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WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
7AND TOXIC SUBSTANCES

May 5, 2000

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MEMORANDUM

SUBJECT: Nitrapyrin (second Review) - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

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The Cancer Assessment Review Committee met on March 8, 2000 to evaluate the carcinogenic potential of Nitrapyrin. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

014131

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

NITRAPYRIN (SECOND REVIEW)

P.C. CODE: 069203

FINAL REPORT

5-MAY-2000

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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DATA PRESENTATION: Guruva B. Reddy, Toxicologist

DOCUMENT PREPARATION: Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam	
Marion Copley	
Kerry Dearfield	
Virginia Dobozy	
Yiannakis Ioannou	
Nancy McCarroll	
Esther Rinde	
Joycelyn Stewart	
Clark Swentzel	
Linda Taylor	
Yin-Tak Woo	See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John Pletcher	Pathologist	
Lori Brunsman	Statistician	

Nitrapyrin (Second Review) Cancer Assessment Document Final Report

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Guruva B. Reddy, Toxicologist

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Yin-Tak Woo Yin tak Woo

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EXECUTIVE SUMMARY

Previously, the Cancer Peer Review Committee (CPRC, 1992) classified nitrapyrin into category Group D- not classifiable as to human carcinogenicity. This decision was based on a statistically significant increase in renal tubular adenomas and adenocarcinomas in male rats. The Committee concluded that the renal tumors induced by alpha-2u-globulin in male rats were not relevant to assess the cancer risk in humans. No apparent increase in tumors was noted in female rats. The Committee also determined that the dosing in the mouse study was not adequate for assessing the carcinogenic potential and therefore, recommended that a new 2-year chronic/carcinogenicity feeding study in mice be performed at higher dose levels.

On March 8, 2000, the Cancer Assessment Review Committee (CARC) met to evaluate the newly completed mouse chronic/carcinogenicity study. In this study, Charles River $B_6C_3F_1$ mice (50/sex/dose) received nitrapyrin at dietary levels of 0, 125, or 250 mg/kg/day for 24 months. The CARC concluded that nitrapyrin was carcinogenic to male and female mice because: 1) There was a significant increase by pair-wise comparisons of the 125 (females) and 250 mg/kg/day (males and females) groups with the controls for hepatocellular adenomas and combined adenomas/carcinomas; 2) The incidence of hepatocellular adenomas was outside the range of the historical controls and the combined incidence was driven by the adenomas; 3) There was also a significant increasing trend for hepatocellular adenomas and combined adenomas/carcinomas; and 4) There was a significant increase by pair-wise comparisons of the 125 and 250 mg/kg/day (in both sexes) groups for nonglandular mucosa squamous cell papillomas and combined papillomas/carcinomas of the stomach. A significant increase in carcinomas was also noted in males at 250 mg/kg/day. There was also a significant increasing trend for stomach papillomas, carcinomas and combined papillomas/carcinomas. The stomach tumors were considered by the CARC as rare tumors. The incidences of these tumors were outside the historical control range.

In addition, males at 250 mg/kg/day had a significant increase in malignant epididymal sarcomas. A significant increase in Harderian gland adenomas was noted in females from 125 and 250 mg/kg/day dose groups. A dose-related increasing trend was evident for epididymal sarcomas and Harderian gland adenomas and their incidences exceeded that of historical controls. The CARC determined that the epididymal tumors were treatment-related and the evidence for Harderian gland adenomas was supportive of the carcinogenic potential in mice.

The dosing at the highest dose in both sexes was considered to be adequate and not excessive, based on increased mortality in high-dose males during the later part of the study period, decreases in body weight gains and non-neoplastic changes in the liver and stomach (both sexes). The CARC considered the liver and stomach tumors in males and females, epididymal tumors in males, and Harderian gland tumors in females to be treatment-related.

Under the Draft Guidelines for Carcinogen Risk Assessment (July, 1999), nitrapyrin is classified as **"Likely to be carcinogenic in humans."** The CARC's decision was based on the following:

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1. Tumors at multiple sites were induced in both sexes of mice.

2. Nitrapyrin was not mutagenic in submitted studies; however, NTP reported that the compound was mutagenic in *Salmonella typhimurium* assay in the presence of S9 activation.

3 Although the mechanistic data are inadequate, the available mutagenicity data are supportive of a mutagenic mode of action.

The Committee recommended a linear low-dose (Q_1*) extrapolation approach for the quantification of human cancer risk based on the most potent liver tumors in female mice. This approach is supported by the lack of adequate data on the mode of action and a mutagenic concern for nitrapyrin.

