UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Nitrapyrin

Tox. Chem. No. 217
PC No. 069203

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Attached are Sections C, D, E, F and H for incorporation into the Peer Review Document on Nitrapyrin.

The issues of concern are benign and malignant renal tubule tumors occurring in male Fischer 344 rat and support for an α_{2u}-globulin mechanism of nephrotoxicity/renal tubule tumor formation.

(1)

TABLE OF CONTENTS

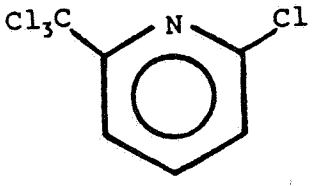
A. and B. <u>Not in this document</u> (to be prepared by the Peer Review Committee).

C. Background Information:

Nitrapyrin [2-chloro-6-(trichloromethyl) pyridine; trade name N-Serve] is a nitrogen stabilizer that acts to delay nitrification of ammoniacal and urea nitrogen fertilizer by selective bacteriostatic activity against <u>Nitrosomonas</u> <u>sp</u>. bacteria. It is a white crystalline solid with a molecular weight of 230.9 and melting point of 62-63°C. Nitrapyrin is relatively insoluble in water (0.004 g/100 g water) but is soluble in some organic solvents, including acetone (198 g/100 g), ethanol (30 g/100 g) and methylene chloride (185 g/100 g) and has a vapor pressure of 2.8 x 10^{-3} mm Hg at 23°C.

Registered uses for nitrapyrin include corn, cotton, sorghum, strawberries and wheat. Tolerances have been established for these commodities and for meat (cattle, goats, hogs, horses, poultry and sheep), based upon the combined residues of nitrapyrin and its major metabolite, 6-chloropicolinic acid. The registered products are approximately 90% technical active ingredient and are supplied as concentrates to be soil injected or incorporated during application at 0.25 to 1.0 pounds active ingredient/acre.

The chemical structure of nitrapyrin is:



The Caswell No. is 217, PC No. is 069203 and the CAS No. is 1929-82-4.

D. Evaluation of Carcinogenicity Evidence:

1. Fischer 344 Rat 2-Year Feeding/Carcinogenicity Study

Reference: Szabo, J.R., Landenberger, B.D. and Rachunek, R.L. (1989) Nitrapyrin (N-Serve): Two-Year Chronic Toxicity and Oncogenicity Study in Fischer 344 Rats: Dow Chemical Company, Health and Environmental Science, Study No. TXT:K-031304-023. MRID No. 413454-03.

a. <u>Experimental Design</u>

Fifty male and fifty female Fischer 344 rats per treatment group were administered nitrapyrin (technical grade, 93.3%) in their diet at concentrations giving doses of 0, 5, 20 and 60 mg/kg body weight/day for two years, beginning at age 4 weeks. An

additional ten males and ten females were included in the study for interim sacrifice at 12 months. Animals were examined daily for clinical signs of toxicity and mortality and received a thorough weekly examination by a veterinarian. Animals were weighed weekly for the first three months, then monthly for the duration of the study. Hematology, clinical chemistry and urinalysis parameters were assayed pretest and at 6, 12, 18 and 24 months. Organ weights, gross pathology and microscopic pathology were determined following death of moribund animals or scheduled sacrifice.

b. Discussion of Tumor Data

The incidence of neoplastic lesions observed in male rat kidney at terminal sacrifice and unscheduled sacrifice is shown below in Table 1:

TABLE 1: INCIDENCE OF NEOPLASTIC LESIONS IN MALE RAT KIDNEY, TERMINAL (24 MOS.) AND UNSCHEDULED SACRIFICE AFTER WEEK 53 (# ANIMALS)

	DOSE, MG/KG/DAY			
LESION	0	5	20	60
No. Animals	50	50	50	50
Renal Tubule:				
Adenoma	0**	0	0	3(6)[1]*
Adenocarcinoma	0**	0	0	3(6)*
Combined Incid.	0**	0	0	6(12)*

1 ( ) - Percent incidence

* $p < 0.05$; ** $p < 0.01$

Pair-wise comparison denoted at Dose level and trend denoted at Control; Fisher's Exact Test and Cochran-Armitage test, statistical analysis prepared by Bernice Fisher in Qualitative Risk assessment memo dated 4/1/92 (memo appended to this document)

A small but statistically significant increase and trend in the incidence of renal tubule neoplasms was observed in high dose males only. The incidence of renal tubule adenoma and adenocarcinoma in male rats at 24 months was 6% each (3/50; combined incidence 12%) compared to 0% in controls, low and mid dose males or females at any dose. No renal tumors were noted in either sex at 12 months. Other neoplasms that were observed in control and treatment groups but which were not treatment-related included hepatocellular adenoma/carcinoma, mammary adenoma/fibroadenoma/adenocarcinoma, pituitary adenoma and pheochromocytoma.

The incidence of renal adenoma and adenocarcinoma in high dose males exceeded historical control values (included in Attachment 1). Historical control data was provided by the registrant for renal histopathology including tumor incidence in Fischer 344 for studies conducted in the same laboratory between 1983 and 1991. Data for the individual experiments is appended to this document. Only three studies were available from the registrant. Incidences

of renal adenoma/carcinoma were 0% in all studies except for one, where 2% (1/50) of female rats developed adenocarcinoma by 2 years. The total number of animals examined was 300 for 2 year and unscheduled sacrifice and 60 for 1 year interim sacrifice.

c. Non-neoplastic Lesions

The primary target organs of nitrapyrin were kidney and liver. Histopathological findings at terminal and unscheduled sacrifice are presented below in Table 2.

