PINOXADEN – POSITION ON THE LEIOMYOSARCOMAS IN THE CHRONIC/CARCINOGENICITY STUDY IN THE RAT

Introduction

Syngenta agrees with the UK HSE that the slightly increased incidence of leiomyosarcoma of the non-glandular stomach in male rats in the chronic/carcinogenicity study is not a specific, treatment related effect of pinoxaden. Details of Syngenta's position are given below:

Incidence of stomach leiomyosarcomas in chronic studies

(a) Incidence of stomach leiomyosarcomas in chronic studies with pinoxaden

Leiomyosarcoma is a malignant mesenchymal tumour. The incidence of stomach leiomyosarcomas in the 2-year study in rats with pinoxaden is low (Table 1)

Table 1: Percent incidence of stomach leiomyosarcoma in the pinoxaden 2 year rat study

	% Incidence per Dose Group						Historic Control Values[1]		
Finding	**0 mg/kg**	**1 mg/kg**	**10 mg/kg**	**100 mg/kg**	**250 mg/kg**	**500 mg/kg**	**%**	**Min**	**Max**
Stomach Leiomyosarcoma Males	0.0% (0/59)	0.0% (0/60)	0.0% (0/60)	0.0% (0/59)	3.3%♦ (2/60)	N/A	RCC: 0.0% RENI: <0.1% Bomhard: 0.0% Walsh: <0.4%	0.0% 0.0% 0.0% nd	0.0% 2.0% 0.0% nd
Stomach Leiomyosarcoma Females	0.0% (0/60)	0.0% (0/60)	1.7% (1/59)	0.0% (0/60)	0.0% (0/60)	0.0% (0/57)	nd	nd	nd

Statistics for tumour incidence: ♦ $p \leq 0.05$ by Peto trend test (1-sided), from original report.
N/A – animals terminated week 61 due to excessive mortality
nd = not determined

In males, a weekly statistically significant one sided Peto trend test for stomach leiomyosarcomas was observed based on an incidence of 2/60 in the 250 mg/kg/day group vs. 0/60 in the control and all other treated groups. Using a pairwise comparison (Fisher's Exact Test) the incidence in males at 250 mg/kg was not statistically significantly different from the controls ($p > 0.05$).

There was a single incidence of leiomyosarcoma of the stomach in females which was present at an intermediate dose level (10 mg/kg) with no incidence at the higher dose levels of 100 or 250mg/kg/day.

No malignant mesenchymal tumours were observed in the stomach of mice in two 80 week oncogenicity studies where pinoxaden was administered by gavage or the diet (Report no. 20001394, 2003. Report no. PM1280, 2004).

[1] See Appendix 1

(b) Incidence of spontaneous stomach leiomyosarcomas in rats

Comparison of the 2-year rat study to historic control values show that the percentage incidence in 250 mg/kg/day males (3.3%) is just above the range of the RITA control database (max. 2%), based on occurrence in 2 animals/group size of 60 in the pinoxaden study vs. 1 animal/group size of 50 in the RITA database (Appendix 1). In review of spontaneous tumours in the Wistar rat, Bomhard and Rinke (1994) did not report any tumours of this particular type and Walsh and Poteracki (1994) did not report any but they only reported tumours that occurred in 3 animals or more.

Historical control data from the NTP (Table 2) suggests that stomach leiomyosarcomas occur sporadically in animals dosed either dermally or orally in diet or by gavage.

Table 2: Incidence of stomach leiomyosarcoma vs. route of exposure: NTP database in Fischer 344 rat[2]

Dose Route	Number of studies	Maximum incidence	
		Forestomach	Glandular stomach
Dermal	10	0/50	1/50
Gavage corn oil	8	1/50	0/50
Gavage methyl cellulose	2	0/50	0/50
Gavage water	1	0/50	0/50
Inhalation	2	0/50	0/50
Magnetic field	1	0/100	0/100
Oral diet	20	1/50	0/50
Oral water	6	0/50	0/50

In the pinoxaden study, a single incidence (1/59) occurred in an intermediate dose group of females (10 mg/kg/day), which illustrates that sporadic occurrences of this tumour type are seen in rats without evidence of relationship to treatment.