I. INTRODUCTION

Nitrapyrin was initially reviewed by the Cancer Peer Review Committee (CPRC) on May 6, 1992. At this meeting, a combined chronic/carcinogenicity feeding study in F344 rats and a carcinogenicity study in $B_6C_3F_1$ mice were evaluated. The rat study was acceptable and served as the basis for a Group D classification. There was a statistically significant increase in renal tubule adenomas and adenocarcinomas at 60 mg/kg/day (adenomas: 6% vs 0% in controls, carcinomas: 6% vs 0% in controls and combined: 12%, $p<0.05$ vs 0% in controls) in male rats. A statistically significant increasing trend ($p<0.01$) was also noted for adenomas, carcinomas and adenomas/carcinomas combined. The CPRC determined that the carcinogenicity study in mice was unacceptable because the MTD was not achieved.

II. EVALUATION OF CARCINOGENICITY AND OTHER EVIDENCE

The rat data are discussed in the CPRC document on nitrapyrin (HED Doc# 009760) and are not reiterated here.

On March 8, 2000, the CARC met to evaluate the newly submitted acceptable mouse study and reconsider the cancer classification for nitrapyrin. The study was submitted in response to CPRC's recommendation to fulfill the guideline requirement. The mouse data were evaluated by the Committee and are discussed below.

1. Carcinogenicity Study with Nitrapyrin in $B_6C_3F_1$ Mice

A. Experimental Design

In a 2-year dietary study, Charles River $B_6C_3F_1$ mice (50/sex/dose) received nitrapyrin at dietary levels of 0, 125, or 250 mg/kg/day for 24 months. An additional 10 mice per sex per dose were designated for interim sacrifice at 12 months.

B. Discussion of Tumor Data and Comparison with Historical Control Data

Male mice had significant differences in the pair-wise comparisons of the 250 mg/kg/day dose group with the controls for hepatocellular adenomas (45/98, 94% vs 12/49, 24% in controls), and adenomas/carcinomas combined (46/49, 94% vs 17/49, 35% in controls), both at $p < 0.01$. A significant ($p< 0.01$) increasing trend for these tumors was also evident. Although the combined incidence was driven by the incidence of adenomas, it had a malignant component (carcinomas: 12/49, 24% vs 7/49, 14% in controls). The incidence of adenomas at 250 mg/kg/day was outside the range for the historical control (14%-54%).

There were also significant differences in the pair-wise comparisons of the 125 and 250 mg/kg/day dose groups with the controls, for stomach nonglandular mucosa squamous cell papillomas (9/49,

18% and 12/36, 33%, respectively, vs 1/43, 2% in controls), and papillomas/carcinomas combined (9/49, 18% and 15/38, 39%, respectively, vs 1/43, 2% in controls), both at $p < 0.01$. A significant difference in the pair-wise comparison of the 250 mg/kg/day dose group with the controls, was also seen for stomach nonglandular mucosa squamous cell carcinomas (3/38, 8% vs 0/43, 0% in controls), at $p < 0.05$. The incidences of papillomas at 125 and 250 mg/kg/day and carcinomas at 250 mg/kg/day were outside the historical controls (0%). A significant ($p < 0.01$ or < 0.05) increasing trend was also evident for papillomas, carcinomas and papillomas/carcinomas combined. The Committee noted that these were all rare tumors.

A significant difference in the pair-wise comparison of the 250 mg/kg/day dose group with the controls at $p < 0.05$ was noted for testicular undifferentiated epididymal sarcomas (4/33, 12% vs 0/40, 0% in controls). An increased incidence of epididymal sarcomas at 125 mg/kg/day (2/48, 4% vs 0/40, 0% in controls) was considered biologically significant. These again are rare tumors. Although there was a high mortality at 250 mg/kg/day, the tumors appeared late during the study period. The incidence of these tumors was outside the historical control (0%) and a dose-related significant ($p < 0.01$) increasing trend was noted.

Female mice had significant differences in the pair-wise comparisons of the 125 and 250 mg/kg/day dose groups with the controls for hepatocellular adenomas (27/48, 56% and 32/48, 67%, respectively vs 6/47, 13% in controls), and adenomas/carcinomas combined (28/48, 58% and 33/48, 69%, respectively, vs 6/47, 13% in controls), at $p < 0.01$. The combined incidence was driven by the incidence of adenomas. The incidences of adenomas at 125 and 250 mg/kg/day were outside the range for the historical controls (4%-26%). A significant ($p < 0.01$) increasing trend was also evident for these tumors.

In females, there were significant differences in the pair-wise comparisons of the 125 and 250 mg/kg/day dose group with the controls for stomach nonglandular mucosa squamous cell papillomas (8/48, 17%, $p<0.05$ and 21/48, 44%, $p<0.01$, respectively, vs 1/47, 2% in controls), and papillomas/ carcinomas combined (8/48, 17%, $p<0.05$ and 22/48, 46%, $p<0.01$, respectively, vs 1/47, 2% in controls). The incidence of papillomas at 125 and 250 mg/kg/day was outside the historical control range (0%-1%). A dose-related significant ($p < 0.01$) increasing trend was also noted for papillomas and papillomas/carcinomas combined. An increase in carcinomas at 250 mg/kg/day (2/48, 4% vs 0/47, 0% in controls) was considered biologically significant and was outside the historical controls (0%).

