CYPROCONAZOLE POSITION ON STOT-RE IN RESPONSE TO CLH DOSSIER:

A classification for STOT-RE category 2 is considered not required by Syngenta. Although it is acknowledged that the target organ in all the mammalian toxicology species is the liver and effect levels are within the 'Guidance Values' for classification, the effects reflect adaptive responses due to xenobiotic metabolism and are not of toxicological concern; thus these findings in the liver do not meet the criteria triggering STOT-RE classification.

STOT-RE 2 H373 (May cause damage to organs (liver))

Syngenta acknowledges that the repeat dose toxicity studies in rats, mice and dogs have NOAEL values based on effects in the liver below the 'Guidance values' applicable to STOT-RE 2 classification. However, in the CLH report, throughout Section 4.7 Repeated dose toxicity, the cyproconazole data are consistently referred to as demonstrating '*Significant/severe hepatotoxicity', 'toxic effects on the liver'.* In addition, the CLH report, Section 4.8 describes the rationale for proposal of STOT-RE 2 H373 classification for cyproconazole and is detailed below. Syngenta disagrees with this emphasis on the changes in the liver and the subsequent conclusions reached and sets out to describe the adaptive nature of the findings.

Text from CLH report: *"Adverse effect levels were generally low, with evidence of significant hepatotoxicity seen at dose levels below the cut-off levels in the rat (28-day (oral and dermal), 90-day oral, and inhalation (16-day), mouse (90-day) and dog (90-day/1 year). Increases in relative liver weight were seen with histopathological change such as hepatocellular hypertrophy, vacuolation, fatty change, and single cell necrosis. These effects were generally accompanied by evidence of functional impairment such as altered clinical chemistry and marker enzymes. The significant adverse effects in the three species are consistent with the criteria for STOT RE 2 H373 (May cause damage to organs (liver)) with the exception of the 28 day rat study where the findings were more severe (at 1000 ppm: 96.18/127.9 mg/kg). The findings of the inhalation and dermal route studies in the rat also support this conclusion."*

Findings in tissues/organs other than the liver, along with haematology and urinanalysis results, are generally inconsistent between studies and species and do not provide sufficient evidence for a STOT-RE classification for the other tissues. Therefore, this document details effects on the liver and associated markers of impairment (clinical chemistry) and considers their potential alignment to the STOT-RE classification criteria.

The dose levels used in the cyproconazole repeat dose studies are generally at or below the 'Guidance Values' (≤100 mg/kg bw/day for 90 day; ≤300 mg/kg bw/day for 28 day duration or less), therefore, dose levels at which the findings occur are not detailed in this document. In addition, liver weights are consistently increased for all species (rat, mouse and dog) for all study durations and therefore are not described further. A summary of effects on clinical chemistry and liver macro- and histopathology are summarized in Table 1 (rats and dogs) and Table 2 (mice). The tables include endpoints which are consistently affected across timepoints and/or at terminal sacrifice, considered treatment-related in the study reports and achieve statistical significance.

In rats, clinical chemistry effects are inconsistent between studies conducted over differing durations or at different times. There is evidence to suggest plasma cholesterol levels are increased in females and liver enzymes (ALT, ALP, AST and G-GT) are also elevated after administration of cyproconazole. Effects on the liver at gross necropsy were limited to only 2/16 rats in a 28 day study with visible changes to liver architecture; however, this finding was not observed in a subsequent 28 day rat study (Warren et al., 1995), in two 90 day studies or in repeat dose inhalation or dermal studies. Histopathological examination in the rat consistently demonstrated hepatocyte hypertrophy at all-time points following repeat doses and was also associated with hepatocyte vacuolation in a number of studies, probably reflecting reversible lipid storage. In the 90 day rat study by Skinner et al. (1985), all of the observed liver changes, resolved after a 4 week recovery period. In all of these rat studies there was no evidence of either necrosis or inflammation or any other adverse findings which would have affected liver function. The hepatocyte hypertrophy, vacuolation and associated changes in clinical chemistry in rats are considered to represent an adaptive response of the liver to xenobiotic metabolism, have been demonstrated to be completely reversible and are thus considered not adverse.

In dogs following exposure for 90 days or 1 year, there were consistent decreases in plasma albumin and cholesterol (females only) and increases in ALP. A pronounced lobular pattern was observed in the liver upon gross post mortem examination in the 1 year study. Histopathological examination revealed hepatocyte hypertrophy, eosinophilic intrahepatocyte bodies, pigmentation and canalicular bile plugs. Eosinophilic intrahepatocyte bodies and hepatocyte hypertrophy are associated with proliferation of smooth endoplasmic reticulum (reflecting xenobiotic metabolism) and considered adaptive responses. The observed increased ALP (higher at early timepoints) showed some degree of recovery over the course of the study, further supporting an adaptive response.

Cyproconazole administration to mice has been extensively studied through a number or regulatory repeat dose and investigative studies. Evaluation of clinical chemistry parameters are limited and were only conducted in investigative studies, where up to 14 day exposures to cyproconazole resulted in decreases in plasma cholesterol and increases in liver enzymes (↑ALP, ALT, AST). Effects on gross pathology of the liver were only noted in one of the two 90 day studies and consisted of an accentuated lobular pattern. Histopathological examination consistently showed hepatocyte hypertrophy, hepatocyte vacuolation and increased hepatocyte lipid content. These histopathological and clinical chemistry observations are considered to represent an adaptive response, similar to the dog and rat. In addition, single cell necrosis was observed in mice only, in one of the two 90 day studies and consistently in the investigative studies (≤28days duration). No multifocal or diffuse necrosis was reported. In two of the investigative studies, the necrosis was described as single cell with small groups of necrotic hepatocytes, frequently accompanied by inflammatory cells (granulocytes). The study pathologist concluded that the pattern of necrosis, time of onset and dose concordance corresponded to the induction of hepatocellular hypertrophy and the cell loss was a consequence of functional or structural disturbances in the liver e.g. hypertrophy related physiological or architectural changes. Single cell necrosis in a regenerative organ such as the liver is generally viewed as not significantly adverse. In addition, hepatocyte hypertrophy, vacuolation and single cell necrosis were not observed when cyproconazole was administered to CAR knockout mice (Milburn 2006b). Further information on the proposed mode of action for cyproconazole and human non-relevance is described in Cowie, 2011 and in the Public Comments submitted to the proposed Carcinogenicity classification. Based on the nature of the histopathology changes observed and the association with hepatocellular hypertrophy, all of the

findings are considered to be associated with the adaptive response to xenobiotic metabolism in the liver and consequently are not adverse.

CONCLUSIONS

The weight of evidence shows that the liver effects in all 3 species are very clearly indicative of an adaptive effect, both in terms of the type of observations, and the chronological pattern, with effects often being seen more clearly in shorter term studies than in longer term.
None of these effects meet the CLP criterion of *"significant toxic effects, of relevance to human health",* which the guidance expands to *"In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant".*

Further reference to the guidance demonstrates that none of the effects seen in the liver meet the criteria supporting classification under Annex 1: 3.9.2.7.3:

> *(a) morbidity or death resulting from repeated or long-term exposure.*
>
> *(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell).*
>
> *(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters.*
>
> *(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.*
>
> *(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.*
>
> *(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver).*
>
> *(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.*

The CLP guidance (Annex I: 3.9.2.8.1) provides further illustration of the type of effects considered <u>not</u> to warrant classification:

It is recognised that effects may be seen in humans and/or animals that do not justify classification. Such effects include, but are not limited to:

(d) Adaptive responses that are not considered toxicologically relevant.

In summary, although there are clear effects in the liver in rats, mice and dogs, all effects are considered adaptive and reversible, and thus are not toxicologically adverse. The effects do not meet the criteria for a STOT-RE classification.

Table 1: A summary of effects on clinical chemistry and liver pathology following repeat dose administration of cyproconazole to rats and dogs

Study	Species	Endpoint			
		Clinical chemistry	Liver macropathology	Liver histopathology	Comments
28 day (Skinner et al 1985a)^	Rat	↑ Cholesterol (f) ↑ ALT ↑ AST (m)	Liver architecture visible 2/16 m	Hepatocellular vacuoles Hepatocyte hypertrophy (centrilobular)	**Adaptive** ↑ AST was >2 wks vs 4 wks.
90 day (Skinner et al 1985)	Rat	↑ creatinine	None	Vacuolated hepatocytes Distinct lobular pattern of liver	**Adaptive** All changes were reversible during 4 week recovery
90 day (Gerspach, 1999)	Rat	↓ bilirubin ↑ protein, globulin, AST ↑ cholesterol, ALP (f) ↑ G-GT, ALT (m) ↓ triglycerides (m)	None	Fatty changes Hepatocyte hypertrophy (centrilobular)	**Adaptive**
90 day (Warren, 1986)	Dog	↓cholesterol, albumin ↑ALP, G-GT, GLDH	None	Hepatocyte hypertrophy 1 m &1 f degeneration single hepatocytes	**Adaptive** Degeneration single hepatocytes is due to metabolic demand as is followed by regeneration
1 year (Warren, 1988)	Dog	↓cholesterol (f), albumin ↑ALP	Pronounced lobular pattern	Eosinophilic intrahepatocytic bodies Canaliclar bile plugs Intrahepatocytic pigment	**Adaptive** Eosinophilic intrahepatocytic bodies assoc with hypertrophy of ER and represent an adaptive change
16 day inhalation (Bernstein et al 1987)	Rat	None	None	Hepatocellular hypertrophy	**Adaptive**
28 day dermal (Sommer, 2000)	Rat	↑ Cholesterol (f)	None	Hepatocellular hypertrophy (m)	**Adaptive** Only effects at dose levels ≤ 600 mg/kg/day are described
Investigative studies					
28 day (Warren et al, 1995)	Rat	↑sorbitol dehydrogenase, lactate dehydrogenase, AST	None	Hepatocyte hypertrophy Hepatocyte vacuolation	**Adaptive**

m= males; f = females

^In the 28 day rat study (Skinner et al., 1984), changes in clinical chemistry were more pronounced at week 2 vs week 4, which is indicative of an adaptive response due to induction of metabolizing enzymes.

Table 2: A summary of effects on clinical chemistry and liver pathology following repeat dose administration of cyproconazole to mice

Study	Endpoint			
	Clinical chemistry	**Liver macropathology**	**Liver histopathology**	**Comments**
90 day (Warren 1987)	Not determined	Accentuated lobular pattern	Hepatocyte vacuolization Hepatocyte eosinophilia Hepatocyte single cell necrosis	**Adaptive**
90 day (Warren 1989) (interim sacrifice from carcinogenicity study)	Not determined	None	Periacinar hepatocyte hypertrophy Hepatocyte vacuolation (m) Hepatocytic fat vacuolation (large vacuoles) (f)	**Adaptive**
Investigative studies				
14 day (Milburn, 2006a) males only	↓cholesterol ↑ALP, G-GT, AST	None	Single cell necrosis Hepatocyte hypertrophy Hepatocyte vacuolation ↑ hepatocyte lipid content	**Adaptive**
7 day (Milburn, 2006b) males only	↓cholesterol, triglycerides ↑ALP, ALT, AST	None	Single cell necrosis +/- inflammatory cell infiltrate Hepatocyte hypertrophy Hepatocyte vacuolation ↑ hepatocyte lipid content	**Adaptive**
7 day (Milburn, 2006b) CAR KO males only	HIGH DOSE ONLY: Slight ↓ triglycerides ↑ALP	None	HIGH DOSE ONLY: Slight ↑ hepatocyte lipid content	**No adverse effects**
28 day (Warren et al, 1995)	No stats sig changes	None	Hepatocyte hypertrophy Hepatocyte vacuolation *single cell necrosis & small groups necrotic hepatocytes, frequently accompanied by inflammatory cells (granulocytes)	**Adaptive** Necrosis - the pattern and dose concordance corresponds to the induction of hepatocellular hypertrophy and it seems likely that the cell loss is a consequence of functional or structural disturbances, e.g. hypertrophy-related physiological or architectural changes.
14 day (Trendelenburg, 2001)	Not determined	None	Hepatocyte hypertrophy Hepatocyte vacuolation Single cell necrosis & small groups necrotic hepatocytes, frequently accompanied by inflammatory cells (granulocytes)	**Adaptive** Necrosis - the pattern and dose concordance corresponds to the induction of hepatocellular hypertrophy and it seems likely that the cell loss is a consequence of functional or structural disturbances, e.g. hypertrophy-related physiological or architectural changes.

m= males; f = females

* Single cell necrosis was not identified in the original study. A pathology re-evaluation (Weber, 1999) reported this finding.

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