The historic control data (including RCC, RITA and NTP data) may slightly underestimate the true occurrence of this tumour type due to the differing terminologies that have been used to describe it. Pathology reviews have noted that: "benign and malignant mesenchymal tumours of the stomach….have been variously described as leiomyomatous tumour, leiomyoma, leiomyosarcoma, fibrosarcoma, hemangiosarcoma, spindle cell proliferation, spindle cell sarcoma, neurinoma and sarcoma" (Takahashi, 1985).

Characteristics of chemically-induced gastric leiomyosarcomas.

Literature data suggests that chemicals known to cause gastric leiomyosarcomas either do so in conjunction with an increased incidence of sarcomas in other locations, suggestive of a systemic effect (PPAR agonists -Tannehill-Gregg 2007/Toyohiko 2007) or in conjunction with other tumours of the stomach, suggestive of a local irritant effect (MNNG administered with gastric irritants – Cohen 1983).

When considering the biological plausibility of a relationship to treatment of the leiomyosarcomas seen in the pinoxaden study, evidence for a systemic effect of pinoxaden on mesenchymal tissue, particularly smooth muscle tissue and evidence for a direct irritant effect on the gastric mucosa were considered.

[2] National Toxicology Programme, Department of Health and Human Services: http://ntp-server.niehs.nih.gov/

(a) Combined incidence of all mesenchymal tissue tumours

Leiomyosarcomas of the stomach arise from smooth muscle cells in the stomach wall, as opposed to the epithelial mucosal lining. When considering their relevance for human risk assessment, smooth muscle tumours are usually considered in the same way as endothelial tumours (angiomas/haemangiomas etc) in that they are counted wherever they arise and the total incidence of tumours is considered as a unit.

In the case of the pinoxaden chronic/carcinogenicity study the US EPA took this approach and evaluated smooth muscle tumours as a group (Table 3).

Table 3: US EPA evaluation of male smooth muscle tumor rates+ and Peto's prevalence test results

Dose (mg/kg bwt/day)

	0	1	10	100	250
Leiomyomas (%)	0/63 (0)	0/64 (0)	0/67 (0)	1[a]/60 (2)	0/44 (0)
P=	0.60076	-	-	0.26354	-
Leiomyosarcomas (%)	0/76 (0)	1/74 (1)	1/77 (1)	0/77 (0)	3[b]/76 (4)
P=	0.13923	0.16918	0.11966	-	0.11703
Combined (%)	0/76 (0)	1/74 (1)	1/77 (1)	1/77 (0)	3/76 (4)
P=	0.18218	0.16918	0.11966	0.14442	0.11703

+ number of tumour bearing animals/number of animals examined, excluding those that died before observation of the first tumour.

a First leiomyoma observed at week 97, dose 100mg/kg

b First leiomyosarcoma observed at week 59, dose 250 mg/kg

Interim sacrifice/accidental kill animals not included – no tumours

Significance of trend denoted at control

Significance of pair-wise comparison with control denoted at dose level. * p= <0.05, ** p= < 0.01

The US EPA[3] concluded

"There were no significant trends or pair-wise comparisons with controls for leiomyomas, leiomyosarcomas, or leiomyomas and/or leiomyosarcomas combined in male rats. There were no significant smooth muscle tumors in female rats."

The approach taken by Syngenta was to consider the combined incidence of malignant mesenchymal tumours in any organ in males and females across all dose groups. When considered in this way there is no evidence of a treatment related effect (Tables 4 and 5).

[3] United States Environmental protection Agency (2005)
Pinoxaden: Report of the Cancer Assessment Review Committee PC Code:147500

Table 4: Incidence of malignant mesenchymal tumours in any organ – Males

Dose Group (mg/kg/day):	0	1	10	100	250
Organ/Tumour					
Body Cavity:					
Leiomyosarcoma	0	0	0	0	1
Osteosarcoma	1	0	0	0	0
Heart:					
Schwannoma	0	0	1	0	0
Large Intestine					
Fibrous histiocytoma	0	1	0	0	0
Nasal Cavity:					
Sarcoma NOS	1	0	0	0	0
Salivary Gland					
Fibrosarcoma	0	0	0	1	0
Skin/subcutis:					
Fibrous histiocytoma	0	0	1	1	1
Liposarcoma	0	0	0	1	0
Sarcoma NOS	1	0	1	0	0
Spleen:					
Leiomyosarcoma	0	1	1	0	0
Sarcoma NOS	1	0	0	0	0
Stomach (includes forestomach and glandular):					
Leiomyosarcoma	0	0	0	0	2
Sarcoma NOS	0	0	0	1	0
Totals:	4	2	4	4	4

