6-17-96

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

JUN 17 1996

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of (4th) Baygon (Propoxur)

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Review Section II
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Science Analysis Branch
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Registration Division (7505C)
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Special Review and Reregistration Division (7508W)

THROUGH: Stephanie R. Irene Ph.D. Stephanie R. Irene 6/12/96
Acting Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on April 05,1995 to discuss and evaluate the weight-of-the-evidence on Baygon with particular reference to its carcinogenic potential.

The CPRC evaluated additional data from "special studies" submitted by the Registrant, in response to requirements and recommendations made in the previous Peer Reviews. The CPRC concluded that these additional data were inconclusive and did not support re-classification of Baygon. The registrant also submitted a new study in the mouse, in which administration of Baygon was associated with significant increases in hepatocellular adenomas and combined adenoma/carcinoma in males. Based on these data and in consideration of the full weight-of-the-evidence, the CPRC concluded that the classification of Baygon should remain as Group B2 - probable human carcinogen, with the low dose extrapolation model applied to the animal data for the quantification of human risk (Q_1^*).

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SUMMARY

Previous Meetings

Baygon was first evaluated by the Toxicology Branch Peer Review Committee as a Group B2 Carcinogen in 1986 [Memo: Rinde to Ellenberger, 9/4/86]. This classification was based on a 1984 2-year chronic feeding study in Wistar rats, in which there was an unusually high incidence of urinary bladder papillomas and carcinomas (considered to be relatively rare) in both sexes. There was also an increase in the incidence of uterine carcinomas, associated with early dose-related deaths.

Baygon was re-evaluated by the CPRC as a Group B2 Carcinogen in 1991 [Memo: Rinde to Edwards, 7/15/91]. The registrant had conducted a subsequent (1988) 2-year dietary chronic study using female Wistar rats[1]. The CPRC concluded that the results of this 1988 study confirmed the association of Baygon with bladder tumors (there were again increases in urinary bladder carcinomas and papillomas). Baygon in Altromin^{R} pulverized feed was also tested in the SPF mouse (1984), in which it appeared to be negative for carcinogenicity; however, concern was expressed by the Committee as to the validity of the study and the Registrant agreed to redo the study. Based on the additional information presented, the CPRC believed that there was insufficient evidence to change the classification of Baygon (Group B2). However, it was agreed, that if species and diet specificity for Baygon could be established and genotoxicity dismissed, 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.

Present Meeting on Baygon (4th)

A new mouse study, a reevaluation of the tumors from the 1988 rat study, and various "special studies" to further investigate the mechanism of action for Baygon were presented to the CPRC for consideration. In the new mouse study (1992), administration of Baygon in Altromin^{R} diet to B6C3F1 mice resulted in statistically significant increases in liver adenomas and combined adenomas/carcinomas in male mice. There were also statistically significant positive trends for the adenomas and combined adenomas/carcinomas in male mice. No apparent increases in tumor incidence were noted in female mice. The CPRC concluded that dosing in this study was adequate for carcinogenicity testing in both sexes.

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[1]The test material was administered in the food (Altromin^{R} 1321).

In the re-evaluation of the uterine tumors from the 1988 rat study, carcinomas occurred in two female rats in the highest dose group (a total of 6 uteri were examined at this dose level following final sacrifice). This re-evaluation[2], did not include all animals and may need further analysis, especially in light of the findings for uterine tumors in the 1984 study. A re-evaluation of the urinary bladder tumor data from the 1988 rat study confirmed the association of Baygon administration with urinary carcinomas and papillomas.

Most of the mutagenicity data are negative, but there is some evidence for clastogenic activity. Still there are major data gaps (if there is mitotic stimulation). Required are an in vivo cytogenetics assay and in vivo UDS/S-Phase (Bladder tissue) - cell proliferation studies.

The "special studies"[3] submitted by the registrant were evaluated by the CPRC and determined to be inconclusive, and could not support a re-classification of Baygon. Furthermore, the additional data from the 1992 mouse study add to the weight-of-the evidence for Baygon. Therefore, the CPRC concluded that the Classification of Baygon should remain as Group B2, with a (Q_1^*).

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[2]A requirement, from the 3rd Peer Review.

[3]Another requirement from the 3rd Peer Review.

A. Individuals in Attendance at the meetings:

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

William Burnam	
Karl Baetcke	
Marcia Van Gemert	retired
Kerry Dearfield	
Elizabeth Doyle	
Marion Copley	
Hugh Pettigrew	
Esther Rinde	

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

Byron Backus[4]	
Clark Swentzel	
Bernice Fisher	
Lucas Brennecke[5] (PAI/ORNL)	

3. Other Attendees:

Lori Brunsman, Albin Kocialski, Yung Yang (HED) and Amber Aranda (OGC)

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[4]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[5]Signature indicates concurrence with pathology report.

