PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Summary of Evidence on the Absence of Hepatotoxicity of IQOS

Response to the article entitled "Possible hepatotoxicity of IQOS" by *Chun et al., 2018*[1]

by Gizelle Baker, Patrick Picavet, Maurice Smith, Patrick Vanscheeuwijck and Manuel C. Peitsch[2]

Philip Morris International R&D

[1] Department of Medicine, University of California, San Francisco, San Francisco, California, USA

[2] Philip Morris Products S.A., PMI Research and Development, Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland. Authors are listed in alphabetical order.

Table of Contents

1 EXECUTIVE SUMMARY

The Department of Medicine, University of California, and San Francisco, California, USA has recently published a letter in Tobacco Control (Chun et. al., 2018) claiming that "The preclinical and clinical data PMI submitted to FDA indicate that IQOS exposure may be associated with unexpected liver toxicity. We reviewed preclinical studies conducted by PMI scientists and clinical studies of 5 and 90 days of exposure to IQOS and IQOS menthol included in PMI's Modified Risk Tobacco Product application submitted to the US FDA."

In summary, we have assessed these claims based on a careful review of our scientific data submitted to FDA. The scientific data is in the FDA submission and requires knowledge of the design and conduct of toxicology and clinical studies, together with a careful and detailed review of the resulting data to reach accurate, science-based conclusions. Such an analysis was not performed by the authors of the letter and therefore the conclusions they have drawn are incorrect and misleading.

We have prepared a point-by-point assessment of the claims made by the authors and this detailed analysis can be found below.

In conclusion, based on an analysis of our toxicological studies and clinical studies performed according to international standards of good practice, there is no evidence that IQOS use leads to hepatotoxicity

2 INTRODUCTION

The Department of Medicine, University of California, San Francisco, San Francisco, California, USA has recently published a letter in Tobacco Control (Chun et. al., 2018) raising concerns on possible hepatotoxicity of IQOS based on results reported in the context of Philip Morris International's (PMI) Modified Risk Tobacco Product Application on IQOS. From their analysis of PMI non-clinical and clinical studies, the authors highlighted that in Sprague Dawley rats after 90 days of exposure to IQOS aerosol, mainstream smoke from 3R4F research cigarettes, or room air (sham), the levels of alanine aminotransferase (ALT) and liver weights were significantly higher with IQOS than with conventional cigarette smoke. Furthermore hepatocellular vacuolization, was significantly increased in IQOS-exposed female rats, an effect not seen in cigarette-exposed animals.

The authors also reported on increases of individual markers such as bilirubin and ALT, which are linked to liver function, in PMI's 5-day and 3-month clinical Reduced Exposure Studies. Based on these findings the authors concluded that IQOS aerosol is possibly hepatotoxic.

This report aims to clarify these findings and to provide context by summarizing the scientific data available on THS (marketed to date in various countries under the brand name IQOS) to date with

regards to liver function, including all relevant medical safety data, which was also reported in PMI's MRTPA.

3 SUMMARY OF NON-CLINICAL DATA AND RESULTS

The liver-related findings observed in the non-clinical studies are related to exposure to very high nicotine concentrations (at a maximum tolerated dose) and induction of xenobiotic metabolism. They are adaptive changes that are transient in nature and therefore not adverse effects indicative of hepatotoxicity of THS.