In females, there were significant differences in the pair-wise comparisons of the 125 and the 250 mg/kg/day dose groups (at $p < 0.05$ and <0.01, respectively), with controls for Harderian gland adenomas (8/48, 17% and 9/48, 19%, respectively, vs 1/47, 2% in controls). In addition, a dose-related significant ($p < 0.05$) increasing trend was noted. The incidence of these tumors was above the concurrent controls and was more than double the historical control mean (3.5%) of the Dow Laboratory. The Harderian gland tumors lacked a malignant component and compared to the incidence of hepatocellular adenomas the incidence of Harderian gland adenomas was low and,

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therefore, the CARC concluded that the evidence for these tumors, although not as strong as the other tumors (lack of carcinomas and lower incidence than liver tumors), was supportive of carcinogenic potential of nitrapyrin in mice.

The statistical analyses of the male mice were based upon Peto's prevalence test since there was a statistically significant increasing trend for mortality with increasing doses of nitrapyrin. The statistical analyses of the female mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. Refer to Tables 1 through 6 for the results of tumor analysis.

Table 1. Nitrapyrin (N-Serve™) - $B_6C_3F_1$ Mouse Study
Male Liver Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)		
	0	125	250
Adenomas (%) p =	12/49 (24) 0.000**	19/50 (38) 0.107	45[a]/48 (94) 0.000**
Carcinomas (%) `p =	a7/49 (14) 0.176	3/50 (6) n.a.	12[b]/49 (24) 0.188
Combined (%) p =	17[c]/49 (35) 0.000**	20[c]/50 (40) 0.244	46[d]/49 (94) 0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First adenoma, not in an interim sacrifice animal, observed at week 75, dose 250 mg/kg/day.
[b]First carcinoma observed at week 72, dose 250 mg/kg/day.
[c]Two animals in each of the 0 and 125 mg/kg/day dose groups had both an adenoma and a carcinoma.
[d]Eleven animals in the 250 mg/kg/day dose group had both an adenoma and a carcinoma.
Note: There were 2 animals in the control group, and 3 animals in the 250 mg/kg/day dose group, of the interim sacrifice group with liver adenomas. There were no liver carcinomas observed in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level. If*, then $p < 0.05$. If **, then $p < 0.01$.

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Table 2. Nitrapyrin (N-Serve™) - $B_6C_3F_1$ Mouse Study

Male Stomach Nonglandular Mucosa Squamous Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)		
	0	125	250
Papillomas (%)	1/43 (2)	9/49 (18)	12[a]/36 (33)
p =	0.000**	0.009**	0.000**
Carcinomas (%)	0/43 (0)	0/49 (0)	3[b]/38 (8)
p =	0.021*	n.a.	0.042*
Combined (%)	1/43 (2)	9/49 (18)	15/38 (39)
p =	0.000**	0.009**	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First papilloma observed at week 105 in an animal that died on study, dose 250 mg/kg/day.
[b]First carcinoma observed at week 101, dose 250 mg/kg/day.

Note: There were no stomach nonglandular mucosa squamous cell papillomas or carcinomas observed in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Nitrapyrin (N-Serve™) - $B_6C_3F_1$ Mouse Study

Male Testes Undifferentiated Epididymal Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)		
	0	125	250
Sarcomas (%)	0/40 (0)	2[a]/48 (4)	4[a]/33 (12)
p =	0.0096**	0.097	0.012*

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

[a]First testicular undifferentiated epididymal sarcoma observed simultaneously in final sacrifice animals at week 105, doses 125 and 250 mg/kg/day.

Note: There were no testicular undifferentiated epididymal sarcomas observed in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Nitrapyrin (N-Serve™) - $B_6C_3F_1$ Mouse Study

Female Liver Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)		
	0	125	250
Adenomas (%)	6/47 (13)	27[a]/48 (56)	32/48 (67)
p =	0.000**	0.000**	0.000**
Carcinomas (%)	0/47 (0)	1[b]/48 (2)	2[b]/48 (4)
p =	0.150	0.505	0.253
Combined (%)	6/47 (13)	28/48 (58)	33[c]/48 (69)
p =	0.000**	0.000**	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 75, dose 125 mg/kg/day.
[b]First carcinomas observed simultaneously at week 105 in final sacrifice animals, doses 125 and 250 mg/kg/day.
[c]One animal in the 250 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: There were no liver adenomas or carcinomas observed in any interim sacrifice animals.

Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Nitrapyrin (N-Serve™) - $B_6C_3F_1$ Mouse Study

Female Stomach Nonglandular Mucosa Squamous Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)		
	0	125	250
Papillomas (%)	1/47 (2)	8[a]/48 (17)	21/48 (44)
p =	0.000**	0.017*	0.000**
Carcinomas (%)	0/47 (0)	0/48 (0)	2[b]/48 (4)
p =	0.111	1.000	0.253
Combined (%)	1/47 (2)	8/48 (17)	22[c]/48 (46)
p =	0.000**	0.017*	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First papilloma observed at week 81, dose 125 mg/kg/day.
[b]First carcinoma observed at week 105 in a final sacrifice animal, dose 250 mg/kg/day.
[c]One animal in the 250 mg/kg/day dose group had both a papilloma and a carcinoma.

Note: There were no stomach nonglandular mucosa squamous cell papillomas or carcinomas observed in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Nitrapyrin (N-Serve™) - $B_6C_3F_1$ Mouse Study

Female Harderian Gland Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)		
	0	125	250
Adenomas# (%)	1/47 (2)	8[a]/48 (17)	9[a]/48 (19)
p =	0.011*	0.017*	0.008**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 81, dose 125 mg/kg/day.

#No Harderian gland carcinomas were observed.

Note: There were no harderian gland adenomas observed in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Lesions and Other Findings:

Treatment-related increases in absolute and relative (to body weight) liver weights were seen in both sexes at both dose levels after 12 and 24 months of treatment; liver weights ranged from 111 to 129% of control values ($p < 0.05$) at 125 mg/kg/day and 131 to 201% ($p<0.05$) of control value at 250 mg/kg/day. Increased incidences of pale or dark liver foci were seen in the liver of low- and high-dose males and in high-dose females after 24 months. Microscopic examination of the liver showed that centrilobular or panlobular hepatocyte hypertrophy occurred in all treated mice at 12 months of treatment at both doses. Centrilobular or panlobular hepatocyte hypertrophy was also seen in ≥92% of all treated groups compared with 0% in controls after 24 months of treatment. The incidence of single-cell hepatocellular necrosis was increased after 24 months in treated males (96% at both doses vs 10% for controls, $p < 0.05$) and females (22 and 38%, respectively, vs 6% for controls, $p < 0.05$). At both doses, the incidence of mitotic figures increased 16 to 18% ($P < 0.05$) in males vs 0% in controls. Liver inflammation was observed in 88 to 90% ($p < 0.05$) of treated males compared to 10% in controls at 24 months. Other treatment-related liver effects included centrilobular multi focal pigment, cytoplasmic inclusions, and bile duct hyperplasia in high dose males; hepatocellular foci of altered cells and hepatocellular vacuolation in both sexes at the high dose.

Hyperkeratosis and/or hyperplasia of the stomach occurred in 26 to 40% ($p<0.05$) of treated mice compared to 2-8% in controls. The duodenum was pale in ≥68% of both sexes of mice at both dose levels. Epithelial cell vacuolation and/or hyperplasia/hypertrophy was seen in the duodenum and jejunum of 54 to 96% ($p < 0.05$) of all treated males and in 11 to 78% ($p<0.05$) of all treated females compared with 0% in controls. Increased extramedullary hematopoiesis in the spleen was also observed in high-dose males (38% vs 12% in controls, $p < 0.05$).

After 12 months of treatment with nitrapyrin, liver cell proliferation in the centrilobular region was significantly increased in male mice at 125 and 250 mg/kg/day. The increases seen in the centrilobular region of the liver of females and the periportal region of both sexes did not achieve statistical significance. A special 2-week study (MRID 44231803) with higher doses showed an increased liver cell proliferation index in the centrilobular region at 200 and 400 mg/kg/day and in the periportal regions at 400 mg/kg/day in males; increases in the periportal region in females did not achieve statistical significance. No statistically significant differences were seen in liver cell apoptosis or in cell proliferation of the nonglandular stomach of treated mice compared to control animals.

The statistical evaluation of mortality (Bronfman, 2000) indicated a significant increasing trend with increasing doses of Nitrapyrin in male mice. Female mice showed no significant incremental changes in mortality with increasing doses of nitrapyrin.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dose selection for the 2-year study (0, 125 and 250 mg/kg/day) was based upon the results of a subchronic dietary study (MRID 44231802) in mice. Liver was identified as target organ; absolute and relative liver weights increases were associated with centrilobular to panlobular hepatocellular hypertrophy at or above 200 mg/kg/day in both sexes. At 400 mg/kg/day decreased body weight gain, change in the hematological parameters, increased alanine aminotranferase levels, increased absolute and relative liver weights, and hepatocellular hypertrophy and single cell necrosis of hepatocytes were observed in both sexes. Mice administered 600 to 800 mg/kg/day that did not survive until study termination, had decreased body weights, food consumption, enlarged livers and hepatocellular hypertrophy.