TABLE 2: HISTOPATHOLOGICAL FINDINGS AT TERMINAL (24 MOS.) AND UNSCHEDULED SACRIFICE (# ANIMALS AFFECTED)

LESION	SEX	DOSE, MG/KG/DAY 0	5	20	60
No. Animals		50	50	50	50
Kidney:					
Chronic progressive glomerulonephropathy					
slight	M	33	33	35	7
	F	27	34	27	31
moderate	M	13	19	12	10
	F	4	4	5	12*
severe	M	2	4	2	33*
total	M	48	46	49	50
	F	34	41	35	45
Cyst	M	0	1	0	2
Liver:					
Hypertrophy, centrilobular,	M	0	0	2	30*
slight	F	2	2	1	15*
Vacuolization (fatty change),	M	1	0	3	28*
slight	F	2	2	1	19*
Thyroid:					
cystic follicles	M	3	3	2	15*

* $p < 0.05$

The severity of chronic progressive glomerulonephropathy increased significantly at high dose in males but not females. All males with renal tubule tumors also had moderate to severe chronic progressive glomerulonephropathy. High dose males also had an increase in cystic follicles of the thyroid, which the study authors attributed to compensation resulting from renal failure. Slight hepatic centrilobular hypertrophy and vacuolization, consistent with fatty change, were observed in males and females at

5

high dose.

Histopathology results for control and high dose males and females from the 1-year interim sacrifice group are shown below in Table 3:

TABLE 3: HISTOPATHOLOGIC LESIONS AT 12 MONTH INTERIM SACRIFICE (# ANIMALS AFFECTED)

		DOSE, MG/KG/DAY			
LESION	SEX	0	5	20	60
No. examined		10	10	10	10
Kidneys:					
Mineralization	M	0	0	0	9
	F	1	1	0	0
Tubule degeneration/ regeneration, slight	M	10	6	8	10
Dilated tubule, proteinaceous casts, very slight	M	2	9	9	0
	F	0	0	1	6
slight	M	7	0	1	9
Protein droplet nephropathy, slight	M	0	0	9	1
moderate	M	0	0	0	9
Liver:					
Hypertrophy, centrilobular, slight	M	0	0	0	6
	F	0	0	0	2
moderate	M	0	0	0	4
	F	0	0	0	8
Vacuolization (fatty change) very slight	M	0	0	5	0
slight	M	0	0	0	6
	F	0	0	0	2
moderate	M	0	0	0	4
	F	0	0	0	8

At high dose, increased incidences of mineralization of the loops of Henle and hyaline protein droplet accumulation in the P2 section of proximal tubules in males and slight increase of dilated tubules with proteinaceous casts in females were observed. Slight hyaline protein droplet nephropathy was also noted in mid dose males. The incidence of dilated tubules with proteinaceous casts increased only among low and mid dose males.

Slight-to-moderate diffuse hepatic centrilobular hypertrophy and vacuolization consistent with fatty change were observed in all high dose males and females. Some mid dose males also showed vacuolization classified as very slight.

Immunoperoxidase localization of α_{2u} globulin (α_{2u}-g), a male rat urinary protein, in hyaline droplets was performed on kidney from control and high dose males and females sacrificed at 12 months. Results for individual males are shown below in Table 4:

TABLE 4: IMMUNOPEROXIDASE STAIN FOR ALPHA$_{2u}$ GLOBULIN (MALES)

CONTROL		HIGH DOSE	
ANIMAL NO.	RESULTS	ANIMAL NO.	RESULTS
2778	+/-	2958	++++
2779	+/-	2959	++++
2780	+/-	2960	++++
2781	+/-	2961	+++
2782	+/-	2962	ND
2783	+/-	2963	+++
2784	+/-	2964	+++
2785	+/-	2965	+++
2786	+/-	2966	++++
2768	+/-	2967	+++

+/-	Slight, scattered response	+++	Moderate response
ND	Not determined	++++	Strong response

Control and high dose females showed scattered immunoperoxidase staining in a few proximal convoluted tubules similar to the pattern observed in control males. In contrast, all high dose males showed clearly positive staining in the P2 section of the proximal tubules.

d. Other Signs of Toxicity

Mean body weight/weight gain were statistically significantly decreased in high dose males and females and mid dose males. Cumulative body weight gains relative to initial weight for each group are presented below in Table 5. Percent weight gain relative to concurrent controls was calculated for mid and high dose animals (in parentheses):

TABLE 5: BODY WEIGHT GAIN RELATIVE TO DAY 0, GRAMS (% CHANGE FROM CONTROLS)

		NITRAPYRIN, MG/KG/DAY			
WEEK:		0	5	20	60
53	♂	300.1	298.8	295.6 (-0.7)[1]	295.5 (-0.4)
	♀	154.2	159.1	152.9 (-0.4)	146.2 (-5.1)*
69	♂	321.4	322.1	319.8 (+0.6)	313.0 (-1.1)*
	♀	179.8	186.7	172.5 (-4.3)	165.6 (-8.2)*
81	♂	314.6	313.3	305.3 (-2.3)	291.3 (-6.0)*
	♀	178.3	186.3	170.4 (-4.4)	161.1 (-9.9)*
93	♂	312.6	313.9	302.9 (-2.7)	275.0 (-11)*
	♀	185.1	188.6	181.5 (-2.1)	161.2 (-13)*
105	♂	297.5	288.2	270.7 (-8.4)*	237.6 (-19)*
	♀	184.0	190.3	174.7 (-5.3)	156.0 (-15)*