3.1 Clarification on Exposure Levels

The exposure to nicotine in THS-exposed rats is much higher than in 3R4F smoke-exposed rats in the PMI studies. Therefore effects related to the nicotine exposure levels are expectedly more prominent in THS-exposed than in smoke-exposed rats: The THS-exposed rats in the cited studies were exposed to 50 µg/l nicotine in the test atmospheres. This exposure concentration is near to Maximum Tolerated Dose (MTD) as required for toxicity studies and by the OECD Test Guidelines 412/413 (OECD 2009). In the same studies, the exposure concentration of 3R4F Reference Cigarette smoke was up to MTD as well and up to 23 µg/l nicotine in the test atmospheres. Upon 3R4F smoke exposure, the MTD is driven by the CO concentration in the test atmosphere, and not by the nicotine because of the much higher CO levels in smoke than in THS aerosol. Thus the nicotine exposure levels in the THS groups were up to twice as high as in the smoke-exposed groups. When exposures are extrapolated from the nicotine concentrations in inhalation studies to human exposure levels using the Alexander formula (Alexander et al. 2008) and the Guidance Document from the FDA (FDA 2005) based on the body surface area, THS exposure levels reach the equivalent of more than 100 sticks per day, whereas for 3R4F smoke exposure levels reach half of this. For a detailed calculation, refer to Wong et al. (Wong et al. 2016). In addition, due to the irritation caused by cigarette smoke constituents, the respiratory minute volume of the 3R4F smoke-exposed rats was decreased by approximately 40 %, whereas no reduction was observed upon THS exposure, even at twice the nicotine test atmosphere concentration (see table 2 in Wong et al.2016). This results in much higher nicotine exposure levels in THS aerosol exposed than in 3R4F smoke exposed rats, as evidenced by the quantity of total urinary nicotine metabolites in 24-hour urine. For example, in the urine of the male rats, the total nicotine metabolites was 5146.1 ± 648.86 nmol in the THS high dose group, whereas it was 1548.4 ± 355.49 nmol (means ± SD) in the 3R4F high dose group. At equal nicotine test atmosphere concentration, i.e. approximately 23 µg/L, the total nicotine metabolites in the THS group was 2619.3 ± 306.20 nmol vs. 1548.4 ± 355.49 nmol in the 3R4F group (see table 4 in Wong et al. 2016).

3.2 Increases in alanine aminotransferase (ALT), liver weight and liver vacuolization

Increases in the activity of alanine aminotransferase (ALT), liver weights relative to body weight and the occurrence of hepatocellular vacuolization containing glycogen are not related to THS aerosol exposure per se, but to nicotine. These findings have been observed in a study investigating the nicotine concentration-response relationship and reported in detail in a previously published article (Phillips et al. 2015). In this study, test atmosphere nicotine concentrations were similar to those used in the two THS studies the authors refer too. Also the nicotine uptake in the THS studies is similar to the nicotine uptake in the nicotine inhalation study as evidenced by the total nicotine metabolites recovered in 24-hour urine. As explained above, in our experimental rat models, the maximum nicotine uptake is much larger, up to 4-fold, upon exposure to non-irritant aerosols (THS and nicotine aerosols) than upon exposure to smoke, thus the nicotine exposure effects are lower when rats are exposed to cigarette smoke. **Figure 1** shows the ALT activities and relative liver weights for female and male rats as a function of the nicotine uptake from 3 studies (Phillips et al. 2015; Oviedo et al. 2016; Wong et al. 2016). Similar changes have also been found in a study using another heat-not-burn platform, namely CHTP1.2 (Phillips et al. 2018), as well as when assessing the effect of nebulized nicotine-containing e-liquid aerosols (Phillips et al. 2017). It should also be noted that in the studies cited above, when a 42-day recovery post-inhalation phase was included, the liver-related changes were transient, and thus fully reverted to base-line levels at the end of the post-inhalation period. The effect of nicotine on ALT and other liver-derived enzyme activity accompanied by oxidative stress and lipid peroxidation has furthermore been demonstrated in rats after subcutaneous injection on a high dose of nicotine (Balakrishnan and Menon 2007; Kalpana et al. 2007).

