks.

b Final Sacrifice at week 104.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison of control denoted at Dose level.
If * then $p<.05$ and if ** then $p<.01$.

E. Additional Information (contd.)

2. Other Data: Metabolism

The Registrant has taken the position that the bladder tumors are species-specific to the rat, and has submitted studies indicating that even in the rat these bladder tumors do not develop unless the animals are also fed an "Altromin®" diet. In one metabolism study submitted to the Agency, groups of 5 female rats received 50, 250 or 5000 ppm Baygon in either casein or Altromin® diet for 5 months, then received a single dose of 1 mg/kg radiolabeled Baygon. **There were no significant quantitative or qualitative differences in urinary metabolites between rats fed Baygon in the casein diet, and those receiving the same Baygon exposure in Altromin® diet.**

3. Other Data: SAR

Baygon has some structural similarity (weak) to Urethane (ethyl carbamate), which is carcinogenic in rats and mice, and to methyl carbamate which is carcinogenic in Wistar rats but not in Sprague-Dawley rats or in mice. However, none of these two carbamates has been shown to induce bladder tumors. Furthermore, the mechanism of action of these alkyl carbamates (methyl carbamate may serve as an alkylator whereas urethane may be activated by dehydrogenation to vinyl carbamate) is expected to be quite different from that of Baygon, an aryl carbamate.

F. Weight of Evidence Considerations:

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

Administration of Baygon in the diet to SPF-fed Wistar female rats was associated with:

A significant increase in urinary bladder carcinomas ($p<0.05$), papillomas ($p<0.01$) and in combined carcinoma/papilloma ($p<<.01$) at the HDT (8000 ppm).

There were also significant increases in papillomas and in combined carcinoma/papilloma at 3000 ($p<0.05$) and at 5000 ($p<<0.01$) ppm. The trend for carcinoma, papilloma and combined carcinoma/papilloma was also highly significant ($p<<0.01$).

Statistically significant increases and trends for urinary bladder carcinoma, papilloma and combined carcinoma/papilloma were also reported in both sexes in the previous 1984 study)[3].

The incidence of these tumors in Control animals was zero.

This again bears out the rarity of this tumor type, as it did in the **1984 study, in which the same zero incidence was reported for control animals.**

A dose-related occurrence of papillary and/or nodular hyperplasia of the bladder was also observed in female SPF-bred Sprague Dawley rats administered 3000 and 8000 ppm dietary Baygon for one year.

Uterine carcinoma occurred in two female rats in the highest dose group (a total of 6 uteri were examined at this dose level following final sacrifice).

A borderline statistically significant increase in uterine carcinoma was also reported in female rats in the 1984 study.

There was only a weak structural relationship of Baygon to Urethane and methyl carbamate, and these (weak) analogs are expected to have different mechanisms of action than Baygon.

The Peer Review Committee (PRC) considered all the new evidence presented above and additional data from the 1989 FAO/WHO "Pesticide Residues in Food, Evaluations - Part II Toxicology" were referred to.

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[3]The dose-related occurrence of papillary and/or nodular hyperplasia of the bladder was also observed in female SPF-bred Sprague Dawley rats administered 3000 and 8000 ppm dietary Baygon for one year in another study (conducted by Bayer AG Toxicology Division; review in Caswell File 8191).

Included in the FAO/WHO Evaluation are summaries of the following:

Long Term Carcinogenicity Study

A 1974 feeding study in rats which was reported as negative for bladder neoplasia at Baygon doses up to 6000 ppm (diet was not identified). This study was also mentioned in the original Peer Review of Baygon; the PRC had insufficient information with which to evaluate this study (limited available information, including pathology data) [Memo to Ellenberger, Sept. 4, 1986].

Strain Specificity

There was no apparent difference in sensitivity between Wistar and Sprague Dawley rats with regard to the formation of urinary bladder hyperplasia in Baygon in Altromin® 1321 diet feeding studies at doses up to 8000 ppm.

