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WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

September 28, 2004

Subject: EPA Id # 069203. Nitrapyrin: Revised Toxicology Chapter for the RED.

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Attached is the revised Toxicology Chapter for the RED for nitrapyrin. Revisions were made in response to comments provided by the DOW Chemical Company dated August 27, 2004 (refer to letter from Michael D. Culy of the DOW Company to Stephanie Plummer, SRRD/OPP/USEPA, MRID No.: 46353401).

Nitrapyrin

PC Code: 069203

Revised

Toxicology Disciplinary Chapter for the Reregistration Eligibility Decision (or Registration Support) Document

Date revised: September 29, 2004

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Introduction to the September 29, 2004 revision

The DOW Company presented (August 27, 2004, MRID # 46353401) provided a response to the Nitrapyrin Evaluation of Preliminary Human Health and Ecological Risk Assessments. In their response, the DOW Company indicated several areas of the original Toxicology Chapter for the RED (DP Barcode D295599, May 13, 2004). In response to the DOW Company's comments, ReRegistration Branch III has revised the Toxicology Chapter to account for several typos and inconsistencies.

After giving consideration to the comments provided by the DOW Company, the following items were not changed from the original Toxicology Chapter for Nitrapyrin:

1. Commentary regarding the mouse carcinogenicity study.

HED's CARC considered the study by Quast (1990, MRID # 41651601) and determined that the dose levels were too low for adequate carcinogenicity assessment and specifically requested that a replacement study be provided. This replacement study (1997, MRID # 44231803 and 44231801) was considered the definitive study for defining the carcinogenic potential of nitrapyrin in mice and the basis for the classification. The DOW Company has indicated that "A pathology peer review is scheduled for early October, 2004 to evaluate all relevant studies and information on the carcinogenic potential of Nitrapyrin". However, HED cannot alter its conclusions regarding the classification of Nitrapyrin at this time based on an unseen document. When the peer review report is submitted to the Agency, HED will consider its content and if deemed appropriate, convene a meeting of the CARC to consider reclassifying Nitrapyrin for carcinogenicity.

2. NOEL for the mouse subchronic study.

The DOW Company indicated that a "NOEL" for a subchronic mouse study can be established from the Barna-Lloyd et al study at a dose level of 45 mg/kg/day". No change in the Toxicology Chapter of the RED for this comment was made since this study was not included as a critical endpoint in risk assessment and also because current HED guidance does not require that hepatocellular hypertrophy and slight liver weight change be included in the LOAEL for a study.

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EPA Secondary EPA Reviewer): Pamela Hurley _____, Date _____
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1.0 HAZARD CHARACTERIZATION

Nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) is a nitrification inhibitor that is used to prolong nitrification of ammonium ions when applied with ammonical fertilizers (urea, anhydrous ammonia, etc) and liquid animal wastes. It is used for land to be planted with corn, wheat and sorghum. It acts by inhibiting *Nitrosomonas* bacteria.

Acute toxicity and sensitization. Nitrapyrin is classified as Toxicity III for acute oral (LD_{50} 1.07 gm/kg for males and 1.23 gm/kg for females), dermal (LD_{50}> 2000 mg/kg) and inhalation (LC_{50} > 0.03 mg/L) and is not considered a dermal irritant (Toxicity Category IV) but does have some properties as an ocular irritant (Toxicity Category II). Nitrapyrin was demonstrated to be positive in a modified Maguire dermal sensitization study.

Dermal toxicity. Following dermal application for 21 days, the liver was demonstrated to be increased in weight but without concurrent histopathological changes.

Subchronic and chronic oral toxicity. In addition to body weight decreases, the liver was demonstrated to be the principle target organ in rat, mouse and dog subchronic and chronic studies. In dogs liver toxicity was indicated by changes in cholesterol and alkaline phosphatase and liver weight. and hypertrophy. In rats, liver weight was increased and this was associated with clinical chemistry changes such as increases in albumin, alanine aminotransferase and cholesterol and hepatocellular hypertrophy and centrilobular vacuolization consistent with fatty change. In mice, the liver weight changes were associated with several histopathological findings ranging from centrilobular and panlobular hepatocyte hypertrophy, mitotic figures and eventual necrosis and including bile duct proliferation. The stomach was also demonstrated to be a target organ as indicated by hyperkeratosis and/or hyperplasia Epithelial cell vacuolation and/or hyperplasia/hypertrophy was also seen in the duodenum and jejunum. Increased extramedullary hematopoiesis in the spleen was also seen in high dose group mice.

In addition to the liver, the kidney was also demonstrated to be a target organ in male rats only and the weight of evidence indicated that nitrapyrin affects the kidney in a manner consistent with the alpha 2µ globulin model. Consistent with this model, kidney non-neoplastic pathology was evident and there were increases in kidney tumors in male rats. Induction of kidney pathological changes in the alpha 2µ globulin model is not relevant to human risk assessment.

Reproductive and Developmental toxicity. Nitrapyrin did not demonstrate increased sensitivity to fetuses and offspring. Maternal toxicity consisted of decreases in body weight and liver effects and kidney effects in the rat reproduction study. There were no indications of impaired reproductive performance. Fetal and offspring toxicity was slight and included increased incidence of crooked hyoid in rabbits, ossification decreases in rats and body weight decreases and evidence of hepatic centrilobular hypertrophy with fatty changes in rats in the reproduction study.

Carcinogenicity. Nitrapyrin is classified as "likely to be a human carcinogen" based on the mouse study which demonstrated liver tumors, stomach tumors and Harderian gland neoplasm. The Q1* was determined to be 4.25×10^{-2} human equivalents.

Mutagenicity. There is no mutagenicity concern with nitrapyrin. The mutagenicity data base for nitrapyrin satisfies the current recommendations for testing and the submitted studies do not indicate a mutagenicity concern. However, there is one study (unreviewed) that reports that nitrapyrin is mutagenic in the *Salmonella typhimurium* strains TA97, TA98 and TA100 in the presence of S9 metabolic activation. The results of the NTP study are in contrast with the submitted study which did not demonstrate positive mutagenicity effects in these strains.

Neurotoxicity, Endocrine Disruption and Immunotoxicity. . Neither the subchronic, chronic, developmental or reproductive rat, mouse, dog or rabbit studies indicated that nitrapyrin was associated with either a specific or an indirect neurotoxic or immunotoxic response or endocrine disruption.

Metabolism. Absorption and elimination of labeled nitrapyrin was rapid and essentially complete by 72 hours. Most (79.56% to 85.48%) of the radioactivity was recovered in the urine with a smaller amount (11.04% to 13.63%) in the feces. Very little (0.51% to 0.95%) remained in the tissue. There was no unchanged nitrapyrin in the urine and 6-chloropicolinic acid and its glycine conjugate were identified as the metabolites. The glycine conjugate was more common in females and for both sexes following multiple dosing.