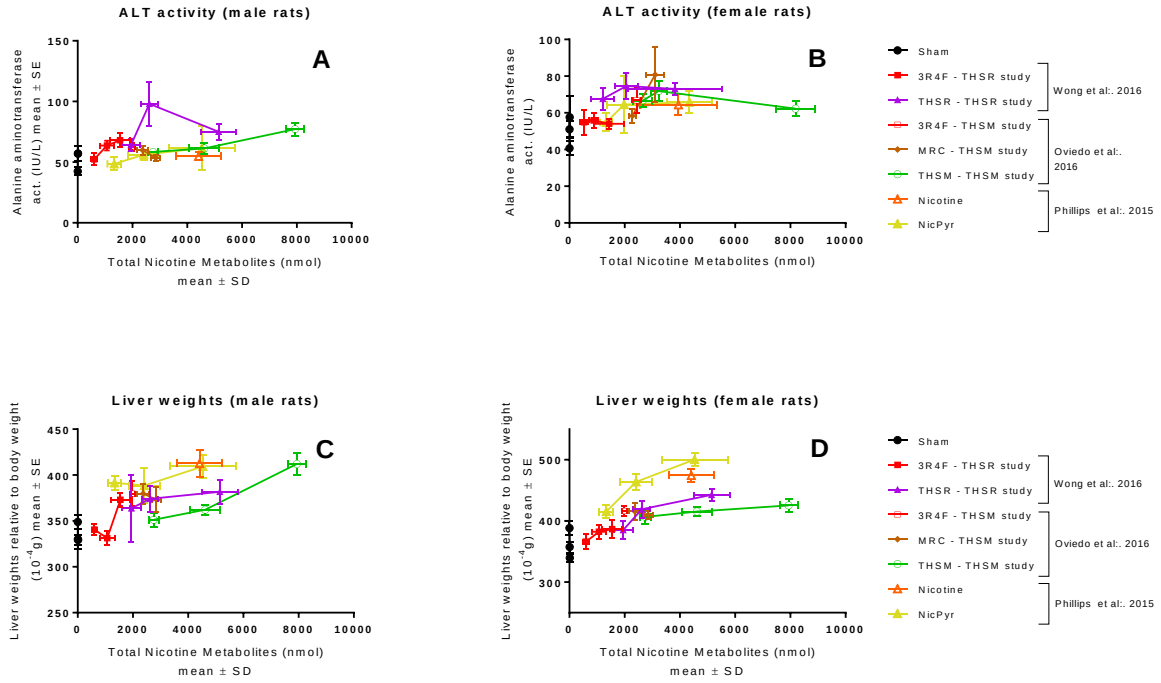

Figure 1 : Blood ALT activity in male (panel A) and female (panel B) rats, and liver weights relative to body weight in male (panel C) and female rats (panel D) as a function of total nicotine exposure, shown as the total nicotine metabolites measured in 24-hour urine from 3 different studies with cigarette smoke (3R4F and MRC- mentholated reference cigarettes), THS aerosols and nebulized nicotine or nicotine salt (NicPyr) solutions. THSR refers to Wong et al., 2016 using the regular, non-mentholated variant of THS, THSM refers to Oviedo et al., 2016 using the mentholated THS variant, Nicotine and NicPyr refer to Phillips et al., 2015. The Sham refers to the sham groups of the 3 studies mentioned above. Means are shown ± variance. Statistically significant differences are not shown but can be retrieved in the original publications.

Significance of the increased ALT activity, liver weights and hepatocellular vacuolization: The increased ALT activities and increased liver weights relative to body weight in the rat studies are considered adaptive, non-adverse changes with little relevance to man for risk assessment. It is true that ALT activity increase is frequently used as an indicator of liver injury, (Ennulat et al. 2010) while liver enlargement as a sensitive measure of hepatocellular hypertrophy, may be linked to hepatotoxicity (Hall et al. 2012). However, when ALT activity increases are below 2- to 3-fold, even when the changes are statistically significant, when there is reversibility and when there is no association with structural degenerative or necrosis changes in rat studies, their significance is low, i.e. they are an adaptive response to a xenobiotic (Hall et al. 2012). In our studies, ALT activity increases are approximately 1.5 fold without histological correlates in the liver. In a study where Apoe -/- mice were exposed to THS aerosol for up to 8 months, liver effects have been studied in great detail, including systems biology approaches and histopathological assessment. Neither degenerative liver changes nor molecular changes have been found as alerts indicative for hepatotoxicity (Phillips et al.

2016; Lo Sasso et al. 2016). Finally, in an 18-month A/J mouse inhalation study focusing on chronic toxicity and lung cancer, THS-induced liver-related changes were minimal and no non-proliferative or proliferative liver changes were found (unpublished results).

Hepatocyte vacuolization has been considered an adverse effect because it is seen after mild and subacute liver injury (Ennulat et al. 2010). However, several lines of evidence point to the possibility that hepatocyte swelling with non-lipidic vacuolization reflect a cellular adaptation rather than a degenerative change. Observations in animal and human livers suggest vacuolated hepatocytes during liver injury are cells adaptively altered to resist further insult (Nayak et al. 1996).

