REGULATORY TOXICOLOGY

Subject:

Bayer CropScience Comment on the CLH Dossier on Spiroxamine of April 24, 2015

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Introduction

Spiroxamine is currently reviewed for harmonised classification and labelling by the European Chemicals Agency Committee for Risk Assessment (ECHA RAC). In the additional CLH dossier of April 24, 2015 on repeated dose toxicity a possible classification of spiroxamine for hyperkeratosis with STOT-RE 1 (H372) is discussed. With regard to classification for developmental effects it is concluded that the toxicity observed in the repeated dose toxicity studies is not severe enough to lead as a secondary, unspecific consequence to the observed slight increase in cleft palates in developmental toxicity studies with spiroxamine in rats.

The document at hand by Bayer CropScience provides additional data on the severity (grading) of hyperkeratosis in the repeated dose toxicity studies on spiroxamine as a basis for adequate classification with STOT-RE. In addition, all necessary facts for the assessment of the slightly increased incidence of cleft palates in rats are summarized.

1. Possible classification of spiroxamine for hyperkeratosis with STOT-RE 1

Incidences and severity (grading) of hyperkeratosis, hyperplasia and hypertrophy of the tongue, oesophagus, forestomach / stomach, urinary bladder epithelium or renal pelvis in rodent studies with spiroxamine are summarized in Annex I.

In most of the cases the findings were graded as minimal to moderate. Marked or severe findings were only observed in the following cases:

- 13-week dietary study in rats ([redacted], 1992, M-006484-01-1): marked oesophagus hyperkeratosis in 4/10 males and 6/10 females at 32.81 and 43.04 mg/kg bw/day and marked forestomach hyperkeratosis in 1/10 males and 2/10 females at the same doses.
- 13-week dietary study in mice ([redacted], 1992, M-008032-01-1): marked hyperplasia of the urinary bladder epithelium in 1/10 males and 2/10 females at 366.2 and 413.7 mg/kg bw/day.
- 2-year dietary study in rats ([redacted], 1994, M-006861-01-1): marked transitional cell hyperplasia of the urinary bladder in 1/48 females at 75.1 mg/kg bw after 2 years of treatment.
- 2-year dietary mouse ([redacted], 1995, M-006925-01-1): hyperkeratosis of the oesophagus: marked in 3/50 males and 4/47 females, severe in 1/50 males and 2/47 females at 59.3 and 102.6 mg/kg bw/day.

In conclusion, marked or severe findings were noted after subchronic oral exposure in rats at doses > 10 and < 100 mg/kg bw/day, thus leading to a classification with STOT-RE 2 (H373). The cases from the subchronic study in mice and from the chronic/carcinogenicity studies in rats and mice were observed at higher doses exceeding the trigger values for a STOT-RE classification.

2. Cleft palates and maternal toxicity in developmental toxicity studies on spiroxamine

Cleft palates (palatochisis) occurred in the developmental toxicity study in rats in the high dose of 100 mg/kg bw/day in 3 fetuses from 3 litters. In this study, apart from a perforating gastric ulcer in one dam no pronounced maternal toxicity was reported (also in this dam no clinical signs were noted). However, with the study running over Christmas (necropsies starting on January 3) clinical observation of the animals might have been insufficient to detect clinical signs of abdominal pain. Cleft palates in 3 fetuses from 2 litters were also seen in a dose range finder at 100 mg/kg bw/day. Both dams showed severe signs of toxicity (ruffled fur, lateral recumbency, dyspnoe, sedation, hunched posture; additionally slightly

reduced food consumption and body weight gain). In a second dose range finder there were 3 fetuses from 2 litters with cleft palates at 150 mg/kg bw/day. At this dose all 25 dams showed severe symptoms like ruffled fur, ataxia, ventro-lateral recumbency, paddling and rolling movements, spasms, dyspnoe, sedation, hunched posture, cometose state (in addition also mucous diarrhea, distinctly reduced food consumption and body weight gain), and 21 of the 25 dams died. No malformations occurred in dose range finding studies up to 75 mg/kg bw/day of spiroxamine.

All three studies were conducted in 1990 at RCC. Especially in this year, but also in the years thereafter, the rat strain used in these studies showed repeatedly cleft palate in untreated control rats (see Table 1 below). Thus, cleft palate is a common spontaneous malformation this rat strain – this is somewhat unusal for rats since generally mainly mice are known to commonly show this type of malformation in untreated control animals.

