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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No.: 0053329

MEMORANDUM

DATE: March 26, 2005

SUBJECT: **NITRAPYRIN:** Report of the Cancer Assessment Review Committee
PC Code: 069203

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

Jessica Kidwell

TO: John Doherty, Toxicologist (RRB3)
Seyed Tadayon, Risk Assessor (RRB3)
Health Effects Division (7509C)

Stephanie Plummer and
Laura Parsons
RRB1, Special Review & Reregistration Division (7508C)

The Cancer Assessment Review Committee met on February 9, 2005 to evaluate the carcinogenic potential of NITRAPYRIN. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

MAY 0 4 2005

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF *NITRAPYRIN*

PC CODE 069203

FINAL REPORT

March 26, 2005

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

NITRAPYRIN CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: _____
John Doherty, Toxicologist

DOCUMENT PREPARATION: _____
Jessica Kidwell, Executive Secretary

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

Karl Baetcke _____

Lori Brunsman _____

William Burnam _____

Marion Copley _____

Vicki Dellarco _____

Kit Farwell _____

Abdallah Khasawinah _____ for AK

Nancy McCarroll _____

Esther Rinde _____ for ER

Jess Rowland _____

Linda Taylor _____

Yin-Tak Woo _____ for YT

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist _____

OTHER ATTENDEES: Yon-Hwa Kim, Jin-Sup Shin (HED/RRB1), Seyed Tadayon (HED/RRB3), Laura Parsons (SRRD), Stephanie Plummer (SRRD), Whang Phang (HED/RRB1), Cathy Eiden (HED/RRB3)

TABLE OF CONTENTS

EXECUTIVE SUMMARY 1

I. BACKGROUND 2

II. ISSUES 3
A. Mutagenicity 3
B. Harderian Gland Tumors in Female Mice 8
C. Epididymal Tumors 9
D. Papilloma and Squamous Cell Carcinomas in the Forestomach 10
E. Liver Tumors 12

III. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE 14

IV. CLASSIFICATION OF CARCINOGENIC POTENTIAL 16

V. QUANTIFICATION 16

VI. REFERENCES 17

EXECUTIVE SUMMARY

The Health Effects Division (HED) Carcinogenicity Assessment Review Committee (CARC) met on February 9, 2005 to consider the DOW Chemical Company's request to re-evaluate the cancer classification of nitrapyrin. The CARC previously classified nitrapyrin as "Likely to be Carcinogenic to Humans" based on tumors noted in a mouse carcinogenicity study with the B6C3F1 strain mouse. The CARC reconsidered the significance of the four tumor types (liver and stomach tumors in both sexes, Harderian gland tumors in females and epididymal tumors) and the DOW Company's analysis of the significance of these tumors based on a Scientific Advisory Group review prepared at the DOW Company's expense (2004, MRID No.: 46428301).

The CARC determined that insufficient data were provided to support a cytotoxic mode of action for the induction of liver tumors. In particular, there were no temporal data showing time to onset of non-neoplastic conditions relative to the tumors and there was no reversibility data for the non-neoplastic lesions. The CARC did not agree with the DOW Company that the epididymal tumors in the high dose group were spontaneous and a result of natural variation. Thus, the CARC determined that the epididymal tumors are biologically significant and cannot be dismissed from the weight-of-the-evidence for carcinogenicity evaluation since they are malignant and a rare tumor in mice. In addition, the CARC did not eliminate the possibility that there was a mutagenic effect of nitrapyrin based on the positive response in the *Salmonella* tests conducted by the National Toxicology Program as well as structure activity relationships. The CARC, however, concurred with the DOW Company and determined that the Harderian gland tumors in females were not treatment-related. The significance of the stomach tumors in both sexes was determined by the CARC not to be relevant to human health risk assessment because the structure and physiology of the mouse forestomach is different from the human and does not serve as an appropriate model.

In summary, the CARC maintained the classification of nitrapyrin as "Likely to be Carcinogenic to Humans". The quantification of human cancer risk is recommended.