1 Value in parentheses = [(% body weight gain relative to initial weight, treatment - % body weight gain relative to initial weight, controls) ÷ % body weight gain relative to initial weight, controls] x 100

* $p < 0.05$

Mean body weights of males were comparable to controls during the first year of treatment, but were statistically significantly decreased from Day 438 to termination at high dose and for most measurements from Day 595 to termination at mid dose. In high dose females most mean body weight measurements from Day 49 to termination showed statistically significant decreases. Body weights of mid dose females were slightly decreased but no statistically significant differences were observed. Percent total body weight/body weight gain (relative to concurrent controls) at termination were: -15%/-19%, high dose males; -9%/-15%, high dose females and -8%/-8.4%, mid dose males.

Mean absolute and relative organ weights for liver and kidney are shown below in Table 6:

TABLE 6: ABSOLUTE AND RELATIVE ORGAN WEIGHTS (GM AND GM/100 GM BODY WT)

	NITRAPYRIN, MG/KG/DAY							
	0		5		20		60	
ANIMAL GROUP: ORGAN	♂	♀	♂	♀	♂	♀	♂	♀
1 YR:								
KIDNEY G	2.578	1.654	2.570	1.575	2.652	1.674	2.666	1.705*
G/100	0.648	0.694	0.638	0.686	0.655	0.731	0.691	0.763*
LIVER G	10.083	5.939	10.173	5.567	10.684	5.907	12.839*	6.699*
G/100	2.535	2.288	2.522	2.424	2.639	2.578	3.322*	2.994*
2 YR:								
KIDNEY G	2.949	2.165	2.969	2.140	3.068*	2.241	3.457*	2.391*
G/100	0.754	0.822	0.776	0.805	0.845*	0.878	1.039*	1.022*
LIVER G	11.246	7.930	10.994	7.913	12.296*	8.167	15.862*	11.579*
G/100	2.858	3.000	2.878	2.977	3.383*	3.199	4.760*	4.932*
ADRENALS G	0.064	0.065	0.063	0.066	0.093	0.064	0.108*	0.061
G/100	0.017	0.025	0.017	0.025	0.026	0.025	0.032*	0.026

* $p < 0.05$

Both absolute and relative liver weights were increased significantly at the high dose in males and females at 12 and 24 months. At 24 months absolute/relative liver weights were increased by 41%/67% (males) and 46%/64% (females) relative to controls. Absolute and relative kidney weights were increased significantly in males and females at 24 months but at 12 months, only relative kidney weight in females was elevated. Absolute/relative kidney weights were increased by 17%/38% (males) and 24%/25% (females). Absolute and relative adrenal weights were increased at 24 months in males only, probably due to renal failure resulting from chronic progressive glomerulonephropathy.

8

Small but statistically significant depressions (3% - 9%) of red blood cell parameters were noted in high dose males but not females at 6, 12 and 18 months. Platelets were also significantly decreased in males at 18 months (13%) and females at 24 months (23%).

Clinical chemistry values that showed possible treatment-related changes for males and females at 24 months are presented below in Table 7:

TABLE 7: CLINICAL CHEMISTRY VALUES, 24 MONTHS

NITRAPYRIN	0 MG/KG/DAY		5 MG/KG/DAY		20 MG/KG/DAY		60 MG/KG/DAY	
PARAMETER:	♂	♀	♂	♀	♂	♀	♂	♀
SGPT, U/L	31.8	44.8	31.5	50.1	41.7	50.8	39.0*	82.9*
BUN, MG/DL	17.1	14.3	14.0	13.9	19.9	14.9	36.8*	16.6*
CREAT KIN, U/L	423.8	233.8	150.0*	156.9*	245.4*	132.8*	266.7*	161.4*
TOT BILI, MG/DL	0.30	0.16	0.15*	0.13	0.16*	0.11	0.24	0.30*
CHOL, MG/DL	130.4	175.0	171.9	160.8	144.2	174.3	160.0	245.0*
ALB, G/DL	2.98	3.61	3.01	3.74	2.80	3.53	2.58	3.04*
TOT PROT, G/DL	6.21	6.59	6.25	6.50	5.94	6.43	5.73*	6.20
PHOS, MG/DL	4.5	4.3	4.5	4.4	4.8	4.6	6.4*	4.5

* $p < 0.05$

Damage to liver in high dose males and females was reflected in several clinical chemistry values to varying degrees. SGPT was elevated in males and females (23% and 85%), as was serum cholesterol (40% and 23%). Other parameters indicative of possible liver damage were slightly decreased albumin and total protein. High dose males (but not females) showed evidence of kidney damage as increased BUN (116%) and increased phosphorus (42%). Creatinine kinase was also decreased at all doses in both males and females.

Mortality in the two-year study is shown below in Table 8:

TABLE 8: PERCENT MORTALITY DURING STUDY (INCLUDING MORIBUND SACRIFICE, ANIMALS FOUND DEAD) AND SCHEDULED 2-YEAR SACRIFICE

DOSE	NO. ANIMALS	MALES	FEMALES
0	50	24%**	36%
5	50	20%	47%
20	50	24%*	32%
60	50	46%	38%

* $p < 0.05$; ** $p < 0.01$

Pair-wise comparison denoted at Dose level and significant trend at Controls; Cox or generalized K/W Test, statistical analysis prepared by Bernice Fisher in Qualitative Risk Assessment memo dated 4/1/92.