Table 1: Incidences of palatoschisis in vehicle controls of developmental rat studies in WIST HanIbm: WIST (SPF) rats conducted at RCC between 1988 and 1995.

Malformation		Incidences of palatoschisis
Year	Studies	[no. of studies, affected fetuses]
1988	7	0
1989	12	0
1990	7	3 (1 fetus in one litter, 4 fetuses in 1 litter and 2 fetuses in 1 litter)
1991	6	1 (1 fetus in 1 litter)
1992	4	0
1993	2	1 (2 fetuses in 1 litter)
1994	1	0
1995	1	2 (2 fetuses from 2 litters with palatoschisis + multiple malformations)

The fetal incidences in the main developmental toxicity study in rats with spiroxamine (3 out of 265 fetuses) were covered by the 1990 historical controls (4 out of 280 fetuses); only the litter incidences of the developmental toxicity study with 3 out of 25 litters were slightly exceeding the historical control range (2 out of 24 litters).

These data show that only a minimal increase in spontaneously occurring cleft palates in rat fetuses was observed, and this occurred only after treatment with very high doses of spiroxamine in the maternal sublethal to lethal dose range.

A 2 % solution of spiroxamine in water has a pH of 9.9. The alkalinity of the compound is the reason for the strong irritant action of spiroxamine on skin, oesophagus, stomach mucosa and/or urinary bladder epithelium, which is evident in the *in vivo* toxicity studies.

Especially in the case of bolus applications by gavage the local irritation caused by higher doses will lead to abdominal indisposition or pain. Depending on individual sensitivity possible reactions in individual animals varied from showing apparently no symptoms to signs like hunched posture, ventro-lateral recumbency, dyspnoe and ruffled fur. It should be noted that small animals like rats are prey animals, which try to hide signs of disease as long as possible in order to avoid attracting attention of predators.

The strong irritant action on epithelia and mucous membranes is evidenced in the range finding rat developmental toxicity studies by slight erosions of gastric glandular mucosa at 10 and 25 mg/kg bw/day. Histology of the stomach mucosa was not conducted at higher doses but it is assumed that higher doses were affected to a stronger degree. From other rat studies it is known that already a single dose of 30 mg/kg bw caused abdominal pain (decrease in landing foot splay, considered to be a typical expression of a slight spasmodic condition of the hind legs, was noted at $\geq$ 30 mg/kg bw in the acute neurotoxicity study). Repeated doses of 10 mg/kg bw/day caused mild clinical signs like salivation; higher doses led to digging and preening activities, transient tremor and also hyperkeratosis of the forestomach.

As a further consequence of abdominal irritation, higher doses caused marginally reduced food consumption at 75 mg/kg bw/day and clinical signs and mortality with a very steep dose-effect-relationship at doses from 100 to 250 mg/kg bw/day. 100 mg/kg caused severe clinical signs like ruffled fur, lateral recumbency, dyspnoe, sedation and hunched posture in some of the animals. Mortality was observed after 150 mg/kg bw/day (amounting to 84% in one study), a dose of 250 mg/kg bw/day of spiroxamine led to 100% mortality.

Based on these facts it is concluded that despite the lack of reported clinical symptoms at 100 mg/kg/day of spiroxamine in the main developmental study in rats there was pronounced maternal toxicity (caused by the irritant action of spiroxamine) also in these animals. This is supported by the fact that one dam had a perforating gastric ulcer, and that food consumption of the animals was markedly reduced at this dose.

It is further concluded that the slightly increased incidence of cleft palates in the highly maternally toxic (even sublethal to partially lethal) dose range of 100-150 mg/kg bw/day of spiroxamine reflects an enhancement of a (in this rat strain) common spontaneous malformation secondarily to the strong irritant action of spiroxamine and maternal pain and toxic stress, and not a specific or direct teratogenic effect of the compound.