4 SUMMARY OF CLINICAL DATA AND SAFETY EVENTS RELATED TO LIVER INJURY

To assess if a product presents a specific risk or raises a specific safety concern such as hepatotoxicity, clinical and medical practice require to consider a set of standard parameters and diagnostic tools to be used to come to a conclusion. To identify a safety concern with regards to hepatotoxicity based on laboratory assessments, at minimum a liver diagnostic standard laboratory panel (a group of clinical tests that are performed together to detect, evaluate and monitor organ disease or damage) would need to be considered. The FDA Guidance for Industry "Drug-Induced Liver Injury: Premarketing Clinical Evaluation" (2009) provides a framework for assessing hepatocellular injury by evaluating and following over time a set of clinical tests including liver enzymes and serum bilirubin together with physical symptoms such as fatigue and nausea [18].

Each of these parameters would provide an insight on the potential cause, level and location of any potential injury of the liver and the seriousness thereof.

The standard liver panel we monitored in PMI clinical studies to assess liver-function included, among others, total bilirubin, direct (conjugated) bilirubin, ALT, aspartate transaminase (AST), AST/ALT ratio, alkaline phosphatase (AP) and gamma glutamyl transpeptidase (GGT). Therefore a good set of data exists to investigate if a potential safety concern for THS with regards to hepatotoxicity exists on a clinical level.

Several levels of data would need to be considered for this assessment:

- All Adverse Events (AEs) reported based on each of the parameters listed above in all study groups.
- The relationship between these AEs and their development or resolution over time in all study group.

- Changes in these laboratory parameters not considered as AE but which may have shown an increase during the study period (in all groups).

The sections below provide a detailed outline of all three sets of data and provide a clear picture and conclusion why no hepatotoxicity exists related to THS use based on the data available.

4.1 Adverse Events Reported related to Liver Function Parameters in PMI Clinical Studies

The following sections provide details on the AEs reported based on abnormal liver-function test in PMI clinical studies as well as all changes in liver-function parameters observed independently if they needed to be reported as AEs or not as per the study protocol and safety management plans of PMI clinical studies.

To assess levels of safety laboratory parameters, such as liver-function related parameters, in a standardized way, PMI uses the grading scheme of the Common Terminology Criteria for Adverse Events and Common Toxicity Criteria (CTCAE) (in version 4.03 for the 8 PMI clinical studies). This grading scheme is widely used in pharmacovigilance and well accepted across various industries. Based on this grading and the judgement of the Investigator of a PMI clinical study the following rules are applied to determine if an abnormal safety laboratory value such as a liver-function parameter must be reported as AE:

- All Grade 1 (as per CTCAE) abnormal laboratory values will be evaluated by the Investigator with respect to baseline value and clinical relevance. If considered to be clinically relevant the Investigator or designee must report it as an AE. All Grade 2 and higher abnormal laboratory values must be reported as or linked to an AE/concomitant disease.
- If a subject has Grade 2 and higher abnormal laboratory values at Screening it is at the discretion of the Investigator or designee to enroll the subject or not.
- If there is any worsening in grade from Grade 2 and above during the study the Investigator or designee must report this worsening as an AE.
- Where there is no grading available, the abnormal laboratory value will be evaluated by the Investigator or designee and assessed for clinical relevance. If considered to be clinically relevant, the Investigator will report it as an AE.
- Any other abnormal clinical laboratory result (including those that are not part of the core safety assessments) can, at the discretion of the Investigator or designee, be reviewed and assessed. Even if they do not meet the criteria of the CTCAE grading scheme, the Investigator or designee may consider them to be of clinical relevance and, if they are, must report them as AEs.

All AEs reported for safety laboratory parameters will be assessed for relationship to IP, intensity, seriousness and outcome.

4.1.1 Adverse Events Reported related to Liver Function Parameters in PMI Single Use Pharmacokinetic Studies

Among the 264 subjects included in the safety population in the four single use cross-over pharmacokinetic studies, a total of 85 AEs were reported in 57 subjects (21.6%) with 22 AEs being assessed by the principal investigators as investigational product (IP)-related.