Mortality of high dose males showed a statistically significant increase and trend due primarily to the increased severity of chronic progressive glomerulonephrotoxicity during the second year of treatment. Most deaths occurred during the last 5-6 months of treatment. It did not appear that any deaths were due to the tumors themselves. Female mortality was higher than males at all doses except high dose and was largely due to Fischer rat leukemia.

e. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dose levels were chosen based on the results of two previous 90-day feeding studies in Fischer 344 rats. In a 1962 study by Greenhoe, rats were given doses of 0, 1.5, 5, 15, 50 and 150 mg/kg/day nitrapyrin in the diet. Rats fed 50 and 150 mg/kg/day nitrapyrin showed decreased weight gain, hepatocellular fatty change and necrosis, renal tubule epithelial cell swelling and increasingly severe interstitial nephritis. A 1986 study by Szabo et al. (MRID No. 00163217) gave similar results and also showed decreased hematocrit, hemoglobin and RBC and increased serum bilirubin at 120 mg/kg/day (dose levels 0, 10, 40 and 120 mg/kg/day).

The high dose of 60 mg/kg/day in the 2 year study produced definite signs of toxicity in males and females (toxicity was more pronounced in males), including decreased body weight gain, decreased absolute and relative kidney weight, clinical chemistry changes, liver histopathology and in males, kidney histopathology/chronic progressive glomerulonephropathy and increased mortality. The kidney effects in males may not be applicable as end points for toxicity (see Section H).

Based on the toxicity observed in the preliminary studies and the primary study, the dosing regimen was considered adequate (to excessive in males) to properly test carcinogenic potential of nitrapyrin.

2. B6C3F1 Mouse 2 Year Oncogenicity Study

Reference: Quast, J.F., Cosse, P.F. and Corley, R.A. (1990) Nitrapyrin (N-Serve): Two-Year Dietary Oncogenicity Study in B6C3F1 Mice. Testing Facility - Health and Environmental Sciences-Texas, Lake Jackson Research Center, The Dow Chemical Company, Freeport, TX; Study No. K-031304-027; MRID No. 416516-01.

a. Experimental Design

Nitrapyrin (technical grade, 93.3%) was administered in the diet at doses of 0, 5, 25 and 75 mg/kg/day. Fifty male and fifty female mice were assigned to each test group. Ten males and ten females per dose were assigned to the interim (12 months) sacrifice group. Mice were examined at least once daily for signs of toxicity and mortality. Body weights were taken weekly up to Week 13 and monthly thereafter. Food consumption was determined weekly. Hematology parameters were analyzed at 12 and 24 months and clinical chemistry was analyzed only in the interim sacrifice group just prior to sacrifice. Organ weights, gross pathology and histological pathology were determined upon necropsy. No urinalysis was performed.

b. Discussion of Tumor Data

No treatment-related neoplastic lesions were observed. The following liver and kidney neoplasms were observed in this study:

TABLE 9: NEOPLASTIC LESIONS OBSERVED AT NECROPSY[1]

		DOSE, MG/KG/DAY			
LESION	Sex	0	5	25	75
12 Mo. Sacrifice:					
No. Examined		10	10	10	10
Liver:					
HC[2] adenoma	M	0	1	1	1
	F	0	0	1	0
24 Mo. and Unsched. Sacrifice:					
No. Examined		50	50	50	50
Liver:					
HC adenoma	M	13	9	13	15
	F	9	5	6	13
HC carcinoma	M	4	4	4	2
	F	1	1	0	0
Kidney:					
Adenoma,cortex	M	0	0	1	1
Transit. cell carcin., prim.	F	0	0	1	0

1 Data taken from study Table 39 and not DER
2 HC - Hepatocellular

11

The single incidence of renal pelvis transitional cell carcinoma in mid dose females was not considered significant since the incidence was very low (2%), the tumor type was different than the tumors of concern in rats and no dose-response was observed. Single incidences of renal cortical adenoma in mid and high dose males were also not considered significant because of their low incidence (2%).

c. Non-neoplastic Lesions

Incidence data for non-neoplastic histopathological lesions is presented below in Table 10:

TABLE 10: NON-NEOPLASTIC HISTOPATHOLOGY, 12 MONTH AND 24 MONTH (AND UNSCHEDULED) SACRIFICES

		Dose Administered, mg/kg/day			
Lesion	Sex	0	5	25	75
12 Mo. Sacrifice					
No. examined		10	10	10	10
Duodenum, mucosa:					
altered staining	M	0	1	8	9
	F	0	0	2	9
Jejunum, mucosa:					
altered staining	M	0	0	0	2
Liver:					
altered cytopl.					
homogeneity	M	0	0	3	9
	F	1	0	0	3
24 Mo. and Unsched. Sacrifice					
No. Examined		50	50	50	50
Duodenum, mucosa:					
altered staining	M	1	0	27	41*
	F	0	0	9	32*
Liver:					
altered cytopl.					
homogeneity	M	8	4	11	43*

* $p < 0.05$

The following non-neoplastic lesions were observed: 1) duodenum of mid and high dose animals at 12 and 24 months appeared pale upon gross inspection and showed altered tinctorial properties of the mucosa, and 2) livers of high dose males showed increased incidence of altered cytoplasmic tinctorial properties with centrilobular distribution at 12 and 24 months. No treatment-related renal gross or histopathology was noted.

d. Other Signs of Toxicity

No treatment-related decreases in body weight or weight gain

were observed in males or females in the primary study except for sporadic incidences. Food consumption was also similar among all treatment groups. Kidney absolute/relative weights were increased at 24 months in high dose males by 5.5%/7% (statistically significant) and liver absolute/relative weights were increased by 11%/14% (not statistically significant). Liver absolute/relative weights in high dose females sacrificed at 24 months showed statistically significant increases of 7.8%/9.1% and absolute and relative kidney weights showed non-statistically significant increases of 5.9%/6.7%. Increases for both kidney and liver weights were also noted in high dose males and females at 12 months but were in part due to the lower terminal body weights observed in control animals.

