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AND TOXIC SUBSTANCES

April 11, 2002

MEMORANDUM

SUBJECT: Iprovalicarb - Report of the Cancer Assessment Review Committee

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

TO: Ed Budd, Toxicologist
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The Cancer Assessment Review Committee met on February 6, 2002 to evaluate the carcinogenic potential of Iprovalicarb. Attached please find the Final Cancer Assessment Document.

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

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HED DOC. NO. 0050652

CANCER ASSESSMENT DOCUMENT

**EVALUATION OF THE CARCINOGENIC POTENTIAL OF
IPROVALICARB**

P.C. Code: 098359

FINAL REPORT

11- April, 2002

**CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS**

I PROVALICARB

CANCER ASSESSMENT DOCUMENT

FINAL REPORT

DATA PRESENTATION:

Edwin Budd

Edwin Budd, Toxicologist

DOCUMENT PREPARATION:

Sanjivani Diwan

Sanjivani Diwan, Executive Secretary

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

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See attached sheet

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

John M. Pletcher, Pathology Consultant

See attached sheet

Virginia Fornillo, Statistical Analysis

Virginia Fornillo

The meeting was also attended by Cameron Bowes, Felix Omara, and Catherine Adcock from Pest Management Regulatory Agency (PMRA), Health Canada, Canada by teleconference

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EXECUTIVE SUMMARY

On February 6, 2002, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs met to evaluate the carcinogenic potential of iprovalicarb. This meeting was attended by Pest Management Regulatory Agency (PMRA), Health Canada, Canada. The studies evaluated included a combined chronic toxicity and carcinogenicity study in Wistar rats, a carcinogenicity study in B₆C₃F₁ mice, a chronic toxicity study in Beagle dogs, subchronic toxicity studies in mice, rats and dogs, metabolism studies, and a battery of mutagenicity studies. Mechanistic studies related to the mechanism of tumor induction in rats were also available for review.

In the chronic toxicity/carcinogenicity study, iprovalicarb was administered in the diet to 50 Wistar (Hsd/WIN:WU) rats/sex/dose level at dose levels of 0, 500, 5000 or 20000 ppm (equivalent to 0, 26.0, 262.5 or 1109.6 mg/kg/day in males and 0, 31.7, 326.3 or 1379.7 mg/kg/day in females) for 24 months. In a carcinogenicity study, 50 B₆C₃F₁ (SPF) mice/sex/dose level were fed iprovalicarb at dose levels of 0, 280, 1400 or 7000 ppm (equivalent to 0, 58.5, 283.4 or 1566.8 mg/kg/day in males and 0, 97.4, 503.1 or 2544.0 mg/kg/day in females) for 105 weeks.

The CARC concluded that iprovalicarb was carcinogenic to male and female rats based on the following:

- In male rats, 1) there was a statistically significant positive trend for osteosarcomas of the femur and of the lower jaw (both $p < 0.05$) and the combined osteosarcomas at the two sites ($p < 0.01$). There was also a statistically significant ($p < 0.05$) increase by pair-wise comparison of the 20000 ppm (1109.6 mg/kg/day) dose group with the controls for combined osteosarcomas. The combined incidence at the high dose exceeded the range for the historical controls from the Bayer laboratory and RITA database. **These tumors are rare and malignant and were considered by the Committee to be treatment-related.** 2) A single incidence of chondrosarcoma of the nasal cavity was observed in the high dose group. Although chondrosarcomas and osteosarcomas have a common etiology, their incidences could not be combined for statistical analyses since not all animals were examined histopathologically for chondrosarcomas. The Committee could not ascertain the statistical and biological significance of the chondrosarcoma at the high dose. 3) The occurrence of other tumors including tumors of the thyroid, pituitary and mammary glands as well as Leydig cell adenomas of the testes were not considered by the Committee to be treatment-related because there was no dose-response, and the incidences were within the range of historical controls for both the in-house laboratory (Bayer AG) and RITA database.
- In female rats, 1) there was an increase in the incidence of mixed Mullerian tumors of the uterus in mid and high dose females. The incidence of mixed Mullerian tumors at the high dose exceeded the range of historical controls for both the in-house laboratory (Bayer AG) and RITA database. Although, the increase was not statistically significant by trend analysis or by pair-wise comparison with the controls, **these tumors are malignant and rare and were considered by the CARC to be treatment-related.** There was also an increase in the

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incidence of adenocarcinomas of the uterus in all treated groups compared to controls. However, the incidence at the high dose fell within the range of historical controls for both the in-house laboratory (Bayer AG) and RITA database. The biological significance of this numerical increase in these tumors could not be determined. Furthermore, these tumors are not rare and, therefore, were not considered by the CARC to be treatment-related. The adenocarcinomas of the uterus and mixed Mullerian tumors do not share a common etiology since they originate from different cell types. Therefore, they were not combined for statistical analyses. 2) Additionally, there was an increase in the incidence of benign transitional cell papillomas of the urinary bladder in high dose females, but the increase was not statistically significant by trend analysis or by pair-wise comparison with the controls. **Nevertheless, these tumors are rare and the incidence at the high dose exceeded the range for the historical controls from both the Bayer laboratory and RITA database, therefore, they were considered by the CARC to be treatment-related.** 3) There was a statistically significant ($p < 0.05$) positive trend for combined incidence of thyroid follicular cell adenomas/carcinomas. However, the increased incidence at the high dose was not statistically significant by pair wise comparison with the controls. The combined incidence at the high dose was slightly outside the range for the historical controls from the Bayer laboratory but was within the historical control range for the RITA database. **Since these tumors are uncommon in Wistar rats, the Committee considered them to be treatment-related.** 4) Tumors of the clitoral gland were noted in 2 of 2 high dose females examined. Since not all animals were examined for histopathology, the significance of the occurrence of these tumors could not be determined. 5) Female rats developed tumors at other sites including those of mammary and pituitary glands. These were not considered to be treatment-related because there was no dose-response, and the control groups also had higher incidences of these tumors..

Although the animals were tested above the limit dose, there was no adverse effect on their survival. Based on the results of metabolism studies there was no deviation from metabolic pathway at the highest dose tested which would have suggested that the highest dose was excessive. **The dosing at the highest dose was considered by the CARC to be adequate and not excessive** based on decreases in body weight gain and food efficiency, increases in liver weights and liver hypertrophy in females, and bile duct and Leydig cell hyperplasia in males, which were not severely adverse.

- **The CARC concluded that iprovalicarb was not carcinogenic to male and female mice. The dosing at the highest dose exceeded the limit dose. The CARC considered the highest dose to be adequate and not excessive** because at the highest dose, there were decreased kidney weights, clinical chemistry and histopathological changes suggestive of impairment of kidney function and increased liver weight as well as changes in liver pathology in males and/or females, which were not severely adverse and the survival of the animals was not adversely affected by the treatment.
- Iprovalicarb was not mutagenic in *in vitro* and *in vivo* standard battery of guideline assays. It also gave a negative response in a DNA adduct assay, and was negative in a liver tumor initiation assay. The results from the latter studies suggest that iprovalicarb may not directly

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react with DNA. However, these studies did not establish the mode of action for tumor induction in rats.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified iprovalicarb into the category **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence considerations:

- Iprovalicarb induced rare and infrequently occurring tumors in rats. At the high dose, males developed osteosarcomas and females developed transitional cell papillomas of the urinary bladder. At the mid and high doses, females developed mixed Mullerian tumors of the uterus and follicular cell adenomas/carcinomas of the thyroid gland. Although the incidences of these tumors are low, they are either rare or uncommon in this strain of rat. Most of these tumors were induced above the limit dose which was not excessively toxic. In mice, no treatment-related increase in tumors was observed in animals treated above the limit dose which was adequate and not excessively toxic.
- Iprovalicarb is not mutagenic. Although mechanistic studies suggested that iprovalicarb is not a tumor initiator, these studies did not establish the mode of action for tumor induction in rats.

The Committee further recommended using a linear low-dose extrapolation approach for estimating the human cancer risk based on the most potent tumor in rats. This approach is supported by the lack of confirmation of the mode of action of iprovalicarb.

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I. INTRODUCTION

The TM-210 (SZX 0722) Fungicide Task Force, comprised of Tomen Agro, Inc. and Bayer Corporation (EPA Consortium No. 72273), has requested establishment of tolerances for residues of the fungicide iprovalicarb (P. C. Code: 098359, CAS No. 140923-25-7) also known as TM-210 and as SZX 0722, in/on imported grapes and raisins. Iprovalicarb is a new active ingredient not registered by EPA.

II. BACKGROUND INFORMATION

Data submitted by the TM-210 (SZX 0722) Fungicide Task Force was reviewed jointly by Health Canada's Pest Management Regulatory Agency (PMRA) and the U.S. EPA. With regard to the potential carcinogenicity of iprovalicarb, HED has conducted an independent evaluation of selected toxicology data. On February 6, 2002, the CARC met to review and further evaluate these data to determine the carcinogenic potential of iprovalicarb. Mr. Edwin Budd of the Registration Action Branch 2 presented the results of the rat and mouse carcinogenicity studies, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, and the adequacy of the dose levels tested in addition to the relevant toxicology, metabolism, mutagenicity, and mechanistic data.

III. EVALUATION OF CARCINOGENICITY EVIDENCE

1. Combined Chronic Toxicity/Carcinogenicity Study in Wistar Rats

Reference:

Schladt, L. and E. Hartmann. 1998. SZX 0722: Chronic toxicity and cancerogenicity investigations in Wistar rats (administration in the feed over 24 months). Bayer AG Institute of Toxicology, Wuppertal, Germany. Laboratory Study Number T 1055425. Bayer Report No. PH-27160. February 4, 1998. Submitted by TM-210 (SZX 0722) Fungicide Task Force. MRID 44865723. Unpublished.