Species Specificity

No effect on the urinary bladder epithelium was reported in two oral short-term toxicity studies in dogs fed Baygon in Ssniff-HH sole diet; in a long-term feeding study in CF1/W74 mice fed Baygon in Altromin® pulverized feed at doses up to 6000 ppm; in another 53 week feeding study in NMRI mice fed Baygon in Altromin® 1321 diet at doses up to 8000 ppm; in a 53 week feeding study in female Syrian golden hamsters fed Baygon in Ssniff H-Mehl diet at doses up to 8000 ppm; or in 6 Rhesus monkeys receiving 40mg/kg Baygon by oral intubation and Altromin® GmbH 6014 ad libitum along with fresh fruits and vegetables for 13 weeks.

Effect of Vitamin C supplementation

A group of female Wistar rats fed Vitamin C, at 1% in the diet, concomitantly with Baygon in Altromin® 1321 diet at doses up to 8000 ppm were reported to show the same degree of hyperplastic effects on the urinary bladder epithelium when compared to a group fed Baygon at the same level, but without Vitamin C supplementation.

Effect of Ammonium chloride supplementation

In a 15 week study in female Wistar rats were fed Baygon at 8000 ppm in Altromin® 1321 diet, with or without the addition of 2% ammonium chloride. The pH of the urine was monitored and after feeding of Baygon (without ammonium chloride) was found to be slightly alkaline; when 2% ammonium chloride was given, the pH shifted (approximately 2 units) to slightly acid. Bladder hyperplasia was reported in 4/10 (at 4 weeks) and 8/14 (at 15 weeks) in animals fed Baygon alone and in **0/10 (at 4 weeks) and 1/15 (at 15 weeks) in animals fed Baygon with ammonium chloride.** [Details provided by G. Burin (personal communication) and will be added to the 1990 revision of the FAO/WHO document.]

Effect of Diet

Altromin[R] 1321 standard diet was used in the rat studies which were positive for urinary bladder neoplasia.

Casein semi-synthetic diet no. 1/0 containing Baygon was fed to female Wistar rats in 2 additional studies. In one study the dosed animals received 8000 ppm Baygon for 4, 8 or 14 weeks. In the other, Baygon was fed at 3000 or 8000 ppm for 100 weeks. **No histopathologic changes in the urinary bladder were reported in either study.**

Possible differences in the absorption of Baygon from the Altromin[R] diet and the Casein diet, and differences in the renal metabolite pattern of Baygon attributable to either diet, were reportedly ruled out in studies with ^{14}C-labeled Baygon.

A more complete summary of additional information on Baygon, not available to the Committee at the time of the meeting, is provided in Appendix II. (The complete discussion document, on which the summary is based, is attached to the file copy of this report.)

G. Classification of Carcinogenic Potential:

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

The Peer Review Committee concluded that although the cancer data on female rats confirms the association of Baygon with bladder tumors, other studies presented in the 1989 FAO/WHO document (as summarized in the previous section) suggest, but do not prove that this association may be species and diet specific. In addition, the PRC discussed new evidence on sodium saccharin carcinogenicity (previously, the sodium saccharin induced bladder tumors were not thought to be associated with crystals in the bladder; Electron Micrography (EM) evaluation has shown otherwise). These findings may be significant in light of the study reported in the FAO/WHO document, in which supplementation of Altromin[R] diet with ammonium chloride reduced or eliminated the appearance of bladder hyperplasia (and lowered the urine pH) in Baygon-fed female Wistar rats.

Based on the additional information presented (including the 1989 FAO/WHO data) the PRC believed that there was insufficient evidence to change the classification of Baygon (Group B2 carcinogen) and method of quantification at this time. However, it was agreed, that if the species and diet specificity could be established and genotoxicity dismissed for Baygon, the use of the conventional low-dose quantitative risk assessment method (Q1*) might not be appropriate. Studies designed to further investigate the mechanism of action and genotoxic potential were recommended.

PRC Requirements and Recommendations

Requirements

To answer questions raised about the most recent (1988) female Wistar rat study the registrant should be **required** to:

1. Re-cut the bladder sections and have a pathologist (with expertise in bladder neoplasia) read these (and re-read the original) bladder slides.[4]

2. A pathologist should also look at sections from all groups for uterine pathology.

3. The registrant should submit historical control data from their testing facility and information on the diet composition (Altromin^{R} 1321 vs other diets).