2.0 REQUIREMENTS - The requirements (CFR 158.340) for nitrapyrin will be addressed by the risk assessor who is more familiar with the exact nature of the uses and exposure of this chemical.

3.0 DATA GAP(S)

There are no data gaps for nitrapyrin at this time. The HIARC recommended that a 28 day inhalation toxicity study be conducted with nitrapyrin to provide a better endpoint for inhalation risk assessment. However, the waxy physical nature of technical nitrapyrin precludes generating aerosols of appropriate atmospheric concentrations to meaningfully assess the inhalation toxicity. Please refer to the memo dated December 8, 1991 from Linnea Hansen (TXR # 009040) addressing the problems associated with assessing nitrapyrin in inhalation studies.

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of data base for acute toxicity: The data base for acute toxicity of nitrapyrin is considered complete and is summarized in the Table 2. No additional studies are required at this time. Nitrapyrin is classified as Category III or IV for acute oral and dermal toxicity and for dermal irritation. However, nitrapyrin is classified as Category II for ocular irritation. Nitrapyrin cannot be classified for inhalation toxicity because of the waxy physical nature of this chemical precludes assessing inhalation toxicity in unformulated products.

Table 2. Acute Toxicity of Nitrapyrin (PC Code - 069203)

Guideline No.	Study Type	MRID #(s)	Results	Toxicity Category
81-1	Acute Oral - rat	00037519 (1972)	LD_{50} = 1.07 gm/kg ♂ 1.23 gm/kg ♀	III
81-2	Acute Dermal - rabbit	(1986)	LD_{50} > 2000 mg/kg	III
81-3	Acute Inhalation	00158901 (1986)	LC_{50} > 0.03 mg/L no effects (technically limited atmospheric conc.)	Cannot classify
81-4	Primary Eye Irritation	00158902 (1986)	Corneal opacity to day 14 (2/6), conjunctiv-itis today 21, iritis to day 7.	II
81-5	Primary Skin Irritation	00037519 (1972)	Very slight erythema and slight exfoliation.	IV
81-6	Dermal Sensitization	00158903 (1986)	Positive in the modified Maguire method.	

4.2 Subchronic Toxicity

Adequacy of data base for subchronic toxicity: The data base for subchronic toxicity is considered complete. Subchronic feeding studies in rats and dogs were completed years ago. The more current chronic feeding studies can be used to assist in the evaluation of the subchronic toxicity of nitrapyrin. The mouse subchronic study demonstrated increased liver weight and hepatocellular hypertrophy at the lowest dose tested and at higher doses there was poor survival. The 21-day dermal toxicity study with rabbits demonstrated liver weight effects at the highest dose tested. There is no subchronic inhalation study and it is currently not required. No additional studies are required at this time.

870.3100 90-Day Oral Toxicity - Rat

Refer to the rat chronic feeding/oncogenicity study below.

870.3100 90-Day Oral Toxicity - Mouse

Executive Summary. In a subchronic toxicity study (1995, MRID 44253802), nitrapyrin (90.0 and 92.05% a.i.) was administered in the diet continuously to 10 male B6C3F_1 mice per group at dose levels of 0, 200, 300, 400, or 600 mg/kg/day and to 10 female B6C3F_1 female mice per group at dose levels of 0, 200, 400, 600, or 800 mg/kg/day for up to 95 or 96 days. Dietary concentrations were adjusted each week so as to deliver constant doses throughout the study.

No animals administered 600 or 800 mg/kg/day of the test material survived to study termination. One male and one female in the control groups died, but all animals administered the test material at concentrations ≤400 mg/kg/day survived to study termination. No clinical signs of toxicity were observed in animals administered ≤400 mg/kg/day; clinical signs observed in the 600- and 800-mg/kg/day groups were associated with imminent death. Mice administered 600 or 800 mg/kg/day showed pronounced decreases in body weights, body weight gain, and food consumption prior to death. Male and female mice administered 400 mg/kg/day weighed up to 9% ($p<0.05$ or <0.01) less than the controls from day 54 to study termination. Body weight gain at 400 mg/kg/day was reduced by 37% for males and 25% for females over the first 75 days of the study and by 21 and 16% for males and females, respectively, over the entire study. There were no clear dose-related effects on food consumption or food utilization for groups that survived to study termination. No statistically significant effects were observed on body weights, body weight gain, food consumption, of food efficiency in mice administered 200 or 300 mg/kg/day of the test material.

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

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

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

incidences of green or brown intracytoplasmic pigment in Kupffer cells and mixed inflammatory cell infiltrate of the liver were observed in males and females administered 400 mg/kg/day. In addition, hypoplasia/atrophy of the ovary and uterus occurred in all females administered 400 mg/kg/day. The degree, but not the incidence, of extramedullary hematopoiesis in the spleen was increased at 400 mg/kg/day in both sexes as compared with controls. **The lowest observed effect level (LOEL) for male and female mice administered nitrapyrin is 300 and 400 mg/kg/day, respectively, based on liver toxicity (increased liver weights and hepatocellular hypertrophy). A no-observed-effect level (NOEL) is 200 mg/kg/day supported by elevated liver enzymes.**

This subchronic toxicity study is classified **acceptable (guideline)** and does satisfy the guideline requirement for a subchronic oral study (82-1b) in mice.

870.3150 90-Day Oral Toxicity - Dog

Refer to the chronic dog study below.

870.3200 21/28-Day Dermal Toxicity – Rat

Executive Summary: In a 21-day dermal toxicity study (1992, MRID No.: 42239301), four groups of 5 male and 5 female New Zealand White Rabbits were dosed with 0, 100, 500 or 1000 mg/kg/day for 6 hours/day, 5 days/week over a three week period.

At 1000 mg/kg/day there were increased liver absolute (17% for males and 20% for females, $p < 0.05$) and relative (20% for males and 22% for females, $p < 0.05$) weights. There were no concurrent histological or clinical chemistry changes associated with the liver weight increases. Local site of application dermal irritation was noted in all treated dose groups and was described as very slight to well-defined erythema and/or very slight edema. **The LOAEL is 1000 mg/kg/day based on liver weight effects. The NOAEL is 500 mg/kg/day.**

Classification. This 21-day dermal toxicity study is classified as ACCEPTABLE/Non-Guideline. Since the study assessed only 5 animals/sex/dose rather than the 10/sex/dose currently required by the 870.3200 guidelines the study does not satisfy the guideline requirement for a 21 day dermal toxicity study.

870.3465 90-Day Inhalation – Rat

There is no subchronic inhalation toxicity study with nitrapyrin.

4.3 Prenatal Developmental Toxicity

Adequacy of Data Base for Prenatal Developmental Toxicity: The data base for prenatal developmental toxicity is considered complete. No additional studies are required at this time. Neither the rat or rabbit developmental toxicity studies demonstrated increased sensitivity to the fetuses.

