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

SEP 14 1992

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MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Nitrapyrin

FROM: Linnea Hansen, Ph.D.
Section IV Toxicology Branch I
HED (H7509C)
Linnea J. Hansen 7/16/92

and
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Health Effects Division (H7509C)

TO: Walter Waldrop
Product Manager # 71
Special Review and Reregistration Division (H708W)

The Health Effects Division Carcinogenicity Peer Review Committee met on 5/6/92 to discuss and evaluate the weight-of-the-evidence on nitrapyrin with particular reference to its carcinogenic potential.

The Peer Review Committee agreed that nitrapyrin should be classified as Group D - not classifiable as to human carcinogenicity.

A. Individuals in Attendance:

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

Karl Baetcke
Reto Engler
William L. Burnam
Julie Du
George Ghali
Marion Copley

(1)

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Kerry Dearfield

Hugh Pettigrew

Esther Rinde

William Sette

Yin-Tak Woo

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

Linnea Hansen[1] 7/16/92

Bernice Fisher 8/3/92

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

Penelope Fenner-Crisp

Marcia Van Gemert

Lucas Brennecke

Richard Hill —

Jean Parker

John Quest

4. Other Attendees:

Eve Andersen (Clement)
Lori Brunsman (HED)
Flavic Puga (Instituto Biologico)
Michael Stedham (PAI, attended for Lucas Brennecke)

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

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B. Material Reviewed:

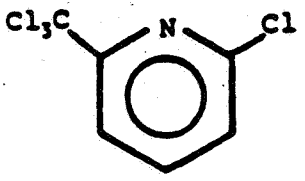
The material available for review consisted of DER's, one-liners, and other data summaries prepared by Linnea Hansen; tables and statistical analysis by Bernice Fisher. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by Dow Chemical Co.

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 Nitrosomonas sp. 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 2.8×10^{-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 Caswell (or Tox Chem) Number of nitrapyrin is 217.
The Chemical Abstracts Registry Number (CAS No.) is 1929-82-4.
The PC Number is 069203.

The structure of nitrapyrin is



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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. Experimental Design

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

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

* $p < 0.05$; ** $p < 0.01$
Pair-wise comparison denoted at Dose level and trend denoted at Control; Fisher's Exact Test and Cochran-Armitage tests were used.

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. 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 and Other Observations

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

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TABLE 2: NUMBER OF RATS WITH HISTOPATHOLOGICAL FINDINGS AT TERMINAL (24 MOS.) AND UNSCHEDULED SACRIFICE

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, slight	M	0	0	2	30*
	F	2	2	1	15*
Vacuolization (fatty change), slight	M	1	0	3	28*
	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 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: NUMBER OF RATS WITH HISTOPATHOLOGIC LESIONS AT 12 MONTH INTERIM SACRIFICE

LESION	SEX	DOSE, MG/KG/DAY 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, 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 ANIMAL NO.	RESULTS	HIGH DOSE 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
ND Not determined
+++ Moderate response
++++ 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)	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)*

Value in parentheses = [(percent body weight gain relative to initial weight for treatment group minus percent body weight gain relative to initial weight for controls) ÷ percent body weight gain relative to initial weight for 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							
ANIMAL GROUP:	0		5		20		60	
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.

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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 in both sexes 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

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%); however, spontaneous renal cortical adenomas are very rare in mice.

c. Non-neoplastic Lesions and Other Observations

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

Lesion	Sex	Dose Administered, mg/kg/day 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). 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 carcinogenicity study, the highest dose tested (75 mg/kg/day) produced minimal toxicity in mice. The PRC decided that the highest dose tested (HDT) was not adequate for assessing carcinogenicity of nitrapyrin in mice. The HDT was far from the MTD shown in the 2 week and 13 week studies.

A table summarizing renal effects observed in rats and mice treated with nitrapyrin in each study is given in Table 11.