A. Experimental Design

In a combined chronic toxicity/carcinogenicity study (MRID 44865723), SZX 0722 (batch no. 05013/0194, 95.8-98.5% purity) was administered in the feed to 50 Wistar (Hsd/WIN:WU) rats/sex/dose level for 24 months at dose levels of 0, 500, 5000 or 20000 ppm (equivalent to 0, 26.0, 262.5 or 1109.6 mg/kg/day in males and 0, 31.7, 326.3 or 1379.7 mg/kg/day in females). An additional 10 rats/sex/dose level were sacrificed at 12 months for interim evaluations.

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

Slightly increased incidences of several rare and infrequently observed tumor types were observed in multiple organs/tissues in male and female rats at the highest dose level tested (20000 ppm) and in some instances also in female rats at the next highest dose level tested (5000 ppm).

In male rats, the rare tumors of concern were 3 malignant osteosarcomas of the bone (2 in the femur and 1 in the lower jaw) and 1 malignant chondrosarcoma of the nasal cavity, all of which were observed at the highest dose level tested (20000 ppm). No osteosarcoma or chondrosarcoma was observed in any other male or female rat in this study. Incidences and statistical analyses of the osteosarcomas in the male rats are presented in Table 1 (Fornillo, 2002). The incidence of combined osteosarcomas for censored data (Fornillo, 2002) was 0/59 (0%), 0/60 (0%), 0/56 (0%) and 3/60 (5%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively. There was a statistically significant increasing ($p < 0.05$) trend for osteosarcomas of the femur and lower jaw. For combined osteosarcomas, the male rats had a statistically significant ($p < 0.01$) increasing trend and a statistically significant ($p < 0.05$) increase by pair-wise comparison of the 20000 ppm group with the control group. The incidence of combined osteosarcomas for uncensored data (reported by the performing laboratory) was 0/50 (0%), 0/50 (0%), 0/49 (0%) and 3/50 (6%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively. A statistical analysis of the data on the chondrosarcoma is not reported here because the nasal cavity is not a protocol organ and only a single animal at the high dose level of 20000 ppm (which had a gross lesion in the nasal cavity at week 101) was examined microscopically. From a pathological point of view (based largely on histogenesis), the CARC considered the 3 osteosarcomas and the chondrosarcoma observed in the male rats in this study to have a common etiology since they are derived from differentiated osteoblasts and chondroblasts, respectively, which in turn stem from pluripotent mesenchymal cells of mesodermal origin. However, since not all the nasal cavity tissues were examined microscopically in this study, and additional small chondrosarcomas (not grossly visible) may have been present in the control and/or test groups, the CARC did not consider it appropriate to combine the osteosarcomas and the chondrosarcoma for purposes of statistical analysis. Nevertheless, the occurrence of a single chondrosarcoma at the highest dose level tested in this study was duly noted by the CARC.

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Table 1. Iprovalicarb - Hsd/WIN:WU(SPF) Rat Study (Fornillo, 2002)Male Bone Tumor Rates⁺ and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	500	5000	20000
Osteosarcomas (Femur) (%)	0/41 (0)	0/39 (0)	0/44 (0)	2 ^a /47 (4)
p =	0.0319*	---	---	0.0868
Osteosarcomas (Lower Jaw) (%)	0/59 (0)	0/60 (0)	0/56 (0)	1 ^b /60 (2)
p =	0.0436*	---	---	0.0984
Combined (%)	0/59 (0)	0/60 (0)	0/56 (0)	3/60 (5)
p =	0.0003**	---	---	0.0127*

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

^a First osteosarcoma (femur) observed at week 89, dose 20000 ppm.

^b First osteosarcoma (lower jaw) observed at week 53 not in an interim sacrifice animal, dose 20000 ppm.

Note: Significance of trend denoted at control.

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

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

Incidences of additional selected neoplastic lesions observed in the male rats in this study are presented in Table 2. Tumors of the pituitary, mammary and thyroid glands were noted in male rats. However, these were not considered by the CARC to be treatment-related because the incidences were within the historical control range and no dose-response was evident (see Table 7). Additionally, a slight increase in Leydig cell adenomas of the testes (2%, 6%, 2% and 8% in the control, low, mid and high dose level groups, respectively) was not considered to be biologically significant because the increase was not dose-related and the highest incidence of 8% fell well within the historical control range of 0-22% at the performing laboratory and was similar to the average percent of 9.2% (see Table 7). No other tumors of concern were observed in the male rats in this study

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Table 2. Incidence of Selected Neoplastic Lesions in Male Wistar ⁽¹⁾ Rats in Iprovalicarb Study ⁽²⁾ (Uncensored Data)

Study performed at Bayer AG Institute of Toxicology (Wuppertal, Germany). In-life phase completed in February, 1996. b = benign; m = malignant.

ORGAN/TISSUE Tumor	Dose	MALES							
		0 ppm		500 ppm		5000 ppm		20000 ppm	
		inci ⁽³⁾	% ⁽⁴⁾	incid	% ⁽⁴⁾	incid	% ⁽⁴⁾	incid	% ⁽⁴⁾
TESTES									
Leydig Cell Adenoma	b	1/50	2	3/50	6	1/49	2	4/50	8
PITUITARY GLAND									
Adenoma/Pars Distalis	b	11/50	22	6/50	12	10/49	20	12/50	24
Adenocarcinoma	m	1/50	2	1/50	2	0/49	0	0/50	0
MAMMARY GLAND									
Fibroadenoma	b	0/50	0	0/50	0	0/49	0	1/49	2
Adenoma	b	0/50	0	0/50	0	0/49	0	0/49	0
Adenocarcinoma	m	0/50	0	0/50	0	0/49	0	0/49	0
THYROID GLAND									
Adenoma, Follicular Cell	b	1/50	2	1/50	2	2/49	4	0/50	0
Carcinoma, Follicular Cell	m	0/50	0	0/50	0	0/49	0	0/50	0

⁽¹⁾ Wistar substrain used in this study was Hsd/WIN:WU.

⁽²⁾ Data extracted from MRID 44865723.

⁽³⁾ Number of animals with lesion/Number of animals histopathologically evaluated

⁽⁴⁾ Statistically significant trend denoted at control (0 ppm).

Statistically significant pairwise difference from control shown at dose level.

* p < 0.05; ** p < 0.01

Historical control data from the performing laboratory (Bayer AG Institute of Toxicology) for osteosarcomas of the bone (all sites) and chondrosarcomas of the nose/nasal cavity for both male and female rats are presented in Table 7a, which tabulates selected tumor data for fourteen 2-year studies completed between April, 1991 and April, 1998 on Wistar rats (MRID 45048705). In none of 698 male and 700 female control rats was any osteosarcoma or chondrosarcoma reported in any tissue.

The CARC noted that additional historical control data from the performing laboratory were submitted to the Agency for 9 studies on Wistar rats with in-life phases between 1990 and 1999 (MRID 45493401; Table 7b). The CARC noted that only 1 osteosarcoma of the bone in 1 male control rat was reported in 1 of 9 studies. No chondrosarcoma was reported in any of the studies (in any group).

Historical control data for selected tumors in Wistar rats from the RITA database (Hannover, Germany) are presented in Table 8. The RITA database is a collection of histopathological diagnoses of neoplastic and pre-neoplastic lesions and related data from control animals involved

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in long-term carcinogenicity or toxicity studies. The data have been collected since 1988 exclusively from various European companies. For osteosarcomas of the bone (all sites) in male Wistar rats, 7 incidences were noted out of 2807 male rats examined. The highest percent incidence reported was 2%. For osteosarcomas of the bone (all sites) in female Wistar rats, 3 incidences were noted out of 2707 female rats examined. The highest percent incidence reported was 4%. Historical control data on chondrosarcomas were not reported in the RITA database. Presumably, this lesion was not observed in any of the studies in Wistar rats in the RITA database.

The incidence of osteosarcomas of the bone observed in male rats in the iprovalicarb study (3/60 or 5% for censored data; 3/50 or 6% for uncensored data) exceeded the highest historical control incidence rate in both the in-house laboratory (Bayer AG) historical control database (0% in males; 0% in females for uncensored data) and the RITA historical control database (2% in males; 4% in females for uncensored data). One osteosarcoma of the bone was noted in 1 male control rat in additional in-house historical control data (MRID 45493401).

In female rats, the tumors of concern were 3 malignant mixed Mullerian tumors of the uterus (2 at 20000 ppm and 1 at 5000 ppm), 2 benign transitional cell papillomas of the urinary bladder (both at 20000 ppm), 3 benign follicular cell adenomas of the thyroid gland (2 at 20000 ppm and 1 at 5000 ppm), 2 malignant follicular cell carcinomas of the thyroid gland (1 at 20000 ppm and 1 at 5000 ppm), and 2 malignant squamous cell carcinomas of the clitoral gland (both at 20000 ppm). All of the aforementioned types of tumors were considered to be rare tumors except for the follicular cell adenomas and carcinomas of the thyroid gland which were considered to be infrequently observed (uncommon) tumors. No additional tumors (of the types described above) were observed in the female rats in this study. Incidences and statistical analyses of the mixed Mullerian tumors of the uterus, the transitional cell papillomas of the urinary bladder, and the follicular cell adenomas and carcinomas of the thyroid gland are presented in Tables 3, 4, and 5, respectively. A statistical analysis of the data on the 2 malignant squamous cell carcinomas of the clitoral gland is not reported here because the clitoral gland is not a protocol organ and only two animals at the high dose level of 20000 ppm (which had a gross lesion in the clitoral gland) were examined microscopically. Since not all the clitoral glands were examined microscopically in this study, and additional small carcinomas (not grossly visible) may have been present in the control and/or test groups, the CARC did not consider it appropriate to perform a statistical analysis of these data. Nevertheless, the occurrence of 2 malignant squamous cell carcinomas of the clitoral gland at the highest dose level tested in this study was duly noted by the CARC.

The incidence of mixed Mullerian tumors of the uterus for censored data (Fornillo, 2002) was 0/49 (0%), 0/49 (0%), 1/48 (2%) and 2/48 (4%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively. There was no significant trend and no significant difference in the pair-wise comparisons of the dosed groups with the control group. The incidence of mixed Mullerian tumors of the uterus for uncensored data (reported by the performing laboratory) was 0/50 (0%), 0/50 (0%), 1/48 (2%) and 2/50 (4%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively.