In the 2-year carcinogenicity study, the body weights of high dose males were decreased by 3 to 13% ($p < 0.05$) from day 19 to study termination, and overall body weight gains were decreased by 15 to 34% ($p < 0.05$). At 125 and 250 mg/kg/day, increased absolute and relative liver weights, and increased incidence of pale or dark liver foci, hepatocellular hypertrophy, single cell necrosis of hepatocytes, hyperkeratosis and/or hyperplasia of stomach in both sexes were observed. NOAEL for systemic toxicity was not established in this study. There was some discussion regarding the high-dose in males being excessive or not. There was a significant increase in mortality in this group but the increased death was noted towards the end of the study and in this case the study was of 24 month duration (instead of usual 18 months). In addition, since most of the tumors appeared late and the mortality was later in the study, sufficient number of male mice were considered at risk. The discussion was tempered somewhat by the fact that most of these tumors were present in the lower dose group. Based on these findings, the CARC concluded that the highest dose was adequate and not excessive to assess the carcinogenic potential of nitrapyrin in both sexes.

2. Carcinogenicity Study with 6-Picolinic acid in $B_6C_3F_1$ Mice

A. Experimental Design

In a 2-year dietary study (MRID 40339301), Charles River $B_6C_3F_1$ mice (50/sex/dose) received nitrapyrin at dietary levels of 0, 100, 300 or 900 mg/kg/day for 24 months. An additional 10 mice per sex per dose were designated for interim sacrifice at 12 months.

B. Discussion of Tumor Data and Comparison with Historical Control Data

There was an equivocal and nonsignificant ($p>0.05$) increase in hepatocellular carcinomas in high-dose females. No increase in hepatocellular tumors was noted in treated male mice. Under the conditions of the study 6-picolinic acid was considered not carcinogenic to male and female mice. The study was considered acceptable/guideline.

2. ADDITIONAL TOXICOLOGY DATA

A. Metabolism

In a metabolism study (MRID 44679301) groups of 10 male $B_6C_3F_1$ mice were given single oral doses (25 or 250 mg/kg) of nitrapyrin (Lot no. 1321, 99.9% purity [99+% radiochemical purity]). Additionally, two groups of three male mice were given either the low or high dose to assess acute toxicity and another group given no test material (controls). To assess interspecies variability in urinary metabolite profiles, two male F344 rats were given single oral doses of 60 mg/kg.

No overt signs of toxicity or histopathologic changes were observed. Dose confirmation indicated that the administered doses represented 95-103% of the nominal radioactivity and 102-106% of the nominal nitrapyrin. Mean total recovery of administered radioactivity (% of dose) was 99.38% and 100.82%, respectively, for the low and high dose groups. Total absorption over a 72-hour period, implied from urinary excretion/cage wash and tissue/carcass burden data was 76.89% and 82.76% for the low and high dose groups, respectively. Urinary excretion assessed over a 72-hour period accounted for 76.12% and 82.12%, respectively, of the low- and high-dose, most of which occurred within 36 hours of dosing. Fecal excretion over the 72-hour period accounted for 21.55% and 16.04%, respectively, of the low- and high-dose. Under the conditions of this study, neither absorption nor excretion of nitrapyrin appeared to be saturated. Tissue/carcass burden at 72 hours post dosing was minimal and accounted for only 0.77% (low dose) and 0.64% (high dose) of the administered radioactivity (1.8 and 9.7 μg eq./g, respectively for the low and high dose). At the doses tested, nitrapyrin did not exhibit potential for tissue accumulation following a single oral dose in mice.

The urinary metabolite profile in mice included four distinct peaks as determined by HPLC analysis: 6-chloropicolinic acid, a glycine conjugate of 6-chloropicolinic acid, a taurine conjugate of 6-chloropicolinic acid, and the parent compound. All four components were detected in the urine of high-dose mice but no parent compound was detected in the urine of the low-dose mice. This may be indicative of the high dose representing a near-threshold for saturation of metabolism. Over the 72-hour period, peak 3 (the glycine conjugate of 6-chloropicolinic acid) represented 70.6% of the administered dose in the low-dose group and 69.8% in the high-dose group. Urinary metabolite data from rats showed some qualitative and quantitative differences relative to mice. Peak 2 (6-chloropicolinic acid) in rat urine represented a greater portion of the administered dose (~7-8 fold) than detected in the urine of mice. Peak 1 (taurine conjugate of 6-chloropicolinic acid) and Peak 4 (parent compound) were not detected in the rat urine while Peak 3 (glycine conjugate of 6-chloropicolinic acid)

was detected at levels only slightly greater than that observed for mice. A proposed metabolic pathway for nitrapyrin in mice was provided by the study authors.