Table 11

RENAL PATHOLOGY IN MALE AND FEMALE RATS AND MICE WITH TREATED WITH NITRAPYRIN: CONSISTENCY OF OBSERVATIONS WITH α_{2u}-GLOBULIN-RELATED NEPHROTOXICITY/TUMOR INDUCTION

SPECIES/OBSERVATION	MALE	FEMALE
RAT:		
13 WEEKS		
1) Increased kidney wt.	+	-
2) Histopathology		
-Prox. tubule degen./regen., incr. severity	+	-
-Brown pigment deposition, prox. tubules	-	+[1]
1a) Histopathology - Incr. severity, interstit. nephritis and prox. tubule cell swelling	+	?[2]
1 YR		
1) Increased kidney wt.	-/-	-/+*
2) Histopathology		
-Incr. mineralization, prox. tubules	+	-
-Incr. hyaline droplets, prox. tubules	+	-
-α_{2u}-G present in droplets	+	-
-Incr. proteinaceous casts (slight)	-	+*
3) Clinical chemistry - BUN ↑	-	-
2 YR		
1) Increased kidney wt.; abs./rel.	+/+	+/+*
2) Histopathology		
-Chron. progr. glomer. nephr., incr. severity	+	-
-Renal adenoma/adenocarcinoma	+	-
3) Clinical Chemistry		
-BUN ↑	+*	-
-Phosphorus↑(slight)	+	-
4) Incr. mortality from renal nephropathy	+	-
MOUSE:		
2 WKS		
1) Decreased kidney wts.	+*	+*
2) Histopathology - necrosis of desc. prox. tubules	+*	-
1 YR		
1) Increased kidney wts, abs./rel. (slight)	+/+[3]*	+/+*
2) Clinical chemistry - Incr. blood glucose (sl.)	+*	-
2 YR		
1) Increased kidney wts. abs./rel. (slight)	+/+*	+/+*
2) Histopathology - Renal cortical adenoma[4]	+*	-
3) Clinical chemistry - Incr. blood glucose	+*	+*

1 Bold* indicates observations which may reflect renal effects unrelated to α_{2u}-G accumulation
2 Not clear from study summary whether both males and females were affected
3 Due mostly to low control group terminal body wt.
4 Very rare tumor; single incidences in mid and high dose group

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.

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. MRID 00151627.
b. Unscheduled DNA synthesis, male rat primary hepatocyte- No increase in unscheduled DNA synthesis up to 23 ug/ml (1×10^{-4}M). MRID and 00109456.
c. Mouse micronucleus assay - No mutagenic activity up to 800 mg/kg. MRID 00151628.
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. MRID 00163805.

An NTP study reported that nitrapyrin was mutagenic in the Salmonella 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. The developmental NOEL was 10 mg/kg/day. The maternal LEL was 30 mg/kg/day based upon decreased body weight gain and increased absolute/relative liver weight. The NOEL was 10 mg/kg/day.

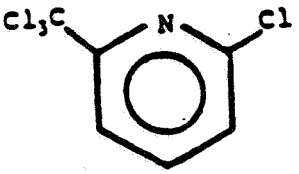
Rat - A developmental toxicity study in rat (MRID Nos. 00163792, 420501-01) was considered unacceptable because the highest dose level (50 mg/kg/day) was considered insufficient to challenge developmental toxicity.

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. The NOEL was 5 mg/kg/day.

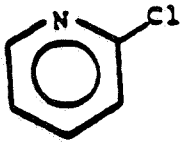
4. Structure-Activity Correlations

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

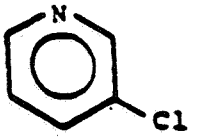
Figure 1: Structurally Related Compounds



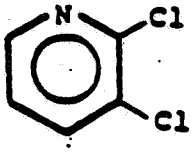
2-CHLORO-6-(TRICHLOROMETHYL)-PYRIDINE
CAS NO. 1929-82-4
M+/-; C-/+ (α_{2u}-g?)



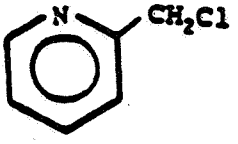
2-CHLOROPYRIDINE
CAS NO. 109-09-1
M+; C?



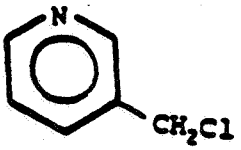
3-CHLOROPYRIDINE
CAS NO. 626-60-8
M+; C?



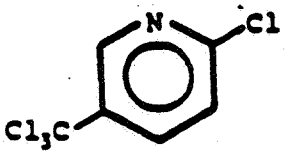
2,3-CHLOROPYRIDINE
CAS NO. 2402-77-9
M+; C?