B. Material Reviewed

The material available for review consisted of 1) a DER from a recent (1992) 24-month carcinogenicity feeding study in B6C3F1 mice with Baygon; 2) a 1992 study of the effect of diet on the pH of urine in male and female B6C3F1 mice (although Baygon was not actually tested in this study); 3) a 1992 study of the effect of diet on the pH of urine in male and female Wistar rats (Baygon was not tested in this study); 4) a position document from Miles presenting the case that the increased incidences of urinary bladder tumors observed in chronic rat feeding studies are limited to one species (rats only), and are the result of epigenetic (non-genotoxic) mechanisms; 5) an evaluation of the urine and urinary bladder from male Wistar rats fed Baygon with and without ammonium chloride (this study included examination of urinary bladders by both SEM and light microscopy and examination for crystalline deposits); 6) a supplemental histopathology report from the 1992 Wistar rat study (utilizing females only) which includes morphometric measurements of the urinary bladders, re-evaluation of the original urinary bladder slides by Dr. S. M. Cohen of the University of Nebraska Medical Center, and historical control data from the Bayer AG Institute of Toxicology (Wuppertal, Germany) for uterine carcinomas in chronic rat studies.
Tables and statistical analysis were provided by Bernice Fisher.
The material reviewed is attached to the file copy of this report.

C. Background Information

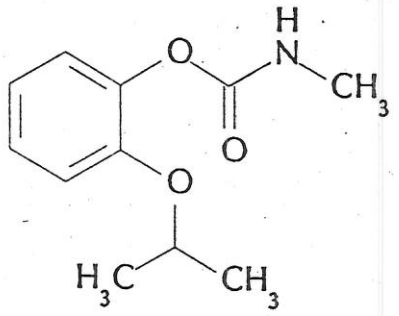


Figure 1. Baygon (Propoxur)

CAS registry #: 114-26-1
P.C. Code #: 047802

Baygon, (also known as Propoxur) was previously classified (first Peer Review meeting, June 26, 1986) as a B2 (probable human) carcinogen on the basis of findings (dose-related and highly significant increased incidence of urinary bladder tumors in both sexes, and a borderline statistically significant increased incidence of uterine carcinomas in high-dose females) from a 1984 rat study conducted at Bayer. There were subsequent Peer Review meetings on November 21, 1990 and April 10, 1991, both confirming the original B2 classification.

The findings of the 1984 study (Tables 1-3) were confirmed in a subsequent 2-year rat feeding study (study completion date: 15 August, 1988) in which groups consisting of 70 female SPF-bred Wistar rats received 0, 50, 250, 1000, 3000, 5000 or 8000 ppm for up to 2 years. In the 1988 study a considerable number of animals (40 or more/group) were sacrificed during the 2-year period. For animals which died between 78 weeks and the final sacrifice and/or which were terminated at final sacrifice the combined incidences of urinary bladder papilloma and carcinoma were 0/29, 0/24, 0/29, 0/26, 6/29, 13/29, and 10/24. The emphasis in this 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 carcinomas were reported from 2 female rats in the highest (8000 ppm) dose group (a total of 6 uteri were examined at this dose level. These were the only two occurrences of uterine carcinomas reported in this study, although a number of adenocarcinomas of the uterus (with no evidence of a dose-related trend) were also reported.

In a 1984 SPF (CF1/W74) mouse study conducted at Bayer, with dietary exposure levels of 0, 700, 2000 and 6000 ppm over a 2-year period, there were no indications of carcinogenicity; however, concern was expressed by the Committee as to the validity of the study and the Registrant agreed to redo the study.

There is an extensive mutagenicity data base on propoxur. In the great majority of the studies, this chemical and/or its metabolites have tested negative. One noteworthy finding in an unscheduled DNA synthesis assay with epithelial cells from the urinary bladders of baygon-dosed rats there was a significant increase in the incidence of cells in S-phase, although (after a reevaluation of the study) it was concluded that there were no indications of an increased incidence of unscheduled DNA synthesis in these cells.

The registrant (Miles Inc., the American subsidiary of Bayer AG) has taken the position that the urinary bladder tumors in the rat are species specific, and are a result not only of dietary exposure to propoxur, but also of the diet (and resultant urinary pH).

There has been a considerable amount of discussion in previous cancer peer review meetings as to the mechanism(s) by which the urinary bladder tumors arise.

As a result of the Third Cancer Peer Review meeting (April 10, 1991) it was stated that the registrant should be **required** to:

1. Re-cut the bladder sections from the 1988 female Wistar rat study and have a pathologist (with expertise in bladder neoplasia) read these (and re-read the original) bladder slides.

2. A pathologist should also look at sections from all groups of the 1988 study 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).

To better understand mechanistic considerations and relate them to the Agency's regulatory position on Baygon, the Cancer Peer Review Committee **suggested** additional studies to clarify Baygon's genotoxic potential. It was noted that the registrant was encouraged to discuss with or submit to the Agency their protocols before initiating studies. These included a repeated-dose study in female rats with the following endpoints: <u>in vivo</u> UDS and S-phase analysis with bladder epithelial cells, including careful dose-response and time-action work; EM for crystalline deposits, with histological evaluation correlating with EM findings; and determination of the effect of pH on crystalline deposits/hyperplasia.

Table 1. Baygon (Propoxur) - 1984 Study: SPF Male Rats, Urinary Bladder Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
Tumors	0	200	1000	5000
Papillomas	0/57	0/60	1/59	26[a]/57
(%)	(0)	(0)	(2)	(46)
p=	0.000**	1.000	0.509	0.000**
Carcinomas	0/57	0/60	0/59	8[b]/57
(%)	(0)	(0)	(0)	(14)
p=	0.000**	1.000	1.000	0.003**
Both	0/57	0/60	1/59	34/57
(%)	(0)	(0)	(2)	(60)
p=	0.000**	1.000	0.509	0.000**

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a] First papilloma observed at week 52, dose 5000 ppm.
[b] First carcinoma observed at week 86, dose 5000 ppm.