Reduced body weight and food consumption were the most evident effects in dams. Slightly reduced fetal weight as well as possible skeletal variations and delayed ossification in rat pups and crooked hyoid in rabbit pups were evident in the offspring.

870.3700a. Prenatal Developmental Toxicity Study - Rat

Executive Summary. In a developmental toxicity study (1994, MRID No.: 43210302), 28 mated Crl:CD(SD) BR rats/dose group received 0, 15, 50 or 120 mg nitrapyrin/kg/day (technical, 92% a.i.) in corn oil by gavage from gestation days 6 through 15, inclusive. These dose levels were selected based on a dose range finding study (1994, MRID No.: 43210301). There were 25, 26, 25 and 23 litters that produced live fetuses for the control, 15, 50 or 120 mg/kg/day dose groups, respectively.

Maternal toxicity. At 120 mg/kg/day, significantly reduced mean maternal body weight gain (35% less than control gain, days 6-16) and reduced body weight (7%) were noted. Food consumption was also reduced during the first three days of treatment (13%). A significant reduction in maternal weight gain (13%) at 50 mg/kg/day was attributed to decreased uterine weight not related to treatment. **The LOAEL for maternal toxicity is 120 mg/kg/day based on decreased body weight gain and reduced food consumption. The NOAEL is 50 mg/kg/day.** *Developmental toxicity*. There were a total of 393, 398, 379 and 356 live fetuses available for evaluation for the control, 15, 50 and 120 mg/kg/day dose groups. At 120 mg/kg/day, slightly reduced (5.5%) mean fetal body weight in female fetuses and increases (not significant) in rudimentary 1st lumbar ribs (about 4 times the control incidence, in litter incidence), and incompletely or unossified 4th, 5th, and 6th sternebrae (about 1.6 to 3 fold over the control litter incidence) were noted. **The LOAEL for developmental toxicity is 120 mg/kg/day based on marginally decreased fetal weight in females and possible increased incidence of skeletal variations and delayed ossification. The NOAEL is 50 mg/kg/day.**

Classification. This developmental toxicity study in rats is classified as ACCEPTABLE/ GUIDELINE and satisfies the requirement for a series 83.3 developmental toxicity study in rats.

In addition to the above study, there is an earlier study (1986, MRID No.: 00163792), that was classified as Supplementary since the dose levels were considered inadequate for testing. The study could not conclusively establish that there was sufficient toxicity at 50 mg/kg/day, the highest dose tested.

870.3700b. Prenatal Developmental Toxicity Study - Rabbit

Executive Summary. In a prenatal developmental toxicity study (1985, MRID 00153543), Nitrapyrin (91.9% a.i., Lot# 840319 [AGR 213226]) in corn oil was administered to artificially inseminated New Zealand White rabbits (25-27/dose) via gavage in a dosing volume of 1 mL/kg at concentrations of 0, 3, 10, or 30 mg/kg/day on gestation days (GD) 6 through 18. Control animals were dosed with corn oil alone. All does were sacrificed on GD 28 and their uterine contents examined. Data were not

included in the study report for food consumption, uterine weights, and adjusted (for gravid uterus) maternal body weights.

There were no effects of treatment on maternal survival, clinical signs, or gross pathology. One 10 mg/kg doe died on GD 25, and one 30 mg/kg doe died on GD 22. These deaths were attributed to inanition resulting from the presence of a hair ball in each case. At 30 mg/kg, maternal body weight gains were decreased ($p<=0.05$) during GD 12-15 (-86 g treated vs -22 g controls), resulting in decreased ($p<=0.05$) body weight gains for the overall (GD 6-19) treatment interval (-275 g treated vs -112 g controls). Absolute and relative (to body) liver weights were increased ($p<=0.05$) by 22-24% over controls. **The maternal LOAEL is 30 mg/kg/day based on decreased body weight gains and increased absolute and relative (to body) liver weights. The maternal NOAEL is 10 mg/kg/day.**

There were no effects of treatment on the number of litters, litter size, resorptions/litter, percent of litters with resorptions, number of fetuses (live or dead), post-implantation loss, fetal body weights, or sex ratio. Incidences of crooked hyoid were increased ($p<=0.05$) at 30 mg/kg (19% fetuses; 57% litters) over concurrent controls (5% fetuses; 32% litters) and historical controls (0-9.4% fetuses; 0-53% litters). There were no treatment-related external or visceral abnormalities in the fetuses. **The developmental LOAEL is 30 mg/kg/day based on increased incidences of crooked hyoid. The developmental NOAEL is 10 mg/kg/day.**

This study is classified **acceptable/guideline** and satisfies the guideline requirements for a developmental toxicity study in the rabbit (OPPTS 870.3700; §83-3[b]).

4.4 Reproductive Toxicity

Adequacy of data base for Reproductive Toxicity: The data base for reproductive toxicity is considered complete. No additional studies are required at this time. There was no indication of increased susceptibility to the offspring. The liver was affected in both the parental and offspring groups. In the parental group, the kidney was also affected.

870.3800 Reproduction and Fertility Effects - Rat

Executive Summary. In a multi-generation reproduction toxicity study (1988, MRID 40952701), Nitrapyrin (93.3% a.i.; Lot #WP 860 516-308B) was administered in the diet to Fisher 344 rats (30/sex/dose) at dose levels of 0, 5, 20, or 75 mg/kg/day. The P animals were dosed for approximately 10 weeks prior to mating to produce the F_1 litters. After weaning at approximately 4 weeks of age, F_1 animals were randomly selected to become the parents of the F_2 generation and were dosed for approximately 12 weeks prior to mating. After weaning of each generation and selection of animals chosen to be parents of the next generation, the original parental animals and 10 weanlings/sex/dose were sacrificed and submitted for gross necropsy and histopathological examination.

Parental Toxicity. There were no treatment-related effects on survival or clinical signs. Absolute and/or relative **liver** weight were increased in the 5 mg/kg/day dose group (9% and 4% for the P group and 4% (ns) and 3%, otherwise all $p < 0.05$ for the F1 group). At 20 mg/kg/day, absolute (17% and 13%) and relative (8% and 12%) and at 75 mg/kg/day absolute (60% and 49%) and relative (51% and 59%) increases in liver absolute and relative liver weight were noted for the P and F1 parental group males. Liver weight was affected in females only at 20 mg/kg/day for the F1 absolute weight (7%, $p <= 0.05$) and in the 75 mg/kg/day dose group (22 to 25% absolute and 27 to 36% relative). Thus, there was a dose response for liver weight effects with males being more sensitive. Pathologically, at necropsy, the liver of nearly all P and all F1 group males dosed at 75 mg/kg/day were described as increased in size. Only one F1 male and 1 each P and F1 female were characterized as being large. Centrilobular diffuse hypertrophy described as slight was present in 28 males and 2 females in the 20 mg/kg/day P group and in 20 males in the F1 group. At 75 mg/kg/day, hepatic hypertrophy was described as moderate for most or all males and most females were affected with either slight or moderate hypertrophy. The liver condition was also characterized as involving the centrilobular region and extended to the midzonal region and consistent with fatty change (either slight or moderate) for all males and 28 of 30 females at 75 mg/kg/day and a single male at 20 mg/kg/day had this condition.