I. BACKGROUND

The Health Effects Division (HED) Carcinogenicity Assessment Review Committee (CARC) previously classified nitrapyrin as "Likely to be carcinogenic to humans" based on tumors noted in a mouse carcinogenicity study with the B6C3F1 strain mouse (Memo, May 5, 2000, HED Document No. 014131; MRID Nos. 44231803, 44231801). In this study, treatment related increases in neoplasms were noted in the liver and stomach of both sexes, in the epididymis, and in the Harderian gland in females at doses of 125 and/or 250 mg/kg/day. Historically, a previous study (MRID No. 41651601) in the same strain of mice tested at dose levels of 0, 5, 20 or 75 mg/kg/day did not indicate treatment related neoplasms. However, the Carcinogenicity Peer Review Committee (CPRC) determined that the high dose of 75 mg/kg/day was not adequate for carcinogenicity assessment (Memo, September 14. 1992, HED Doc. No. 009760). The CARC May 5, 2000 decision on classification was based on the following:

1. Tumors at multiple sites were induced in both sexes of mice (liver and stomach for both sexes and in the epididymis and in the Harderian gland in females at 125 and/or 250 mg/kg/day).

2. Nitrapyrin was not mutagenic in the studies submitted by the DOW Company. However, in studies conducted by the National Toxicology Program (NTP), nitrapyrin was mutagenic in *Salmonella typhimurium* assay in the presence of S9 activation.

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

The DOW Company convened its own Histopathology Peer Review and Scientific Advisory Group (SAG) to address the issues related to the carcinogenicity classification of nitrapyrin and presented both a written (MRID No. 46428301) and oral presentation (January 13, 2005) to OPP for review. The SAG report as prepared by the DOW Company was presented to the CARC for review on February 9, 2005. A discussion of each of the several issues including comments on the DOW Company's position, together with the CARC's interpretation and response on each of the issues, is outlined below.

II. ISSUES

A. Mutagenicity

A.1. Background and DOW Company's position.

The DOW Company has submitted the following genetic toxicity mutagenicity studies.

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)

As noted above, none of these studies was demonstrated to be positive. In particular the

reverse mutation study in *Salmonella typhimurium* was negative.

The reverse mutation assay in *Salmonella typhimurium* in the study conducted by the National Toxicology Program (Zeiger et al. 1988) was determined to be weakly positive in the presence of metabolic activation in strains TA100, TA97 and TA 98. However, only a weak response was indicated.

The DOW Company reviewed the genotoxicity issues and made the following conclusion.

> "Using the weight of evidence approach to evaluating the entire battery of genotoxicity assays available, including systems that measure different endpoints in mammalian cells, metabolically competent cells and in the treated animals, the SAG considered it unlikely that nitrapyrin was acting through a genotoxic mode of action".

Thus, the CARC was requested to address the issue as to whether or not the existing data base supports the original conclusion that "the available mutagenicity data are supportive of a mutagenic mode of action." In particular, is the weak response seen in the presence of metabolic activation that is apparent in the NTP study sufficient to override the lack of a positive response in the same study type conducted by the Microtest Research Company as well as all of the other studies that assess for mutagenicity/genetic toxicity?

A.2. CARC's February 9, 2005 conclusions

Mutagenicity

Nitrapyrin was not mutagenic in the submitted battery [*i.e, S. typhimurium* mammalian microsome reverse gene mutation assay (plate incorporation procedure), Chinese hamster ovary cell(CHO) forward gene mutation assay, mouse micronucleus assay and unscheduled DNA synthesis (UDS) in primary rat hepatocytes]. There was, however, a valid positive response in the NTP-sponsored *S. typhimurium* mammalian microsome reverse gene mutation assay (preincubation modification to the standard plate incorporation procedure) (Zeiger et al. 1988). Revertant colony counts and fold increases for strains TA97 and TA98 are presented below. As shown, strain TA98 responded at 666 µg/plate + 10% rat S9 with a 2.3-fold increase in mutants. Similarly, >2-fold increases were seen at 333 and 666 µg/plate + 30% rat S9. Although the increases at these concentrations were >2-fold, the number of revertant colonies was within the spontaneous range of TA98. These results should, therefore, be considered questionable evidence of mutagenicity.

When the assay was repeated using a liver homogenate derived from hamster livers, 2-fold or approaching a 2-fold increase were seen at 100, 333 and 666 µg/plate + 10% rat S9. This

response increased to >3-fold at the higher levels in the presence of 30% hamster S9 and 2-fold at 100 µg/plate. The effect was concentration-related and the mutant counts were outside of the spontaneous range at the two highest concentrations tested. Zeiger *et al.* (1988) concluded that these results provided clear evidence of mutagenicity.