Recommendations

To better understand mechanistic considerations and relate them to the Agency's regulatory position on Baygon, the registrant is advised to clarify Baygon's genotoxic potential (see Appendix 1)[5] and to resolve the discrepancy created by the 2 diets (refer to: Cohen and Elwein[6]) The registrant is encouraged to discuss with or submit to the Agency their protocols before beginning any studies. The following are suggested:

Repeated-Dose Study in female rats considering the following endpoints:

in vivo UDS with bladder epithelium and S-phase analysis, including careful dose-response and time-action work (a 90 day study for starts).

EM for crystalline deposits (crystalline deposits were detected using EM with other compounds, eg: saccharin, which were also thought not to be associated with bladder crystalline deposits)

Histological evaluation (correlate with EM findings)

Determination of the effect of pH on crystalline deposits/hyperplasia.

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[4]In order to clarify the bladder pathology, ie: increased consistency and/or decreased transparency reported at all doses; the gross findings should be correlated with the bladder histopathology.

[5]An in vivo cytogenetics assays will become necessary.

[6]Cohen, S.M. and Ellwein, L.B. (1989) Cell Growth Dynamics in Bladder Carcinogenesis: Implications for Risk Assessment. J. of Am. Coll. Tox. 8:1103-1114.

APPENDIX I

Additional Comments on the Mutagenicity of Baygon (Propoxur)

CASWELL FILE

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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Comments on Propoxur Mutagenicity Information for Peer Review Document

FROM: Kerry L. Dearfield, Ph.D. 5.24.91
Geneticist
Science Support and Special Review Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Esther Rinde, Ph.D.
Manager, Peer Review Committee
Science Support and Special Review Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

This reviewer has been requested by Dr. Rinde to assemble a perspective from the available mutagenicity data concerning the Peer Review chemical Propoxur. This information will be attached to the Peer Review Document on Propoxur as an addendum since most of this material was not discussed at the Peer Review Committee meeting held April 10, 1991. This reviewer was not present at that Peer Review Committee meeting.

cc: Byron Backus

COMMENTS ON PROPOXUR MUTAGENICITY

I. Test Results

Many studies pertaining to mutagenicity have been submitted by the registrant to the OPP on Propoxur. Acceptable studies have been reviewed to minimally satisfy the three categories of mutagenicity testing, i) gene mutations, ii) structural chromosomal aberrations, and iii) other genotoxic effects.

Studies with microorganisms did not produce any positive results with exposure to the parent compound Propoxur. Negative results were obtained in acceptable in vitro assays for gene mutation with Salmonella (MRID #s 00145741, 00147479, 00149043), E. coli (MRID #s 00145741, 00149043), and S. cerevisiae D7 (MRID #00165001). Also, Propoxur was negative in a bacterial test for DNA damage/repair with the E. coli pol A assay (MRID #00149042). It is reported in the published literature that Propoxur is negative for gene mutations in Salmonella and E. coli, but the N-nitroso derivative of Propoxur is a very active mutagenic compound (Blevins et al., Mutat. Res. 56: 1-6, 1977; Seiler, Mutat. Res. 48: 225-236, 1977; Shirasu et al., Cold Spring Harbor Conf. Cell Proliferation 4: 267-285, 1977). Several B. subtilis rec assays were submitted and reported negative, but none were also performed with metabolic activation (MRID #s 00083550, 00145741, 00149043). Published information states that Propoxur was negative for mitotic gene conversion in the yeast S. cerevisiae D4 (Siebert and Eisenbrand, Mutat. Res. 22: 121-126, 1974; Siebert and Lemperle, Mutat. Res. 22: 111-120, 1974).

Several Propoxur metabolites have been tested in the Salmonella assay. Metabolites M1 (MRID #00144357), M2 (MRID #00142730), M3 (MRID #00142728), M4 (MRID #404256-02) and M8 (MRID #00148224) were all found negative in the Salmonella assay. However, metabolite M5 (MRID #00142729) produced variable, but significant increases without activation for gene mutations in Salmonella strain TA1535 (strain TA100 and the frameshift strains were negative). Urine samples obtained from rats exposed to Propoxur during a chronic exposure study were assayed for mutagenic activity with the Salmonella assay and were found negative (MRID #s 00158419, 00158420). Other studies performed with Propoxur metabolites included negative results for M1 in the E. coli pol A test (MRID #00142727) and for M2 for mitotic recombination in S. cerevisiae D7 (MRID #00142726).