The CARC considered the appropriateness of combining incidences of two types of uterine tumors, mixed Mullerian tumors and adenocarcinomas, in its evaluation of the carcinogenic potential of iprovalicarb. It was concluded, however, that mixed Mullerian tumors are a clearly distinct and

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easily recognizable type of tumor that would not be confused with adenocarcinomas during microscopic examination and that the etiology and histogenesis of the two types of tumors are sufficiently different so as to make it inappropriate to combine them.

The incidence of transitional cell papillomas of the urinary bladder for censored data was 0/49 (0%), 0/48 (0%), 0/48 (0%) and 2/48 (4%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively. For transitional cell papillomas, there was no significant trend and no significant difference in the pair-wise comparisons of the dosed groups with the control group. No transitional cell carcinomas of the urinary bladder were observed in the female rats in this study and no transitional cell adenomas or carcinomas of the urinary bladder were observed in the male rats in this study. The incidence of transitional cell papillomas of the urinary bladder for uncensored data was 0/50 (0%), 0/49 (0%), 0/48 (0%) and 2/50 (4%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively.

In the female rats in this study, 1 follicular cell adenoma and 1 follicular cell carcinoma of the thyroid gland were reported at 5000 ppm and 2 follicular cell adenomas and 1 follicular cell carcinoma were reported at 20000 ppm. The incidence of combined follicular cell adenomas and carcinomas of the thyroid gland for censored data was 0/49 (0%), 0/49 (0%), 2/48 (4%) and 3/48 (6%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively. For combined follicular cell adenomas and carcinomas, the female rats had a statistically significant ($p < 0.05$) increasing trend, but a pair-wise comparison of the 20000 ppm group with the control group was not statistically significant. The incidence of combined follicular cell adenomas and carcinomas of the thyroid gland for female rats for uncensored data was 0/50 (0%), 0/50 (0%), 2/48 (4%) and 3/50 (6%) for the 0 (control), 500, 5000 and 20000 ppm groups, respectively.

The CARC noted that in the male rats, follicular cell adenomas of the thyroid gland were reported in 1 control animal, in 1 low dose (500 ppm) animal, in 2 mid dose (5000 ppm) animals and none in high dose (20000 ppm) animals. No follicular cell carcinomas were observed in any of the male rats in this study.

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Table 3. Iprovalicarb - Hsd/WIN:WU(SPF) Rat Study (Fornillo, 2002)

Female Uterine Mixed Mullerian Tumor Rates* and
Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	500	5000	20000
Mixed Mullerian (%)	0/49 (0)	0/49 (0)	1/48 (2)	2*/48 (4)
p =	0.0596	1.0000	0.4948	0.2423

* Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice.

* First mixed Mullerian tumor observed at week 104, dose 20000 ppm.

Note: Significance of trend denoted at control.

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

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

Table 4. Iprovalicarb - Hsd/WIN:WU(SPF) Rat Study (Fornillo, 2002)

Female Urinary Bladder Transitional Cell Papilloma Rates* and
Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	500	5000	20000
Transitional Cell Papilloma (%)	0/49 (0)	0/48 (0)	0/48 (0)	2*/48 (4)
p =	0.0609	1.0000	1.0000	0.2423

* Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice.

* First transitional cell papilloma observed at week 107, dose 20000 ppm.

Note: Significance of trend denoted at control.

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

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

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Table 5. Iprovalicarb - Hsd/WIN:WU(SPF) Rat Study (Fornillo, 2002)

Female Thyroid Gland Follicular Cell Tumor Rates⁺ and
Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	500	5000	20000
Adenomas (%)	0/49 (0)	0/49 (0)	1/48 (2)	2 ^a /48 (4)
p =	0.0596	1.0000	0.4948	0.2423
Carcinomas (%)	0/49 (0)	0/49 (0)	1 ^b /48 (2)	1/48 (2)
p =	0.1833	1.0000	0.4948	0.4948
Combined (%)	0/49 (0)	0/49 (0)	2/48 (4)	3/48 (6)
p =	0.0228*	1.0000	0.2423	0.1173

⁺ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice.

^a First adenoma observed at week 82, dose 20000 ppm.

^b First carcinoma observed at week 106, dose 5000 ppm.

Note: Significance of trend denoted at control.

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

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

Incidences of additional selected neoplastic lesions observed in the female rats in this study are presented in Table 6. An increased incidence of uterine adenocarcinomas was observed at 20000 ppm in this study. For these uterine tumors, the percent incidence rates were 4%, 6%, 6% and 12% for the control, 500, 5000 and 20000 ppm groups, respectively. The increased incidence of adenocarcinomas at the highest dose level tested (12%), however, fell within the upper range of historical control rates for both the in-house laboratory database (14%, Table 7a) and the RITA database (14.3%, Table 8). Except for the uterine adenocarcinomas, mixed Mullerian and thyroid tumors and urinary bladder papillomas, as noted above, an increased incidence of other tumor types was not observed in this study. Incidence rates of the following tumor types were decreased in the treated female rats at 20000 ppm: malignant adenocarcinomas of the mammary gland (no pair-wise comparison performed, but statistically significant negative trend, $p < 0.01$), fibroadenomas of the mammary gland, and adenomas of the pars distalis of the pituitary gland. No osteosarcoma of the bone or chondrosarcoma of the nasal cavity was observed in the female rats in this study. The nasal cavity is a non-protocol organ, hence the number of nasal cavities examined is not known. No other tumors of concern were observed in the female rats in this study.

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Table 6. Incidence of Selected Neoplastic Lesions in Female Wistar ⁽¹⁾ Rats in Iprovalicarb Study ⁽²⁾ (Uncensored Data)

Study performed at Bayer AG Institute of Toxicology (Wuppertal, Germany). In-life phase completed in February, 1996. b = benign; m = malignant.

ORGAN/TISSUE Tumor	Dose	FEMALES							
		0 ppm		500 ppm		5000 ppm		20000 ppm	
		inci ⁽³⁾	% ⁽⁴⁾	incid	% ⁽⁴⁾	incid	% ⁽⁴⁾	incid	% ⁽⁴⁾
PITUITARY GLAND									
Adenoma/Pars Distalis	b	20/50	40	19/50	38	20/48	42	14/50	28
Adenocarcinoma	m	1/50	2	0/50	0	0/48	0	0/50	0
MAMMARY GLAND									
Fibroadenoma	b	8/50	16	6/49	12	11/48	23	3/50	6
Adenoma	b	0/50	0	1/49	2	0/48	0	0/50	0
Adenocarcinoma	m	6/50	12**	3/49	6	2/48	4	0/50	0 ⁽⁵⁾
THYROID GLAND									
Adenoma, Follicular Cell	b	0/50	0 *	0/50	0	1/48	2	2/50	4
Carcinoma, Follicular Cell	m	0/50	0	0/50	0	1/48	2	1/50	2
[Adenoma, Follicular Cell	b	0/49	0	0/49	0	1/48	2	2/48	4
Carcinoma, Follicular Cell	m	0/49	0	0/49	0	1/48	2	1/48	2
Combined ⁽⁶⁾	b/m	0/49	0 *	0/49	0	2/48	4	3/48	6]
URINARY BLADDER									
Papilloma, Transitional Cell	b	0/50	0 *	0/49	0	0/48	0	2/50	4
Carcinoma, Transitional Cell	m	0/50	0	0/49	0	0/48	0	0/50	0
UTERUS									
Adenocarcinoma	m	2/50	4	3/50	6	3/48	6	6/50	12
Mixed Mullerian Tumor	m	0/50	0 *	0/50	0	1/48	2	2/50	4
CLITORAL GLANDS ⁽⁷⁾									
Carcinoma, Squamous Cell	m	0/0	0	0/0	0	0/0	0	2/2	--- ⁽⁷⁾
Adenocarcinoma	m	0/0	0	0/0	0	0/0	0	0/2	--- ⁽⁷⁾
BONE (ALL SITES)									
Osteosarcoma	m	0/50	0	0/50	0	0/48	0	0/50	0
NOSE/NASAL CAVITY ⁽⁸⁾									
Chondrosarcoma	m	0/0	0	0/0	0	0/0	0	0/0	0

⁽¹⁾ Wistar substrain used in this study was Hsd/WIN:WU.

⁽²⁾ Data extracted from MRID 44865723.

⁽³⁾ Number of animals with lesion/Number of animals histopathologically evaluated

⁽⁴⁾ Statistically significant trend denoted at control (0 ppm).

Statistically significant pairwise difference from control shown at dose level.

⁽⁵⁾ No pairwise group comparison performed.

⁽⁶⁾ Additional data analysis extracted from Fornillo, V. (2002). Iprovalicarb: Revised Qualitative Risk Assessment Based on Hsd/WIN:WU (SPF) Rat Dietary Study. HED Memorandum, dated February 21, 2002. TXR No. 0050482.

⁽⁷⁾ Non-protocol organ. Two squamous cell carcinomas observed at 20000 ppm. No pair-wise group comparison performed.

⁽⁸⁾ Non-protocol organ. No chondrosarcomas observed at any dose.

* p < 0.05; ** p < 0.01

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¹The incidence of mixed Mullerian tumors of the uterus observed in rats at 20000 ppm (2/48 or 4% for censored data; 2/50 or 4% for uncensored data) exceeded the highest historical control incidence rate in both the in-house laboratory historical control database (0% for uncensored data; Table 7a) and the RITA historical control database (2% for uncensored data; Table 8). In a study completed in the performing laboratory after finalization of the report on iprovalicarb (MRID 45493401; Table 7b), only 1 mixed Mullerian tumor of the uterus in 1 treated rat was noted.