Response of Select *S. typhimurium* Strains to S9-activated Concentrations of Nitrapyrin

	TA98				TA98			
µg/plate	Rat (10%)		Rat (30%)		Hamster (10%)		Hamster (30%)	
	Revertants	Fold Inc.	Revertants	Fold Inc	Revertants	Fold Inc	Revertants	Fold Inc
0	23		17		25		21	
10			29				22	
33	26		23		26		20	
100	30	1.3	27	1.6	40	1.6	44	**2.0**
166	32	1.4			34	1.4		
333	30	1.3	47	**2.8**	46	1.8	70	**3.3**
666	54	**2.3**	45	**2.7**	51	2	73	**3.5**

Spontaneous (+S9) 20-50

For strain TA97, there was a <2-fold response at 333 and 666 µg/plate in the presence of 10% rat S9 and a response approaching 2-fold when the S9 mix was derived from hamsters. This response should be characterized as weakly positive. Findings for strain TA100 would also be considered weak or questionable evidence of a positive response since increases approaching 2-fold were seen but not in a concentration-related manner. The data for strain TA98 indicate mutagenicity and are supported primarily by the results with strain TA97. It is of interest to note that both strains TA97 and TA98 detect frameshift mutations. Additionally, these responses occurred at concentrations that were not cytotoxic in the presence of the different S9 fractions but were near cytotoxic levels in the absence of S9 activation and in the presence of lower S9 levels (5%).

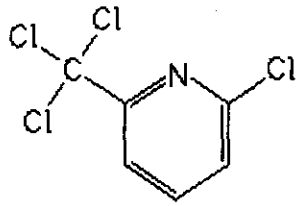
Response of Select *S. typhimurium* Strains to S9-activated Concentrations of Nitrapyrin

TA97					TA97			
µg/plate	Rat (10%)		Rat (30%)		Hamster (10%)		Hamster (30%)	
	Revertants	Fold Inc.	Revertants	Fold Inc	Revertants	Fold Inc	Revertants	Fold Inc
0	181				195			
10	186				220			
33	186				248			
100	203	1.1			262	1.3		
166								
333	237	1.3			332	1.7		
666	255	1.4			360	1.9		

Spontaneous (+S9) 75-200

For the above reasons, nitrapyrin was listed as mutagenic based on the positive NTP study in the Carcinogenicity Peer Review Committee (CPRC) report on Nitrapyrin, dated September 14, 1992 (TXR 0009760), which was reiterated by the CARC in March 2000. Nevertheless, the registrant dismissed these findings because they were not reproduced in the submitted Ames assay. The Dow Ames test did not include a preincubation component and did not test the pesticide against a hamster S9 liver fraction. The registrant also stated that , "Reasons for the opposing conclusions between the Dow and the NTP Ames studies might include purity of the test article or differences in the experimental procedure." No purity information was listed in the Dow Ames test while the purity of the test material in the NTP study was 99%. The issue of different test protocols (*i.e.*, plate test vs preincubation modification to the plate test) may account for the conflicting responses since the preincubation protocol is generally more sensitive than the plate incorporation procedure.

Structure Activity Relationship (SAR)



Nitrapyrin

From the SAR point of view, nitrapyrin expected to have some genotoxic potential. The chlorine at the *ortho*position of pyridine is expected to be reactive. The positive Ames mutagenicity of nitrapyrin is supported by the positive Ames mutagenicity of *ortho*-chloropyridine tested by the preincubation method. Nitrapyrin could also be compared to benzotrichloride, which is thought to be a genotoxic carcinogen. In addition, SAR was shown between nitrapyrin and other chlorinated pyridines that are mutagenic in the Ames test and carcinogenic in mouse and the rat (see CPRC Review of Nitrapyrin, dated September 14, 1992).

More specifically, nitrapyrin can be looked upon as a combination of three carcinogenic and/or genotoxic compounds: (a) 2-chloro-pyridine (without the trichloromethyl moiety), (b) benzotrichloride (without the 2-chloro moiety and substituting a ring C with heterocyclic N), and (c) pyridine without either the *ortho* chloro or the trichloromethyl group. Each of these analogies could provide some support for carcinogenic and/or genotoxic concern for nitrapyrin.