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

If * then $p<0.05$ and if ** then $p<0.01$.

Table 2. Baygon (Propoxur) - 1984 Study: SPF Female Rats, Urinary Bladder Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
Tumors	0	200	1000	5000
Papillomas	0/47	0/46	0/47	28[a]/48
(%)	(0)	(0)	(0)	(58)
p=	0.000**	1.000	1.000	0.000**
Carcinomas	0/47	0/46	0/47	5[b]/48
(%)	(0)	(0)	(0)	(10)
p=	0.000**	1.000	1.000	0.030*
Both	0/47	0/46	0/47	33/48
(%)	(0)	(0)	(0)	(69)
p=	0.000**	1.000	1.000	0.000**

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a] First papilloma observed at week 67, dose 5000 ppm.
[b] First carcinoma observed at week 75, dose 5000 ppm.

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

If * then $p<0.05$ and if ** then $p<0.01$.

Table 3. Baygon (Propoxur) - 1984 Study: SPF Female Rats, Incidences of Carcinoma of the Uterus

	Dose (ppm)			
Tumor	0	200	1000	5000
Carcinoma of the Uterus	3/48	4/48	3/47	8/48
(%)	(6)	(8)	(6)	(17)

By the Cochran-Armitage Trend Test there was a significant ($p = 0.024$) dose-related trend associated with these incidences.

In the first Peer Review Memorandum (September 4, 1986) it was stated that:

° In the uterus, there was an increased incidence of carcinoma associated with early dose-related deaths.

° There was an earlier onset of uterine carcinoma at the highest dose tested (5000 ppm).

D. Evaluation of Carcinogenic Evidence

1. B6C3F1 Mouse Carcinogenicity Study. Reference: Bomhard E.: BOQ 5813215 Study for Carcinogenicity in B6C3F1 Mice (Twenty-four Month Feeding Study): July 27, 1992. MRID No. 425977-01.

Testing Facility: Bayer AG, Fachbereich Toxikolgie, Friedrich-Ebert-Strasse 217-333, D-5600 Wuppertal 1, Germany.

a. Experimental Design: In this study, groups of 50 male and 50 female B6C3F1 mice received Baygon in Altromin 1321 diet for a 2-year period at 0, 500, 2000 and 8000 ppm (reported as being equivalent to 0, 114.3, 472.4 and 2080.6 mg/kg/day for males, and 0, 150.4, 591.4 and 2671.1 mg/kg/day for females; these values appear to be relatively high and food consumption values are reported as ranging from 7-8.5 g/animal/day, but there may have been considerable wastage as the usual value for food consumption in the mouse is 3 g/animal/day; recalculating then on this basis would give 47.6, 202.5 and 879.1 mg/kg/day for males and 0, 53.1, 219 and 977.2 mg/kg/day for females).

b. Discussion of tumor data

The only unequivocal neoplastic finding associated with exposure to Baygon involved hepatocellular adenomas (but not carcinomas) in male mice, with the following incidences:

Table 4. Baygon (Propoxur) - 1992 Study: B6C3F1 Male Mice
Incidences of Hepatocellular Adenomas and Carcinomas

Tumor	Dose (ppm)			
	0	500	2000	8000
Hepatocellular adenoma (%)	10/49 (20)	10/49 (20)	15/49 (31)	21/50 (42)
	p= 0.0029**	0.5987	0.1771	0.0174*
Hepatocellular adenoma/ carcinoma (%) Combined	15/49 (31)	16/49 (33)	23/49 (47)	26/50 (52)
	p= 0.0073**	0.5000	0.0731	0.0249*

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denote at Dose level.
If * then $p<0.05$ and if ** then $p<0.01$.

c. Non-Neoplastic Lesions

Among non-neoplastic lesions, the incidence of urinary bladder epithelial hyperplasia was increased in male and female mice at 2000 and 8000 ppm which died during the study or were terminally sacrificed. This urinary bladder epithelial hyperplasia was classified as minimal and diffuse in all instances and it is reported that there was no evidence of any dose-related increase in severity.

Table 5. Baygon (Propoxur) - 1992 Study: B6C3F1 Mice
Incidences of Urinary Bladder Hyperplasia

	Dose (ppm)			
	0	500	2000	8000
Males with urinary bladder hyperplasia (%)	2/49 (4)	2/49 (4)	5[a]/49 (10)	20[b]/50 (40)
Females with urinary bladder hyperplasia (%)	1/48 (2)	1/48 (2)	6[c]/47 (13)	31[d]/48 (65)

[a] $p = 0.218$ compared to control incidence by Fisher's Exact Test.
[b] $p < 0.001$ compared to control incidence by Fisher's Exact Test.
[c] $p = 0.052$ compared to control incidence by Fisher's Exact Test.
[d] $p < 0.001$ compared to control incidence by Fisher's Exact Test.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The CPRC concluded that dosing in this mouse study was adequate for carcinogenicity testing in both sexes, based on signs of clinical pathology and weight gain depression.

2. Additional Histopathology Data for a 2-Year Chronic Study Conducted with Female Wistar Rats. Reference: Hahnemann, S. & Ruhl-Fehlert, C.: Propoxur - Chronic Feeding Test on Female Wistar Rats Over 2 Years (Dose-Effect-Time Relationship) - Supplemental Submission. Study completion date December 15, 1992. MRID No. 426154-06.