Note: 500 mg/kg/day male group was terminated at week 61 due to excessive mortality

Table 5: Incidence of malignant mesenchymal tumours in any organ – Females

Dose Group (mg/kg/day):	0	1	10	100	250	500
Organ/Tumour						
Body Cavity: Schwannoma	0	2	2	1	1	0
Bone: Osteosarcoma	0	0	0	1	0	0
Kidney Malignant mesenchymal tumour	0	0	0	1	0	0
Skin/subcutis: Sarcoma NOS	1	0	0	0	0	0
Spinal Nerve Schwannoma	0	1	0	0	0	1
Stomach (includes forestomach and glandular): Leiomyosarcoma	0	0	1	0	0	0
Uterus: Leiomyosarcoma Schwannoma	1 0	0 0	0 0	0 0	0 1	0 1
Vagina: Schwannoma	0	0	0	0	0	1
Totals:	2	3	3	3	2	3

(b) Influence of gastric irritation on tumour development

Signs of significant mucosal irritation in the forestomach of the rat would be expected to include an increase in the incidence of erosion/ulceration accompanied by inflammatory cell infiltration and, with continued dosing, basal cell or squamous cell hyperplasia. Any tumours resulting from significant, persistent irritation would be expected to be associated with the stomach epithelium (squamous cell papilloma or carcinoma). The pattern observed in the 2-year rat study with pinoxaden was not consistent with such an effect.

There is no evidence of any preneoplastic findings in any dose group or any recorded instance of tumours of the gastric epithelium.

In males dosed with pinoxaden, histopathological examination of the stomach showed little or no evidence of significant irritation in any dose group. There was no evidence of a dose response in any finding, with the possible exception of a slight increase in ulceration of the forestomach at terminal sacrifice (Table 6). However, this was not accompanied by any other signs of irritation.

Table 6: Histopathology of the stomach (all animals) – chronic/carcinogenicity study males

Findings Number examined	0 mg/kg 60	1 mg/kg 60	10 mg/kg 60	100 mg/kg 59	250 mg/kg 60
FORESTOMACH					
Cyst epidermal	1	-	-	-	1
Oedema inflammatory	2	1	1	1	-
Hyperkeratosis	-	-	-	1	-
Hyperplasia basal cell	-	-	1	-	-
Hyperplasia squamous cell	4	-	2	1	3
Infiltration inflammatory cells	-	-	-	1	-
Infiltration lymphocytic	-	-	-	1	-
Ulceration	1	-	3	3	5
GLANDULAR STOMACH					
Adenocarcinoma	-	1	-	-	-
Dilatation glandular	21	24	15	17	15
Erosion	-	-	-	1	3
Hyperplasia epithelial	-	-	-	-	1
Hyperplasia lymphoid	9	8	5	4	6
Hyperplasia papillary	-	-	-	-	1
Infiltration inflammatory cells	2	-	-	-	-
Mineralisation	-	-	-	1	-
Ulceration	-	-	-	1	-
STOMACH					
Leiomyosarcoma	-	-	-	-	2
Mineralisation metastatic	4	-	1	1	10
Sarcoma NOS	-	-	-	1	-
Lymphoma malignant	-	-	-	1	1

Examination of individual animal data shows that in the high dose males there was little evidence of associated mucosal irritation in animals which were recorded as having forestomach ulcers (Table 7). In addition, the two animals with leiomyosarcomas of the stomach (animals 366 and 406) were not recorded as having any additional histopathological findings in the forestomach.