This metabolism study in mice is Acceptable (Non Guideline). Although it did not satisfy the requirements for a metabolism and Pharmacokinetics study (85-1), the study provided supplemental data in mice to augment Guideline 85-1 requirements. It was designed to determine the metabolism and disposition in the mouse following a high dose (tumor-producing) and low dose (non-tumorigenic) oral exposure to nitrapyrin.

B. Mutagenicity

The studies submitted by the registrant were discussed in the previous review (CPRC, 1992). These acceptable studies fulfill all three categories for both the pre- 1991 and new minimum initial mutagenicity testing requirements. The results of the submitted studies indicate that nitrapyrin is not mutagenic. The NTP, however, reported that nitrapyrin was mutagenic in the *Salmonella* assay with metabolic activation in strains TA 97, TA98 and TA100 (CPRC, 1992). The halogenated pyridines especially with the halogen at the 2-position and on a methyl substituent, have mutagenic activity (discussed fully in CPRC, 1992; also Dearfield et al., 1987; 1993). 6-Chloropicolinic acid, a major metabolite of nitrapyrin, possesses a similar structural alert which is suggestive of a mutagenic concern but there is no evidence of carcinogenicity in $B_6C_3F_1$ mice (MRID # 40339301; HED Doc. # 007622).

C. Structure-Activity Relationship:

Seven chlorinated pyridine analogues were reviewed in the previous cancer peer review (CPRC, 1992) that have shown mutagenic activity in various assays. 3-Chloromethyl-(but not 2-chloro methyl-) pyrimidine induced stomach carcinomas in mice and rats when administered orally.

E. Subchronic Toxicity

In a subchronic toxicity study (MRID 44253802), nitrapyrin (90.0 and 92.05% a.i.) was administered in the diet continuously to 10 male $B6C3F_1$ mice per group at dose levels of 0, 200, 300, 400, or 600 mg/kg/day and to 10 female $B6C3F_1$ female mice per group at dose levels of 0, 200, 400, 600, or 800 mg/kg/day for up to 95 or 96 days. Dietary concentrations were adjusted each week so as to deliver constant doses throughout the study.

No animals administered 600 or 800 mg/kg/day of the test material survived to study termination. One male and one female in the control groups died, but all animals

administered the test material at concentrations ≤400 mg/kg/day survived to study termination. No clinical signs of toxicity were observed in animals administered ≤400 mg/kg/day; clinical signs observed in the 600- and 800-mg/kg/day groups were associated with eminent death. Mice administered 600 or 800 mg/kg/day showed pronounced decreases in body weights, body weight gain, and food consumption prior to death. Male and female mice administered 400 mg/kg/day weighed up to 9% ($p<0.05$ or <0.01) less than the controls from day 54 to study termination. Body weight gain at 400 mg/kg/day was reduced by 43% for males and 25% for females over the first 75 days of the study and by 21 and 16% for males and females, respectively, over the entire study. There were no clear dose-related effects on food consumption or food utilization for groups that survived to study termination. No statistically significant effects were observed on body weights, body weight gain, food consumption, of food efficiency in mice administered 200 or 300 mg/kg/day of the test material.

Treatment-related effects were observed on hematologic and clinical chemistry parameters. Hemoglobin and hematocrit levels were decreased by 6 to 11% ($p<0.01$) in male and female mice at 400 mg/kg/day. Other statistically significant ($p<0.05$ or <0.01) findings in females at 400 mg/kg/day included 16% reduction in platelet counts, 199% increase in the white blood cell count, a concomitant 234% increase in lymphocyte count, and a 127% increase in the reticulocyte count. Treatment-related effects on clinical chemistry parameters included statistically significant increases in serum alanine aminotransferase levels at 300 mg/kg/day in males (181%) and at 400 mg/kg/day in both sexes (435% in males and 235% in females) and a decrease in fasting glucose (-30%) at 400 mg/kg/day in females.

Statistically significant changes ($p<0.05$ or <0.01) in absolute and relative (to terminal body weights and to brain weights) weights were noted for several organs. Absolute and relative kidney weights were decreased by 8 to 14%, testes weights by 12 to 18%, and brain weights by 9 to 10% at the 400-mg/kg/day dose level; these decreases were probably due to decreased body weights. Absolute and relative liver weights showed dose-related, statistically significant increases at all doses in both sexes; liver weight increases ranged from 125 to 129% at 200 mg/kg/day, 146 to 148% at 300 mg/kg/day (males only), and 158 to 189% at 400 mg/kg/day.