Kidney weights were also increased in the 20 mg/kg/day males (12% and 5% for the P group and 14% and 5% for the F1 group for both absolute and relative weight respectively) in males but only 4 to 6% in females. At 75 mg/kg/day, absolute and relative kidney weights were increased 10 to 18% in males and 7 to 16% in females. Pathologically, at the 75 mg/kg/day dose group only, the kidney weight increases were associated with intratubular mineralization and multifocal necrosis of intratubular epithelium in the P group and there was also multifocal dilated tubules with proteinaceous casts in the F1 group. **The LOAEL is 20 mg/kg/day based on increases in liver and kidney weight and on the presence of hepatic centrilobular diffuse hypertrophy. The NOAEL is 5 mg/kg/day.**

Offspring toxicity. At 75 mg/kg/day, pup weights were decreased ($p<=0.05$) by 7-21% in the F1 generation from post-natal day (PND) 4 to 28 and from 6-24% in the F2 generation from PND 1 to 28. Note that pup weights were increased ($p<=0.05$) by 31% in the F1 generation on PND 1. While litter size was unaffected by treatment, there was a slight increase in the number of runts at 75 mg/kg/day (7.4-15.4% fetuses; 21.7-23.5% litters) compared to controls (1.8-3.3% fetuses; 5.6-9.1% litters). Enlarged liver was noted in the F2 males (7/10) and females (10/10). Very slight to slight diffuse centrilobular vacuolation consistent with fatty change was observed at 75 mg/kg/day in both sexes and generations (7-10/10 each treated vs 0/10 each controls). **The LOAEL for offspring toxicity is 75 mg/kg/day based on decreased body weights and increased hepatic centrilobular vacuolation consistent with fatty change in both sexes and generations. The NOAEL for offspring toxicity is 20 mg/kg/day.**

Reproductive toxicity. There were no effects of treatment on mating, conception, gestation, gestation survival (live birth), or lactation survival (viability and lactation) indices or on sex ratio or gestation duration in either generation. **The LOAEL for reproductive toxicity was not observed. The NOAEL for reproductive toxicity is 75 mg/kg/day.**

This study is classified **acceptable/guideline** and satisfies the guideline requirements for a multi-generation reproductive toxicity study in the rat (OPPTS 870.3800; OECD 416).

4.5 Chronic Toxicity

Adequacy of data base for chronic toxicity: The data base for chronic toxicity is considered complete. No additional studies are required at this time. The dog and the rat were about equally sensitive to nitrapyrin with the liver being affected in the dog (liver weight, enzyme changes and hypertrophy and cholesterol). In the rat, body weight was affected and there were liver and kidney effects including indications that nitrapyrin fits the alpha 2µ globulin model for kidney toxicity.

870.4100a (870.4300) Chronic Toxicity – Rat

Executive Summary. In a chronic toxicity and carcinogenicity study (1989, MRID 41345403), 50 Fischer 344 rats/sex/dose were exposed to nitrapyrin (93.3% a.i.; Batch #: AGR229141) in the diet at concentrations of 0, 5, 20, or 60 mg/kg/day for up to 24 months. An additional group of 10 rats/sex/dose were similarly treated and sacrificed at 12 months.

Clinical signs, food consumption, hematology, and urinalysis for both sexes at all doses were unaffected by treatment. No treatment-related differences in any parameter were observed in the 5 mg/kg/day group.

In the 20 mg/kg/day males, body weights were generally similar to controls. Body wight at Week 105 was decreased ($p<=0.05$) by 7%. Body wight gain (Weeks 53-105) was decreased severely (loss of 24.6 g in treated vs a loss of 0.8 g in controls). Overall body weight gain (Weeks 0-105) was decreased by 9%. At 60 mg/kg/day, body weights were often decreased ($p<=0.05$) by 2-15% in males during Weeks 69-105 and by 2-10% in females during Weeks 7-105. Body weight gain in the last year of the study was decreased severly in males (loss of 55.8 g in treated vs a loss of 0.8 g in controls) and females (13 g treated vs 33.2 g controls). Overall body wight gain (Weeks 0-105) was decreased by 15-20% in both sexes. Evidence of nephrotoxicity was observed. Blood urea nitrogen levels were increased ($p<=0.05$) by 12-115% in males at Months 6, 18, and 24 and in females by 16% at Month 24. At Month 24, relative kidney to body wight increased ($p<=0.05$) by 7% in males at Month 18 and by 13-16% in both sexes at Month 24. In females at Month 24, alanine aminotransferase, cholesterol, and total bilirubin were increased 40-88%. Absolute and relative to body liver weights were increased ($p<=0.05$) in both sexes by 13-31% at Months 12 and by 41-67% at Month 24. At Month 12, incidences of slight to moderate hepatocentrilobular hypertrophy and centrilobular vacuolization, consistent with a fatty change, were increased in both sexes (20 treated vs 0 controls, each lesion, n=10/sex). At Month 24, the liver surface appeared roughened upon gross examination in the females (11 treated vs 4 controls; n=50). At Month 24, the incidence of very slight to slight hepatocentrilobular hypertrophy was increased ($p<=0.05$) in both sexes (20-30 treated vs 0-3 controls, n=50), and the incidence of very slight to slight hepatocentrilobular vacuolation, consistgent with a fatty change, was also increased ($p<=0.05$) in both sexes (23028 treated vs 1-3 controls). **The**

LOAEL is 20 mg/kg/day, based on decreased body weight gain in males. The NOAEL is 5 mg/kg/day.

In the control and 60 mg/kg/day groups at Month 12, immunoperoxidase staining for $\alpha_{2\mu}$-globulin in the cytoplasm of cells lining the renal proximal convoluted tubules resulted in moderate to numerous cell staining in 9/10 males vs 0/10 in controls and in females. At Month 12, proteinaceous casts and protein droplet nephropathy were observed dose-dependently in the males in incidence and severity. At Month 24, chronic nephropathy prevented accurate measurement of $\alpha_{2\mu}$-globulin, but chronic progressive glomerulonephropathy, tubule adenoma, and tubule adenocarcinoma were observed dose-dependently in males in incidence and severity. Furthermore, the increased mortality observed in the 60 mg/kg/day males was due to chronic progressive nephropathy (10 treated vs 1 control, and 13 rats died of other causes in treated vs 11 in controls). An increase in renal tubular tumors was observed only in the 60 mg/kg/day males, which were also shown to have an excess of $\alpha_{2\mu}$-globulin in the renal tubules. Adenomas (3 treated vs 0 controls) and adenocarcinomas (3 treated vs 0 controls) were observed in the 60 mg/kg/day males, and a total of 6 of the 60 mg/kg/day males had at least one primary renal tumor (vs 0 in all other dose groups). Overall, nitrapyrin can be classed in the α2u globulin model for a male rat specific kidney lesion that is not related to humans.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4300; OECD 453) for a combined chronic toxicity/carcinogenicity study in rats.