Mortality among treated animals was comparable to controls. Among mid and high dose satellite males cholesterol was decreased (19% and 30%, respectively) and glucose was elevated (21% and 17%, respectively), respectively; SGPT was elevated (37%) only in high dose males. Elevated SGPT may have been related to the mild liver histopathology.

e. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dose selection for the 2-year study was based upon the results of a two-week dietary range finding study and a 13-week dietary study in mice. The two-week study showed no treatment-related effects at doses of 150 mg/kg/day or lower. Statistically significantly decreased body weights were observed at 400 and 600 mg/kg/day (highest does) in males and at 600 mg/kg/day in females. Liver and kidney were identified as target organs: absolute and relative liver weight increased at or above 200 mg/kg/day, absolute and relative kidney weights decreased in males treated with 200 and 400 mg/kg/day and in females treated with 400 and 600 mg/kg/day. At 400 and 600 mg/kg/day, hepatocellular hypertrophy, fatty change and cell necrosis were noted, and at 600 mg/kg/day, necrosis of the renal descending proximal tubule was observed in males only.

No treatment-related effects were observed in the 13-week study up to 45 mg/kg/day. At 90 mg/kg/day, absolute and relative liver weights increased in males and females, and microscopic hypertrophy of centrilobular and midzonal hepatocytes was observed. At 180 mg/kg/day (highest dose) these effects were more pronounced. No kidney effects were observed at any dose.

In the 2-year oncogenicity study, the highest dose tested (75 mg/kg/day) produced minimal toxicity in mice. The peer review committee is asked to decide whether the toxicity observed at the highest dose tested was adequate for assessing carcinogenicity of nitrapyrin in mice and if not, should the study be downgraded to supplementary and/or repeated.

E. Additional Toxicology Data on Nitrapyrin:

1. Metabolism

An acceptable metabolism study in Fischer 344 rat was submitted (MRID No. 403055-01). ^{14}C-nitrapyrin administered orally at doses of 1 and 60 mg/kg was completely metabolized and excreted primarily in the urine (80-85%) and to a lesser extent, the feces (11-14%). Two metabolites of nitrapyrin were identified in the urine: 6-chloropicolinic acid (6-CPA) and its glycine conjugate (6-CPA-gly). A slightly higher percentage of 6-CPA-gly was excreted from 0-12 hr than from 12-24 hr. Females excreted more 6-CPA-gly than males, particularly during the 0-12 hr period.

Nitrapyrin was not retained to any significant degree in any tissue: less than 1% of the radioactivity remained in total tissue after 72 hr. The bulk of radioactivity in tissues was found in the liver, with traces in kidney, lung, RBC and plasma. No significant sex differences for these parameters were observed and distribution was not affected by dose level or by repeated administration. Absorption half-life was lower at low dose than high (1.22 and 3.19 hrs, respectively). Pharmacokinetic data suggested a two-compartment model for compound distribution and excretion.

Nitrapyrin is also metabolized to 6-CPA in plants, although some parent compound remains. Since the plant and animal metabolite is the same, the current policy as outlined in the registration standard is to regulate using the parent compound. The parent compound is also of concern for applicator exposure risk. An RfD for nitrapyrin of 1.5×10^{-3} (safety factor = 1000) was originally determined based on studies using 6-CPA. The RfD for nitrapyrin was recently reevaluated using results from newer studies with nitrapyrin and determined to be 3×10^{-2} (safety factor = 100). The RfD committee will determine whether an individual or combined RfD for nitrapyrin and 6-CPA are most appropriate.

2. Mutagenicity

Acceptable studies for mutagenicity fulfill all three categories for mutagenicity testing (under old guidelines) and there are no data gaps. Nitrapyrin was tested for mutagenicity in the following assays:

a. Salmonella assay - No evidence of mutagenicity in presence or absence of S9 up to 500 ug/plate
b. Unscheduled DNA synthesis, male rat primary hepatocyte - No increase in unscheduled DNA synthesis up to 23 ug/ml (1×10^{-4}M)
c. Mouse micronucleus assay - No mutagenic potential up to 800 mg/kg.
d. CHO cell gene mutation, HGPRT locus - No evidence of

mutagenicity up to 100 ug/ml in the absence of S9 and 200 ug/ml in the presence of S9.

It should be noted that an NTP study reported that nitrapyrin was mutagenenic in the Ames test with metabolic activation in strains TA97, TA98, TA100 (Environmental and Molecular Mutagenesis 11 (Supplem. 12): 1, 1988).