Table 7: Individual animal data - histopathology of the stomach in males dosed with 250 mg pinoxaden/kg bw/day

Animal number	Leiomyosarcoma	Forestomach Ulceration	Forestomach Squamous Cell Hyperplasia
368	-	-	Y
372	-	-	Y
378	-	Y	-
392	-	Y	Y
394	-	Y	-
401	-	Y	-
408	-	Y	-

In short term studies in rats dosed by gavage with pinoxaden, and in the animals terminated after 1 year in the chronic study, there was no evidence of gastric irritation (Tables 8 and 9). There were no reported histopathological findings in the stomach of rats dosed in the diet for 90 days. (Report no.CTL/P/1254, 2003)

Table 8: Microscopic findings - Forestomach - 90 day gavage study[4] (n=10)

	0 mg/kg	1 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
MALES						
Hyperplasia squamous cell	3	2	3	2	2	3
Infiltration lymphatic	0	0	0	2	0	0
FEMALES						
Hyperplasia squamous cell	0	2	1	2	1	1
Infiltration lymphatic	0	0	0	0	0	0

Table 9: Microscopic findings - Forestomach - 1 year interim kill from chronic/carcinogenicity study (n=10)

	0 mg/kg	1 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
MALES						
Hyperplasia squamous cell	0	0	0	0	0	1
FEMALES						
Hyperplasia squamous cell	1	0	0	0	0	0

There was no evidence of treatment related gastric irritation in 18-month bioassays of pinoxaden in mice, dosed by gavage or in diet (Report no. 20001394, 2003. Report no. PM1280, 2004)

Conclusion

The stomach leiomyosarcomas in the pinoxaden chronic rat study are considered not to be related to treatment because:

- The finding is of low incidence (not statistically significant using the pairwise Fisher's Exact Test) and confined to one sex (males) in one species (rat).
- Historic control data and data from the female groups in the current study confirm the sporadic nature of this tumour.
- The incidence of spontaneous stomach leiomyosarcomas in rats is low, regardless of exposure route, but the incidence reported in historical control data may underestimate the actual occurrence due to the potential for tumours to be diagnosed as different sarcoma types.
- The tumours are not associated with any other significant non-neoplastic or pre-neoplastic gastric pathology.
- Leiomyosarcomas of the stomach arise from mesenchymal tissue in the stomach wall, as opposed to the epithelial mucosal lining. Neither the combined incidence of smooth muscle tumours not the combined incidence of malignant mesenchymal tumours showed any evidence

[4] Report No 991092, (2001)

of a treatment related effect , confirming a lack of oncogenic potential for the induction of these tumour types within all tissues.

- There is no evidence of significant gastric irritation in any dose group in the study or in studies of shorter duration involving gavage dosing in rats and there is no evidence of treatment related gastric irritation in mice. Moreover, cycles of damage and repair in the stomach mucosa would be expected to progress and to manifest as tumours of the mucosal epithelium and not as tumours of the underlying mesenchymal derived structures.

It is Syngenta's position that the incidence of gastric leiomyosarcomas in male rats at 250 mg/kg is not related to treatment with pinoxaden. It reflects a chance event of slightly higher incidence of one type of mesenchymal tumour.

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Appendix 1

Historical control data for stomach leiomyosarcomas are included in Tables A1. The data were obtained from the following sources, each of which was cited in the original report. The strain of rats and other details for each database are indicated:

RCC Stein data: Wistar rats bred by RCC, Ltd., Fuellinsdorf, Switzerland (Hanlbm:WIST (SPF)). Control groups from studies conducted at RCC Ltd., Toxicology, CH-4332, Stein, Switzerland. Syngenta Study 970043.

RITA database:

- thyroid: Wistar rats, all strains (1991 – 1997)
- stomach leiomyosarcoma (males): Wistar rats, all strains (1991 – 1997, from original report)

Registry of Industrial Toxicology Animal data (RITA), accessed via RENI explorer v1.05.31, September 2004.

Bomhard, 1994: tabulated tumour incidence for all occurrences of neoplasms. Rat strain: Wistar rats bred by Winkelmann, Borchen (Bor:WISW (SPF Cpb)) (Bomhard et al., 1994).

Walsh, 1994: tabulated tumour incidence only for spontaneous neoplasms occurring in 3 or more Wistar rats. Rat Strain: Wistar rats from Charles River (Crl(WI)BR from Kingston, NY or Portage, MI) (Walsh et al., 1994).

STOMACH: LEIOMYOSARCOMA							
data base	**Number of groups**	**sex**	**total number of animals**	**animals with lesions**		**% range**	
				number	**%**	**min.**	**max.**
RCC STEIN	5	M	250	0	0.0	0.0	0.0
RITA	48	M	2325	1	<0.1	0.0	2.0
Bomhard 1994	22	M	1240	0	0.0	0.0	0.0
Walsh, 1994	10	M	685	<3	<0.4	nd	nd

na = not available. nd = not determined.