The chlorine at the 2- (or *ortho*-) position of pyridine is a good leaving group so the compound is expected to have electrophilic and genotoxic potential. Indeed, comparative mutagenicity studies of a series of halogenated pyridines (Claxton et al. 1987; Dearfield et al. 1993) showed that halogen at the 2-position (*ortho* to the ring N) of pyridine is a major determinant for potential genotoxicity. 2-Chloropyridine is positive in the Ames *Salmonella* test (Claxton et al. 1987; NTP data) and in L5178Y mouse lymphoma assay as demonstrated by increases in chromosome aberrations and micronuclei (Dearfield et al. 1993). Metabolic activation increases the mutagenicity/clastogenicity probably by oxidizing the ring nitrogen to N-oxide thereby further increasing the leaving tendency of the chlorine at the 2-position. 2-Chloro-5-trifluoromethylpyridine, a reasonably close analog of nitrapyrin, is also considered positive in the mouse lymphoma assay although its activity appears to be lower than that of 2-chloropyridine.

Benzotrichloride was tested positive in Ames mutagenicity assays (Zeigler et al., Environ. Mol. Mutagen. (Suppl. 12):1-158, 1988) and found to be a potent, multi-target carcinogen when tested in mice (Fukuda et al., Gann, 72:655, 1981). Pyridine has been shown to induce mouse liver tumors in NTP bioassay (NTP TR 470); there appears to be no evidence of genotoxicity. The structural analogy of these two compounds is not as good as 2-chloropyridine

because benzotrichloride is much more active and direct acting whereas the unsubstituted pyridine lacks the reactive chlorine.

Overall Conclusions

Based on these considerations, it was concluded that nitrapyrin was not mutagenic in CHO cells, did not cause micronuclei induction in mice bone marrow and did not induce UDS in primary rat hepatocytes. The registrant submitted a negative *Salmonella* assay conducted in 1985. Nonetheless, 99% nitrapyrin did cause a reproducible increase in gene mutations in *S. typhimurium* TA98 in the NTP study of Zeiger *et al.* (1988; conducted in 1983). The increases in the presence of hamster S9 were concentration-related and ~3-fold at the two highest levels. This weight-of-the-evidence analysis is not a sufficient bases to ascribe a mutagenic mode of action to the carcinogenic response of nitrapyrin. Although nitrapyrin induced a positive response in bacteria and there is support from SAR for nitrapyrin having genotoxic potential (see discussion above), the issue of whether this potential mutagenicity contributes to the mode of action for nitrapyrin-induced carcinogenesis is unresolved.

B. Harderian Gland Tumors in Female Mice

B.1. Background and DOW Company's position.

If the two studies are combined, the reported incidence of Harderian gland adenomas in female control mice is 6/100 (5/50 in the first study but only 1/50 in the second study) and (1/15), (3/10), (0/50), 8/50, and 9/50 in the female treated groups in the two studies for 5, 25, 75, 125, and 250 mg/kg/day dose groups, respectively. (The incidences in the first (low dose) study are shown in parenthesis.) See table below for combined incidences of the low dose (unbolded) and high dose (bolded) studies.

Dose (mg/kg/day)	0 (control)	**0 (control)**	5	25	75	**125**	**250**
Adenoma (%)	5/50 10%	**1/50 2%**	1/15	3/10	0/50 0%	**8/50 16%**	**9/50 18%**

Since only selected Harderian glands were assessed for the 5 and 25 mg/day dose groups (note low denominator) and it is assumed that only those animals with suspect gross necropsy were further examined histologically, the net count of 1 in the 5 mg/kg/day group and 3 in the 25 mg/kg/day group may be considered as representative of the tumors in these groups.

There was a significant increasing trend as well as significant differences in the pair-wise comparison of the 125 and 250 dose groups, with controls. The tumor incidence in both the 125 mg/kg/day (8/50, 16%) and 250 mg/kg/day (9/50, 18%) dose groups exceed the historical control

range for Harderian Gland tumors in the B6C3F1 strain mice as provided by both the DOW company's archives (4-14%) and the NTP archives (0-10%). It is also noted that the Charles River Company archives dated 1989 report a range of 0- 4.3%.