Testing Facility: Bayer AG, Fachbereich Toxikolgie, Friedrich-Ebert-Strasse 217-333, D-5600 Wuppertal 1, Germany.

Material in submission: There are 3 parts to the material in MRID 426154-06: 1) complete histopathological examination of the uteri of female rats which survived past 78 weeks; 2) morphometric investigations of the urinary bladder (in order to clarify the original gross pathology reporting of "increased consistency and/or decreased transparency of the urinary bladder" at all dose levels); and 3) A reevaluation of the urinary bladder slides from the original study. The material in MRID 426154-06 was submitted by the registrant to address some of the requirements and recommendations made by the Cancer Peer Review Committee following the Third Peer Review Meeting on Baygon.

The original study report was reviewed (February 8, 1991; Toxicology file document 008261) and classified as supplementary data, since it did not satisfy the Subdivision F Guideline requirements for either a chronic rat toxicity [83-1(a)] or carcinogenicity [83-2(a)] study. The study utilized only female rats, and complete histopathology was routinely done on only the bladder, kidneys, ureters and liver. Other organs, including the uterus, were examined microscopically only if macroscopic changes were evident. There were 7 dietary exposure groups (0, 50, 250, 1000, 3000, 5000 and 8000 ppm), each initially with 70 animals. Because of the considerable number of interim sacrifices, only 21-29 rats/group survived after 78 weeks (with terminal sacrifice at 104 weeks).

a. Uterine carcinomas:

In the initial report, there were only two reported occurrences of carcinoma of the uterus, both occurring in females of the highest (8000 ppm) group. However, it was difficult to interpret this finding, as only 3 to 9 uteri/dietary exposure level (6 at 8000 ppm) were examined microscopically. The recommendation was made by the Cancer Peer Review Committee that: "A pathologist should look at sections from all groups for uterine pathology."

In MRID 426154-06, the previous reporting of a solid carcinoma of the uterus for one of the two females (animal #436) at 8000 ppm is

stated to be spurious, a result of transcriptional error. The incidence of carcinomas of the uterus then in highest-dose (8000 ppm) rats was 1/17 (0/26 for controls). No other occurrences of carcinoma of the uterus were found, so the only animal affected in this study was #430 of the 8000 ppm group. The histopathological investigations detected one carcinoma in situ (it is stated in the report that this is normally classified as a benign neoplasm; Dr, Brennecke confirmed this) in the uterus of rat 423 of the 8000 ppm group, and this was the only occurrence in this study. Several additional adenocarcinomas of the uterus were detected (including one at 50 ppm and two at 1000 ppm), but there was no indication of any dose-relationship involving this finding.

Table 6. Baygon - 1992 Study: Female Wistar rats
Incidences of Uterine Tumor Findings

Dose, ppm	0	50	250	1000	3000	5000	8000
No. of rats	26	22	25	23	23	25	17
Polyp, stromal	10	2	3	1	8	8	5
Fibroma						2	
Stromal sarcoma					1		
Osteo-plastic sarcoma	1						
Polyp, glandular		3	1		1		
Carcinoma in situ							1
Adenoma	1					1	
Adeno-carcinoma		2		3	4	1	
Carcinoma undiffer-entiated							1

b. Morphometric investigations of the urinary bladder:

The recommendation was made by the Cancer Peer Review Committee that: "In order to clarify the bladder pathology, i.e. increased consistency and/or decreased transparency reported at all doses, the gross findings should be correlated with the bladder."

Urinary bladder wall thicknesses were measured using a Videoplan image analyzer. The results obtained showed no consistent dose-related differences in urinary bladder wall thickness.

c. Re-evaluation of urinary bladder slides:

The previously prepared (and previously examined) urinary bladder slides were re-evaluated by Dr. S. M. Cohen of the University of Nebraska Medical Center. Among his comments were the following:

"The quality of the slides was generally very good. Occasionally, a bladder was obviously not distended, and this was noted... This occasionally made histologic diagnoses difficult but was usually not a problem."

"My diagnoses were in general agreement with those of the study pathologist, although occasionally my diagnosis was of slightly greater severity. However, the differences do not affect the overall interpretation of the study regarding dose-response or no-effect level."

"Clearly, carcinomas were induced at the higher doses (5000 and 8000 ppm). Papillomas were observed at doses of 3000 ppm and above, with one small papilloma observed in a rat fed 1000 ppm. In addition, hyperplasia was observed at these doses. There were clearly chemical-induced changes in all groups from doses 1000 ppm and higher. In the group receiving 250 ppm, there were four rats which showed mild focal simple hyperplasia (also seen in two control rats), but one rat fed 250 ppm propoxur (Baygon) had nodular hyperplasia. Although I am sure that this is not a statistically significant incidence, I cannot exclude the possibility that these slight changes, especially the one instance of nodular hyperplasia, are treatment related. In contrast, the bladders in the rats fed 50 ppm propoxur were completely within the normal range (one rat had mild focal hyperplasia), and is clearly a no-observable effect level in this study."

"There was some indication in the gross descriptions, apparently, of abnormalities, including decreased transparency and/or increased consistency, but I could find no histologic explanation for these gross observations. I would not attribute these to the chemical

treatment, since it is generally accepted in bladder carcinogenesis research that the findings observed by light microscopy in the epithelium are the critical parameter to be assessed."

"I paid particular attention...to the possibility of toxicity or evidence of foreign body formation, such as calculi and/or microcrystals. I could not detect any evidence of an irritative process in the bladder of the rats fed propoxur (Baygon)..."