Several in vitro studies with mammalian cells were performed. Propoxur was negative in an acceptable Chinese hamster ovary (CHO)/hprt assay for gene mutations (MRID #408364-03) and in an acceptable unscheduled DNA synthesis (UDS) assay with primary rat hepatocytes (MRID #411699-01). A sister chromatid exchange (SCE) assay in cultured human lymphocytes found a slight, though not statistically significant increase in SCE without activation (MRID

#00165002; however, the activation portion was originally reviewed negative, but unacceptable as highest concentration used was close, but not quite high enough to produce appropriate toxicity). A chromosomal aberration assay performed with CHO cells produced a significant positive response with activation at the top two concentrations (2500 and 5000 ug/ml) after a 20 hour harvest time (performed at longer harvest time since appeared to affect cell cycle determined from earlier experiment)(MRID #s 409535-01, 417246-01). The company suggests that the response is not relevant due to precipitation of test compound at these two concentrations and thus producing a non-physiological effect. Propoxur was otherwise negative without activation and at lower, non-precipitating concentrations with activation.

Published in vitro studies with mammalian cells reveal mixed results. Propoxur did not produce detectable single strand breaks in DNA in human skin fibroblasts, but the nitroso derivative did (Blevins et al., Mutat. Res. 44: 1-7, 1977). Propoxur produced no SCE in CHO cells (Wang et al., Bull. Inst. Zoo., Acad. Sinica 27: 111-117, 1988) or little increase in SCE in cultured human lymphocytes (Gonzalez-Cid et al., Mutat. Res. 232: 45-48, 1990). However, a significant increase in micronuclei induction without activation was seen in human lymphocytes after Propoxur exposure (Gonzalez-Cid et al., ibid; nitroso-Propoxur produced similar response for micronuclei and similar slight effect for SCE).

The registrant performed an additional UDS and S phase induction assay using female rat bladder epithelial tissue (MRID #405640-03), the apparent target tissue for Propoxur-induced tumors. There was a reported positive UDS response after exposure to Propoxur in the diet. The registrant argues that this effect should be re-evaluated as there were several technical weaknesses with this assay, including no prior experience with this unvalidated assay, an unusual method for reporting grain counts and the slight UDS increase that may not be significant. It is agreed that there are difficulties with the UDS portion of this assay and it may need re-evaluation. On the other hand, there was a dose-related increase in the proportion of S phase epithelial cells which the registrant agrees is occurring. This suggests that Propoxur induces cell proliferation in this target tissue.

In a submitted study reviewed as supplementary (MRID #00142731), Propoxur and metabolite M5 did not have an effect on programmed DNA synthesis on rat spleen cells. There was little suppression of programmed DNA synthesis by two metabolites, M3 and M5. None of the compounds had any effect on suppressed programmed DNA synthesis, repair, or DNA nucleoid sedimentation rates. Binding of DNA from liver was low, if at all. This suggests little activity by Propoxur and these three metabolites for these parameters in spleen and liver.

Several in vivo studies have been submitted to the OPP.

Negative results have been reported for SCE in Chinese hamster bone marrow (MRID #00158427), for aberrations in Chinese hamster spermatogonia (MRID #404256-01), and for aberrations in Chinese hamster bone marrow (MRID #410087-01). In the first two of these three studies with Chinese hamsters, no adverse effects (e.g. clinical signs) were seen at the doses used (up to 150 mg/kg). This suggests higher dosing could have been used. The Chinese hamster bone marrow aberration study was reviewed unacceptable for several reasons, one of which stated that higher dosing (300 mg/kg for instance) should have been tested at appropriate sampling times. The 300 mg/kg dose showed clinical signs, but at a sampling time of 48 hours; sampling should be done at earlier times as well. A submitted negative mouse micronucleus assay (MRID #00149041) was performed by an unacceptable protocol by today's standards (Schmid protocol) and did not show signs of toxicity in the performance of the assay. A negative published study (Seiler, Mutat. Res. 48: 225-236, 1977) also used the same protocol and Propoxur was administered with $NaNO_2$, thereby making interpretation of possible Propoxur effects unclear.