The DOW Company's position is that the incidence of Harderian adenomas in females is not related to treatment but "it is simply a statistical anomaly resulting from the lower than expected incidence in the concurrent control group". In the study at higher doses where the apparent increase in Harderian gland tumors are noted, the control has only one incident in the 50 animals assessed. However, in the previous study, there were 5 out of 50 in the control group. Thus, the DOW Company concluded that the concurrent control group has a "lower than expected incidence" for this tumor type in the second study where there is an appearance of treatment related Harderian gland tumors.

B.2. CARC's February 9, 2005 conclusions.

Although the incidence of Harderian gland tumors in female mice is slightly outside of the historical control range (2nd study), there is a lack of a clear dose response between 125 (16%) and 250 (18%) mg/kg/day and the concurrent control for the second study is considered low relative to the first. Therefore, it is difficult to interpret the significance of this lesion due to the variation in control incidence between the first and second study. The CARC concluded that the Harderian gland tumors were not considered to be treatment-related.

C. Epididymal Tumors

C.1. Background and DOW Company's position.

The incidence of undifferentiated sarcomas of the epididymis was 2/50, 0/50, 0/50, 0/50, 1/50, 2/50, 4/50 in the first study control, second study control, 5, 25, 75, 125 and 250 mg/kg/day dose groups, respectively. See table below for combined incidences of the low dose (unbolded) and high dose (bolded) studies.

Dose (mg/kg/day)	0 (control)	**0 (control)**	5	25	75	**125**	**250**
undifferentiated sarcoma (%)	2/50 4%	**0/50 0%**	0/50 0%	0/50 0%	1/50 2%	**2/50 4%**	**4/50 8%**

The original reading for the first study identified the two tumors in the epididymis in the control group and implied that the single tumor in the 75 mg/kg/day groups as "Leydig Cell Tumor".

However, the reading by the SAG revised the diagnosis as undifferentiated sarcoma and reported that the tumors originally described as Leydig Cell tumors and those described as undifferentiated sarcoma have the same histomorphology.

The overall conclusion of the SAG and the DOW Company's position is that the two incidents in the 125 mg/kg/day and 4 incidents in the 250 mg/kg/day dose groups are considered to be "a spurious finding as the result of biological variation unrelated to treatment with nitrapyrin".

No historical control data were presented to show the spontaneous variation of tumors in the epididymis. Charles River compilation of spontaneous tumors does not list tumors in the epididymis.

C.2. CARC's February 9, 2005 conclusions.

The CARC's consulting pathologist (Dr. John Pletcher) advised that the diagnosis provided by the SAG (undifferentiated sarcoma) is appropriate for the evaluation of the epididymal tumors, therefore, superceding the original diagnosis (Leydig cell tumor).

The CARC determined that the epididymal tumors are biologically significant and should be included in the weight-of-the-evidence assessment of the carcinogenicity evaluation of nitrapyrin. Considerations for this conclusion included that the undifferentiated sarcoma is a rare and malignant tumor. The tumors were late developing and this was a 24-month study (rather than the typical 18-month duration study as required by the Subdivision F guidelines). No historical control data were provided for 24-month studies to assess the spontaneous occurrence of this tumor type seen in aged (2-year) mice.

D. Papilloma and Squamous Cell Carcinomas in the Forestomach

D.1. Background and DOW Company's position.

The DOW Company's position is that the increased incidence of papilloma and squamous cell carcinomas in the forestomach are the result of the irritancy of nitrapyrin and attempt to demonstrate that there is also an increase in forestomach hyperplasia due to the irritant property of nitrapyrin. In support of this contention, the DOW Company cites that "nitrapyrin produced transient irritation of the eyes (Carreon, 1966)". In addition, the DOW Company cited a 21-day repeated dose toxicity study (Cosse et al, 1992) resulted in dermal irritation.

The following table presents data on both the non-neoplastic and neoplastic findings in the forestomach in the mouse carcinogenicity study (1997, MRID No.: 44231803 and 44231801).

Table 1. Non-neoplastic and neoplastic response in the forestomach in mice dosed with nitrapyrin.