870.4100b Chronic Toxicity - Dog

Executive Summary: In a chronic toxicity study (1989, MRID 41345401), nitrapyrin (92.8% a.i.; Lot # WP860516-308B [T19B]) was administered to 4 beagle dogs/sex/dose in the diet for up to 53 weeks at nominal doses of 0, 0.5, 3, or 15 mg/kg/day.

No compound-related effects were observed on mortality, clinical signs, ophthalmology, body weight, body weight gain, food consumption, hematology, urinalysis, or gross pathology. At 15 mg/kg/day, hepatotoxicity was evident. Differences ($p \leq 0.05$) from the control were detected by time-dose interaction in alkaline phosphatase and cholesterol levels. Alkaline phosphatase levels increased with the duration of exposure, and increases were observed in males at 6 and 12 months (96-187%) and females throughout the study (30-137%). Cholesterol levels increased throughout the study in both sexes by 35-64%. Differences ($p \leq 0.05$) from the control were detected in absolute and relative to body liver weights at this dose which were increased by 21-49% in both sexes. Slight diffuse central lobular and midzonal hypertrophy was observed in all males (vs none in controls). Slight diffuse midzonal and centrilobular or panlobular hypertrophy was observed in all females (vs none in controls). **The LOAEL is 15 mg/kg/day, based on increased alkaline phosphatase, cholesterol, absolute and relative to body liver weights, and liver hypertrophy in both sexes. The NOAEL is 3 mg/kg/day.**

This study is acceptable (guideline) and satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in dogs.

4.6 Carcinogenicity

Adequacy of data base for Carcinogenicity: The data base for carcinogenicity is considered complete. No additional studies are required at this time. Please refer to Section 5.3 below for discussion of the cancer issues and classification.

870.4200a Carcinogenicity Study - rat

Refer to chronic feeding - rat study above.

870.4200b Carcinogenicity (feeding) - Mouse

Executive Summary: In a carcinogenicity toxicity study (MRID 44231803), nitrapyrin (95.4% a.i., lot no. TSN 100319) was administered to groups of 50 male and 50 female $B6C3F_1$ mice in the diet at concentrations delivering doses of 0, 125, or 250 mg/kg/day for 24 months. Ten additional mice of each sex per dose were given the same diets for interim evaluations at 12 months. A special study on hepatocyte proliferation and apoptosis (MRID 44231801) was conducted utilizing five $B6C3F_1$ mice of each sex per dose. Nitrapyrin (purity and lot no. not reported) was given in the diet at concentrations delivering doses of 0, 200, or 400 mg/kg/day for 2 weeks.

Systemic toxicity. Mortality was not significantly affected by nitrapyrin treatment. Body weights of high dose males were decreased by 3 to 13% ($p < 0.05$) from day 19 to study termination, and body weight gains were decreased by 15 to 34% ($p < 0.05$). No significant differences were seen in food consumption or efficiency. There were no treatment-related changes in hematology parameters.

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

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

After 12 months of treatment with nitrapyrin, liver cell proliferation in the centrilobular region was significantly increased in male mice at 125 and 250 mg/kg/day. The increases seen in the centrilobular region of the liver of females and the periportal region of both sexes did not achieve statistical significance. A special 2-week study (MRID 44231801) with higher doses showed an increased liver cell proliferation index in the centrilobular region at 200 and 400 mg/kg/day and in the periportal regions at 400 mg/kg/day in males; increases in the periportal region in females did not achieve statistical significance. No statistically significant differences were seen in liver cell apoptosis or in cell proliferation of the nonglandular stomach of treated mice compared to control animals. **The LOEL for this study is 125 mg/kg/day, based on lesions in the liver (hepatocellular hypertrophy and single-cell necrosis) and digestive tract (hyperkeratosis and hyperplasia of the nonglandular stomach and epithelial cell vacuolation and hyperplasia/hypertrophy of the duodenum and jejunum in both sexes). The NOEL was not determined.**

Carcinogenicity assessment. At the doses tested, there was a treatment related increase in the incidence of hepatocellular adenomas and adenomas/carcinomas combined and squamous cell papillomas and papillomas/carcinomas combined in the nonglandular stomach when compared to controls. The masses and nodules noted in the liver and nonglandular stomach were due primarily to neoplasms. At the low and high doses, the incidence of hepatocellular adenomas was 38% (N.S.) and 90% ($p<0.05$) vs 24% in controls for males and 27% ($p<0.05$) and 64% ($p<0.05$) vs 12% in controls for females. The incidence of papillomas in the nonglandular stomach was 18% ($p < 0.05$) and 24% ($p < 0.05$) vs 2% in the controls for males and 16% ($p < 0.05$) and 42% ($p < 0.05$) vs 2% in controls for females. The incidences of carcinomas only in the liver or nonglandular stomach did not achieve statistical significance in treated groups compared with controls. The incidence of Harderian gland adenomas/carcinomas increased 16% and 18% in low and high dose females, respectively, vs 2% in controls ($P < 0.05$). The doses were adequate based ≤12% decrease in body weight and no effect on mortality; both doses, however, resulted in overt toxicity in the target organs: liver and nonglandular stomach.

This carcinogenicity study in the mouse is **acceptable (guideline)** and does satisfy the requirements for a carcinogenicity study (83-2 b) in the mouse. Despite some deficiencies this study demonstrated carcinogenicity of the test material at the doses tested.

4.7 Mutagenicity

Adequacy of data base for Mutagenicity: The data base for mutagenicity is considered adequate based on as per the pre 1991 mutagenicity guidelines. Nitrapyrin is not considered to have a

mutagenicity or genetic toxicity concern.

Gene Mutation

Ames test. Microtest Research, Study No.: DCE 3/S/S5/AF2, January 18, 1985. Accession No.: 259818.	No evidence of mutagenic activity in strains TA-97, TA-98, TA-100 and TA 1535 in the presence or absence of activation. Classification-Acceptable (W. Woodrow reviewer)

Cytogenetics

Mammalian gene mutation. Health and Environmental Sciences, Study No.: TXT:K-031304-022, August 1986. MRID No.: 00163805.	Negative for genotoxic effect in the presence (up to 200 µg/mL) and absence (up to 100 µg/mL) of S9 in an *in vitro* mammalian gene mutation test with cultured (CHO/HGPRT) cells. Classification - Acceptable (W. Dykstra reviewer).
Micronucleus test. Microtest Research Ltd. Study No.: DCE 3/MNT/AR/KF11, March 13, 1985. Accession No.: 259818.	No evidence of mutagenic potential in the mouse micronucleus test at a dose of 800 mg/kg. Classification - Acceptable (W. Woodrow reviewer).