The registrant reports that there is a positive mouse dominant lethal test in the Eastern European literature (Tyrkiel, Rocz. Panstw. Zakl. Hig. 28(6): 601-613, 1977; unavailable to this reviewer). Positive dominant lethal effects were found at 50 mg/kg given to males for 5 consecutive days. The registrant claims the result is surprising, especially if the animals tolerated the dose. The registrant performed their own test (MRID #00128786) at 10 mg/kg (given only once) and reported negative results. But no signs of toxicity were seen and higher dosing should have been used.

II. M1 Metabolite - Catechol

One of the major metabolites, catechol (labelled M1), is recovered in rat urine at a level of about 10%, regardless of the two types of diet used by the registrant in these studies. Catechol has been shown by the registrant to be negative in the Salmonella and E. coli pol A assays (see M1 results above). Catechol has been reported negative in a published Salmonella assay study (Haworth et al., Environ. Mutagen. 5 (Suppl. 1): 1-142, 1983). However, catechol has been found to be a mutagenic agent in mammalian cells. Positive results were obtained in the mouse lymphoma assay for gene mutations without activation (McGregor et al., Environ. Molec. Mutagen. 11: 523-544, 1988; Wangenheim and Bolcsfoldi, Mutagenesis 3: 193-205, 1988). It is recognized that the mouse lymphoma assay is capable of detecting genotoxic effects due to a clastogenic mechanism. Since the majority of catechol effects appear to be due to chromosomal alterations and not specifically to gene mutations, this is the likely reason for the positive mouse lymphoma response. McGregor et al. further demonstrate that the mutagenic potential of catechol is negated by coincubation with superoxide dismutase; however, there was little

effect on the cytotoxicity produced by catechol in mouse lymphoma cells. These results suggest that some of the mutagenic activity may be due to superoxide anion and that mutagenicity and cytotoxicity may be induced by independent chemical species.

Catechol has been shown to induce sister chromatid exchanges (SCEs) and inhibit cell cycle progression in cultured human lymphocytes without activation (Morimoto and Wolff, Cancer Res. 40: 1189-1193, 1980; Erexson et al., Cancer Res. 45: 2471-2477, 1985). Yager et al. (Cancer Res. 50: 393-399, 1990) show that catechol induced a significant concentration-related increase in micronuclei in cultured human lymphocytes by the cytokinesis-block technique. Furthermore, they also observe an increase in the level of kinetochore-positive micronucleated cells, suggesting an aneuploidy-inducing mechanism as well. At 0.005 mg/ml, catechol induced chromatid breaks and exchanges in CHO cells without activation (Stich et al., Cancer Lett. 14: 251, 1981). One mouse micronucleus assay reports a negative response in vivo with catechol at 150 mg/kg p.o., but this was performed with the Schmid protocol (Gad-El Karim et al., Toxicol. Appl. Pharmacol. 85: 464-477, 1986). In two other studies, catechol has been shown to induce slight to moderate increases of micronuclei in vivo in mouse bone marrow and in fetal liver after exposure to their dams (Ciranni et al., Mutat. Res. 208: 61-67, 1988 and Mutat. Res. 209: 23-28, 1988).

III. Metabolite M9A

Another structure has been observed in human and rat urine, which the company identified as metabolite M9A. This compound has a nitro group added to the phenyl ring of metabolite M2. The registrant suggests this compound is formed in the stomach. This is plausible if there is a source of nitrite (perhaps in the diet) in which an intermediate C-nitroso compound is formed under acidic conditions. Reduction of this group could result in an N-hydroxylamine and oxidation could result in the C-nitro compound. Another possible mechanism of action of C-nitroso compounds is through the formation of N-nitroso compounds by transnitrosation (these mechanisms are summarized in Arcos et al., Chemical Induction of Cancer, Vol IIIA, Academic Press, 1982, pp. 608-613). It is found in several mutagenicity studies that the N-nitroso derivative of Propoxur is a very mutagenic compound. The effects of these types of metabolites would increase the level of concern with Propoxur. This possible intermediate metabolism to compounds capable of carcinogenic and/or mutagenic effects is an area that appears to require further investigation. It is noted that antipyrine, another compound capable of undergoing C-nitroso formation, is a weak urinary tract carcinogen (Johansson et al., Carcinogenesis, 10, 105-111, 1989).