Treatment-related gross necropsy findings consisted of enlarged livers in seven male and six female mice administered 600 mg/kg/day. Treatment-related microscopic findings were observed in the liver of both sexes and ovary and uterus of females. The liver lesions consisted of centrilobular or panlobular hepatocellular hypertrophy in all mice administered 200 to 400 mg/kg/day, hepatocyte intracytoplasmic vacuoles (eosinophilic and clear) and single cell necrosis of hepatocytes in males administered 300 and 400 mg/kg/day and in females administered 400 mg/kg/day. Increased

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incidences of green or brown intracytoplasmic pigment in Kupffer cells and mixed inflammatory cell infiltrate of the liver were observed in males and females administered 400 mg/kg/day. In addition, hypoplasia/atrophy of the ovary and uterus occurred in all females administered 400 mg/kg/day. The degree, but not the incidence, of extramedullary hematopoiesis in the spleen was increased at 400 mg/kg/day in both sexes as compared with controls.

The lowest observed effect level (LOAEL) for male and female mice administered nitrapyrin is 200 mg/kg/day based on liver toxicity (increased liver weights and hepatocellular hypertrophy). A no-observed-effect level (NOAEL) cannot be determined, because toxicity occurred at the lowest dose tested.

This subchronic toxicity study is classified **acceptable (guideline)** and does satisfy the guideline requirement for a subchronic oral study (82-1b) in mice, although a NOAEL was not established.

F. Chronic Toxicity

Refer to page 9 for discussion on non-neoplastic changes in various organs in $B6C3F_1$ mice. In this study, after 12 months of treatment with nitrapyrin, liver cell proliferation in the centrilobular region was significantly increased in male mice at 125 and 250 mg/kg/day. The increases seen in the centrilobular region of the liver of females and the periportal region of both sexes did not achieve statistical significance.

The LOAEL for this study is 125 mg/kg/day, based on lesions in the liver (hepatocellular hypertrophy and single-cell necrosis) and digestive tract (hyperkeratosis and hyperplasia of the nonglandular stomach and epithelial cell vacuolation and hyperplasia/hypertrophy of the duodenum and jejunum in both sexes). The NOAEL was not determined.

G. Mechanistic Study

A special 2-week feeding study (MRID 44231801) was conducted to evaluate hepatocyte proliferation and apoptosis in $B_6C_3F_1$ mice. The animals received nitrapyrin at dietary doses of 0, 200 or 400 mg/kg/day. There was a significant increase in the liver cell proliferation index in the centrilobular region at the 200 and 400 mg/kg/day and in the periportal regions at 400 mg/kg/day in males. In females, similar increases were noted but these increases were not statistically significant. There were no statistically significant differences seen in liver cell apoptosis or in cell proliferation of the nonglandular stomach of treated mice compared to the controls (MRID 44231803). The CARC concluded that the available data were limited to determine the mode of action for nitrapyrin.

III. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity:

- **The CARC reaffirmed the CPRC's conclusion that nitrapyrin was carcinogenic in male F344/N rats.** There was a statistically significant increase (both pair-wise and trend) in renal tubular adenomas /adenocarcinomas. The Committee concluded, however, that these renal tumors were induced by alpha-2u-globulin and, therefore, were not relevant for human cancer risk assessment.

- **CARC concluded that nitrapyrin was carcinogenic in male and female $B_6C_3F_1$ mice. Male mice** had significant differences in the pair-wise comparisons of the 250 mg/kg/day dose group with the controls, for hepatocellular adenomas (45/98, 94%, $p<0.01$ vs 12/49, 24% in controls), and adenomas/carcinomas combined (46/49, 94%, $p<0.01$ vs 17/49, 35% in controls). A significant ($p< 0.01$) increasing trend for these tumors was also evident. The combined incidence was driven by the incidence of adenomas. The incidence of adenomas at 250 mg/kg/day was outside the range for the historical controls (14%-54%).

  There were also significant differences in the pair-wise comparisons of the 125 and 250 mg/kg/day dose groups with the controls, for stomach nonglandular mucosa squamous cell papillomas (9/49, 18% and 12/36, 33%, respectively, vs 1/43, 2% in controls), and papillomas/carcinomas combined (9/49, 18% and 15/38, 39%, respectively, vs 1/43, 2% in controls), both at $p < 0.01$. A significant difference in the pair-wise comparison of the 250 mg/kg/day dose group with the controls, was also seen for stomach nonglandular mucosa squamous cell carcinomas (3/38, 8% , $p<0.05$ vs 0/43, 0% in controls). The combined incidence was driven by the incidence of papillomas. The incidence of papillomas at 125 and 250 mg/kg/day was outside the historical controls (0%). A significant ($p < 0.01$ or < 0.05) increasing trend was also evident for papillomas, carcinomas and papillomas/carcinomas combined. The Committee noted that these are rare tumors.