3. Developmental and Reproductive Toxicity

a. Developmental Toxicity: Rabbit - Pregnant rabbits were administered 0, 3, 10 and 30 mg/kg/day nitrapyrin by gavage, administered on days 6-18 of gestation, inclusive (MRID No. 00153543). Developmental LEL for nitrapyrin in rabbits was 30 mg/kg/day, and consisted of increased incidence of crooked hyoid bones (19% fetuses; 57% litters at high dose vs 6% fetuses and 32% litters, control). These fetal and litter incidences exceeded historical controls (9.4%, fetal; 53%, litter) and the increase was statistically significant. A slight, dose-related increase in % implantations resorbed was not considered treatment-related since it was not statistically significant and was within the historical control range. No teratogenic effects were observed. Developmental NOEL was 10 mg/kg/day.

Maternal LEL was 30 mg/kg/day based upon decreased body weight gain and increased absolute/relative liver weight. NOEL was 10 mg/kg/day.

Rat - A developmental toxicity study in rat (MRID Nos. 00163792, 420501-01) was considered unacceptable because the highest dose level (60 mg/kg/day) was considered insufficient to challenge developmental toxicity.

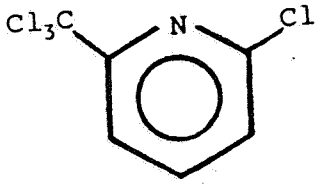
b. Reproductive Toxicity: Male and female rats were administered 0, 5, 20 and 75 mg/kg/day nitrapyrin for 10 weeks prior to mating (MRID No. 409527-01). Decreased pup weight and increased incidence of fetal liver histopathology (hypertrophy and vacuolization) were observed at 75 mg/kg/day. NOEL was 20 mg/kg/day. No effects on reproductive parameters were observed.

Maternal toxicity was observed at 20 mg/kg/day as increased absolute and relative kidney and liver weights in F_0 males. NOEL was 5 mg/kg/day.

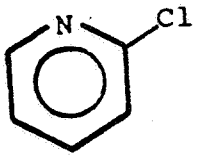
4. Structure-Activity Correlations

A computerized structure-activity search was performed for nitrapyrin (CIS and Chemline). Related structures with known mutagenic and/or carcinogenic activity are shown below in Figure 1.

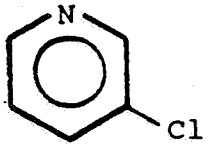
FIGURE 1: STRUCTURALLY RELATED COMPOUNDS



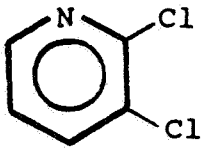
2-CHLORO-6-(TRICHLOROMETHYL)-PYRIDINE
CAS NO. 1929-82-4
M+/-; C-/+ (α_{2u}-g?)



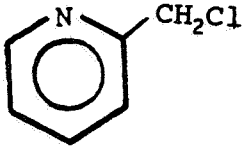
2-CHLOROPYRIDINE
CAS NO. 109-09-1
M+; C?



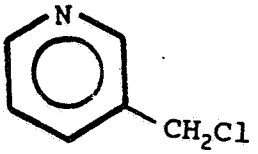
3-CHLOROPYRIDINE
CAS NO. 626-60-8
M+; C?



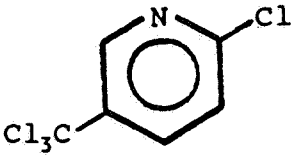
2,3-CHLOROPYRIDINE
CAS NO. 2402-77-9
M+; C?



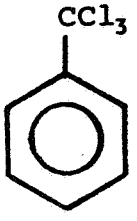
2-CHLOROMETHYL PYRIDINE
CAS NO. 6959-47-3
M+; C-/-



3-CHLOROMETHYLPYRIDINE
CAS NO. 6959-48-4
M+; C+/+



2-CHLORO-5-(TRICHLOROMETHYL)-PYRIDINE
CAS NO. 69045-78-9
M+; C?



TRICHLOROMETHYL BENZENE
CAS NO. 98-07-7
M+; C+/+

1,4-DICHLOROBENZENE
CAS NO. 106-46-7
M-; C+/+ (α_{2u}-g?)

M mutagenic
C carcinogenic mouse/rat
? data not available

a. Mutagenicity: Several chlorinated pyridines have tested positive for mutagenicity. 2-chloropyridine, 2-chloromethyl pyridine and 3-chloromethyl pyridine were mutagenic in the Ames assay with metabolic activation. 2-chloropyridine was also mutagenic with metabolic activation in L5178Y mouse lymphoma cells (3-chloropyridine was very weakly mutagenic) but both were clastogenic in the same assay. 2-chloro-5-trichloromethyl pyridine was mutagenic in the Ames test without activation, in mouse L5178Y lymphoma cells with and without activation, and clastogenic in the human lymphocyte assay with and without activation.

A benzene analog of nitrapyrin, benzotrichloride, was mutagenic in the Ames assay and B. subtilis recombination assays.

b. Carcinogenicity: 3-chloromethyl-(but not 2-chloromethyl-) pyridine was found to induce stomach carcinomas in mice and rats when administered orally. Benzotrichloride caused squamous cell carcinomas of the forestomach and adenocarcinomas of the lung in female mice when administered orally and squamous cell carcinoma of skin, lung adenomas and upper GI tract tumors in female mice when administered dermally. 1,4-dichlorobenzene caused renal tubule tumors in male rats (accompanied by α_{2u}-g accumulation and renal nephrotoxicity) and hepatocellular adenomas/carcinomas in male and female mice when administered orally, but was not mutagenic.