Lesion	Males			Females		
Group	Control	125	250	Control	125	250
Hyperplasia	0	16*	14*	4	18*	20*
Papilloma	1	9*	12*	1	8*	21*
Squamous Cell Carcinoma	0	0	3	1	0	2
Total (a)	1	9*	15*	1	8*	22*

(a) Papilloma and/or Squamous cell carcinoma.
* "Statistically identified difference from control mean by Yate's Chi-square pair-wise test (DOW's SAG report Table 6, page 28).
Note: based on 50 animals per group.

It is obvious that there are treatment related increases in both the non-neoplastic hyperplasia in both sexes as well as increases in papillomas.

The DOW Company maintains that since the function of the forestomach is to serve as a storage site in the rodent, this area in the mouse is subject to the irritancy for a longer period and thus has a higher probability to develop the tumors secondary to the irritancy than in the human which does not store foodstuffs for extended periods.

D.1. CARC's February 9, 2005 conclusions

The CARC concluded that tumors in the forestomach of mice were treatment-related, but are not relevant for human risk assessment based on differences in the structural/physiological function of the forestomach.

Note: Although the CARC determined that the forestomach tumors are not relevant to humans, there is some question regarding the logic provided by the registrant regarding the theory of irritancy due to the caustic nature of nitrapyrin inducing the forestomach tumors. In particular, inspection of the nitrapyrin data base in HED files indicates that although nitrapyrin produces transient ocular irritation (it is classified as Tox Cat. II), nitrapyrin is not considered an irritant to the skin (it is classified as Tox Cat. IV). The local dermal irritation was described as "very slight to well defined erythema and/or very slight edema" (DER TXT # 009637, prepared by L. Hansen, 7/13/92). Also, the NOAEL and LOAEL for the 21-day dermal study in rabbits are 500 and 1000 mg/kg/day based on liver effects. Overall, nitrapyrin does not seem unusually more irritant than other chemicals that do not produce the forestomach tumors.

One other aspect within the toxicity data base for nitrapyrin that raises questions concerning the DOW Company's mechanism of action for the induction of the forestomach tumors is that the cancer study with 6-chloro picolinic acid (the principle metabolite of nitrapyrin) did not demonstrate increased forestomach tumors in the same strain of mouse. 6-chloro picolinic acid because it is an acid, would be expected to be more caustic than the parent nitrapyrin. Hence, it might be expected to also show the forestomach tumors if irritancy to the forestomach were the precursor to development of forestomach papillomas.

E. Liver Tumors

E.1. Background and DOW Company's position.

The DOW Company's position is that the liver tumors are associated with non-neoplastic conditions also occurring in the liver as result of the MTD being exceeded. The following table illustrates the key non-neoplastic lesions and tumors diagnosed in the full term cancer study (1997, MRID No.: 44231803 and 44231801) assessed at doses of 125 and 250 mg/kg/day.

Table 2. Non-neoplastic and neoplastic lesions in the liver of mice dosed with nitrapyrin.

Lesion	Males			Females		
Group	Control	125	250	Control	125	250
Non-Neoplastic:						
Necrosis (a)	5	48*	48*	3	11*	19*
Vacuolation	0	4	39*	1	3	15*
Increase size-hepatocytes(b)	0	49*	26*	0	46*	44*
Increased mitotic figures	0	8*	9*	1	0	3
Inflammation (c)	5	44*	45*	4	5	12
Pigment - multi focal	0	1	41*	0	1	3
Basophil foci	0	1	8*	1	1	12*
Eosinophilic foci	5	5	12*	2	2	23*
Neoplastic:						
Hepatocellular adenoma	12	19	45*	6	27*	32*
Hepatocellular carcinoma	7	3	12	0	1	2
Combined (d)	17	20	46*	6	28*	33*

Based on 50/sex/group.
(a) individual cells, focal or multi focal
(b) often accompanied by altered tinctorial properties, centrilobular
(c) subacute to chronic, multi focal or diffuse
(d) animals with both adenoma and carcinoma counted once.
* "Statistically identified difference from control mean by Yate's Chi-square pair-wise test; alpha = 0.10, two sided." (DOW SAG report page 25 and 26).

The above table demonstrates that there are a variety of non-neoplastic lesions in the liver of both males and females but that males seem to be more affected than females. The earlier study assessed at up to 75 mg/kg/day was not reported to be associated with the non-neoplastic conditions listed above nor were liver tumors diagnosed.