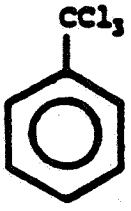
2-CHLOROMETHYL PYRIDINE
CAS NO. 6959-47-3
M+; C-/-



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 of Analogs: Several chlorinated pyridines have tested positive for mutagenicity.. 2-Chloropyridine, 2-chloromethyl pyridine and 3-chloromethyl pyridine were mutagenic in the Salmonella assay with metabolic activation (Mutation Research 176:185-198, 1987).. 2-Chloropyridine was also mutagenic with and without metabolic activation in L5178Y mouse lymphoma cells (higher activity with activation); it was also clastogenic (aberrations and micronuclei) in mouse lymphoma cells (greater activity seen with activation) (USEPA sponsored research, in press). 2-Chloro-5-trichloromethyl pyridine was mutagenic in the Salmonella test without activation, in mouse L5178Y lymphoma cells with and without activation, and clastogenic in the human lymphocyte assay with and without activation (TSCA 8(e) submission 8EHQ-1084-05335). A benzene analog of nitrapyrin, benzotrichloride, was mutagenic in the Salmonella assay and B. subtilis recombination assays.

The mutagenic activity exhibited by nitrapyrin (NTP Salmonella test) and its 2-substituted chlorine analogues are consistent with the hypothesis that nucleophilic displacement of halogens at the 2 position can occur through addition-elimination reactions. Results are seen with activation (or greatly enhanced by activation) and this is consistent with an N-oxidation reaction which is a microsomal activity. It is possible that the electron-attracting property of the N-oxide would make the halogen in the 2-position more labile and susceptible to nucleophilic attack. Activity by the trichloromethyl group is also possible as evidenced by the positive Salmonella result by benzotrichloride. These data suggest that nitrapyrin has genotoxic potential and would be part of the consideration for a carcinogenicity concern.

b. Carcinogenicity of Analogs: 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.

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 considered the following facts regarding the toxicology data on nitrapyrin in a weight-of-the-evidence determination of carcinogenic potential:

1. Nitrapyrin was associated with an increased incidence of renal tubular adenomas and adenocarcinomas (significance of trend, $p<0.01$, significance of pairwise comparison of combined incidence at high dose, $p<0.05$) 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}-globulin 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}-globulin mechanism for male rat nephrotoxicity and tumor formation.

3. The PRC reviewed the data on renal toxicity in the female rat, including very slight presence of proteinaceous casts in the renal tubule (see Table 3), moderate glomerular nephropathy (see Table 2), increased kidney weights (see Table 6) and increased BUN (see Table 7). The PRC determined that these were isolated findings and that they did not detract from the proposed α_{2u}-globulin mechanism for renal toxicity in males.

4. B6C3F1 mice of either sex did not show evidence of treatment-related renal pathology when fed up to 75 mg nitrapyrin/kg/day, but single incidences of renal cortex adenomas were seen in males at 25 and 75 mg/kg/day. The incidence of these tumors in this study was not significant (2% at each dose) but these are normally very rare tumors. The highest dose tested (75 mg/kg/day) caused only minimal toxicity in mice and may not have adequately tested the carcinogenic potential of nitrapyrin. The PRC determined that dosing in this study was not adequate and requested both an additional range-finding study and a chronic study.

5. There was no evidence of genotoxicity in the submitted mutagenicity tests conducted using nitrapyrin. The NTP however reported positive activity in the Salmonella assay with activation. This is consistent with activity seen with several closely related analogues that have shown mutagenic activity in

various assays. Activity by these chlorinated pyridines is consistent with nucleophilic displacement of halogen at the 2 position through addition-elimination reactions. This suggests that nitrapyrin has genotoxic potential. In relation to the tumor data obtained with nitrapyrin, it is not clear if this potential genotoxicity would play a role in the α_{2u}-globulin mechanism is male rats. However, with the uncertainty of renal associated lesions in the mouse study (therefore necessitating a required repeat), alternative mechanism(s) for tumor formation, including a possible role for genotoxicity, cannot be ruled out at this time.

6. Except for questions regarding genotoxicity of nitrapyrin, the above findings are consistent with male rat-specific nephrotoxicity related to chemically-induced α_{2u}-globulin accumulation that has been described (see Section H). Based on these findings, the PRC determined that the available evidence is consistent with the α_{2u}-globulin mechanism for male rat nephrotoxicity and tumor formation by nitrapyrin. The Agency has determined that renal toxicity and neoplasia induced by α_{2u}-globulin in male rats are not considered toxicological end-points to be used in human risk assessment determinations, under certain circumstances.

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 the classification for nitrapyrin should be Group D - not classifiable as to human carcinogenicity due to inadequate evidence.