This conclusion is supported by findings published for the mouse, a species which generally shows a high incidence of spontaneous “background” malformations, especially cleft palate. Peters and Straßburg (1969) stressed pregnant mice in various ways and concluded that during the phenocritical phase of palate closure obviously any unphysiologic exogenous stimulation may produce teratogenic effects (i.e., a strongly increased incidence of cleft palate) provided it has previously caused a stress-situation in the mother animals (for example by withdrawal of food for 24 h or noise (3 x 1 h)).
Golub et al. (2004) describe a high susceptibility of pregnant mice to non-chemical stress factors “In mice, an increased incidence of cleft palate, exencephaly, supernumerary ribs, fused ribs, and resorption can be produced by restraint procedures, depending on the timing, and type of restraint, and the strain of mouse. … Cleft palate induction in restrained mice was as high as 69 % of fetuses as compared to 1 % in controls….”.

Based on our experience, it is typical for changes with a spontaneous inherent biological variability that these background incidences can be increased by unspecific maternal stressors. A “proof of principle” for this unspecific pathomechanism in rats causing an

increased incidence of common malformations secondarily to maternal toxicity is demonstrated by the results of a developmental toxicity study conducted in our laboratory with a different rat strain ([redacted] 1994). In this study, pregnant rats were exposed via inhalation to an irritating compound which caused reflectory induced maternal bradypnea and hypoxia. This maternal hypoxia triggered an enhancement of several spontaneous malformations in this rat strain (umbilical hernia, microphthalmia, vertebral/rib malformations). The unspecificity of this effect is evidenced by the fact that the same dose in that study caused 50 % less malformations if the inhaled air was enriched with oxygen in order to reduce maternal hypoxia. Furthermore, the same compound did not cause malformations after oral application.

Therefore, the increased incidence of cleft palate caused by 100 mg/kg bw of spiroxamine is assessed as an enhancement of a (in this rat strain) common spontaneous malformation secondarily to the strong maternal toxic stress (i.e. the strong irritant action and toxicity of spiroxamine in sublethal to lethal doses) and not as a specific or direct teratogenic effect of spiroxamine (for details see [redacted], 2015). In the present case the rat strain used in the spiroxamine studies obviously behaved "like a mouse" for which it is well known that even relatively minor stressors (like noise) can lead to an enhancement of cleft palates. It is concluded that this constellation of strong maternal toxicity and unspecific secondary developmental toxicity does not trigger any classification for developmental toxic effects.

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References

[redacted] (1993): Dose range-finding embryotoxicity study (including teratogenicity) with KWG 4168 technical in the rat, RCC Project 286648, report no. R6072, M-007009-01-1, October 11, 1993

[redacted] (1995): Range-finding studies with KWG 4168 technical in the rat, RCC Projects 268075, 272610 and 277931, report no. R6343, M-008093-02-1, January 18, 1995

[redacted] (1995): Combined report of embryotoxicity screening study (including teratogenicity) and supplementary study to the embryotoxicity screening study (including teratogenicity) with KWG 4168 technical in the rat, RCC Projects 263068 and 281507, report no. R6355, M-006780-01-1, February 03, 1995

[redacted] (1994): KWG 4168 – Acute oral neurotoxicity screening study in rats, report no. 23503, M-016556-01-1, November 25, 1994

[redacted] (1994), KWG 4168 - Investigations of chronic toxicity and carcinogenicity in Wistar rats (administration in diet over 2 years), Bayer AG, report no. 23580, Doc No. M-006861-01-1, December 28, 1994

[redacted] (1992), KWG 4168 - Subchronic toxicity study in Wistar rats (thirteen-week administration in the diet with a four-week recovery period), Bayer AG, report no. 21627, Doc No. M-006484-01-1, August 18, 1992

[redacted] (1992), KWG 4168 - Subchronic range-finding testing for a two-year study in B6C3F1 mice (administration in the diet over a period of about 13 weeks), Bayer AG, report no. 21022, Doc No. M-008032-01-1, January 15, 1992

[redacted] (1995), KWG 4168 - Summary report of a oncogenicity study and a supplementary six-months chronic toxicity study in B6C3F1 mice, Bayer AG, report no. 23975, Doc No. M-006925-01-1, May 5, 1995

[redacted](1992), KWG 4168 - Subchronic toxicologic study in B6C3F1 mice to examine effects on the skin, kidneys, liver and urinary bladder (thirteen-week administration by gavage and eight-week recovery period), Bayer AG, report no. 21330, Doc No. M-008069-01-1, April 29, 1992

[redacted] (1997), KWG 4168 - Supplementary oncogenicity study in B6C3F1 mice (Administration in diet over 2 years), Bayer AG, report no. 26780, Doc No. M-010987-01-1, October 30, 1997