The incidence of transitional cell papillomas of the urinary bladder observed in female rats at 20000 ppm (2/48 or 4% for censored data; 2/50 or 4% for uncensored data) exceeded the highest historical control incidence rate in both the in-house laboratory historical control database for female rats (0% for uncensored data from Table 7a) and the RITA historical control database for female rats (2.2% for uncensored data; Table 8).

The increased incidence of follicular cell adenomas of the thyroid gland observed in female rats at 20000 ppm (2/48 or 4% for censored data; 2/50 or 4% for uncensored data) exceeded the highest historical control incidence rate in the in-house laboratory historical control database for female rats (2% for uncensored data; Table 7a), but did not exceed the highest historical control incidence rate in the RITA database for female rats (6.1% for uncensored data; table 8). The increased incidence of follicular cell carcinomas of the thyroid gland observed in female rats at 20000 ppm (1/48 or 2% for censored data; 1/50 or 2% for uncensored data) exceeded the highest historical control incidence rate in the in-house laboratory historical control database for female rats (0% for uncensored data; Table 7a), but did not exceed the highest historical control incidence rate in the RITA database for female rats (5.0% for uncensored data; Table 8).

Although no adenocarcinomas of the clitoral gland occurred in the iprovalicarb study, Bayer nevertheless reported incidences for this lesion in the historical control data and suggested it might be appropriate to combine incidences of both squamous cell carcinomas and adenocarcinomas of the clitoral gland when considering background levels of tumors in this organ. The CARC, however, considered squamous cell carcinomas and adenocarcinomas of the clitoral gland to be sufficiently different in etiology and histogenesis to not combine them for the evaluation of carcinogenic potential. The number of squamous cell carcinomas of the clitoral gland observed in rats at 20000 ppm (2 tumors in 1 study) exceeded the highest number of similar tumors (1 tumor in 1 study) in the in-house laboratory historical control database (Table 7a), and MRID 45493401 (Table 7b), as well as the RITA historical control database (Table 8). The CARC concluded that the biological and statistical significance of the findings of malignant squamous cell carcinomas of the clitoral gland in high dose females (2/2 examined) could not be determined due to inadequate examination of animals.

¹NOTE: In the report for this study by the performing laboratory (Bayer AG, MRID 44865723), the historical control data for tumors in Wistar rats in the following reference were cited (pp. 88-91): "BOMHARD E. and RINKE M., Exp. Toxic. Pathol. 46 17, 1994". Examination of this reference, however, revealed that all of the historical control data reported in this reference, although published in 1994, were for 2-year studies initiated at Bayer AG from May, 1975 to December, 1980. HED considers these data (1975 - 1980) to be too far removed from the iprovalicarb study (initiated in January, 1994) to be useful and did not consider historical control data from this reference in its evaluation of the results in the iprovalicarb study.

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**Table 7 a. Historical Control Data for Selected Tumors in Wistar ⁽¹⁾ Rats at Bayer AG ⁽²⁾
(Uncensored Data)**

Data are for fourteen 2-year studies performed at Bayer AG Institute of Toxicology (Wuppertal, Germany) completed between April, 1991 and April, 1998 (a total of 698 male and 700 female rats were examined for histopathology). b = benign; m = malignant.

ORGAN/TISSUE Tumor	MALES			FEMALES		
	Range Percent	Overall Incidence	Average Percent	Range Percent	Overall Incidence	Average Percent
BONE (ALL SITES) Osteosarcoma m	0 - 0	0/698	0.0	0 - 0	0/700	0.0
NOSE/NASAL CAVITY Chondrosarcoma m	0 - 0	0/xxx ⁽³⁾	0.0	0 - 0	0/xxx ⁽³⁾	0.0
TESTES Leydig Cell Tumor b/m	0 - 22	64/698	9.2	-----	-----	-----
PITUITARY GLAND Adenoma b	8 - 24	110/698	15.8	28 - 54	286/700	40.9
Adenocarcinoma m	0 - 2	1/698	0.1	0 - 4	6/700	0.9
MAMMARY GLAND Fibroadenoma b/m	0 - 0	0/698	0.0	2 - 32	118/700	16.9
Adenoma b	0 - 0	0/698	0.0	0 - 10	14/700	2.0
Adenocarcinoma m	0 - 2	3/698	0.4	2 - 22	60/700	8.6
THYROID GLAND Adenoma, Follicular b	0 - 4	5/698	0.7	0 - 2	4/700	0.6
Carcinoma, Follicular m	0 - 2	1/698	0.1	0 - 0	0/700	0.0
URINARY BLADDER Papilloma, Transitional Cell b	0 - 0	0/698	0.0	0 - 0	0/700	0.0
Carcinoma, Transitional Cell m	0 - 0	0/698	0.0	0 - 0	0/700	0.0
UTERUS Adenocarcinoma m	-----	-----	-----	0 - 14	32/700	4.6
Mixed Mullerian Tumor m	-----	-----	-----	0 - 0	0/700	0.0
CLITORAL GLANDS Carcinoma, Squamous Cell m	-----	-----	-----	0 - 2	1/xxx ⁽⁴⁾	0.1

⁽¹⁾ Data are for the following substrains: BOR:WISW (SPF Cpb), Hsd/WIN:WU and Hsd Cpb:WU. Tumor data from all these substrains is considered to be comparable since all these substrains can be traced back to the Cpb:WU rat, bred between 1958 and 1986 at Zeist, Netherlands.

⁽²⁾ Data extracted from Schladt, L. and Eiben, R. (2000). Spontaneous Tumors in Wistar Rats in 2-Year Studies Performed at Bayer AG. Bayer AG, Wuppertal, Germany Bayer Report No. MO-00-000946. January 21, 2000. Unpublished. MRID 45048705.

⁽³⁾ Since the nose/nasal cavity is not a protocol tissue, the number of animals examined is not known.

⁽⁴⁾ Since the clitoral gland is not a protocol tissue, the number of animals examined is not known.

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Table 7b. Historical Control Data for Selected Tumors in Wistar ⁽¹⁾ Rats at Bayer AG ⁽²⁾

Data is for studies performed at Bayer AG Institute of Toxicology (Wuppertal, Germany) with in-life phase between 1990 and 1999. The data "includes lesions found in treated rats but definitely demonstrated no relation to treatment" (quoted from page 4 of MRID 45493401).

"Lesions reported in other studies after finalization of the report on SZX 0722 [iprovalicarb] are marked with an asterisks" (quoted from page 7 of MRID 45493401).

ORGAN/TISSUE Tumor	Con- trol	Dose 1	Dose 2	Dose 3	Dose 4	Report No.	Remarks
URINARY BLADDER Carcinoma, Transitional Cell	1	0	0	0	-	29419	Female
FEMUR/BONE Osteosarcoma	1 *	0	0	0	-	28843	Male
	0	0	1 *	0	0	26610	Female
	0	1 *	0	0	-	28333	Female
	0	0	1 *	0	-	28333	Male, Sarcoma NOS
UTERUS Malignant Mixed Mullerian Tumor	0	1 *	0	0	0	27637	----
CLITORAL GLANDS Carcinoma, Squamous Cell	0	0	1 *	0	0	26610	----
	0	0	1	0	-	25522	----
	1 *	0	0	0	0	27480	----
Adenocarcinoma	0	1 *	0	0	-	28843	----
	0	1	0	0	0	25426	----
	1 *	0	0	0	0	27637	----

⁽¹⁾ Wistar substrains(s) used in these studies was not identified.

⁽²⁾ Data extracted from Hartmann, E. (2000), Update of Historical Control Data on Rare Tumors in Wistar Rats in a 2-Year Carcinogenicity Study with SZX 0722, Bayer AG, Wuppertal, Germany, Bayer Report No. 27160; MO-01-016359, December 1, 2000, Unpublished, MRID 45493401.

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Table 8. Historical Control Data for Selected Tumors in Wistar ⁽¹⁾ Rats in RITA Database ⁽²⁾ (Uncensored Data)

Data are from RITA database (Hannover, Germany) for 54-69 studies of 24-31 months duration started between May, 1984 and August, 1997. With a few exceptions, 50 or 100 male and 50 or 100 female rats were histopathologically evaluated from each study. N.R. = Not Reported to EPA.

ORGAN/TISSUE Tumor		MALES			FEMALES		
		Range Percent	Overall Incidence	Average Percent	Range Percent	Overall Incidence	Average Percent
BONE (ALL SITES)							
Osteosarcoma	m	0 - 2.0	7/2807	0.2	0 - 4.0	3/2707	0.1
NOSE/NASAL CAVITY							
Chondrosarcoma	m	0 - 0	0/xxx ⁽³⁾	0.0	0 - 0	0/xxx ⁽³⁾	0.0
TESTES							
Leydig Cell Tumor	b/m	N.R.	N.R.	N.R.	-----	-----	-----
PITUITARY GLAND							
Adenoma	b	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
Adenocarcinoma	m	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
MAMMARY GLAND							
Fibroadenoma	b/m	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
Adenoma	b	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
Adenocarcinoma	m	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
THYROID GLAND							
Adenoma, Follicular	b	N.R.	N.R.	N.R.	0 - 6.1	N.R.	N.R.
Carcinoma, Follicular	m	N.R.	N.R.	N.R.	0 - 5.0	N.R.	N.R.
URINARY BLADDER							
Papilloma, Transitional Cell	b	0 - 15.0 ⁽⁴⁾	38/3571	1.1	0 - 2.2	7/3456	0.2
Carcinoma, Transitional Cell	m	0 - 1.0	4/3571	0.1	0 - 2.0	1/3456	<0.1
UTERUS							
Adenocarcinoma	m	-----	-----	-----	0 - 14.3	98/3011	3.3
Mixed Mullerian Tumor	m	-----	-----	-----	0 - 2.0	4/3485	0.1
CLITORAL GLANDS							
Carcinoma, Squamous Cell	m	-----	-----	-----	----- ⁽⁵⁾	2/xxx ⁽⁵⁾	----- ⁽⁵⁾

⁽¹⁾ Data are for several substrains of Wistar rats (obtained from several breeders) used in studies by various European companies.