"Although papillary and nodular hyperplasia frequently occurred together, it is noteworthy that there was a preponderance of nodular hyperplasia in the rats rather than papillary hyperplasia. I cannot provide an explanation for this, but it should be noted that this is somewhat unusual for chemicals affecting rats whereas it is much more common in mice..."

Historical Control Data for Uterine Carcinomas

Historical Control Data for Uterine Carcinomas in 2-Year Feeding Studies. Reference: Karbe, E.: BOQ 5812315, Two Year Feeding Study in Rats Supplement Title: Historical Data on Uterine Carcinomas of Control Rats in Two-Year Feeding Studies. Original Report: August 20, 1984; Supplement: December 15, 1992. MRID No. 426154-04.

The registrant has submitted control uterine carcinoma incidence data from 32 studies (12 published, 20 unpublished) at the Bayer Research Center in Wuppertal, Germany. In 6 of these studies the incidences were relatively high, ranging from 14.4 to 20.0% (as compared to 8/48, or 16.7%, in the 5000 ppm females in the 1984 rat study).

From the way the unpublished data are presented, what was probably the same tumor type was classified as an adenocarcinoma in 16 of the studies, and as a carcinoma in 4. In the publication by Bomhard et al. the tumor type is designated as a carcinoma/adenocarcinoma.

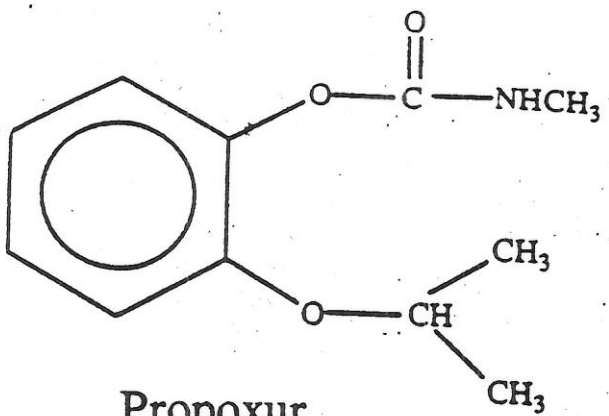
Other studies

The other studies submitted by the registrant relate largely to urinary pH associated with different diets in the rat (and mouse), as well as effects on rat urinary pH associated with addition of ammonium chloride to the diet. The position of the registrant is that the urinary bladder effects (hyperplasia, papillomas, carcinomas) observed in the rat result not only from the presence of Baygon (and/or its metabolites) but from an alkaline urinary pH.

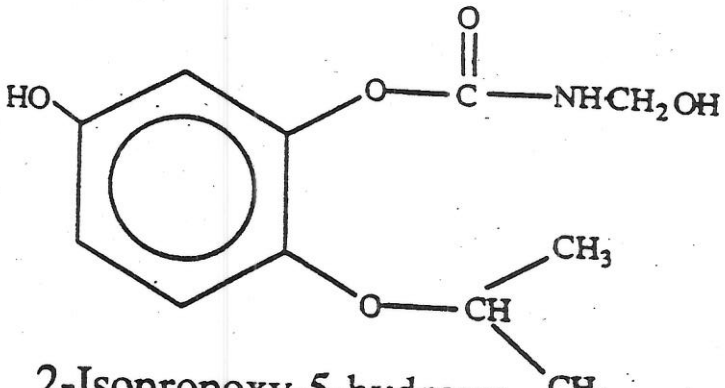
The position of the registrant is that the urinary bladder effects (hyperplasia, papillomas, carcinomas) observed in the rat result not from the presence of Baygon (and/or its metabolites) but also from an alkaline urinary pH. The registrant's argument is that the tumors are species-specific to the rat, develop by way of an epigenetic mechanism, and as a result, the occurrence of urinary bladder tumors in rats is not relevant to any human hazard.

E. Additional Toxicology Data on Baygon

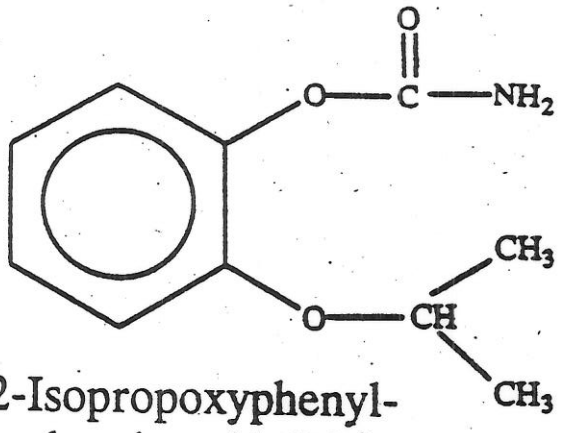
1. Metabolism: The following are structures of the parent compound, some of the more prevalent degradation products, and some (particularly 1-Hydroxy-2-isopropoxy-4-nitrobenzene or M 9A) of the more interesting metabolites observed in rat, monkey, and human urine. M S3 (2-Isopropoxy-5-hydroxy phenyl hydroxymethyl carbamate) has been observed only in rat and mouse urine. 2-Isoproxy-3-hydroxyphenyl methylcarbamate and 1,3-Dihydroxy-2-iso propoxybenzene have been observed only in rat, mouse and hamster urine. 2-Isopropoxy-5-hydroxyphenyl carbamic acid (M6 CII has been only observed in rat urine. The other metabolites have been detected in all five species.