IV. Overall Evaluation and Recommendations

Propoxur and its metabolites, including catechol, do not appear to produce detectable gene mutations, with the exception of metabolite M5. Propoxur itself appears to have some clastogenic potential and the metabolite M1, catechol, has been shown to be genotoxic (primarily via a clastogenic mechanism) in several studies.

For regulatory purposes, the Salmonella and CHO/hprt assays satisfy the category of gene mutations for Propoxur mutagenicity testing. The UDS assay in rat hepatocytes satisfies the category of other genotoxic effects. The studies that make up the structural chromosome aberrations category do not provide an entirely clear picture of the potential genotoxic activity of Propoxur. The CHO/aberrations study with both submissions would minimally satisfy this category.

All of the submitted in vivo cytogenetic studies were either rated unacceptable, or performed at dose levels where there were no signs of toxicity noted. This indicated that higher dosing could have been used (highest doses in Chinese hamsters were 150 mg/kg (although one study went to 300 mg/kg and saw clinical signs, but at only one sampling time and other sampling times appear necessary); in mice went to 10 mg/kg). With the evidence of micronuclei formation in human lymphocytes without activation from a published report and possible aberrations at high levels in CHO cells with activation (although at precipitating concentrations; also company submitted report showed a negative response in CHO cells without activation), a possible clastogenic mechanism needs to be investigated with an adequately performed in vivo cytogenetics assay. Furthermore, the reported positive dominant lethal assay provides some additional information that adds to the overall concern (although this report is unavailable from the Eastern European literature). The clastogenic activity of one of the Propoxur metabolites, catechol, (and possibly the mutagenic activity of M5) adds to this concern as well. Discussion with the OPP regarding protocols and target tissues should be done before initiation of such testing.

Based on the available mutagenicity evidence, it is not clear how Propoxur may be contributing to the tumor response seen in the rat bladder epithelium. If Propoxur is indeed clastogenic, then this may be a leading candidate mechanism; however, available studies indicate that any clastogenic activity is not easy to discern with the parent compound Propoxur. On the other hand, one metabolite, catechol, produces slight to moderate genotoxicity in vivo and any effect by catechol needs to be evaluated in terms of Propoxur metabolism and amount of catechol available at the appropriate target(s). There are certainly detectable levels of catechol in the urine, but are the amounts available capable of inducing detectable genotoxic and/or carcinogenic effects. The

possibility of the nitroso formation as discussed above would add concern and this should be investigated.

The induction of S phase in these bladder epithelial cells shows that Propoxur is capable of inducing cell proliferation. Also, catechol is reported to produce cytotoxicity. Perhaps a complex interaction of moderate genotoxic activity, cell proliferation and cytotoxicity in the target tissue contributes to tumor formation. This may be influenced by the pH of the urine, the type of diet in which Propoxur is administered, and the concentration of Propoxur and metabolites coming together in the bladder, among many factors. These are parameters that can be investigated.

As a final thought, it has recently been shown that human bladder cancers have been identified with p53 gene mutations (Sidransky et al., Science 252: 706-709, 1991). Evidence suggests that p53 acts as a tumor-suppressor gene. Inactivation of this gene by mutation or deletion may play a role in the pathogenesis of many human cancers. Chromosome 17p deletions were noted by these investigators in many transitional cell carcinomas of human bladder tumors and these deletions are suspected to reflect underlying mutations in the p53 suppressor gene. The catechol induced kinetochore-positive micronuclei suggest a possible chromosomal alteration mechanism that could lead to loss of possible tumor suppressor genes. Generation of active oxygen species could contribute additional effects. Cell proliferation could enhance the loss of these genes and help facilitate clonal expansion of a cancerous outgrowth.

APPENDIX II

Summary of Additional Information on Baygon

(Provided by Dr. Byron Backus - The complete Document Memo (Backus to Rinde, 6/7/91) on which this summary is based, is attached to the file copy of this report.)

SUMMARY OF ADDITIONAL INFORMATION ON BAYGON

This summary represents a condensation of supplementary information relating to toxicological issues associated with Baygon (propoxur).