⁽²⁾ Data extracted from letter from Dr. E. Hartmann (Bayer AG) to EPA dated 9/5/01 (MRID 45493400) and from Hartmann, E. (2000), Update of Historical Control Data on Rare Tumors in Wistar Rats in a 2-Year Carcinogenicity Study with SZX 0722, Bayer AG, Wuppertal, Germany, Bayer Report No. 27160; MO-01-016359, December 1, 2000, Unpublished, (MRID 45493401) and from study report (MRID 44865723).

⁽³⁾ Historical control data not available. Presumably, no chondrosarcoma was reported in any study in Wistar rats in the RITA database. Since the nasal cavity is not a protocol tissue, the number of animals examined is not known.

⁽⁴⁾ One study with 15.0 percent incidence. Next highest percent incidence was 8.0 percent in another study.

⁽⁵⁾ Non-protocol organ. One lesion observed in each of 2 studies (1993 and 1995) out of 69 studies.

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C. Non-neoplastic Lesions and Other Related Changes

The statistical evaluation of mortality in this study (Fornillo, 2002) indicated a statistically significant decreasing trend with increasing doses of iprovalicarb in male rats. There were only a few non-neoplastic treatment-related effects observed in the male rats in this study. A statistically significant increased alkaline phosphatase activity at 20000 ppm during week 53 and 105 of the study and a statistically significant increased incidence of bile duct hyperplasia in the liver at 5000 and 20000 ppm at termination indicated the liver was the main target organ. Slightly increased Leydig cell hyperplasia in the testes at 20000 ppm was also noted (statistically significant positive trend; but no pairwise comparison performed); the incidences were 2/50, 2/50, 4/49 and 6/50 in the control, 500 ppm, 5000 ppm and 20000 ppm groups, respectively.

In female rats, there were no statistically significant incremental changes in mortality with increasing doses of iprovalicarb. Non-neoplastic treatment-related effects observed in the female rats in this study at 5000 and 20000 ppm were statistically significant decreased body weight gain (after 3 months at 5000 ppm and throughout the study at 20000 ppm), statistically significant increased serum cholesterol levels (after 1 year), statistically significant increased absolute and/or relative liver weights, and statistically significant increased hepatocellular hypertrophy in the liver. Additional non-neoplastic effects observed at 20000 ppm only included increased vaginal bleeding, slightly decreased food efficiency, and increased hematopoiesis in the bone marrow (femur and sternum). Increased incidences of hyperplastic lesions of the transitional epithelium of the urinary bladder were not observed in the female (or male) rats in this study. Pre-neoplastic lesions in the thyroid gland (focal hyperplasia of the follicular epithelium) occurred at incidences of 2/50, 0/50, 1/48 and 3/50 in the control, 500 ppm, 5000 ppm and 20000 ppm groups, respectively. Other lesions indicating an effect on the thyroid gland, such as hypertrophy, were not observed.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Although the highest dose tested exceeded the limit dose for carcinogenicity testing, no treatment-related adverse effect on the survival of male and female rats was noted. In male rats, there was an increase in alkaline phosphatase activity at week 53 and 105 at 20000 ppm, as well as a statistically significant increase in the incidence of bile duct hyperplasia at ≥ 5000 ppm and a slight increase in Leydig cell hyperplasia of the testes at 20000 ppm. In female rats, the treatment-related effects observed included decreased body weight gains ($\geq 10\%$), increased absolute and/or relative liver weights, and statistically significant increased hepatocellular hypertrophy in the liver at ≥ 5000 ppm. Increased vaginal bleeding, slightly decreased food efficiency, and increased hematopoiesis in the bone marrow (femur and sternum) were also noted at 20000 ppm. None of these effects occurred to a degree that was excessively adverse. Based on the results of metabolism studies there was no deviation from metabolic pathway at the highest dose tested which would have suggested that the highest dose was excessive. **Based on these findings, which were not severely adverse, the CARC concluded that the highest dose level of 20000 ppm was adequate and not excessive for carcinogenicity testing.**

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2. Carcinogenicity Study in B6C3F₁ Mice

Reference:

Leser, K.H. and O. Vogel. 1997. SZX 0722: Oncogenicity study in B6C3F₁ mice (administration in the feed over 2 years). Bayer AG Institute of Toxicology, Wuppertal, Germany. Laboratory Study Number T 0055442. Bayer Report No. 26450. November 7, 1997. Submitted by TM-210 (SZX 0722) Fungicide Task Force. MRID 44865722. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID 44865722), SZX 0722 (batch no. NLL 4812-6.1, 95.8-98.5% purity) was administered in the feed to 50 B6C3F₁ (SPF) mice/sex/dose level for 105 weeks at dose levels of 0, 280, 1400 or 7000 ppm (equivalent to 0, 58.5, 283.4 or 1566.8 mg/kg/day in males and 0, 97.4, 503.1 or 2544.0 mg/kg/day in females). An additional 10 mice/sex/dose level were sacrificed at 52 weeks for interim evaluations.

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

There were no treatment-related adverse effects on survival. There was no evidence of treatment-related increase in tumors in either sex at any of the dose levels tested.

Incidences of selected neoplastic lesions in male and female mice are presented in Table 9. In male mice, an increased incidence of hepatocellular adenomas and of hepatocellular carcinomas was observed at 280 ppm, but not at 1400 ppm or 7000 ppm (7/50, 15/50, 7/50 and 7/50 for hepatocellular adenomas and 7/50, 10/50, 6/50 and 7/50 for hepatocellular carcinomas at 0, 280 ppm, 1400 ppm and 7000 ppm, respectively). The percent incidence rates in the historical control database for hepatocellular neoplasms in male B6C3F₁ mice are reported to range from 7% to 58% (page 771 in study report, MRID 44865722). Since the increased incidences observed at 280 ppm of both the adenomas and carcinomas were clearly not dose-related and the incidences of both tumor types at both the higher dose levels of 1400 ppm and 7000 ppm were nearly identical to the control incidences, and all the observed incidences fell within the historical control range, the hepatocellular tumors at 280 ppm were not considered to be treatment-related. In addition, a liver foci test for tumor initiating effect, conducted in male Wistar rats (MRID 44908616), failed to demonstrate that iprovalicarb is a liver tumor initiator. No other tumors of concern were observed in the male mice in this study.

In female mice, a slightly increased incidence of hepatocellular adenomas, but not of hepatocellular carcinomas was observed at 7000 ppm (1/50, 1/50, 2/50 and 4/50 for hepatocellular adenomas and 2/50, 1/50, 1/50 and 0/50 for hepatocellular carcinomas at 0, 280 ppm, 1400 ppm and 7000 ppm, respectively). The percent incidence rates in the historical control database for hepatocellular neoplasms in female B6C3F₁ mice are reported to range from 0% to 21% (page 771 in study report, MRID 44865722). Since the increased incidence of hepatocellular adenomas at 7000 ppm was small, and the incidences at all the dose levels fell well within the historical control range, the hepatocellular tumors at 7000 ppm were not considered to be treatment-related. No other tumors

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of concern were observed in the female mice in this study.

Table 9. Incidence of Selected Neoplastic Lesions in Male and Female B6C3F₁ Mice in Iprovalicarb Study ⁽¹⁾

Study performed at Bayer AG Institute of Toxicology (Wuppertal, Germany). In-life phase completed in March, 1996.

ORGAN/TISSUE Tumor	Dose (ppm)	MALES				FEMALES			
		0	280	1400	7000	0	280	1400	7000
LIVER # exam		50	50	50	50	50	50	50	50
Adenoma, hepatocellular b		7	15	7	7	1	1	2	4
Carcinoma, hepatocellular m		7	10	6	7	2	1	1	0
LUNGS # exam		50	50	50	50	50	50	50	50
Pulmonary adenoma b		6	6	5	4	2	2	0	0
Pulmonary carcinoma m		1	0	1	1	1	0	0	0
PITUITARY GLAND # exam		46	50	49	48	49	50	50	47
Adenoma b		0	0	0	0	7	5	2	5
HEMATOPOIETIC # exam		50	50	50	50	50	50	50	50
Lymphoma m		5	2	1	6	13	23	14	11
Histiocytic sarcoma m		1	2	1	0	0	3	2	2
HARDERIAN GLAND # exam		50	50	50	50	50	50	50	50
Adenoma b		3	3	4	3	4	1	7	2

¹ Data extracted from MRID 44865722.

C. Non-neoplastic Lesions

Non-neoplastic treatment-related effects observed in the male mice in this study at 7000 ppm and 1400 ppm suggested an impairment of kidney function at these dose levels as evidenced by increased concentrations of blood urea nitrogen (BUN), statistically significant decreased absolute and relative kidney weights, and greatly decreased incidences of tubular vacuolization in the kidney (6% at 7000 ppm and 48% at 1400 ppm vs 100% in controls). Increased absolute and relative liver weights (not statistically significant) were also observed at these same dose levels. Additional non-neoplastic effects observed at 7000 ppm included increased fatty changes in the liver (42% vs 10% in controls) and statistically significant increased triglyceride concentrations (at 52 weeks), and relative liver weights (at 104 weeks).

Non-neoplastic treatment-related effects observed in the female mice in this study at 7000 ppm and 1400 ppm also suggested a slight impairment of kidney function at these dose levels as evidenced by increased BUN levels. Additional non-neoplastic effects observed at 7000 ppm included increased fatty changes in the liver (14% vs 4% in controls) and increased relative liver weights ($\geq 10\%$ of controls).