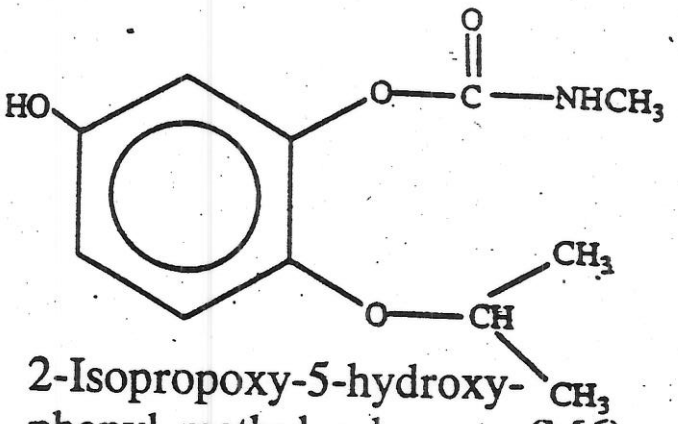
Propoxur



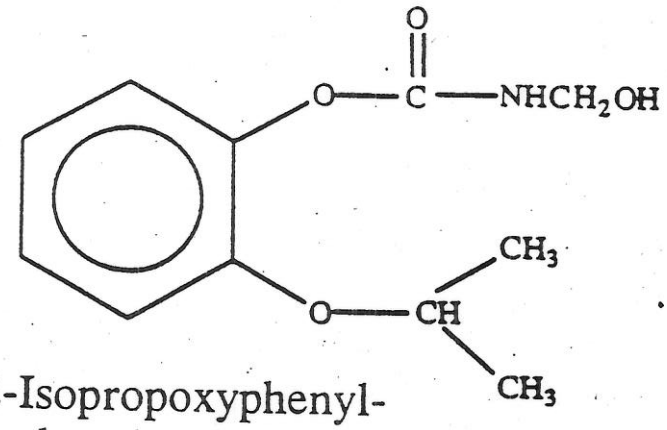
2-Isopropoxy-5-hydroxy-phenyl-hydroxymethyl-carbamate (M S3)



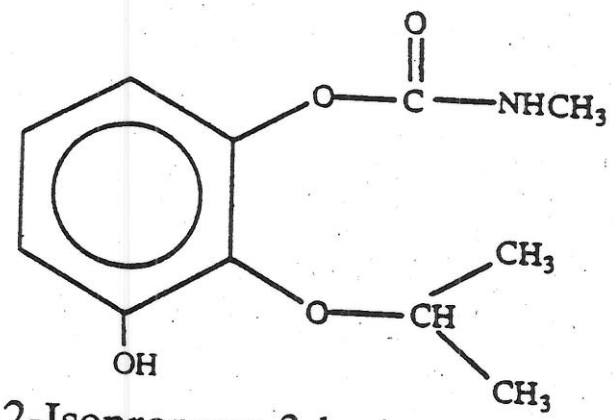
2-Isopropoxyphenyl-carbamic acid (M4)



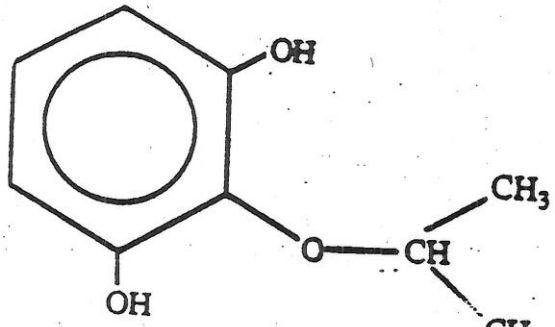
2-Isopropoxy-5-hydroxy-phenyl-methylcarbamate (M6)



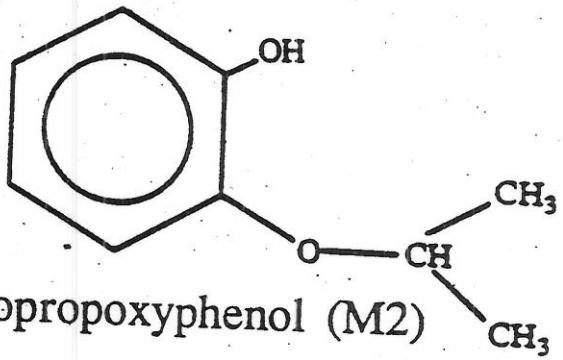
2-Isopropoxyphenyl-hydroxymethylcarbamate (M5)



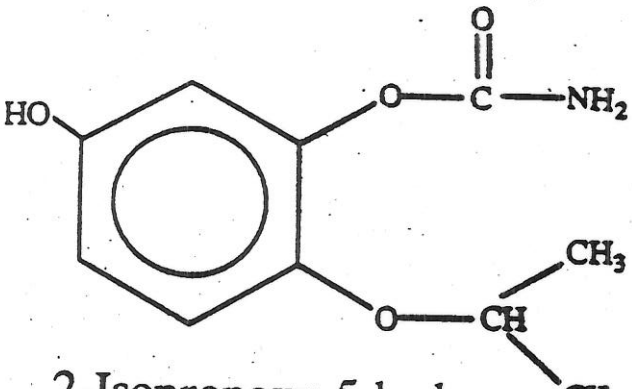
2-Isopropoxy-3-hydroxy-phenyl-methylcarbamate