In addition to the full term cancer studies with nitrapyrin, there are a special two-week mechanism study (1996, MRID No. 44231801) assessed at 0, 200 and 400 mg/kg/day) and data from the one-year sacrifice of the cancer study (assessed at 0, 125 and 250 mg/kg/day). The two week study demonstrated males were especially responsive to cell proliferation at both 200 mg/kg/day (17 fold increase) and 400 mg/kg/day (9-105 fold increase depending on region of the hepatic lobule). However, females were less responsive (little or no increase at 200 mg/kg/day but 2 fold increase at 400 mg/kg/day). The interim sacrifice group for the 12 month study also indicated increased hepatocyte proliferation especially in males at 250 mg/kg/day, where there was a 10 - 12 fold increase and at 125 mg/kg/day where these was 2.5 to 5 fold increase. Female mice, which already have a lower hepatocyte proliferation index than males in the controls, also showed large increases in the hepatocyte proliferation index.

The DOW Company's position is that the short term and cancer studies demonstrate that nitrapyrin causes necrosis and regenerative hepatocyte proliferation and eventual neoplasia. Thus, the dose levels were excessive.

E.2. CARC's February 9, 2005 conclusions

The CARC concluded that the liver tumors, which were primarily benign, were treatment-related in both sexes of the mouse. The CARC reaffirmed its earlier position that the high dose of 250 mg/kg/day was adequate, but not excessive, to assess the carcinogenicity potential of nitrapyrin. This was because the increased mortality in high dose males was seen only at the end of the study period (and in this case the study was of 24 months duration instead of the usual 18 months), there were only minimal decreases in overall body weight gains, and non-neoplastic changes in the liver and stomach (both sexes) that included single cell necrosis of hepatocytes and inflammation were not considered critical. The latter is because no data were presented on the severity of the necrosis or other lesions/conditions (i.e., the extent of liver damage). In addition, there was a lack of concordance between the increase of non-neoplastic and neoplastic lesions in female mice.

The CARC also concluded that insufficient data were provided to accept a cytotoxic mode of action. Deficiencies included a lack of temporal data and reversibility studies in mice. The data did not show a dose-response concordance with key events and tumors. In addition, although the evidence is not compelling, the genotoxic potential cannot be discounted.

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

The Committee's assessment of the current weight-of-the-evidence (WOE) is discussed below:

1. Carcinogenicity

Mouse

▸ If the two studies are combined, the reported incidence of Harderian gland adenomas in female control mice is 6/100 (5/50 in the first study but only 1/50 in the second study) and (1/15), (3/10), (0/50), 8/50, and 9/50 in the female treated groups in the two studies for 5, 25, 75, 125, and 250 mg/kg/day dose groups, respectively. (The incidences in the first (low dose) study are shown in parenthesis.) Although the incidence of Harderian gland tumors in female mice is slightly outside of the historical control range (2nd study), there is a lack of a clear dose response between 125 (16%) and 250 (18%) mg/kg/day and the concurrent control for the second study is considered low relative to the first study. Therefore, it is difficult to interpret the significance of this lesion due to the variation in control incidence between the first and second study. Ultimately, the CARC concluded that the Harderian gland tumors were not considered to be treatment-related.

▸The CARC determined that, although the forestomach tumors in male and female mice are clearly associated with treatment, this tumor type is not relevant to human health risk assessment based on structural/physiological differences between the rodent and human gastric function.

▸The incidence of epididymal undifferentiated sarcomas in male mice was 0/40, 2/48 (4%), and 4/33 (12%) for the control 125, and 250 mg/kg/day dose groups, respectively. There was a significant increasing trend and a significant difference in the pair-wise comparison of the 250 mg/kg/day dose group with the controls. The tumors appeared late in this 24-month study (usually the duration of a mouse carcinogenicity study is 18 months). The CARC determined that the epididymal undifferentiated sarcomas are biologically significant and cannot be dismissed from the weight-of-the-evidence for carcinogenicity evaluation since they are malignant and a rare tumor in mice.