STUDIES ON SPECIES OTHER THAN THE RAT

The registrant has submitted a number of Baygon (propoxur) feeding studies involving species other than the rat. These include a mouse carcinogenicity study, a 12-month dog study, a one-year mouse (female only) study, a one-year hamster (female only) study, and a 13-week rhesus monkey (gavage at 40 mg/kg/day) study. In none of these studies was there any indication of hyperplasia in the urinary bladder, although in the one-year mouse and hamster studies there were "changes in mural condition (increased consistency, decreased transparency)" which were not associated with any histopathological findings. The one-year mouse and hamster studies used the same diet (Altromin 1321) received by the rats in some of the positive (for hyperplasia and tumors of the urinary bladder) studies.

There were no indications of bladder effects or carcinogenicity in the mouse 2-year feeding study (dated May 12, 1981, in Acc. 070831), at levels of 0, 700, 2000, and 6000 ppm. The study was classified as core minimum data by HED; however, Mobay has stated that a new mouse carcinogenicity study will be submitted to satisfy re-registration data requirements. This is probably because the California Department of Food and Agriculture classified the 1981 study as unacceptable. Since the text of the histopathology report states that the thymus and mesenteric nodes were apparently not sampled regularly, the HED classification would probably now be core supplementary. An additional problem is that (from the report text): "The animals in the study showed a comparatively high level of infection...and inflammatory infiltrations of varying severity were common. When the obvious inflammations, localised or generalised (septicaemias) had been eliminated, there remained a miscellaneous group of lymphocytic proliferations; these were whenever possible classified as reactive hyperplasias or malignant lymphomas... A few liver infiltrations which could have been lymphomas were too autolyzed to diagnose, some other diagnoses of lymphoma are at best uncertain because of autolysis or lack of adequate tissues (thymus and mesenteric nodes were apparently not regularly sampled, yet these are the sites where lymphoma usually begins in the mouse)."

12-Month Dog Study (April 11, 1984, in Acc. 256151). Initial dietary exposure levels (each group with 6M, 6F) were 0, 200, 600, and 1800 ppm. The dietary level for high-dose animals was raised to 3600 ppm for weeks 41-44, and was then raised again, to 5400 ppm, for weeks 45-52. There were no bladder effects. At termination, high-dose animals showed thymic atrophy, along with (in 7/12 dogs) reduced splenic weights.

In the chronic (1-year) feeding study in Syrian hamsters (females only) and 1-year feeding study in NMRI mice, dose levels were 0, 3000 and 8000 ppm (in Altromin 1321 feed), with no evidence of either hyperplasia or neoplastic changes in the bladder.

The registrant conducted a subchronic study utilizing 3M and 3F rhesus monkeys, each of which received a 40 mg/kg dose of propoxur once a day for 13 weeks (the diet consisted of Altromin 6014 breeding diet for monkeys, along with fresh vegetables and fruits). At this dose level there was considerable (>50%) plasma ChE inhibition in blood taken one hour after the test material was given, along with transient symptoms ("twitching in the head, limb and chest areas, salivation") in four animals, which tended to appear 15 minutes after test material administration. From the report text: "Histopathological examination of kidneys, ureters, and urinary bladders did not detect any indications of treatment-related organ alterations, in particular no urothelial hyperplasia was observed."

PROPOXUR AND AMMONIUM CHLORIDE FEEDING STUDY IN RATS

The registrant has conducted a 15-week subchronic feeding study with female Wistar rats, in which 0 and 8000 ppm propoxur were administered in the diet with and without 2% ammonium chloride. At 4 weeks, bladder hyperplasia was found in 4/10 rats at 8000 ppm without NH_4Cl, and in 0/10 with. At 15 weeks, these incidences were 8/14 and 1/15 respectively.

SACCHARIN ISSUE

In rats, chronic dietary exposure to appropriate levels of either sodium saccharin or propoxur results in urothelial hyperplasia and subsequent bladder tumor formation. Development of the urothelial hyperplasia (and tumors) also depends on the diet; bladder effects are observed when propoxur is administered in an "Altromin" diet (in a recently reviewed 2-generation rat reproduction study urothelial hyperplasia of the bladder was also observed in Baygon-dosed rats fed "Standard Kliba 343 Rat Diet"), but not in a semisynthetic casein diet.