  A significant difference in the pair-wise comparison of the 250 mg/kg/day dose group with the controls, was noted for testicular undifferentiated epididymal sarcomas (4/33, 12% , $p<0.05$ vs 0/40, 0%

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in controls). The increased incidence of these tumors at 125 mg/kg/day was considered biologically significant. The incidences of these tumors were outside the historical control (0%) and a dose-related significant ($p < 0.01$) increasing trend was noted. These again are rare tumors.

Female mice had significant differences in the pair-wise comparisons of the 125 and 250 mg/kg/day dose groups with the controls, for hepatocellular adenomas (27/48, 56% and 32/48, 67%, respectively, vs 6/47, 13% in controls), and adenomas/carcinomas combined (28/48, 58% and 33/48, 69%, respectively, vs 6/47, 13% in controls), at $p < 0.01$. The combined incidence was driven by the incidence of adenomas. The incidences of adenomas at 125 and 250 mg/kg/day were outside the range for the historical controls (4%-26%). A significant ($p < 0.01$) increasing trend was also evident for these tumors.

There were significant differences in the pair-wise comparisons of the 125 and 250 mg/kg/day dose groups with the controls, for stomach nonglandular mucosa squamous cell papillomas (8/48, 17%, $p<0.05$ and 21/48, 44%, $p<0.01$, respectively, vs 1/47, 2% in controls), and papillomas/ carcinomas combined (8/48, 17%, $p<0.05$ and 22/48, 46%, $p<0.01$, respectively, vs 1/47, 2% in controls). An increased incidence of carcinomas at 250 mg/kg/day (2/48, 4% vs 0/47, 0% in controls) was considered biologically significant and was outside the historical controls (0%). The combined incidence was driven by the incidence of papillomas. The incidences of papillomas at 125 and 250 mg/kg/day were outside the historical control range (0%-1%). A dose-related significant ($p < 0.01$) increasing trend was also noted for papillomas and papillomas/carcinomas combined.

In females, there were significant differences in the pair-wise comparisons of the 125 and the 250 mg/kg/day dose groups (at $p < 0.05$ and <0.01, respectively), with the controls for Harderian gland adenomas (8/48, 17% and 9/48, 19%, respectively, vs 1/47, 2% in controls). In addition, a dose-related significant ($p < 0.05$) increasing trend was noted. The incidences of these tumors were above the concurrent controls and more than twice that of historical control mean (3.5%) of the Dow Laboratory. The CARC concluded that the evidence for these tumors, although not robust as the other tumors, was supportive of carcinogenic potential of nitrapyrin in mice.

The CARC determined that the dosing at the highest dose was adequate and not excessive based on decreased overall body weight gain (15 to 34%) in high dose males, increased absolute and relative liver weights, and increased incidence of pale or dark liver foci, hepatocellular hypertrophy, single cell necrosis of hepatocytes, hyperkeratosis and/or hyperplasia of stomach in both sexes at 125 and 250 mg/kg/day. There was a significant increase in mortality among males in this dose group but the increased deaths occurred towards the end of the study and in this case the study was of 24 months duration instead of usual 18 months. Since most of the tumors appeared late and the mortality was later in the study, sufficient number of male mice were considered at risk. Many of these tumors were also present in the next lower dose group. **The CARC concluded that the newly submitted mouse study is acceptable.**

2. Mutagenicity

- The review of submitted mutagenicity studies indicate a non-mutagenic effect. However, NTP reported that nitrapyrin was mutagenic in the *Salmonella* assay with metabolic activation in strains TA97, TA98 and TA100. In addition, 6-chloropicolinic acid, a major metabolite of nitrapyrin, has a structural alert but is not carcinogenic $B_6C_3F_1$ mice.

3. Structure Activity Relationship

- Seven chlorinated pyridine structural analogs have been shown to be mutagenic and/or carcinogenic in mice and rats (CPRC, 1992).

4. Mode of Action

- Although the mechanistic data are inadequate, the mutagenicity data are supportive of a mutagenic mode of action.

IV. CLASSIFICATION OF CARCINOGENIC POTENTIAL

Under the Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the Committee classified nitrapyrin as **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence:

1. There was an increase (both pair-wise and trend) in **liver and stomach** tumors in $B_6C_3F_1$ male and female mice, **epididymal sarcomas** in male mice and **Harderian gland tumors** in female mice.

2. Nitrapyrin was not mutagenic in submitted studies; however, NTP reported that the compound was mutagenic in *Salmonella typhimurium* strains TA97, TA98 and TA100 in the presence of S9 activation.

3. Nitrapyrin is structurally related to chlorinated pyridines which are mutagenic and carcinogenic in mice and rats.

V. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose (Q_1*) extrapolation approach for the quantification of human cancer risk based on the most potent liver tumors in female mice. This approach is supported by the lack of adequate data on the mode of action and a mutagenic concern for nitrapyrin.

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