5. Acute, Subchronic and Chronic Toxicity Studies

a. Acute Toxicity Studies: The acute oral LD_{50} of nitrapyrin in rats is 1.23 g/kg, females and 1.07 g/kg, males (Tox. Category III). The acute dermal LD_{50} is > 2000 mg/kg (Tox. Category IV) in rabbits. An LC_{50} could not be determined for inhalation in a rat study due to technical limitations on the concentration of respirable nitrapyrin that could be produced. Nitrapyrin is a Tox. Category II primary eye irritant in rabbits and a Tox. Category IV dermal irritant in rabbit. Nitrapyrin was also found to be a positive skin sensitizer in the guinea pig.

b. Subchronic Toxicity Studies: 13-week studies in rat and dog were submitted by the registrant but have not yet been reviewed by Tox. I. The results are summarized below:

i. Rats were treated for 13 weeks with 0, 10, 40 and 120 mg/kg/day nitrapyrin in the diet (MRID No. 00163217). High dose animals showed slightly decreased total body weights (statistically significant only during last 1-3 weeks of study for males and females), decreased RBC count, increased serum bilirubin, increased absolute and relative liver weights, and vacuolization of the liver (liver effects were also seen at 40 mg/kg/day). Increased absolute and relative kidney weight (120 mg/kg/day) and increased severity of renal nephropathy including renal tubule degeneration/regeneration, (40 and 120 mg/kg/day) were noted only in males. Increased incidence of

brown pigment deposit in the proximal tubule epithelium in females was noted at mid and high dose.

ii. Dogs were treated for 13 weeks with 0, 15, 40 and 75 mg/kg/day nitrapyrin in the diet (MRID No. 409184-01). Decreased body weights were noted at 40 and 75 mg/kg/day and decreased total protein and albumin at high dose only. Alkaline phosphatase activity, increased absolute and relative liver weights and liver histopathology (vacuolization consistent with fatty change) were noted at all doses; therefore, a NOEL could not be determined for this study.

c. Chronic Toxicity Studies: A one-year chronic feeding study in dog (MRID No. 413454-01) gave a LEL of 15 mg/kg/day (high dose) with increased levels of cholesterol and alkaline phosphatase at 6 - 12 months, increases in absolute and relative liver weight and panlobular/ centrilobular hepatocellular hypertrophy. A NOEL of 3 mg/kg/day was determined.

F. Weight of Evidence Considerations:

The committee is asked to consider the following facts regarding the toxicological data on nitrapyrin in a weight-of-the-evidence determination of carcinogenic potential.

1. Nitrapyrin was associated with an increased incidence of renal neoplasms in male but not female F344 rats when fed at 60 mg/kg/day. The renal tumors were not fatal or metastatic and developed during the second year of treatment. No other treatment-related neoplasms were observed.

2. Non-neoplastic renal lesions noted at 12 months included hyaline protein droplets containing α_{2u}-g in the proximal tubules and mineralization of the loops of Henle. Severe chronic progressive glomerulonephrotoxicity occurred at high incidence in high dose males and caused increased mortality. All rats with renal tumors also had moderate-to-severe chronic progressive glomerulonephropathy. There were no changes in urinalysis parameters in treated rats and no increased nephrotoxicity in treated females. In addition, male-specific nephrotoxicity was observed in a 90-day feeding study in F344 rats. These lesions are consistent with an α_{2u}-g mechanism for male rat nephrotoxicity and tumor formation.

3. B6C3F1 mice of either sex did not develop renal neoplasms and did not show treatment-related renal pathology when fed up to 75 mg/kg/day; however, dosing in this study may not have been adequate. No renal toxicity was observed.

4. There was no evidence of genotoxicity in the mutagenicity tests conducted using nitrapyrin. However, some related

chlorinated pyridines have been demonstrated to be mutagenic in various assays. There are also published reports that nitrapyrin is mutagenic with metabolic activation in the Ames test. Direct assessment of the metabolite 6-CPA in mutagenicity tests has not been performed.

Except for questions regarding genotoxicity of nitrapyrin, the above findings are consistent with male rat-specific nephrotoxicity related to chemically-induced α_{2u}-g accumulation that has been described (see Section H). Based on these findings, the PR Committee is asked to consider the following:

1. Whether the available evidence supports the α_{2u}-g mechanism for male rat nephrotoxicity and tumor formation by nitrapyrin. According to the EPA Risk Assessment Forum, renal toxicity and neoplasia induced by α_{2u}-g in male rats are not considered toxicological end-points to be used in human risk assessment determinations.

2. Whether the doses were adequate in the mouse study to test for oncogenicity of nitrapyrin in that species.

G. <u>Not in this document</u> (to be prepared by the Peer Review Committee).

H. Support for α_{2u}-g Mechanism of Renal Toxicity and Tumorigenesis

1. Consideration of the use of the α_{2u}-g model for nitrapyrin:

When considering nitrapyrin, the Committee is requested to consider the possibility of using the α_{2u}-g model for renal nephrotoxicity/tumorigenesis. The EPA Risk Assessment Forum recently prepared a summary document entitled "Alpha$_{2u}$ -Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat" (Document No. EPA/625/3-91/1019F) which provides background information, evaluation of the hypothesis and a science policy for α_{2u}-g-related nephrotoxicity and neoplasia. This document is referred to in the following discussion of nitrapyrin.