Other Genotoxicity

Unscheduled DNA synthesis. DOW Chemical Co.. No study number provided, June 5, 1982. MRID No.: 00109456.	No increase in unscheduled DNA synthesis in the rat hepatocyte UDS assay at up to 10^{-4} moles/liter. Classification - Acceptable (W. Woodrow)

4.8 Neurotoxicity

Adequacy of data base for Neurotoxicity: These studies are not required at this time.

870.6100 Delayed Neurotoxicity Study - Hen

This study is not required since nitrapyrin is not an organophosphate insecticide or cholinesterase inhibitor.

870.6200 and 870.6300. Acute and Subchronic Neurotoxicity Screening Battery and Developmental Neurotoxicity Study.

There are no 870.6200 acute and subchronic neurotoxicity screen or 870.6300 developmental neurotoxicity study. The HIARC (12/11/03) recommended that these studies should not be required since there is no evidence of neurotoxicity in the subchronic, chronic, reproductive or developmental toxicity studies. Also nitrapyrin does not belong to a class of chemicals known to affect the nervous system.

4.9 Metabolism

Adequacy of data base for metabolism: The data base for metabolism is considered to be complete. No additional studies are required at this time. Nitrapyrin is absorbed from the gastro-intestinal tract and metabolized to 6-chloropicolinic acid and its glycine conjugate eliminated in the urine.

870.7485 Metabolism - Rat

Executive Summary. In a metabolism study (1987, MRID No.: 40305501) with Fischer 344 strain rats, ^{14}C-nitrapyrin labeled in the 2 and 6 positions was administered to three groups as a) single dose of 60 mg/kg; b) single dose of 1 mg/kg; and c) 14 daily doses of non-labeled nitrapyrin at 1 mg/kg/day and a single oral dose of radiolabeled nitrapyrin at 1 mg/kg/day on day 15. Urine and feces were collected for 72 hours. After 72 hours, the rats were sacrificed and samples of bone, liver, kidneys, fat, gonads, lung, heart, blood (RBC and plasma), skeletal muscle, spleen, skin and remaining carcass were analyzed for radioactivity.

Total recovery ranged from 94.81% to 99.24%. Absorption and elimination was rapid and essentially complete by 72 hours. Most (79.56% to 85.48%) of the radioactivity was recovered in the urine and a smaller amount (11.04% to 13.63%) in the feces. Very little (0.51% to 0.95%) remained in the tissue. There was no unchanged nitrapyrin in the urine and 6-chloropicolinic acid and its glycine conjugate were identified as the metabolites. The glycine conjugate was more common in females and for both sexes following multiple dosing.

This study is classified as ACCEPTABLE/GUIDELINE and satisfies the guideline requirement for a series 85-1 general metabolism study in rats.

870.7485 Metabolism - Mouse

Executive Summary: In a metabolism study (1998, MRID 44679301) groups of 10 male B6C3F$_1$ mice were given single oral doses (25 or 250 mg/kg) of Nitrapyrin (Lot no. 1321, 99.9% purity [99+% radiochemical purity]). Additionally, two groups of three male mice were given either the low or high dose to assess acute toxicity and another group given no test material (controls). To assess interspecies

variability in urinary metabolite profiles, two male F344 rats were given single oral doses of 60 mg/kg.

No overt signs of toxicity or histopathologic changes were observed. Dose confirmation indicated that the administered doses represented 95-103% of the nominal radioactivity and 102-106% of the nominal Nitrapyrin. Mean total recovery of administered radioactivity (% of dose) was 99.38% and 100.82%, respectively, for the low and high dose groups. Total absorption over a 72-hour period, implied from urinary excretion/cage wash and tissue/carcass burden data was 76.89% and 82.76% for the low and high dose groups, respectively. Urinary excretion assessed over a 72-hour period accounted for 76.12% and 82.21%, respectively, of the low- and high-dose, most of which occurred within 36 hours of dosing. Fecal excretion over the 72-hour period accounted for 21.55% and 16.04%, respectively, of the low- and high-dose. Under the conditions of this study, neither absorption nor excretion of Nitrapyrin appeared to be saturated. Tissue/carcass burden at 72 hours post dosing was minimal and accounted for only 0.77% (low dose) and 0.64% (high dose) of the administered radioactivity (1.8 and 9.7 µg eq./g, respectively for the low and high dose). At the doses tested, Nitrapyrin did not exhibit potential for tissue accumulation following a single oral dose in mice.

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

This metabolism study in mice is Acceptable/Non-Guideline. It partially fulfills the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (85-1)]. By design, the study was to determine metabolism and disposition in the mouse following a high dose (tumor-producing) and low dose (non-tumorigenic) oral exposure to Nitrapyrin. The study achieved this objective.

870.7600 Dermal Absorption - Rat

Executive Summary. In a dermal penetration study (1997, MRID 44282501), ^{14}C-nitrapyrin (99% a.i., Lot # B-844-118A, label position not reported, specific activity 5.2 mCi/mmole) was administered to two groups of four male Fischer 344 rats. The rats were exposed to 1.0 mg/cm^2 radiolabeled test material on a 10 cm^2 shaved anterior intrascapullary area. Twenty-four hours after treatment, the skin of all rats was washed and four rats (Group I) were sacrificed. The remaining four rats (Group II) were

sacrificed 48 hours after skin wash (72 hours after application). From both treatment groups, excreta were collected at 24 hour intervals and the liver, kidney, blood, skin at the treatment site, skin from a remote site, and carcass were collected at sacrifice, and bandages and other application apparatuses were collected for radioanalysis.

Total recovery for Group I (24 hour sacrifice) was approximately 90% and for Group II (72 hour sacrifice) was 83%. Based on the amount of radiolabel recovered in the excreta, tissues and carcass, ~24.6±6.4%% of the ^{14}C-nitrapyrin was absorbed during the first 24 hours of treatment and ~34.6±11% of the dose was absorbed after 72 hours. Since excess material was washed from the skin at 24 hours, the amount remaining on the skin after washing has high potential to be absorbed. Most of the absorbed radiolabel (>78%) was excreted in the urine.

This study in the rat is **Acceptable/Non-guideline** but satisfies the intent of determining dermal penetration of the test material in male rats. The study does not satisfy the guideline requirements of sample collection 1 and 10 hours after dosing; the location of the radiolabel in the test molecule was not given; and determining the residual radioactivity remaining in the dose application syringe for mass balance purposes.