With both propoxur and sodium saccharin, the bladder tumors (and urothelial hyperplasia) appear to be species-specific, having been observed only in the rat. For both propoxur and sodium saccharin, dietary administration of NH_4Cl (which acidifies the urine) inhibits development of at least the hyperplasia. However, in studies in which sodium saccharin is fed to rats, only the males have an increased incidence of bladder tumors (possibly associated with higher levels of urinary protein, particularly $\alpha_{2\mu}$globulin, which may provide a substrate for precipitation of silica); in the propoxur studies there is no obvious difference between sexes with respect to development of the urothelial hyperplasia or bladder tumors. Further, bladder effects with propoxur at 0.5% of the diet

are more severe than those of sodium saccharin at 5% (threshold effects of sodium saccharin occur at about 1%).

Effects of sodium saccharin are similar to those induced by sodium ascorbate, sodium monoglutamate, and sodium citrate, and may involve precipitation of silicate crystals in the bladder (possibly resulting from formation and absorption of sodium silicate in the intestine); it is not obvious how silicate crystal deposition would result from dietary exposure to propoxur. The rat bladder effects (including tumor formation in females) observed with dietary exposure to propoxur are similar to those of feeding studies in which a small dose (not normally resulting in bladder tumor formation) of an initiator is administered to rats before they receive sodium saccharin in their diet, suggesting propoxur and/or its metabolites include initiators as well as substances capable of causing rat bladder cell proliferation.

METABOLITES OF PROPOXUR

Catechol is a metabolite (M1) of both propoxur and benzene, although in the case of propoxur it is reached as part of a break-down process; with benzene it apparently involves oxidation (with benzene dihydriol as an intermediate?). Effects would not necessarily be the same (target organs for benzene toxicity may correlate with peroxidase activity). Catechol has been identified in a National Toxicology Program report as a cocarcinogen and there are a number of studies in the literature indicating that catechol has some clastogenic activity. In rats, approximately 10% of the activity from ring-labelled propoxur is excreted in the urine as catechol. The molecular weight of propoxur is 209, that of catechol is 110 (110/209 = 0.5263), so that if 10% of 5000 ppm propoxur was converted to catechol, this would be equivalent to 5000 ppm x 0.1 x 0.5263 = 263 ppm. Some non-neoplastic effects observed in chronic propoxur feeding studies (mouse carcinogenicity study: comparatively high levels of infection; dog 1-year feeding study: thymic and splenic atrophy) may be related to catechol's toxicity to the immune system (from p. 134 of the 3rd edition of Casarett and Doull's Toxicology: "...the question as to the mode of action of benzene in inducing leukemia in specific occupational groups remains open, but may also involve an indirect immuno-suppressive action by a benzene metabolite such as catechol..."). In addition, the M9A "metabolite" (with a NO_2 group attached to the phenyl ring) may also be mutagenic. Either catechol or the M9A metabolite (or perhaps both acting together) may then be an initiator.

MOBAY'S POSITION

Mobay, and its parent firm, Bayer, have taken the position that propoxur is nongenotoxic, and that an "epigenetic" threshold mechanism, such as that involving dietary exposure to sodium saccharin, is responsible for the development of rat urinary bladder tumors in chronic feeding studies. Mobay's position is that the bladder tumors are species-specific (although not strain-specific, as they can be induced in both Wistar and Sprague-Dawley rats).

HED CONCERNS

There is nothing in the Mobay documents that addresses the clastogenic activity of catechol, and there is nothing relating to the possible activity of the M9A "metabolite." While the Mobay documents do address the rat bladder findings (emphasizing that these appear to be species-specific and also related to the diet), nothing has been found in these documents addressing the increased incidence of uterine carcinomas in the 2-year rat carcinogenicity study.

Since some findings in chronic studies (high levels of infection in the mouse study, thymic and splenic atrophy in the dog study) may be associated with an immunosuppressive effect caused by catechol, the possibility exists that this effect in itself could cause an increased incidence of tumors.

It is noted that (if the mouse study is reclassified) the only acceptable carcinogenicity study on propoxur that the Agency has available is that on the rat. The hamster and NMRI mouse studies utilized only females, and lasted for only one year. This leaves open the possibility that other organs (including the uterus) may be potential targets.

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Chemical:

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