Golub et al., Effects of restraint stress in gestation: Implications for rodent developmental toxicology studies, birth defects., Research (Part B) 71: 26 - 36, 2004. page 34., BCS-Reference No.: M-248213-01-1

[redacted] (2015), Spiroxamine - Additional information on developmental and maternal toxicity, Bayer CropScience AG, January 30, 2015, Doc No. M-508717-01-1; already submitted to RAC

[redacted] (1994): FCR 1272 - Explanatory report on results and mechanistic studies for embryotoxic effects in rats after inhalation, Bayer AG, report no. 23219, Doc No.
M-041671-02-1, August, 12,1994, amended March 28, 1996

[redacted] (1992, amended 1995): KWG 4168 - Subacute oral toxicity study in rats (feeding study), Bayer AG, report no. 21644, Doc No. M-016623-02-1, August 31, 1992, amended August 25, 1995

[redacted] (1992): KWG 4168 – Subacute oral toxicity study in rats, Bayer AG, report no. 21841, Doc No. M-016634-01-1, November 11, 1992

[redacted] (1992), KWG 4168 Aerosol - Study for subacute inhalation toxicity in the rat (according to OECD guideline no. 412, Bayer AG, report no. 21785, Doc No. M-006356-01-1, October 21, 1992

Peters, V.S. and Straßburg, M., Stress als teratogener Faktor (translated: Stress as a teratogenic factor), Arzneimittelforschung 19 (7): 1106 - 1111, (1969)., BCS-Reference No.: M-066928-01-1

Appendix 1 – Incidences and grading of hyperkeratosis, hyperplasia and hypertrophy of the tongue, oesophagus, forestomach / stomach, urinary bladder epithelium or renal pelvis in rodent studies with Spiroxamine

Table 1: [redacted], 1992, KWG 4168 - Subacute oral toxicity study in rats (gavage), M-016634-01-1

	Males				Females			
Dose [*mg/kg bw/day*]	***0***	***10***	***30***	***90***	***0***	***10***	***30***	***90***
Tongue	nad	ni	ni	nad	nad	ni	ni	nad
Oesophagus	nad	ni	ni	nad	nad	ni	ni	nad
Stomach								
Hyperkeratosis	0/5	0/5	0/5	3/5	0/5	0/5	0/5	2/5
minimal				*3*				*1*
slight								*1*
Urinary bladder								
Simple hyperplasia	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5
minimal								*2*

nad: nothing adverse detected
ni: not investigated

Table 2: [redacted], 1992 (amended 1995), KWG 4168 - Subacute oral toxicity study in rats (feeding study), M-016623-02-1

	Males				Females			
Dose [ppm]	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**300**
[*mg/kg bw/day*]	***0***	***3.4***	***10.8***	***33.6***	***0***	***3.8***	***12.2***	***35.6***
Tongue	nad	nad	nad	nad	nad	nad	nad	nad
Oesophagus								
Hyperkeratosis	0/5	0/5	5/5	5/5	0/5	0/5	1/5	5/5
minimal								
slight			*5*				*1*	
moderate				*5*				*5*
Stomach	nad	nad	nad	nad	nad	nad	nad	nad
Urinary bladder								
Epithelial hyperplasia	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
moderate								*1*

nad: nothing adverse detected

Table 3: [redacted], 1992, KWG 4168 - Subchronic toxicity study in Wistar rats (thirteen-week administration in the diet with a four-week recovery period), M-006484-01-1

	Males				Females			
Dose [ppm]	**0**	**10**	**70**	**490**	**0**	**10**	**70**	**490**
[mg/kg bw/day]	***0***	***0.61***	***4.22***	***32.81***	***0***	***0.77***	***5.67***	***43.04***
Tongue								
Hyperkeratosis	0/10	0/10	0/10	7/10	0/10	0/10	0/10	10/10
minimal								*2*
slight				*3*				*4*
moderate				*4*				*4*
Oesophagus								
Hyperkeratosis	1/10	0/10	9/10	10/10	0/10	0/10	5/10	10/10
minimal	*1*		*3*				*3*	
slight			*4*	*1*			*2*	
moderate			*2*	*5*				*4*
marked				***4***				***6***
Hyperplasia / - trophy	1/10	0/10	9/10	10/10	0/10	0/10	5/10	10/10
minimal	*1*		*2*	*2*			*5*	
slight			*7*	*4*				*4*
moderate				*4*				*6*
Stomach								
Forestomach hyperkeratosis	0/10	0/10	1/10	3/10	0/10	0/10	0/10	8/10
minimal								
slight			*2*	*1*				*1*
moderate				*1*				*5*
marked				***1***				***2***
Urinary bladder								
Hyperplasia multifocal	0/10	0/10	0/10	3/10	0/10	0/9	0/9	4/10
minimal								
slight				*3*				*4*
	Recovery groups							
Tongue								
Focal necrosis	0/10			1/10	0/10			0/10
minimal								
slight				*1*				
Oesophagus								
Hyperkeratosis	0/10			2/10	2/9			1/10
minimal				*2*	*2*			*1*
Hyperplasia / - trophy	0/10			2/10	2/9			1/10
minimal				*2*	*2*			*1*
Stomach								
Forestomach hyperkeratosis	0/10			1/10	0/10			0/10
minimal								
slight								
moderate				*1*				
Urinary bladder								
Hyperplasia multifocal	0/10			0/10	0/10			0/10

Table 4: [redacted], 1992, KWG 4168 - Subchronic range-finding testing for a two-year study in B6C3F1 mice (administration in the diet over a period of about 13 weeks), M-008032-01-1

	Males					Females				
Dose [ppm] ***[mg/kg bw/day]***	**0** ***0***	**20** ***6.2***	**80** ***24.9***	**320** ***88.4***	**1280** ***366.2***	**0** ***0***	**20** ***7.3***	**80** ***28.5***	**320** ***126.3***	**1280** ***413.7***
Tongue	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
Oesophagus acanthosis & hyperkeratosis	nad	ni	ni	ni	nad	nad	ni	ni	ni	nad
Stomach acanthosis & hyperkeratosis	nad	ni	ni	ni	nad	nad	ni	ni	ni	nad
Urinary bladder Simple hyperplasia *minimal* *slight* *moderate* ***marked***	0/10	0/10	0/10	0/8	9/10 *1* *7* ***1***	0/10	0/9	0/10	0/10	9/10 *1* *1* *5* ***2***
Kidneys Hyperplasia pelvic urothel: present	0/10	0/10	0/10	0/10	4/10	0/10	0/10	0/10	0/10	7/10

ni: not investigated
nad: nothing adverse detected

Table 5: [redacted], 1992, KWG 4168 - Subchronic toxicologic study in B6C3F1 mice to examine effects on the skin, kidneys, liver and urinary bladder (thirteen-week administration by gavage and eight-week recovery period), M-008069-01-1

Dose [*mg/kg bw/day*]	Males				Females			
	0	***60***	***180***	***240***	***0***	***60***	***180***	***240***
Tongue	nad	nad	nad	nad	nad	nad	nad	nad
Oesophagus	nad	nad	nad	nad	nad	nad	nad	nad
Stomach								
Hyperkeratosis	0/5	0/5	1/5	3/5	0/5	0/5	0/5	3/5
minimal								
slight			*2*	*1*				
moderate				*2*				*3*
Urinary bladder								
Simple hyperplasia	0/5	0/5	1/5	4/5	0/5	0/5	0/5	1/5
minimal				*1*				
slight			*1*	*2*				*1*
moderate								
Hypertrophy	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5
slight			*1*					*1*
Kidneys								
Hyperplasia pelvic urothel	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Recovery groups							
Tongue	nad		nad	nad	nad		nad	nad
Oesophagus	nad		nad	nad	nad		nad	nad
Stomach								
Hyperkeratosis	2/5		0/5	2/5	0/5		0/5	2/5
minimal								
slight	*2*			*2*				*1*
moderate								*1*
Urinary bladder								
Simple hyperplasia	0/5		0/5	1/5	0/5		0/5	0/5
minimal				*1*				
Kidneys								
Hyperplasia pelvic urothel	0/5		0/5	0/5	0/5		0/5	0/5

nad: nothing adverse detected

Table 6: [redacted], 1992, KWG 4168 Aerosol - Study for subacute inhalation toxicity in the rat (according to OECD guideline no. 412), M-006356-01-1