4.10 Special/Other Studies

There are several studies with 6-chloropicolinic acid, a metabolite of nitrapyrin and these can be found under PC Code 069206.

5.0 TOXICITY ENDPOINT SELECTION

5.1 See Section 9.2 for Endpoint Selection Table.

5.2 Dermal Absorption

Dermal Absorption Factor: 46 % based upon the dermal absorption study (1997, MRID 44282501) in rats (refer to section 4.9 above). Not upper limit of the dose absorbed after 72 hours was selected.

5.3 Classification of Carcinogenic Potential

As per the CARC report dated May 5, 2000, nitrapyrin is classified as **"likely to be a carcinogen in humans"** using the criteria in the Draft Guidelines for Carcinogen Risk Assessment (July, 1999) based on the following weight-of-the-evidence:

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

2. Nitrapyrin was not mutagenic in submitted studies, however, NTP reported that the compound was mutagenic in *Salmonella typhimurium* strains TA97, TA98 and TA 100 in the presence of S9 activation.

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

5.3.3 Quantification of Carcinogenic Potential

Nitrapyrin is classified as "likely to be a human carcinogen" based on the mouse study which demonstrated liver tumors, stomach tumors and Harderian gland neoplasm. The Q1* was determined to be 4.25×10^{-2} human equivalents.

6.0 FQPA CONSIDERATIONS

6.1 Special Sensitivity to Infants and Children

The HIARC concluded that there is not a concern for pre- and/or postnatal toxicity resulting from exposure to nitrapyrin.

A. Determination of Susceptibility

Neither the rat or rabbit developmental toxicity studies or the rat multi generation reproduction toxicity study demonstrated increased susceptibility of the fetuses or offspring.

B. Degree of Concern Analysis and Residual Uncertainties

N/A

C. Special FQPA Safety Factor(s):

The HIARC determined that the Special FQPA Safety Factor should be 1 X since there was no evidence of increased susceptibility.

The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

6.2 Recommendation for a Developmental Neurotoxicity Study

The HIARC concluded that there is not a concern for developmental neurotoxicity resulting from exposure to nitrapyrin.

A. Evidence that suggest requiring a Developmental Neurotoxicity study:

None.

B. Evidence that do not support a need for a Developmental Neurotoxicity study:

There is no evidence of neurotoxicity in the rat or dog subchronic studies or in the rat or rabbit developmental toxicity studies with nitrapyrin.

Based on the weight of evidence presented, the HIARC concluded that a developmental neurotoxicity study is not required for nitrapyrin.

7.0 OTHER ISSUES

There are no outstanding issues with nitrapyrin at this time.

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9.0 APPENDICES

Tables for Use in Risk Assessment

9.1 Toxicity Profile Summary Tables

9.1.1 Acute Toxicity Table - See Section 4.1

9.1.2 Subchronic, Chronic and Other Toxicity Tables

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100 90-Day oral toxicity rats	Refer to 870.4300 rat chronic feeding/carcinogenicity study below	
870.3100 90-Day feeding - mouse. Pharmaco LSR, Study No.: 93-2278, May 25, 1995	44231802 (1995) 0, 200, 300 (♂ only), 400, 600 or 800 (♀ only) mg/kg/day. Acceptable/Guideline	NOAEL = 200 mg/kg/day LOAEL = 300 mg/kg/day in males and 400 mg/kg/day in females based on increased liver weight and hepatocellular hypertrophy and enzyme changes. .
870.3150 90-Day oral toxicity in nonrodents	Refer to 870.4100 dog chronic study below.	
870.3200 21/28-Day dermal - rats Health and Environmental Sciences, Study No.: K-031304-031, Feb. 13, 1992.	42239301 (1992) 0, 100, 500 or 1000 mg/kg/day. Acceptable/Non-Guideline.	NOAEL = 500 mg/kg/day LOAEL = 1000 mg/kg/day based on liver weight effects.
870.3250 90-Day dermal toxicity	No study available and not required at this time.	
870.3465 90-Day inhalation toxicity	No study available and not required at this time.	

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3700a Prenatal develop-mental - rats. Pharmaco LSR, Study No.: 93-4050, April 14, 1994.	43210302 (1994, main study) and 43210301 (pilot study). 0, 15, 50 or 120 mg/kg/day (main study). Acceptable/Guideline	**Maternal** NOAEL = 50 mg/kg/day LOAEL = 120 mg/kg/day based on reduced body weight/weight gain and reduced food consumption. **Developmental** NOAEL = 50 mg/kg/day (threshold). LOAEL = 120 mg/kg/day based on possible increase incidence of skeletal variations and delayed ossification and on marginally decreased fetal body weight in female pups only.
870.3700b Prenatal develop-mental - rabbits Dow Laboratory, Study No.: HET 031304-012, October 23, 1985	00153543 (1985) 0, 3, 10 or 30 mg/kg/day Acceptable/Guideline	**Maternal** NOAEL = 10 mg/kg/day LOAEL = 30 mg/kg/day based on decreased body weight gains and increased absolute and relative liver weights. **Developmental** NOAEL = 10 mg/kg/day LOAEL = 30 mg/kg/day based on increased incidence of crooked hyoid.
870.3800 Reproduction. Health and Envir-onmental Sciences, Study No.: TXT:K-030304-025, Dec. 27, 1988.	40952701(1988) Acceptable/Guideline 0, 5, 20 or 75 mg/kg/day.	**Parental/Systemic** NOAEL = 5 mg/kg/day LOAEL = 20 mg/kg/day based on increases in liver and kidney weight and presence of hepatic centrilobular diffuse hypertrophy. **Reproductive** NOAEL = 75 mg/kg/day. LOAEL = not observed - no effects a the highest dose tested. **Offspring** NOAEL = 20 mg/kg/day LOAEL = 75 mg/kg/day based on decreased body weight and increased hepatic centrilobular vacuolation consistent with fatty changes in both sexes and generations.
870.4100a Chronic toxicity rodents	Refer to 870.4300 rat chronic feeding/carcinogenicity study below	
870.4100b Chronic toxicity dogs. DOW Chemical Co. Study # TXT:K-031304-029, Dec. 27, 1989.	41345401(1989) Acceptable/Guideline 0, 0.5, 3 or 15 mg/kg/day	NOAEL = 3 mg/kg/day LOAEL = 15] mg/kg/day based on increased alkaline phosphatase, cholesterol, absolute and relative liver weights and liver hypertrophy in both sexes. .