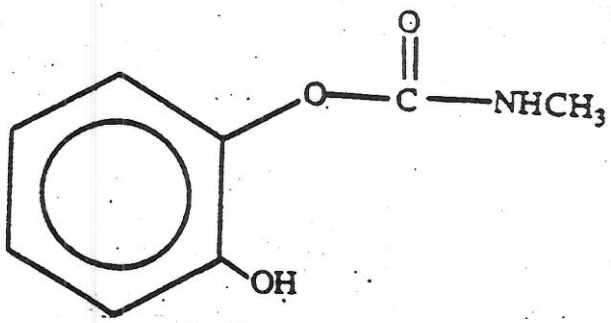
1,3-Dihydroxy-2-iso-propoxy-benzene (pyrogallol derivative)



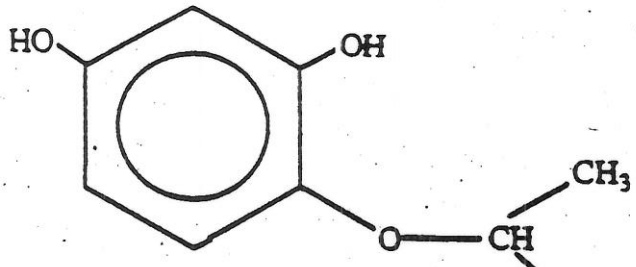
2-Isopropoxyphenol (M2)



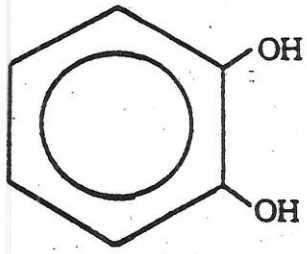
2-Isopropoxy-5-hydroxy-phenyl-carbamic acid (M6 CII)



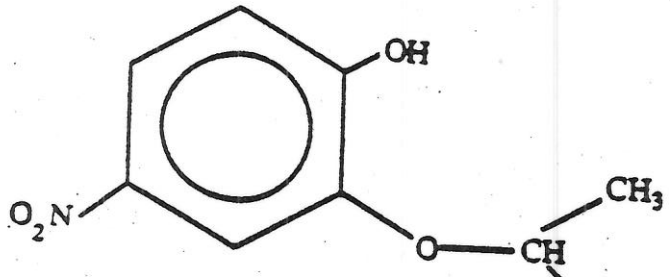
2-Hydroxyphenyl-methylcarbamate (M3)



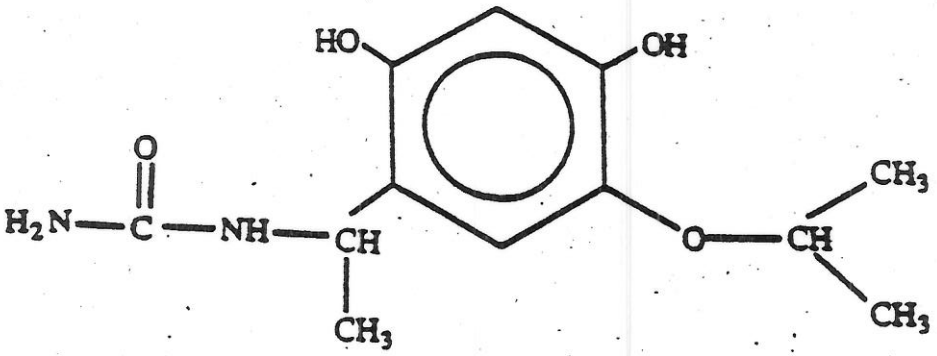
1,5-Dihydroxy-2-iso-propoxy-benzene hydroxyhydroquinone derivative



1,2-Dihydroxybenzene (Catechol) (M1)



1-Hydroxy-2-isopropoxy-4-nitrobenzene (M 9A metabolite)



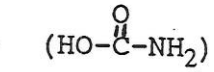
1,5-Dihydroxy-2-isopropoxy-(α-methyl)-benzylurea (M 12)

2. Mutagenicity: Refer to the attached memorandum dated 5/24/91 from Kerry L. Dearfield for an overview of Baygon's mutagenicity data. In a subsequent review of a reevaluation (conducted by Microbiological Associates) of microscope slides from the urinary bladder unscheduled DNA synthesis (UDS) study there was no indication (from the mean net nuclear grain counts) of an increase in UDS. However, in the second assay from the study, concurrent positive controls (urinary bladder cells from rats which had been dosed with 100 mg MMS/kg for 7 consecutive days) showed only a slight increase in mean net nuclear grain count (2.3 vs. a concurrent control value of 0.0). The first assay from this study did not include a concurrent appropriate positive control.

It is noted that the registrant has not submitted any of the CPRC's previously suggested additional studies to clarify Baygon's genotoxic potential, including a repeated-dose study in female rats with the following endpoints: in vivo UDS and S-phase analysis with bladder epithelial cells, including careful dose-response and time-action work. However, the registrant has apparently committed to perform these additional studies and indicated that they have worked on them. The registrant did some initial work on these additional studies, but there is no indication that these studies were completed.

3. Structure Activity Relationships:

Baygon is structurally related to nine other carbamate insecticides possessing the carbamic acid moiety:



As an N-methyl carbamate, it is expected to behave in a biologically similar manner to the other structurally related carbamates. The limited information provided by the one-liners indicates the following carcinogenic and mutagenic activities for these carbamates.