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D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Although the highest dose tested exceeded the limit dose for carcinogenicity testing, no adverse effects on the survival of mice was noted. The treatment-related effects observed in male mice at ≥ 1400 ppm suggested an impairment of kidney function as evidenced by increased concentrations of blood urea nitrogen (BUN), statistically significant decreased absolute and relative kidney weights, and decreased incidences of tubular vacuolization in the kidney (6% at 7000 ppm and 48% at 1400 ppm). Increase in BUN level at ≥ 1400 ppm was also noted in female mice. In addition in males at ≥ 1400 ppm, there was a statistically non-significant increase in absolute and relative liver weights in males and at 7000 ppm increased fatty changes in the liver. In females, increased relative liver weights and increased fatty changes in the liver were also observed at 7000 ppm. **Based on these findings, which were not severely adverse, the CARC concluded that the dosing was adequate and not excessive for carcinogenicity testing in male and female mice.**

IV. TOXICOLOGY DATA

1. Metabolism

The following summary was extracted from the PMRA review of the study. Iprovalicarb is chemically composed of two (S,R and S,S) diastereoisomers. In a series of metabolism studies on male and female Wistar rats (MRID 44865733, 44865734, 44865735), ^{14}C -iprovalicarb was administered by gavage at single doses of 2 mg/kg, or 150 mg/kg, or 2 mg/kg following 14 days of repeated doses of 2 mg/kg/day of non-labeled iprovalicarb. Bile canulation and whole-body autoradiography experiments were also conducted. Iprovalicarb was rapidly absorbed, widely distributed, extensively metabolized and quickly excreted regardless of sex or dose or frequency administered. An enterohepatic circulation was observed. There was little bioaccumulation. Less than 21% of the administered dose was excreted unchanged. Twelve metabolites were identified. The major metabolite was a pair of diastereoisomers of iprovalicarb-carboxylic acid. In the bile, iprovalicarb-carboxylic acid and two conjugates thereof accounted for about 87% of the radioactivity in the bile. Based on the results of metabolism studies there was no deviation from metabolic pathway at the highest dose tested.

In an additional metabolism study, single doses of 2 mg/kg of ^{14}C -iprovalicarb were orally administered to Wistar rats following 13 weeks of feeding of non-labeled iprovalicarb in the diet at dose levels of 0, 500 or 20000 ppm. Results were very similar to those determined in the standard metabolism study and there was little difference in parameters examined between the animals given 0, 500 or 20000 iprovalicarb.

2. Mutagenicity

There are five acceptable genotoxicity studies on technical grade iprovalicarb and an additional study on p-methyl-phenethylamine, a metabolite of iprovalicarb in rats. Together, they satisfy the revised mutagenicity guidelines of 1991 (OPP Pesticide Assessment Guidelines, Subdivision F, Series 84, Addendum 9) which are applicable to all new active ingredients. **Results of all six studies were negative for mutagenic potential.** The following summaries were extracted from the PMRA reviews

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of these studies.

Gene Mutations

Salmonella typhimurium Reverse Mutation Assay (Herbold, B.A., 1994, MRID 44865724)

In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* were exposed to iprovalicarb technical at concentrations of 0, 8, 40, 200, 1000 or 5000 ug/plate in an initial trial and to concentrations of 0, 125, 250, 500, 1000 or 4000 ug/plate in a confirmatory trial. There was no evidence of induced mutant colonies over background without or with S9 metabolic activation.

Mammalian Cells in Culture Forward Gene Mutation Assay (Brendler-Schwab, S., 1995, MRID 44865727)

In an in vitro mammalian (cell) point mutation study, cultures of Chinese hamster lung fibroblasts (V79 cells) were exposed to iprovalicarb technical at concentrations of 0, 7.8, 15.6, 31.3, 62.5 125 or 150 ug/ml without S9 metabolic activation and to concentrations of 0, 12.5, 25, 50, 75, 100 or 125 ug/ml with S9 metabolic activation. There was no evidence of induced point mutations over background without or with S9 metabolic activation.

Salmonella typhimurium Reverse Mutation Assay on p-methyl-phenethylamine, a metabolite of iprovalicarb in rats. (Herbold, B.A., 1996, MRID 44865725)

In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA 102, TA1535 and TA1537 of *S. typhimurium* were exposed to p-methyl-phenethylamine, a metabolite of iprovalicarb in rats at concentrations of 0, 16, 50, 158, 500 1581 or 5000 ug/plate. There was no evidence of induced mutant colonies over background without or with S9 metabolic activation.

Chromosome Aberrations

Mammalian Cells in Culture Chromosomal Aberration Assay (Gahlmann, R., 1995, MRID 44865726)

In an in vitro mammalian (cell) chromosomal aberration assay, cultures of Chinese hamster ovary (CHO) cells were exposed to iprovalicarb technical at concentrations of 0, 6, 30 or 150 ug/ml. There was no evidence of chromosomal aberrations induced over background without or with S9 metabolic activation.

In vivo Cytogenetics (Herbold, B., 1995, MRID 44865728)

In an in vivo mouse bone marrow micronucleus assay, male and female mice were treated with iprovalicarb technical at doses of 0 or 2000 mg/kg. No evidence of clastogenic or aneugenic potential of the test material was observed.

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Other Mutagenic Effects

Unscheduled DNA Synthesis in Mammalian Cells in Culture ((Brendler-Schwab, S., 1996, MRID 44865729)

In an in vitro unscheduled DNA synthesis (UDS) assay, cultures of rat primary hepatocytes were exposed to iprovalicarb technical at concentrations of 0, 50, 150, 200, 250, 300, 400 or 500 ug/ml. No evidence of unscheduled DNA synthesis was observed.

3. Structure-Activity Relationships

Iprovalicarb is the prototype member of a new class of fungicides termed amino acid amide carbamates. As such, it has a unique chemical structure. HED is not currently aware of any structural analogs for this chemical.

4. Subchronic and Chronic Toxicity

In subchronic and chronic oral toxicity studies in rats, dogs and mice, there was no evidence suggesting cumulative toxicity. The liver appeared to be the primary target organ with the dog being the most sensitive species. Induction of the liver microsomal enzyme system (LMES) occurred in rats and dogs as demonstrated by increased N-demethylase activity, increased O-demethylase activity, increased cytochrome P-450 levels, increased absolute and/or relative liver weights, and hepatocellular hypertrophy. Additional signs of treatment-related liver damage observed in rats, dogs and/or mice included increased levels of several serum enzymes indicative of liver damage (e.g. ALP, GGT, GLDH, ALT, and AST), increased or decreased serum cholesterol levels, serum triglyceride levels, and serum protein levels, gross pathological changes in the liver (enlargement, discoloration, distinct lobulation), and increased incidences of several histopathological lesions in the liver (cytoplasmic vacuolation, hepatic necrosis, fatty changes, increased hemosiderin in hepatocytes and Kupffer cells, and bile duct hyperplasia). In mice (in the carcinogenicity study), there were also treatment-related effects on the kidneys as demonstrated by increased serum blood urea nitrogen (BUN) levels, decreased absolute and relative kidney weights, and decreased incidences of tubular vacuolization in the kidneys of males.

Subchronic Toxicity

In a 13-week subchronic dietary study in Wistar rats (MRID 44865710), iprovalicarb was administered in the feed to 10 rats/sex/group at dose levels of 0, 1250, 5000 or 20000 ppm. The LOAEL for both sexes was 20000 ppm (1524 mg/kg/day in males and 2586 mg/kg/day in females) based on treatment-related increased food intake with decreased body weight gain and food efficiency, and increased plasma cholesterol levels in females, decreased plasma triglycerides and increased leukocyte counts, alkaline phosphatase levels and incidences of pale livers in males, as well as increased relative liver weights in both sexes. The NOAEL was 5000 ppm (373 mg/kg/day in males and 561 mg/kg/day in females). Increased liver N-demethylase and O-demethylase activities and increased levels of liver cytochrome P-450 levels, observed in males and females, were considered to be indicators of liver microsomal enzyme induction and not necessarily as indicators of overt toxicity.

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In a 13-week subchronic dietary study in beagle dogs (MRID 44865714), iprovalicarb was administered in the diet to 4 dogs/sex/group at dose levels of 0, 250, 2500 or 50000 ppm. The LOAEL for both sexes was 2500 ppm (62.5 mg/kg/day in males and females) based on treatment-related decreased protein (albumin) levels, slightly increased serum alkaline phosphatase levels, increased absolute and relative liver weights and histopathological changes (hepatocellular hypertrophy) in the liver. The NOAEL was 250 ppm (9.1 mg/kg/day in males and females). At 50000 ppm (1250 mg/kg/day in males and females), the following treatment-related effects were also observed (in addition to those observed at 2500 ppm): 1 mortality (moribund sacrifice at 9 weeks), decreased body weight gain, decreased food consumption, altered clinical chemistry parameters indicating liver damage, decreased prostate, testes and thymus weights, gross pathological changes in the liver, and several histopathological changes in the liver. Increased liver N-demethylase and O-demethylase activities and increased levels of liver cytochrome P-450 levels, observed in males and females at ≥ 250 ppm, were considered to be indicators of liver microsomal enzyme induction and not necessarily as indicators of overt toxicity.

In a 13-week subchronic dose-range finding dietary study in B6C3F₁ mice (MRID 44865711), iprovalicarb was administered in the feed to 6 or 10 mice/sex/group at dose levels of 0, 280, 1400, 7000 or 14000 ppm. The LOAEL for both sexes was 7000 ppm (1725 mg/kg/day in males and 3599 mg/kg/day in females) based on increased water consumption and increased mean corpuscular volume (MCV) in males and increased plasma cholesterol levels and relative liver weights in females. The NOAEL for both sexes was 1400 ppm (325 mg/kg/day in males and 696 mg/kg/day in females). At 14000 ppm (3473 mg/kg/day in males and 6869 mg/kg/day in females), the following treatment-related effects were also observed (in addition to those observed at 7000 ppm): increased water consumption in females, changes in several hematological parameters in both males and females, and slight increases in absolute liver weights in males and females.

Chronic Toxicity

In a 24-month combined chronic toxicity/carcinogenicity study (MRID 44865723), dietary administration of iprovalicarb (refer to page 1 for details regarding the dose levels) to male rats caused a statistically significant increased alkaline phosphatase activity at 20000 ppm and increased incidence of bile duct hyperplasia in the liver at ≥ 5000 ppm at termination indicating that the liver was the main target organ. Slightly increased Leydig cell hyperplasia in the testes at 20000 ppm was also noted. The NOAEL in male rats was 500 ppm (26.0 mg/kg/day) and the LOAEL was 5000 ppm (262.5 mg/kg/day), based on increased bile duct hyperplasia in the liver.