This decision was based on the finding of a statistically significant increase in renal tubular adenomas and adenocarcinomas in male rats. Females rats did not have an increase in tumors. Although a mouse study was performed, inadequate doses for assessing carcinogenic potential were used. However, since there appeared to be potential for kidney, and possibly liver, effects in mice, and there is apparent genotoxic potential for nitrapyrin, an acceptable mouse study is required for complete assessment and whether the assessment will totally focus on the α_{2u}-globulin mechanism or not. Whether the single incidences of renal adenoma, a rare tumor, in the mouse study at mid- and high-dose were coincidental or treatment-related should be resolved by repeating the mouse study at higher doses.

The PRC determined that the available evidence for rats only supports the α_{2u}-globulin mechanism for male rat nephrotoxicity and tumor formation by nitrapyrin. The Agency has determined that renal toxicity and neoplasia induced by α_{2u}-globulin in male rats are not considered toxicological end-points to be used in human risk assessment determinations, under certain circumstances.

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

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

When considering nitrapyrin, the Committee considered the possibility of using the α_{2u}-globulin 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}-globulin-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}-globulin 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}-globulin, 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}-globulin is found in male rat urine at relatively high concentrations and accumulation of α_{2u}-globulin 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}-globulin accumulation

The Risk Assessment Forum document 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 weighed the evidence supporting α_{2u}-globulin-induced nephrotoxicity and renal tumor formation by nitrapyrin in male rats. The three required criteria are listed below and discussed with regard to nitrapyrin:

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

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. "Accumulating protein in the hyaline droplets is α_{2u}-globulin."

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

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

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}-globulin or from other possible causes:

a. Genotoxicity: many α_{2u}-globulin-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. However, since

the doses used in the mouse bioassay were considered to too low, an adequate mouse study is not available.

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}-globulin-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 are negative, several related chloropyridine compounds have been shown to be mutagens in various mutagenicity assays and nitrapyrin itself tested positive in the Salmonella assay in an NTP study. There may also be uncertainty regarding species-specificity of renal tumors if the doses at which mice were tested are considered inadequate.

Other types of information that can be used to help support the plausibility of the α_{2u}-globulin 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}-globulin, 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}-globulin, there is a high degree of confidence that the α_{2u}-globulin syndrome, alone, accounts for the renal tubule tumors."

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

The science policy statement for evaluating data on male rat renal tubule tumors and α_{2u}-globulin related nephropathy is as follows:

> "(1) Male rat renal tubule tumors arising as a result of a process involving α_{2u}-globulin 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}-globulin 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}-globulin accumulation in male rat kidney are still used to evaluate risk if they occur together with the α_{2u}-globulin events.

Appendix

DOW CHEMICAL U.S.A.

TEXAS OPERATIONS
FREEPORT, TEXAS 77541

February 5, 1992

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Indianapolis, IN 46268-1189

RE: NITRAPYRIN REGISTRATION STANDARD
REQUEST FOR ADDITIONAL TOXICOLOGICAL INFORMATION

The information which was requested by the United States Environmental Portection Agency concerned historical control data for male and female Fischer 344 rats as pertains to renal histopathology. In a telephone conversation on 4 February 1992 with Ms. Linea Hanson of the USEPA, in order for the Agency to act on the alpha-2u-globulin issue with regard to Nitrapyrin, historical control data for all renal histopathology is needed. "This historical control data should 1) be given by study, 2) include laboratory historical control data collected for 3 years prior to and 3 years following the 2-year rat study, and 3) include incidence data for renal adenoma, renal adenocarcinoma and combined renal adenoma and adenocarcinoma."

Only three other rat chronic toxicity/oncogenicity studies in Fischer 344 rats have been conducted at this laboratory. They are:

DOWCO 290 (3,6-dichloropicolinic acid): 2-Year Chronic Toxicity and Oncogenicity study in Rats
(TXT: K-038252-25)
Study Start Date: 25 August 1983
Termination Date: 25 August 1985
Report Data: 14 July 1986

Interim (1-Year) Data	Male	Female
Within Normal Limits	0/10	2/10
Aggregate(s) of Mononuclear (Predominately Lymphoid) Cells, Multifocal: - Very Slight	10/10	3/10
Altered Tinctorial Properties - Increased Basophilia, Tubule(s), Multifocal: - Very Slight	7/10	5/10
Dilated, Tubule(s), Multifocal: - Very Slight	10/10	6/10
Mineralization, Tubule(s), Multifocal: - Very Slight	4/10	0/10