▸ Male mice had significant increasing trends and significant differences in the pair-wise comparisons of the 250 mg/kg/day dose group with the controls for hepatocellular adenomas (45/48 (94%) vs 12/49 (24%), and adenomas and/or carcinomas combined (46/49 (94%) vs 17/49 (35%) in controls.) The combined incidence was driven by the incidence of adenomas. The incidence of adenomas at 250 mg/kg/day was outside the range for the historical controls (14-54%). The CARC considered the increased incidence of liver tumors in male mice at the high dose to be treatment-related.

▸ Female mice had significant increasing trends and significant differences in the pair-wise comparisons of the 125 and 250 mg/kg/day dose groups with the controls, for hepatocellular adenomas (27/48 (56%) and 32/48 (67%), respectively vs 6/47 (13%) in controls), and adenomas and/or carcinomas combined (28/48 (58%) and 33/48 (69%), respectively vs 6/47 (13%) in controls). The combined incidence was driven by the incidence of adenomas. The CARC considered the increased incidence of liver tumors in female mice at 125 and 250 mg/kg/day to be treatment-related.

▸ The CARC reaffirmed its earlier position that the high dose of 250 mg/kg/day was adequate, but not excessive, to assess the carcinogenicity potential of nitrapyrin in both sexes of the mouse. This was because the increased mortality in high dose males was seen only at the end of the study period (and in this case the study was of 24 months duration instead of the usual 18 months), the decreases in overall body weight gains were minimal, and non-neoplastic changes in the liver and stomach (both sexes) that included single cell necrosis of hepatocytes and inflammation were not considered to be critical. The latter is because no data were presented on the severity of the single cell necrosis and other lesions (i.e., the extent of liver damage). In addition, there was a lack of concordance between the increase of non-neoplastic and neoplastic lesions in female mice.

Rat

No tumors relevant to human risk assessment were noted in rats at doses that were considered adequate in both sexes to assess the carcinogenic potential of nitrapyrin.

2. Mutagenicity and SAR

▸The CARC concluded that nitrapyrin was not mutagenic in CHO cells, did not cause micronuclei induction in mice bone marrow and did not induce UDS in primary rat hepatocytes. The registrant submitted a negative *Salmonella* assay conducted in 1985. Nonetheless, 99% nitrapyrin did cause a reproducible increase in gene mutations in *S. typhimurium* TA98 in the NTP study of Zeiger *et al.* (1988; conducted in 1983). The increases in the presence of hamster S9 were concentration-related and ~3-fold at the two highest levels. This weight-of-the-evidence is not a sufficient basis to ascribe a mutagenic mode of action to the carcinogenic response of nitrapyrin. Although nitrapyrin induced a positive response in bacteria and there is support from SAR for nitrapyrin having genotoxic potential (as discussed earlier), the issue of whether this potential mutagenicity contributes to the mode of action for nitrapyrin-induced carcinogenesis is unresolved.

3. Mode of Action

The CARC concluded that insufficient data were provided to accept a cytotoxic mode of action. Deficiencies included a lack of temporal data and reversibility studies in mice. The data did not show a dose-response concordance with key events and tumors. In addition, although the evidence is not compelling, the genotoxic potential cannot be discounted.

IV. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), the CARC classified Nitrapyrin as **"Likely to be Carcinogenic to Humans"** based on the following:

(i) The treatment-related mouse liver tumors occurred in both sexes and, although benign, showed a robust increase in incidence compared to the control.
(ii) There was insufficient data to accept the cytotoxic mechanism for the mode of action for induction of liver tumors.
(iii) There were rare, malignant tumors in the mouse epididymis at the high dose.
(iv) Although there was insufficient data to classify nitrapyrin as a mutagenic carcinogen, there remains a concern based on a weak response in the *Salmonella* assay and structural activity relationships.

Note: Although the majority of the CARC voted for "likely to be carcinogenic in humans", there were several members of the CARC that voted for the classification of "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential". Support for the "suggestive" classification was based on: 1)The mouse liver tumors were benign; 2) The epididymal tumors were difficult to interpret since they were seen only after two years in the terminal sacrifice. The study was run for 24 months and the guidelines require only 18 months. Thus, the longer exposure time put the mice at a greater risk for development of epigenetic neoplasms associated with secondary effects of the high doses of the test material. 6-chloro-picolinic acid, the principle metabolite of nitrapyrin in mammals did not also cause similar tumors in the same strain or mice.

V. QUANTIFICATION

The quantification of human cancer risk is recommended.

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