The female rats at ≥ 5000 ppm had a statistically significant decreased body weight gain, increased serum cholesterol levels (after 1 year), statistically significant increased absolute and/or relative liver weights, and statistically significant increased hepatocellular hypertrophy in the liver. Increased vaginal bleeding, slightly decreased food efficiency, and increased hematopoiesis in the bone marrow (femur and sternum) were also noted at 20000 ppm. The NOAEL in female rats was 500 ppm (31.7 mg/kg/day) and the LOAEL was 5000 ppm (326.3 mg/kg/day), based on possibly decreased body weight and body weight gain, increased cholesterol levels, increased relative liver weights, and increased hepatocellular hypertrophy. Refer to page 14 for further details.

In a 105-week carcinogenicity study (MRID 44865722), dietary administration of iprovalicarb (refer to page 15 for details regarding the dose levels) to B6C3F₁ (SPF) mice at ≥ 1400 ppm caused changes

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indicative of an impairment of kidney function in males; these included increased concentrations of blood urea nitrogen (BUN), statistically significant decreased absolute and relative kidney weights, and greatly decreased incidences of tubular vacuolization in the kidney. Additional changes noted at 7000 ppm included increased fatty changes in the liver and statistically significant increased triglyceride concentrations, and relative liver weights. The NOAEL in male mice was 280 ppm (58.5 mg/kg/day) and the LOAEL was 1400 ppm (283.4 mg/kg/day), based on increased BUN, decreased absolute and relative kidney weights, and decreased tubular vacuolization in the kidney. Refer to page 16 for further details

The female mice at ≥ 1400 ppm also had a slight impairment of kidney function as evidenced by increased BUN levels. Additional effects observed at 7000 ppm included increased fatty changes in the liver and increased relative liver weights. The NOAEL in female mice was 280 ppm (97.4 mg/kg/day) and the LOAEL was 1400 ppm (503.1 mg/kg/day), based on increased BUN. Refer to page 16 for further details.

In a 53-week chronic dietary study in beagle dogs (MRID 44865721), iprovalicarb was administered in the diet to 4 dogs/sex/group at dose levels of 0, 80, 800 or 8000 ppm. The LOAEL for both sexes was 800 ppm (25 mg/kg/day in males and 28 mg/kg/day in females) based on increased serum ALT and ALP activities, increased absolute and relative liver weights, increased gross pathological changes in the liver (enlargement, distinct lobulation, discoloration), increased histopathological changes in the liver (hepatocellular hypertrophy, fatty changes, increased iron storage), and adhesive mucous in the gall bladder in males and females. The NOAEL was 80 ppm (2.6 mg/kg/day in males and 2.7 mg/kg/day in females). At 8000 ppm (256 mg/kg/day in males and 262 mg/kg/day in females), the following treatment-related effects were also observed (in addition to those at 800 ppm): severe emaciation in 1 male, decreased body weight gain in males, increased serum AST, GLDH, and GGT activities and increased plasma albumin in males and females, additional histopathological changes in the liver (focal necrosis, multi lamellar inclusions, binucleated hepatocytes, fibrosis) in males and females, inactive prostate gland in 2 males, and decreased spermatogenesis in the testes of 1 male. Increased liver N-demethylase and O-demethylase activities and increased levels of liver cytochrome P-450 levels, observed in males and females at ≥ 80 ppm, were considered to be indicators of liver microsomal enzyme induction and not necessarily as indicators of overt toxicity.

5. Mode of Action Studies

A. Liver Foci Test for Tumor Initiating Effect

The following summary was extracted from the PMRA review of this study. In a liver foci test for tumor initiating effect (MRID 44908616), technical grade iprovalicarb was administered by gavage to groups of 10 male Wistar rats at concentrations of 0 (vehicle only) or 1000 mg/kg/day for 28 days, followed by eight weeks of promotion treatment with phenobarbital. Foci of altered hepatocytes (FAH) were microscopically assessed by four different histochemical marker reactions [glycogen storage, glucose-6-phosphatase (G-6Pase), gamma-glutamyl transpeptidase (GGT), and glycerin-3-phosphate dehydrogenase (G3PDH)]. The number of FAR observed per unit surface in the liver of animals exposed to the test substance was similar to the number in the control group of

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animals. Under the conditions of this study, iprovalicarb did not demonstrate tumor initiating potential in the livers of male rats. The assay methodology used in this study, however, may not have been sufficiently sensitive to detect initiation. Therefore, the lack of a positive response in this study does not necessarily indicate the absence of an initiation potential for iprovalicarb.

B. *In Vivo* ³²P Post-Labeling Assay in Rat Uterus and Urinary Bladder Epithelial Tissues

The following summary was extracted from the PMRA review of this study. In an *in vivo* (Phosphorus-32) post-labeling assay (MRID 44865730), female Wistar rats were given diets containing 10000 or 20000 ppm of iprovalicarb for 7 days and then treated with ³²P; the positive controls received a single dose of 2-acetylaminofluorene (2-AAF) or 7,12-dimethylbenzanthracene (DMBA). The rats were subsequently examined for formation of covalently formed DNA adducts in the epithelium of the uterus, the epithelium of the urinary bladder, and whole urinary bladder. No evidence of treatment-related DNA adduct formation was observed. The study author concluded that the lack of effect was not due to the inappropriateness of the test performance but due to the fact that nuclease P1 enrichment is not suitable for the detection of 2-AAF specific adducts in urinary bladder epithelium. However, in the absence of any accepted guidelines one cannot attest to the significance of the study author's conclusions or to the validity of the study.

C. (Lack of) Studies Relating to Disruption of Thyroid-Pituitary Homeostasis in Rats

With respect to the increased incidences of follicular cell tumors observed in the thyroid glands of the female rats at the higher dose levels tested in the carcinogenicity study on iprovalicarb, although evidence of liver microsomal enzyme induction was observed in the same study, no data or studies were presented by the TM-210 (SZX 0722) Fungicide Task Force suggesting that these tumors may have been the result of a disruption in thyroid-pituitary homeostasis.

V. ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that iprovalicarb was carcinogenic to male and female rats, but not to mice based on the following:

- A. Some rare and uncommon tumors were observed in multiple organs/tissues in male and female rats at the highest dose level tested (1109.6 mg/kg/day in males and 1379.7 mg/kg/day in females), a dose which exceeded the limit dose of 1000 mg/kg/day, which was determined to be adequate but not excessive. . Some of these tumor types were also noted in female rats at the next highest dose level tested (326.3 mg/kg/day).

1) In male rats, the rare tumors of concern were osteosarcomas of the bone and a chondrosarcoma of the nasal cavity. There was a statistically significant increasing trend for osteosarcomas of the femur and of the lower jaw (both at $p < 0.05$) as well as for the combined osteosarcomas ($p < 0.01$). There was also a statistically significant ($p < 0.05$) difference by pair-

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wise comparison of the high dose group with the controls for combined osteosarcomas. In addition, one chondrosarcoma of the nasal cavity was observed in the high dose group. Although osteosarcomas and chondrosarcomas have a common etiology, they were not combined for statistical analyses because of inadequate examination of animals for chondrosarcomas. The biological significance of the finding of chondrosarcoma could not be determined. There were no osteosarcomas (0/698) and no chondrosarcomas observed in the male historical controls from the Bayer Laboratory; only one osteosarcoma was noted in MRID 4549401. The incidence of osteosarcomas at the highest dose was outside the historical control range for males of the in-house (Bayer AG) laboratory and the RITA database (0%-2%); no chondrosarcoma was reported in the RITA database. Only one osteosarcoma and no chondrosarcomas were observed in the male historical controls from the Bayer Laboratory. The incidence of osteosarcomas at the highest dose was outside the historical control range of the RITA database (0%-2% for males); no chondrosarcoma was reported in the RITA database. Osteosarcomas are malignant and rare tumors. Additionally, male rats also developed tumors of thyroid, pituitary, mammary glands and testes. The incidences of these tumors were within the historical control values and lacked the dose-response relationship. Therefore, these tumors were not considered by the CARC to be treatment-related.

2) In female rats, the rare tumors of concern were malignant mixed Mullerian tumors of the uterus, benign transitional cell papillomas of the urinary bladder, and malignant squamous cell carcinomas of the clitoral gland. The infrequently observed tumors of concern were follicular cell adenomas and follicular cell carcinomas of the thyroid gland. All of the aforementioned tumors in female rats were observed at the highest dose and some at the next highest dose.