Terminal (2-Year) Data	Male	Female
Aggregate(s) of Mononuclear (Predominately Lymphoid) Cells, Corticomedullay Junction:	45/50	40/50
Chronic Progressive Glomerulonephropathy: - Slight	37/50	43/50

Terminal (2-Year) Data (continued)	Male	Female
Chronic Progressive Glomerulonephropathy: - Severe	5/50	1/50
Chronic Progressive Glomerulonephropathy: - Total	50/50	47/50
Cyst, Cortex, Focal:	2/50	1/50
Mineralization:	9/50	15/50
Pigment - Hematogenous - Increased, Tubule(s):	4/50	3/50
Adenoma, Cortex, Benign, Primay:	0/50	0/50
Adenocarcinoma, Cortex, Malignant, Primary, No Metastasis:	0/50	0/50

Chlorpyrifos: 2-Year Chronic Toxicity-Oncogenicity Study in Fischer 344 Rats (TXT: K-044793-79)
Study Start Date: 13 February 1986
Termination Date: 1 March 1988
Report Date: 23 December 1988

Interim (1-Year) Data	Male	Female
Aggregate(s) of Mononuclear (Predominately Lymphoid) Cells, Cortex:	7/10	0/10
Atrophy, Tubule(s), Multifocal: - Slight	9/10	0/10
Dilatation, Tubule(s), Multifocal: - Slight	8/10	1/10
Mineralization, Cortex, Multifocal: - Slight	0/10	1/10

Terminal (2-Year) Data	Male	Female
Chronic Progressive Glomerulonephropathy, Bilateral: - Slight	21/50	23/50
Chronic Progressive Glomerulonephropathy, Bilateral: - Moderate	21/50	1/50
Chronic Progressive Glomerulonephropathy, Bilateral: - Severe	7/50	0/50
Chronic Progressive Glomerulonephropathy, Bilateral: - Total	49/50	24/50
Cyst, Cortex:	1/50	1/50
Dilatation, Pelvis, Unilateral: - Slight	0/50	1/50
Dilatation, Pelvis, Bilateral: - Slight	1/50	1/50
Adenoma, Cortex, Benign, Primary:	0/50	0/50
Carcinoma, Cortex, Malignant, Primary, No Metastasis:	0/50	0/50

Chlorpyrifos-methyl: 2-Year Chronic Toxicity/Oncogenicity Study in Rats (TXT: K-046193-31)
Study Start Date: 15 July 1988
Termination Date: 18 July 1990
Report Date: 19 August 1991

Interim (1-Year) Data	Male	Female
Aggregates of Mononuclear Cells (Primarily Lymphocytes and Plasma Cells), Cortex, Multifocal	9/10	1/10
Degeneration/Regeneration, Tubule(s), Cortex, Slight	10/10	5/10
Mineralization	10/10	7/10

Terminal (2-Year) Data	Male	Female
Aggregates of Mononuclear Cells (Primarily Lymphocytes and Plasma Cells), Cortex, Multifocal	35/50	0/50
Chronic Progressive Glomerulonephropathy - Slight (1-50% of the Kidney Affected)	26/50	22/50
Chronic Progressive Glomerulonephropathy - Moderate (51-75% of the Kidney Affected)	17/50	3/50

Terminal (2-Year) Data (continued)	Male	Female
Chronic Progressive Glomerulonephropathy - Total	49/50	26/50
Degeneration/Regeneration, Tubule(s), Cortex, Slight	0/50	14/50
Inflammation, Suppurative (Abscesses), Multifocal	1/50	0/50
Mineralization	27/50	16/50
B-Adenoma, Renal, Benign, Primary	0/50	0/50
M-Adenocarcinoma, Renal, Malignant, Primary, No Metastasis	0/50	1/50

With regard to incidence data for renal adenoma, renal adenocarcinoma, and combined renal adenoma and adenocarcinoma by study, the data are:

DOWCO 290 (3,6-dichloropicolinic acid)

	Male	Female
Renal Adenoma	0%	0%
Renal Adenocarcinoma	0%	0%
Combined	0%	0%

Chlorpyrifos

	Male	Female
Renal Adenoma	0%	0%
Renal Adenocarcinoma	0%	0%
Combined	0%	0%

Chlorpyrifos-methyl

	Male	Female
Renal Adenoma	0%	0%
Renal Adenocarcinoma	0%	2%
Combined	0%	2%

This comprises this laboratory's information with regard to historical control data for renal histopathology and primary renal tumors in the Fischer 344 rat. If I can be of any further help please call.

Sincerely,

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