Four of the 264 subjects experienced 4 AEs related to Liver-Function parameters. This is equivalent to 1.5% of the subjects or 4.7% of all AEs reported. One of the AEs reported for liver-function (hepatic function abnormal) was judged as related to THS by the Investigators. All the 4 AEs related to liver function were mild or moderate in grade (**Table 1**).

The abnormal hepatic function observed in one subject in study ZRHR-PK-02-JP at the discharge visit with a marginal and transient increase above upper limit of normal range (ULN) of alanine aminotransferase (1.5x ULN), aspartate aminotransferase (2.1x ULN) and alkaline phosphatase (1.2x ULN) levels, is not suggestive of a THS induced liver injury given the very limited study duration and amount of exposure to THS.

Table 1 Adverse Events Reported Related to Liver Function Parameters in Single Use Cross-over Studies

Study	THS Variant	Adverse Events Preferred Term (PT)	THS 2.2 - CC	CC - THS 2.2	THS 2.2 - NRT	NRT - THS 2.2	Overall Safety
ZRHR-PK-01-EU	**Regular**	**Safety Population (n)**	**22**	**22**	**9**	**9**	**62**
		Number (%) subjects with any AEs	10 (45.5%)	7 (31.8%)	4 (44.4%)	2 (22.2%)	23 (37.1%)
		No AEs related to Liver Function Parameters	0	0	0	0	0
ZRHR-PK-02-JP	**Regular**	**Safety Population (n)**	**22**	**22**	**9**	**9**	**65**
		Number (%) subjects with any AEs	3 (13.6%)	3 (13.6%)	4 (44.4%)	1 (11.1%)	11 (16.9%)
		Blood bilirubin increased	0	0	1 (11.1%)	0	1 (1.5%)
		Hepatic enzyme increased	1 (4.5%)	0	0	0	1 (1.5%)
		Hepatic function abnormal	0	1 (4.5%)	0	0	1 (1.5%)
ZRHM-PK-05-JP	**Menthol**	**Safety Population (n)**	**22**	**22**	**9**	**9**	**73**

Study	THS Variant	Adverse Events Preferred Term (PT)	THS 2.2 - CC	CC - THS 2.2	THS 2.2 - NRT	NRT - THS 2.2	Overall Safety
		Number (%) subjects with any AEs	1 (4.5%)	2 (9.1%)	0	1 (11.1%)	4 (5.5%)
		Blood bilirubin increased	1 (4.5%)	0	0	0	1 (1.4%)
ZRHM-PK-06-US	**Menthol**	**Safety Population (n)**	**22**	**22**	**9**	**9**	**64**
		Number (%) subjects with any AEs	7 (31.8%)	7 (31.8%)	3 (33.3%)	2 (22.2%)	19 (29.7%)
		No AEs related to Liver Function Parameters	0	0	0	0	0

Source: Appendix A6.1.5.4 of PMIs MRTPA for IQOS. Adverse events from completed clinical studies. Relevant safety summary tables from clinical study reports are compiled in this appendix for convenience purpose.
THS: Tobacco Heating System; CC: cigarettes; NRT; Nicotine Replacement Therapy

4.1.2 Adverse Events Reported related to Liver Function Parameters in PMI 5-Day and 3-Month Reduced Exposure Studies

Overall, and among the 655 subjects (safety population) enrolled in the two 5-day studies or randomized in the two 3-month studies, a total of 526 AEs were reported in 278 subjects (42.4 % of the subjects), with a total of 40 AEs (8% of all AEs) being assessed by the principal investigators as IP-related.

Ten of the 655 subjects experienced 13 AEs (3 AEs in one subject in REXA07) related to Liver-Function parameters. This is equivalent to 1.5% of the subjects or 2.5% of AEs. None of the AEs reported for liver-function was judged as related to THS by the Investigators and all of them were mild or moderate in grade (**Table 2**).