Except for the positive trend in female rats for combined follicular cell adenomas/carcinomas ($p < 0.05$), in no other case in the female rats was there a statistically significant trend or difference by pair-wise comparison of the highest dose group with the controls. The biological and statistical significance of the findings of malignant squamous cell carcinomas of the clitoral gland in high dose females (2/2 examined) could not be determined due to inadequate examination of animals. 3) For all of the following rare tumor types, the increased incidence observed in the iprovalicarb study exceeded the highest value of the historical control incidence rate in both the in-house laboratory (Bayer AG) historical control database and the RITA historical control database: malignant osteosarcomas of the bone in males, malignant mixed Mullerian tumors of the uterus in females, and benign transitional cell papillomas of the urinary bladder in females. In addition, for follicular cell adenomas and carcinomas of the thyroid gland in females, which are considered to be infrequently observed tumors in Wistar rats, the increased combined incidence in the iprovalicarb study exceeded the highest historical control incidence rate in the in-house laboratory (Bayer AG) historical control database, but not the RITA historical control database. **These rare or uncommon tumors in male and female rats were considered by the CARC to be treatment-related.** Although there was an increase in the incidence of adenocarcinomas of the uterus in all treated groups compared to controls, the incidence at the high dose fell within the range of historical control rates for both the in-house laboratory (Bayer AG) historical controls and the RITA historical control database. The biological significance of this numerical increase in uterine adenocarcinomas could not be determined. Furthermore, these tumors are not rare and, therefore, were not considered by the CARC to be treatment-related. Additionally, female rats also developed tumors of the pituitary

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and mammary glands but the incidences were within the range for the historical controls values, these tumors were also in the control groups, and no dose-response was evident. Therefore, they were not considered by the CARC to be treatment-related. 4) **The dosing in this study was considered to be adequate** and not excessive for assessment of carcinogenic potential because the effects seen were not severely adverse, even though the limit dose level of 1000 mg/kg/day was exceeded for both the male rats (1109.6 mg/kg/day) and the female rats (1379.7 mg/kg/day). In male rats, there was an increase in alkaline phosphatase activity at 20000 ppm, as well as a statistically significant increase in the incidence of bile duct hyperplasia at ≥ 5000 ppm and a slight increase in Leydig cell hyperplasia in the testes at 20000 ppm. In female rats, the treatment-related effects observed included decrease in body weight gains ($\geq 10\%$), increased absolute and/or relative liver weights, and statistically significant increased hepatocellular hypertrophy in the liver at ≥ 5000 ppm. Regarding the highest dose level (which was noted by the performing laboratory as being quite high), it was noted in the metabolism studies performed on Wistar rats that there were no biologically meaningful differences in absorption, distribution, metabolism, excretion or qualitative differences in metabolites produced between the low and high dose groups administered iprovalicarb. Based on the results of metabolism studies there was no deviation from metabolic pathway at the highest dose tested which would have suggested that the highest dose was excessive. Thus, the rare and uncommon tumors seen in the rat study occurred at doses which were not excessively toxic.

- B. In the carcinogenicity study in $B_6C_3F_1$ mice (1997), **iprovalicarb did not induce a treatment-related increase in tumors in either sex at any of the dose levels tested.** Although increased incidences of hepatocellular adenomas and of hepatocellular carcinomas were observed in male mice at 280 ppm (the lowest dose level tested), increased incidences of the same tumor types were not observed at the higher dose levels of 1400 ppm or 7000 ppm. The incidences of both tumor types at ≥ 1400 ppm comparable to the control incidences, and the incidences in all the dosed groups fell within the historical control range, the hepatocellular tumors at 280 ppm were not considered to be treatment-related. **The dosing in this study was considered to be adequate and not excessive for assessment of carcinogenic potential** because the effects seen were not severely adverse, even though the animals were tested above the limit dose level of 1000 mg/kg/day (1566.8 and 2544.0 mg/kg/day for males and females, respectively) and no adverse effect on the survival of animals was noted. The treatment-related effects observed in male mice at ≥ 1400 ppm suggested an impairment of kidney function as evidenced by increased concentrations of blood urea nitrogen (BUN), statistically significant decreased absolute and relative kidney weights, and decreased incidences of tubular vacuolization in the kidney (6% at 7000 ppm and 48% at 1400 ppm). Increase in BUN level at ≥ 1400 ppm was also noted in female mice. In addition in males at ≥ 1400 ppm, there was a statistically non-significant increase in absolute and relative liver weights and at 7000 ppm increased fatty changes in the liver. In females, increased liver weights and increased fatty changes in the liver were also observed at 7000 ppm.

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2. Mutagenicity

Iprovalicarb was not mutagenic in a battery of acceptable *in vitro* and *in vivo* mutagenicity assays.

3. Structure-Activity Relationships

There was no information available on any structural analogs for iprovalicarb.

4. Mode of Action Studies

A special liver foci test did not demonstrate tumor initiating potential in the livers of male Wistar rats. It is noted, however, that the methodology employed in this specific study may not have been sufficiently sensitive to detect initiation.

In an *in vivo* ³²P post-labeling assay in female Wistar rats, no evidence of treatment-related DNA adduct formation was observed in the epithelium of the uterus, the epithelium of the urinary bladder or whole urinary bladder. In the absence of any accepted guidelines one cannot attest to the significance of the study author's conclusions or to the validity of the study.

With respect to the increased incidences of follicular cell tumors observed in the thyroid glands of the female rats at the higher dose levels tested in the carcinogenicity study on iprovalicarb, although evidence of liver microsomal enzyme induction was observed in the same study, no data or studies were presented by the TM-210 (SZX 0722) Fungicide Task Force suggesting that these tumors may have been the result of a disruption in thyroid-pituitary homeostasis.

In conclusion, there was insufficient information to clearly identify the mode of action or mechanism of the tumor induction by iprovalicarb (SZX 0722).

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified iprovalicarb into the category "**Likely to be carcinogenic to humans**" based on the following weight-of-the-evidence considerations:

1. Iprovalicarb induced rare and infrequently occurring tumors in rat. At the high dose, males developed malignant osteosarcomas and females developed benign transitional cell papillomas of the urinary bladder. At the mid and high doses, females developed malignant mixed Mullerian tumors of the uterus and follicular cell tumors of the thyroid gland. Although the incidences of these tumors were low, they are rare or uncommon in Wistar rats. Most of these tumors were induced above the limit dose which was not excessively toxic. In mice, no treatment-related increase in tumors was observed in animals treated above the limit dose which was adequate and not excessively toxic.
2. Iprovalicarb is not mutagenic. Although mechanistic studies suggested that iprovalicarb may not be a tumor initiator, these studies were inadequate to establish the definitive mode of action

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for tumor induction in rats...

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee further recommended using a linear low-dose extrapolation approach for estimating the human cancer risk based on the most potent tumor in rats. This approach is supported by the lack of confirmation of the mode of action of iprovalicarb.

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VIII. BIBLIOGRAPHY**MRID****Citation**

- 44865722 Leser, K.; Vogel, O. (1997) SZX 0722: Oncogenicity Study in B6C3F1 Mice (Administration in the Feed Over 2 Years): Lab Project Number: 26450: TMN-0182: T 0055442. Unpublished study prepared by Bayer Ag. 2378 p.
- 44865723 Schladt, L.; Hartmann, E. (1998) SZX 0722: Chronic Toxicity and Cancerogenicity (sic) Investigations in Wistar Rats (Administration in the Feed over 24 Months): Lab Project Number: PH-27160: TMN-018: T 1055425. Unpublished study prepared by Bayer Ag. 1823 p. {OPPTS 870.4300}
- 44865724 Herbold, B. (1994) SZX 0722: Salmonella/Microsome Test: Lab Project Number: 22842: TMN-0194: T 9049312. Unpublished study prepared by Bayer Ag. 52 p. {OPPTS 870.5100}
- 44865725 Herbold, B. (1996) P-Methyl-Phenethylamine: Salmonella/Microsome Test Plate Incorporation and Preincubation Method: Lab Project Number: 24894: TMN-0193: T 7053937. Unpublished study prepared by Bayer Ag. 58 p. {OPPTS 870.5100}
- 44865726 Gahlmann, R. (1995) SZX 0722: In Vitro Mammalian Chromosome Aberration Test with Chinese Hamster Ovary (CHO) Cells: Lab Project Number: 24403: TMN-0190: T 2039217. Unpublished study prepared by Bayer Ag. 33 p.
- 44865727 Brendler-Schwaab, S. (1995) SZX 0722: Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HPRT Assay in Vitro: Lab Project Number: 23858: TMN-0188: T 7039212. Unpublished study prepared by Bayer Ag. 37 p. {OPPTS 870.5300}
- 44865728 Herbold, B. (1995) SZX 0722: Micronucleus Test on the Mouse: Lab Project Number: 24016: TMN-0192: T 5059029. Unpublished study prepared by Bayer Ag. 53 p. {OPPTS 870.5395}
- 44865729 Brendler-Schwaab, S. (1996) SZX 0722: Test on Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures in Vitro: Lab Project Number: 24963: TMN-0189: T 0049322. Unpublished study prepared by Bayer Ag. 32 p. {OPPTS 870.5550}
- 44865730 Brendler-Schwaab, S. (1998) SZX 0722: (Phosphorus-32)-Postlabelling Assay in Female Rat Uterus and Urinary Bladder Epithelium in Vivo: Lab Project Number:

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FINAL REPORT

PH-27184: TMN-0191: T 3053898. Unpublished study prepared by Bayer Ag. 109 p. {OPPTS 870.5999}

44865733 Anderson, C. (1997) (Phenyl-UL-(carbon-14)) SZX 0772: Investigation of the Biokinetic Behaviour and the Metabolism in the Rat: Lab Project Number: MR-805/96: PF-4263: TMN-0080. Unpublished study prepared by Bayer Ag. 227 p. {870.7485}

44865734 Knoell, H.; Anderson, C. (1997) (Carbon-14)SZX 0772: Investigation of the Biokinetic Behaviour and the Metabolism in the Rat Following Subchronic Feeding: Lab Project Number: MR-904/97: PF-4322: ANC96. Unpublished study prepared by Bayer

44908616 Enzmann, H. (1993) SZX 0722: Liver Foci Test for Initiating Effect: Lab Project Number: 22120: TMN-0195. Unpublished study prepared by Bayer AG. 24 p.

45048705 Schladt, L.; Eiben, R. (2000) Spontaneous Tumors in Wistar Rats in 2-Year Studies Performed at Bayer AG: (SZX 0722): Lab Project Number: TMN-0181A: MO-00-000946. Unpublished study prepared by Bayer AG. 14 p. {OPPTS 870.4300}

45493400 Hartmann, E (2001). Letter to EPA, dated September 5, 2001.

45493401 Hartmann, E. (2000). Update of Historical Control Data on Rare Tumors in Wistar Rats in a 2-Year Carcinogenicity Study with SZX 0722, Bayer AG, Wuppertal, Germany, Bayer Report No. 27160; MO-01-016359, December 1, 2000, Unpublished.

Fornillo, V. (2002). Iprovalicarb: Revised Qualitative Risk Assessment Based on Hsd/WIN:WU (SPF) Rat Dietary Study. HED Memorandum, dated February 21, 2002. TXR No. 0050482.