There is evidence suggesting that certain chemicals induce accumulation of the male rat urinary protein α_{2u}-g in hyaline droplets in the male rat kidney. Characteristic progressive renal pathology and, in many instances, development of renal tumors have been described. The tumors may therefore be the result of a sex (male) and species (rat)-specific mechanism whereby certain chemicals promote accumulation of α_{2u} globulin-chemical complexes. The exact mechanism of tumorigenesis has not been demonstrated.

The proposed mechanism of nephrotoxicity and tumorigenesis can be summarized as follows: the chemical binds (reversibly) to α_{2u}-g, making the protein more resistant to lysosomal degradation and causing accumulation of the complex in hyaline droplets in the P2 section of the renal proximal tubule cells with prolonged administration. As the complex accumulates, it causes local necrosis and cell proliferation which, with continued compound administration, may eventually lead to hyperplasia, clonal tumor formation and progression from adenoma to adenocarcinoma.

α_{2u}-g is found in male rat urine at relatively high concentrations and accumulation of α_{2u}-g complexes (or of other urinary protein-chemical complexes) appears to be unique to male rats. The appropriateness of using toxicity endpoints that appear to be related to this accumulation (including tumor formation) as an endpoint for human health hazard assessment has been questioned.

2. Determination of whether male rat kidney neoplasias are due to α_{2u}-g accumulation:

The Risk Assessment Forum outlined three criteria which must <u>each</u> be observed for male rat renal tumors and has listed additional types of data that can be used to further support this hypothesis. The Peer Review Committee is asked to weigh the evidence supporting α_{2u}-g-induced nephrotoxicity and renal tumor formation by nitrapyrin in male rats. The 3 required criteria are listed below and discussed with regard to nitrapyrin:

a. "<u>Increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats.</u>"

Hyaline droplet accumulation of increasing severity in renal proximal tubule cells was observed only in mid and high dose male rats at 12 months;

b. "<u>Accumulating protein in the hyaline droplets is α_{2u}-g.</u>"

Control and high dose rat male and female kidneys were stained for α_{2u}-g using immunohistochemistry. High dose males showed significant increases in **immunochemical staining of α_{2u}-g**, whereas females and control males showed low-level, background staining. Low and mid dose kidneys were not examined;

c. "<u>Additional aspects of the pathological sequence associated with α_{2u}-g nephropathy are present.</u>"

Tubular mineralization was markedly increased by 12 months in high dose males. The observed incidences of some renal lesions typical of this complex, such as single cell necrosis, proteinaceous casts and tubule degeneration/regeneration, were not increased in this study. However, it should be noted that at a single chronic sampling time, not all characteristics may

be evident. In addition, **chronic progressive glomerulonephropathy** was markedly exacerbated in high dose males (and also mid dose males) at termination.

Each of these three required criteria was therefore met for nitrapyrin in rats.

Additional Information: The following additional information can be used to help distinguish between kidney nephrotoxicity/tumor induction resulting from α_{2u}-g or from other possible causes:

a. Genotoxicity: many α_{2u}-g-inducing compounds are not mutagenic or only weakly mutagenic and do not appear to depend on genotoxicity for induction of tumors.

b. Sex and Species-Specificity: renal tumor formation and nephrotoxicity are specific to male rats;

c. Nephrotoxicity: marked exacerbation of the chronic glomerulonephropathy common to aging rats is observed only in male rats;

d. Nature of tumors: renal tumors induced by this mechanism are found in the tubules, usually occur at a low incidence, are not life-threatening, do not metastasize and develop late in life;

e. No other tumors: compound does not induce other tumor types in rats or other species.

In addition to meeting the three required criteria for α_{2u}-g-related tumor induction, the available data on nitrapyrin is consistent with the above additional criteria. However, the issue of genotoxicity of nitrapyrin is not clear-cut: although the in-house data on nitrapyrin is negative, several related chloropyridine compounds have been shown to be mutagens in various mutagenicity assays and nitrapyrin itself tested positive in the Ames assay in an NTP study. There may also be uncertainty regarding species-specificity of renal tumors if the dose at which mice were tested is considered inadequate.

Other types of information that can be used to help support the plausibility of the α_{2u}-g hypothesis for a particular chemical, but for which insufficient data is available for nitrapyrin, include covalent binding to macromolecules like DNA, binding of chemical to α_{2u}-g, retention of the test compound in the male rat kidney, sustained cell division in the P2 segment of the renal tubule/dose-related hyperplasia and nephrotoxic response of male NBR rats or castrated male rats.

According to the Risk Assessment Forum document:

> "Confidence...depends on the comprehensiveness and consistency of available data. If all the data (two species, two sex combination bioassay, all [3 critical elements] and additional information such as that described [above] are consistent with a role for chemical induced α_{2u}-g, there is a high degree of confidence that the α_{2u}-g syndrome, alone, accounts for the renal tubule tumors."

3. Factors to be considered in determining method to be used in estimating the risks of nitrapyrin:

The science policy statement prepared by the EPA Risk Assessment Forum for evaluating data on male rat renal tubule tumors and α_{2u}-g related nephropathy is as follows:

> "(1) Male rat renal tubule tumors arising as a result of a process involving α_{2u}-g accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk.
>
> (2) If a chemical induces α_{2u}-g accumulation in male rats, the associated nephropathy is not used as an endpoint for determining non-carcinogenic hazard. Estimates of non-carcinogenic risk are based on other endpoints."

It should be noted that other types of tumors not related to α_{2u}-g accumulation in male rat kidney are still used to evaluate risk if they occur together with the α_{2u}-g events.