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.4200 Carcinogenicity mice. Dow Toxicology laboratory, Study No.: K-031304-036, Feb. 27, 1997.	44231803 and 44231801(1997). Acceptable/Guideline. 0, 125 or 250 mg/kg/day.	NOAEL < 125 mg/kg/day based on hepatocellular hypertrophy and single cell necrosis and hyperkeratosis and hyperplasia of the non-glandular stomach and epithelial cell vacuolation and hyperplasia/hypertrophy of the duodenum and jejunum in both sexes. LOAEL - not established. **Hepatocellular adenomas and adenomas/carcinomas combined and squamous cell papillomas and papillomas/carcinomas combined in the nonglandular stomach.**
8704300. Combined chronic feeding and carcinogenicity study - rats Health and Environmental Science, Study No.: TXT:K-031304-023, Dec. 26, 1989	41345403 (1989) 0, 5, 20 or 60 mg/kg/day. Acceptable/Guideline	NOAEL = 5 mg/kg/day LOAEL = 20 mg/kg/day based on decreased body weight gain in males. Increase in kidney tumors related to the alpha 2µ globulin model.
Gene Mutation 870.5100. Ames test. Microtest Research, Study No.: DCE 3/S/S5/AF2, January 18, 1985.	Accession No.: 259818. (1985) Acceptable (W. Woodrow reviewer)	No evidence of mutagenic activity in strains TA-97, TA-98, TA-100 and TA 1535 in the presence or absence of activation.
Cytogenetics 870. 5300. Mammalian gene mutation. Health and Environmental Sciences, Study No.: TXT:K-031304-022, August 1986.	MRID No.: 00163805. Acceptable (W. Dykstra reviewer).	Negative for genotoxic effect in the presence (up to 200 µg/mL) and absence (up to 100 µg/mL) of S9 in an *in vitro* mammalian gene mutation test with cultured (CHO/HGPRT) cells.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
Cytogenetics 870.5375. Micro-nucleus test. Microtest Research Ltd. Study No.: DCE 3/MNT/ AR/KF11, March 13, 1985.	Accession No.: 259818. Acceptable (W. Woodrow reviewer).	No evidence of mutagenic potential in the mouse micronucleus test at a dose of 800 mg/kg.
Other Effects 870.5550. Unscheduled DNA synthesis. DOW Chemical Co.. No study number provided, June 5, 1982.	MRID No.: 00109456. Acceptable (W. Woodrow)	No increase in unscheduled DNA synthesis in the rat hepatocyte UDS assay at up to 10^{-4} moles/liter.
870.6200a Acute neurotoxicity screening battery	No studies available and not required.	
870.6200b Subchronic neurotoxicity screening battery		
870.6300 Developmental neurotoxicity		
870.7485 Metabolism and pharmacokinetics DOW Chemical Co. Study No.: K031304, August 11, 1987.	40305501.	Total recovery ranged from 94.81% to 99.24%. Radio-labeled nitrapyrin was rapidly absorbed (T1/2 = 1.2 to 3.2 hours and excreted in urine (~80 to 86%) with excretion essentially complete by 72 hours; < 1% remained in the tissues. 6-chloropicolinic acid and its glycine conjugate identified as urinary metabolites.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.7485. Metabolism study in mice. Health and Environmental Research Labs. Study No.: 971119, Sept. 30, 1998.	44679301 (1998) Acceptable-Guideline Doses tested:25 or 250 mg/kg.	Mean total recovery was 99.38 to 100.82%. && to 83% absorbed based on urinary excretion. < 1% remained in tissues at 72 hours. Four distinct urinary metabolites detected by HPLC: 6-chloropicolinic acid (6-CPA), 6-CPA-glycine conjugate, CPA-taurine conjugate and parent (in high dose group only - possibly indicating saturation of metabolism).
870.7600 Dermal penetration Dow health and Environmental Research Laboratories, Study No.: HET K-031304-039, March 25, 1997.	44282501 radiolabeled nitrapyrin. Acceptable/Non-guideline	~26±6.4% recovered in the excreta in 24 hours. ~34.6±11% recovered after a total of 72 hours. Overall an estimate of dermal absorption in up to 46%.
Special studies	None with parent. It is noted that there are toxicity studies with the metabolite 6-CPA.	

9.2 Summary of Toxicological Dose and Endpoints for Nitrapyrin for Use in Human Risk Assessment[1]

Summary of Toxicological Dose and Endpoints for Nitrapyrin

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary - all populations.	No study was selected for this scenario since neither the rat or rabbit demonstrated definite toxicity following a single dose and developmental toxicity was not a concern for nitrapyrin.		
Chronic Dietary (All populations)	NOAEL= **3** mg/kg/day UF = 100 **Chronic RfD** = **0.03** mg/kg/day	FQPA SF = 1 X **cPAD** = chronic RfD = 0.03 FQPA SF 1 = 0.03 mg/kg/day	Chronic feeding - dog LOAEL = 15 mg/kg/day based on liver effects.
Short-Term Incidental Oral (1-30 days)	NOAEL= 10 mg/kg/day	**Residential** LOC for MOE = 100 **Occupational** = NA	Developmental toxicity - rabbits. LOAEL = 30 mg/kg/day based on body weight and liver weight effects and crooked hyoid in fetuses.
Intermediate-Term Incidental Oral (1- 6 months)	NOAEL= 3 mg/kg/day	**Residential** LOC for MOE = 100 **Occupational** = NA	Chronic feeding - dog LOAEL = 15 mg/kg/day based on liver effects.
Short-Term Dermal (1 to 30 days)	NOAEL= 10 mg/kg/day	**Residential** LOC for MOE = 100 **Occupational** = NA	Developmental toxicity - rabbits. LOAEL = 30 mg/kg/day based on body weight and liver weight effects and crooked hyoid in fetuses.
Intermediate-Term Dermal (1 to 6 months)	NOAEL= 3 mg/kg/day	**Residential** LOC for MOE = 100 **Occupational** = NA	Chronic feeding - dog LOAEL = 15 mg/kg/day based on liver effects.

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Long-Term Dermal (>6 months)	Oral study NOAEL= **3** mg/kg/day Note: dermal absorption rate = 46%.	**Residential** LOC for MOE = 100] **Occupational** LOC for MOE = 100	Chronic feeding - dog LOAEL = 15 mg/kg/day based on liver effects.
Short-Term Inhalation (1 to 30 days)	Oral) study NOAEL= 10 mg/kg/day (inhalation absorption rate = 100%)	**Residential** LOC for MOE = 100 **Occupational** LOC for MOE = 100	Rabbit developmental toxicity LOAEL = 30 mg/kg/day based on body and liver weight effects.
Intermediate (1 to 6 months) and long term (> 6 months)	Oral study NOAEL = 3 mg/kg/day (inhalation absorption rate = 100%)	**Residential** LOC for MOE = 100 **Occupational** LOC for MOE = 100	Chronic feeding - dog LOAEL = 15 mg/kg/day based on liver effects.
Cancer (oral, dermal, inhalation)	Classified as "likely to be a carcinogen in humans" as per May 5, 2000 CARC report. Q1* = 4.25 x 10^{-2} human equivalents (refer to TXR # 0014035, memo dated 3/9/00.		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable