COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

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Substance name: N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide; isoflucypram
CAS number: 1255734-28-1
EC number: n/a
Dossier submitter: United Kingdom

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
19.02.2020	Germany	Bayer AG	Company-Manufacturer	1
Comment received				
Bayer AG would like to take the opportunity of the targeted consultation on Isoflucypram to provide more context to the findings in the rodent liver and thyroid in the frame of the Specific target organ toxicity – repeated exposure (STOT RE) assessment. Please find below a detailed argumentation for both liver and thyroid. Overall, Bayer AG is of the opinion that the liver and thyroid effects were only slight, the MoAs for the effects have been established and that other MoAs can be excluded based on the toxicity profile of isoflucypram established in the standard and mechanistic toxicity assays performed on this molecule. Furthermore, both the liver and the thyroid MoAs are not considered relevant to humans. Just as the available data do not support classification for carcinogenicity, it is our opinion the available data do not warrant classification of isoflucypram with STOT-RE. LIVER: The liver was identified as a target organ in the rat, mouse and dog following dietary exposure to isoflucypram. The effects consisted mainly of increased liver weight and hepatocellular hypertrophy. No adenomas or carcinomas were observed at the end of the rodent cancer bioassays. Several MOAs have been described for liver effects that could potentially lead to tumor formation, including DNA reactivity and increased cell proliferation via key events that are either receptor-mediated or non-receptor mediated (Cohen, 2010). DNA reactivity can be discounted as a possible MOA for the liver effects as isoflucypram is not genotoxic or mutagenic as indicated by the negative results generated in the battery of genotoxicity studies conducted with this molecule. Although the rat liver can be responsive to the carcinogenic action of estrogen (Preston-Martin et al., 1990), this MOA can be discounted as there was no evidence of interference with the estrogen system in the standard rat and mouse studies and the two-generation rat study (e.g. no evidence of decreased fertility in males or in females, alterations in male and female reproductive organ weights, estrus cyclicity, or precocious vaginal opening). Moreover, isoflucypram is not a developmental or reproductive toxicant. Other known MOAs can also be excluded based on liver histopathology (eg. no evidence of iron deposition or fatty change in hepatocytes). Based				

on the general toxicity evaluation of isoflucypram, the liver effects are most likely receptor mediated. In particular, the liver changes observed in rodents consisted of increased weight and hepatocellular hypertrophy. Furthermore, the profile of hepatic P450 enzymes induced in the rodent (increased pentoxyresorufin (PROD) and benzoxyresorufin (BROD)) indicate that the MOA for the isoflucypram-induced liver effects may involve the constitutive androstane receptor (CAR) and pregnane X receptor (PXR). This was confirmed in vitro in CAR/PXR nuclear receptor screens as well as assessing the profile of transcripts associated with Phase I hepatic enzymes induced in primary cultures of rat hepatocytes following exposure to isoflucypram.
Such a MOA has been well established for other non-genotoxic hepatocarcinogens, for example phenobarbital (Elcombe et al., 2014; Holsapple et al., 2006; Whysner et al., 1996), metofluthrin (Deguchi et al., 2009; Yamada et al., 2009) and sulfoxaflor (LeBaron et al., 2013). Furthermore, in a recent publication by Peffer et al (2018), it is indicated that specific data to prove non-relevance to humans is considered not necessary, providing the molecular initiating event (MIE) and the critical key event, namely cell proliferation have been established and that other MoAs can be excluded. In the case of isoflucypram, the MIE has been established as CAR/PXR activation and hepatocellular proliferation was observed in mechanistic studies conducted in both the rat and the mouse. Other MoAs can be excluded based on the toxicity profile of isoflucypram established in the standard toxicity assays performed on this molecule.
References:
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THYROID:
The thyroid was identified as a target organ in the rat following dietary exposure to isoflucypram. Only slight changes in thyroid gland parameters (increased weight and incidence of follicular cell hypertrophy) and thyroid hormone changes in the repeat dose studies were observed. No adenomas or carcinomas were observed at the end of the rat cancer bioassay.
As indicated above, the liver was identified as a target organ with changes consisting of increased liver weight (relative to body weight) and hepatocellular. In addition, significantly increased activity of hepatic Phase II (and Phase I) enzymes was consistently observed in several in vivo and in vitro studies, providing evidence that the minor thyroid effects were liver mediated due to activation of CAR/PXR nuclear receptors. Additional in vitro assays have recently been performed to address alternative MoAs for the observed thyroid effects induced by isoflucypram. Specifically, the potential of isoflucypram to interfere directly with thyroid homeostasis was determined in two in vitro assays (thyroperoxidase (TPO) inhibition in rat thyroid microsomes and inhibition of sodium/iodide symporter (NIS)-mediated iodide uptake in the rat thyroid-derived cell line

Fisher Rat Thyroid Low Serum 5% (FRTL-5)).
No inhibition of TPO or NIS activity was observed. Using a weight of evidence approach, both the in vitro and in vivo data provide strong evidence that the slight thyroid effects observed in the rat repeat dose dietary toxicity studies are liver mediated via activation of CAR/PXR nuclear receptors. Alternative MoAs for the thyroid effects are considered to have been excluded as shown by the absence of interference with TPO and NIS.
This liver mediated MOA for thyroid effects is well recognized as being rodent specific and not relevant to humans, due to intrinsic species differences in thyroid physiology.