Aldicarb - No carcinogenic potential in mice or rats. Some mutagenic (clastogenic) activity reported in the literature.

Bendiocarb - No carcinogenic potential in mice or rats. Positive for genotoxicity; increased chromosomal aberrations with activation, negative without S-9 activation.

Carbaryl - Carbaryl was found to induce tumors (including carcinomas and papillomas of the urinary bladder) at multiple sites in both mice and rats at doses considered to be excessively toxic for carcinogenicity testing. Only hemangiosarcomas (malignant vascular tumors) in CD-1 male mice occurred at a dose which was considered sufficient and not excessive. Mutagenic (clastogenic) activity observed both with and without activation in Chinese hamster cell lines.

Carbofuran - No carcinogenic potential in mice or rats. Positive for genotoxicity in two separate Ames assays using Salmonella strain TA 1535 without activation. In two other Ames assays, a positive response was induced in strain TA 100 with and without activation, and in strains TA 98 and TA 100 with activation. In the mouse lymphoma assay a positive response was induced with and without activation.

Cloethocarb - No carcinogenic potential in mice or rats. Not mutagenic in the assays used.

Methiocarb - No carcinogenic potential in rats; mouse data not available. Not mutagenic in the assays used.

Methomyl - No carcinogenic potential in rats; mouse data not available. Not mutagenic in the assays used.

Mexacarbate - No carcinogenic potential in mice or rats. Positive in Chinese hamster ovary cells with activation.

Trimethacarb - No carcinogenic potential in mice or rats. Positive for genotoxicity, with increase in chromosomal aberrations with and without activation.

The only other carbamate causing urinary bladder tumors in rats is carbaryl, however, as noted in the carbaryl Carcinogenicity Peer Review document of May 12, 1994: "The CPRC...concluded that none of these analogues provided good structure activity relationship (SAR) support for the tumor response seen with carbaryl. Baygon was discussed as a possible analogue for SAR support as it induced urinary bladder tumors (same target as seen at the excessive dose in the carbaryl rat study). However, Baygon was not considered relevant in that there is little evidence that Baygon has genotoxic activity and the induction of urinary bladder tumors appears to have a different mechanism than that considered for carbaryl."

While this is what was stated in the Carbaryl Peer Review document, the genotoxicity of Baygon has not been completely addressed, pending submission of the registrant's additional studies.

F. Weight of the Evidence Considerations

The Committee considered the following observations regarding the toxicology of Baygon for a weight-of-the-evidence determination on its carcinogenic potential:

1. In a 1984 2-year chronic feeding study with Baygon in Wistar rats, there were statistically significant increases in bladder carcinomas (rare), papillomas and combined carcinoma/papilloma in both sexes of the Wistar rat, with positive statistically significant trends and early onset for the tumors, as well as increased incidences of carcinomas of the uterus, which also had an early dose-related onset.

2. In a subsequent (1988) 2-year dietary chronic study with Baygon using female Wistar rats there were again increases in urinary bladder carcinomas and papillomas. The CPRC concluded that the results of this study confirmed the association of Baygon with bladder tumors.

3. In a 1992 B6C3F1 mouse carcinogenicity study conducted at Bayer, with dietary exposure levels of 0, 500, 2000 and 8000 ppm over a 2-year period, there was a statistically significant increased incidence and a statistically significant positive trend for hepatocellular adenomas (but not carcinomas) in males. The dose-related increased incidence in liver adenomas in males was considered by the CPRC as additional evidence (tumorigenic activity in a second species) for a Group B2 classification of Baygon.

4. The CPRC noted that the additional "special studies" submitted by the registrant did not fully address the requests (including those for additional mutagenicity data) expressed in the previous review, particularly as the specific mechanism by which the rat urinary bladder tumors develop was not elucidated. The lack of silica crystalline deposits in the rat bladders indicates that the mechanism differs from that involved with saccharin.

5. In addition, although additional uterine histopathology data and historical control data for rat uterine tumors were submitted, the CPRC was not convinced of the registrant's position that there was no association of uterine tumors with the administration of Baygon.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that Baygon should remain classified as a Group B2 carcinogen.

The B2 classification was based on statistically significant increases in bladder carcinomas (rare), papillomas and combined carcinoma/papilloma in both sexes of the Wistar rat, with positive statistically significant trends and early onset for the tumors, as well as increased incidences of carcinomas of the uterus, which also had an early dose-related onset. The additional "special studies" submitted by the registrant did not provide evidence to change the classification of Baygon. However, the new mouse study, which demonstrated that Baygon was associated with statistically significant increases in hepatocellular adenomas and combined adenoma/carcinoma, with statistically significant positive trends in the male B6C3F1 mouse, added to the weight-of-evidence for Baygon. Therefore the CPRC unanimously agreed that the classification of Baygon remains as Group B2 - probable human carcinogen, with the low dose extrapolation model applied to the animal data for the quantification of human risk (Q_1^*).

H. Induces Cancer Call -- Baygon

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to Baygon resulted in an increased incidence of malignant carcinomas and papillomas in the bladder in both the male and female Wistar rat. Bladder carcinomas are relatively rare, and had an early onset in this study. In addition, there was an increase in uterine carcinoma in female rats, which also had an early onset. In the B6C3F1 mouse Baygon administration resulted in an increased incidence of hepatocellular adenoma and combined adenoma/carcinoma in males.

The Committee concludes that based on this evidence, Baygon induces cancer in animals.

ATTACHMENT

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.