	Males					Females				
Dose [mg/kg bw/day]	**0 air**	**0 v**	**5.1**	**31.1**	**185.1**	**0 air**	**0 v**	**5.1**	**31.1**	**185.1**
[mg/m³ air for 6 h/day]	***0 air***	***0 v***	***14.3***	***87.0***	***518.4***	***0 air***	***0 v***	***14.3***	***87.0***	***518.4***
Nasal and paranasal cavities										
Squamous-cell metaplasia	1/10	1/10	2/10	3/10	8/10**	4/10	5/10	2/10	4/10	3/10
Larynx										
Hyperplasia	0/10	0/10	0/10	3/10	7/10**	0/10	0/10	1/10	2/10	8/10**
Hyperkeratosis	0/10	0/10	0/10	2/10	7/10**	0/10	0/10	1/10	2/10	7/10**
Oesophagus										
Hyperkeratosis	0/10	0/10	0/10	0/10	8/10**	0/10	0/10	0/10	0/10	8/10**
Forestomach										
Hyperplasia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
Hyperkeratosis	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
Urinary bladder										
Urothelial hyperplasia	0/10	0/10	0/10	0/8	4/10	0/10	0/10	0/10	0/10	5/10*
Kidneys, renal pelvis										
Urothelial hyperplasia	0/10	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10

For the findings given above only incidences but no gradings are given in the study report.

0 air: control group inhaling pure air
0 v: control group inhaling vehicle only
*: $p<0.05$
**: $p<0.01$

Table 7: [redacted] , 1994, KWG 4168 - Investigations of chronic toxicity and carcinogenicity in Wistar rats (administration in diet over 2 years), M-006861-01-1

	Males				Females			
Dose [ppm]	**0**	**25**	**125**	**625**	**0**	**25**	**125**	**625**
[mg/kg bw/day]	***0***	***1.9***	***9.3***	***54.9***	***0***	***2.7***	***13.2***	***75.1***
Interim sacrifice after 1 year of treatment								
Tongue								
Acanthosis	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Oesophagus								
Acanthosis	0/10	0/10	0/10	8/10**	0/10	0/10	0/10	9/10*a
minimal				*8*				*6*
slight				*2*				*2*
moderate								*1*
Hyperkeratosis	0/10	0/10	0/10	10/10**	0/10	0/10	0/10	9/10**
minimal				*0*				*1*
slight				*9*				*8*
moderate				*1*				
Stomach								
Keratinised region: hyperkeratosis & acanthosis	0/10	0/10	1/10	0/10	0/10	0/10	0/10	1/10
minimal								
slight								*1*
Urinary bladder								
Transitional cell hyperplasia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Terminal sacrifice after 2 years of treatment								
Tongue								
Acanthosis	0/50	0/49	2/50	0/50	0/50	1/50	1/50	1/49
slight			*2*			*1*	*1*	*1*
Oesophagus								
Acanthosis	0/50	0/49	0/50	40/50**	0/50	0/50	0/50	39/49**
minimal				*35*				*32*
slight				*5*				*7*
Hyperkeratosis	0/50	0/49	0/50	40/50**	0/50	0/50	0/50	39/49**
slight				*31*				*30*
moderate				*9*				*9*
Stomach, keratinised region #: hyperkeratosis & acanthosis	4/50	5/48	5/49	4/49	6/50	10/49	8/50	4/40
slight	*3*	*4*	*3*	*4*	*5*	*6*	6	*4*
moderate	*1*	*0*	*1*		*1*	*3*	2	
marked #		***1#***	***1#***			***1#***		
Urinary bladder								
Transitional cell hyperplasia	0/50	2/48	1/50	0/50	0/50	1/50	0/50	4/48##
slight			*1*					*2*
moderate		*2*				*1*		*1*
marked								***1***

#: The incidences of hyperkeratosis and acanthosis of the keratinised region of the stomach are not dose dependant and occur also in the control groups in relatively high incidences. Therefore, they are not considered as a treatment related effect. This applies also to the three single cases in which this finding was graded as marked.

##: p< 0.05 trend

*: p< 0.01

**: p< 0.001

Table 8: [redacted], 1995, KWG 4168 - Summary report of a oncogenicity study and a supplementary six-months chronic toxicity study in B6C3F1 mice, M-006925-01-1

	Males				Females			
Dose								
[ppm]	**0**	**20**	**160**	**2.5 / 480#**	**0**	**20**	**160**	**2.5 / 480#**
[mg/kg bw/day]	***0***	***4.5***	***36.6***	***59.3***	***0***	***7.8***	***59.5***	***102.6***
Interim sacrifice after 1 year of treatment								
Tongue								
Hyperkeratosis	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Oesophagus								
Acanthosis	0/10	0/10	5/10*	6/8[b]	0/10	0/10	6/10*	9/10**
minimal			*5*	*4*			*5*	*7*
slight				*2*			*1*	*2*
moderate								
Hyperkeratosis	0/10	0/10	2/10	5/8 [b]	0/10	0/10	0/10	9/10**
slight			*2*	*2*				*6*
moderate				*3*				*3*
Stomach, keratinised region: Hyperkeratosis & acanthosis	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Urinary bladder								
Transitional cell hyperplasia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Kidneys, renal pelvis								
Hyperplasia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Terminal sacrifice after 2 years of treatment (480 ppm: 18 months)								
Tongue								
Hyperkeratosis	0/50	0/50	0/50	4/50	0/49	0/50	0/50	7/47[b]
Oesophagus								
Acanthosis	0/50	0/50	1/50	36/50**	0/48	0/50	1/49	22/47**
slight			*1*	*36*			*1*	*22*
Hyperkeratosis	1/50	0/50	7/50	45/50**	0/48	0/50	5/49	41/47**
slight	*1*		*7*	*14*			*4*	22
moderate				*27*			*1*	13
marked				***3***				**4**
severe				***1***				**2**
Stomach, keratinised region: Hyperkeratosis & acanthosis	1/50	0/50	1/50	0/49	0/49	0/50	0/50	0/47
moderate	*1*		*1*					
Urinary bladder								
Transitional cell	0/49	0/50	0/50	0/50	0/49	0/50	0/50	0/47

hyperplasia								
Kidneys, renal pelvis Hyperplasia	0/50	0/50	0/50	0/50	0/49	0/50	0/50	0/47
Additional 6 month study								
Dose [ppm] ***[mg/kg bw/day]***	**0** ***0***		**160** ***36.7***		**0** ***0***		**160** ***59.5***	
Tongue	nad		nad		nad		nad	
Oesophagus	nad		nad		nad		nad	
Stomach Hyperkeratosis	0/7		0/7		0/6		0/6	
Urinary bladder Transitional cell hyperplasia	0/7		0/7		0/7		0/7	
Kidneys, renal pelvis Hyperplasia	0/7		0/7		0/7		0/7	

ni: not investigated
nad: nothing adverse detected
#: the low dose of 20 ppm was increased to 480 ppm from week 32 to 26 months (for further 18 months).
*: $p < 0.05$
[b]: $p<0.01$
**: $p < 0.001$

Table 9: [redacted], 1997, KWG 4168 - Supplementary oncogenicity study in B6C3F1 mice (Administration in diet over 2 years), M-010987-01-1

Dose [ppm] [*mg/kg bw/day*]	Males 0 *0*	Males 160 *41.0*	Males 600 149.8	Females 0 *0*	Females 160 *64.6*	Females 600 *248.1*
Interim sacrifice after 1 year of treatment						
Tongue	ni	ni	ni	ni	ni	ni
Oesophagus	ni	ni	ni	ni	ni	ni
Stomach	ni	ni	ni	ni	ni	ni
Urinary bladder	ni	ni	ni	ni	ni	ni
Kidneys	ni	ni	ni	ni	ni	ni
Terminal sacrifice after 2 years of treatment						
Tongue Hyperkeratosis *slight*	0/50	0/50	0/49	0/50	0/50	5/50* *5*
Oesophagus Hyperkeratosis *minimal* *slight* *moderate*	0/50	0/50	1/49 *1*	2/50 *2*	1/50 *1*	10/50** *5* *3* *2*
Forestomach mucosa: Hyperkeratosis *slight* *moderate*	0/49	ni	0/49	0/50	ni	3/50* *3* *1*
Urinary bladder Transitional cell hyperplasia *slight* *moderate*	0/49	ni	0/49	0/49	ni	2/50 *1* *1*
Kidneys, renal pelvis Transitional cell hyperplasia	0/50	ni	0/49	0/49	ni	0/50

ni: not investigated
*: $p \leq 0.05$
**: $p \leq 0.01$