Table 2 Adverse Events Reported Related to Liver Function Parameters in Reduced Exposure Studies

Study	THS Variant	Adverse Events Preferred Term (PT)	THS 2.2	CC	SA	Overall Safety
ZRHR-REXC-03-EU	**Regular**	**Safety Population (n)**	**80**	**41**	**39**	**169**
		Number (%) subjects with any AEs	50 (62.5%)	29 (70.7%)	24 (61.5%)	112 (66.3%)
		Blood bilirubin increased	1 (1.3%)	0	0	1 (0.6%)
		Hyperbilirubinemia	1 (1.3%)	0	0	1 (0.6%)
ZRHR-REXC-04-JP	**Regular**	**Safety Population (n)**	**80**	**40**	**40**	**166**

Study	THS Variant	Adverse Events Preferred Term (PT)	THS 2.2	CC	SA	Overall Safety
		Number (%) subjects with any AEs	6 (7.5%)	3 (7.5%)	1 (2.5%)	10 (6.0%)
		No AEs related to Liver Function Parameters	0	0	0	0
ZRHM-REXA-07-JP Confinement Phase	**Menthol**	**Safety Population (n)**	**78**	**42**	**40**	**160**
		Number (%) subjects with any AEs	6 (7.7%)	1 (2.4%)	8 (20%)	15 (9.4%)
		Alanine aminotransferase increased	0	0	1 (2.5%)	1 (0.6%)
		Aspartate aminotransferase increased	0	0	1 (2.5%)	1 (0.6%)
		Blood bilirubin increased	1 (1.3%)	0	0	1 (0.6%)
ZRHM-REXA-08-US Confinement Phase	**Menthol**	**Safety Population (n)**	**80**	**41**	**39**	**160**
		Number (%) subjects with any AEs	23 (28.8%)	11 (26.8%)	13 (33.3%)	47 (29.4%)
		No AEs related to Liver Function Parameters	0	0	0	0
ZRHM-REXA-07-JP Ambulatory Phase	**Menthol**	**Safety Population (n)**	**78**	**42**	**40**	**160**
		Number (%) subjects with any AEs	29 (37.2%)	14 (33.3%)	9 (22.5%)	52 (32.5%)
		Gamma-glutamyltransferase increased	2 (2.6%)	0	0	2 (1.3%)
		Alanine aminotransferase increased	1 (1.3%)	0	0	1 (0.6%)
ZRHM-REXA-08-US Ambulatory Phase	**Menthol**	**Safety Population (n)**	**80**	**41**	**39**	**160**
		Number (%) subjects with any AEs	41 (51.3%)	14 (34.2%)	14 (35.9%)	69 (43.1%)
		Alanine aminotransferase	1 (1.3%)	0	0	1 (0.6%)
		Aspartate aminotransferase increased	1 (1.3%)	1 (2.4%)	0	2 (1.3%)
		Blood bilirubin increased	1 (1.3%)	0	1 (2.6%)	2 (1.3%)
		Gamma-glutamyltransferase increased	1 (1.3%)	0	0	1 (0.6%)

Source: Appendix A6.1.5.4 of PMIs MRTPA for IQOS. Adverse events from completed clinical studies. Relevant safety summary tables from clinical study reports are compiled in this appendix for convenience purpose.
THS: Tobacco Heating System; CC: cigarettes; NRT; Nicotine Replacement Therapy

4.1.3 Adverse Events Reported related to Liver Function Parameters in PMI's 6-Month Exposure Response Study

Overall, and among the 1012 subjects included in the safety population of the 6-month Exposure Response study, a total of 883 AEs were reported in 446 subjects (50.5 % of the subjects). Twenty-three (23) of the 1012 subjects experienced 23 AEs related to Liver-Function parameters. This is equivalent to 2.3% of the subjects in the safety population or 2.6% of all AEs occurred. 50% of the AEs were due to increased GGT and none of the different types of liver function parameters reported as AEs were observed in more than 2% of the subjects (**Table 3**).

Table 3 Adverse Events Reported Related to Liver Function Parameters in the 6 Months Exposure Response Study

Study	THS Variant	Adverse Events Preferred Term (PT)	THS 2.2 (n / events) (n %)	CC (n / events) (n %)	Overall Safety (n / events) (n %)
ZRHR-ERS-09-US	**Regular**	**Safety Population (n)**	**477**	**483**	**1012***
		Number (%) subjects with any AEs	214 / 396 (44.9%)	228 / 438 (47.2%)	446 / 838 (44.1%)
		Gamma-glutamyltransferase . increased	10 / 10 (2.1%)	5 / 6 (1.0%)	15 / 16 (1.5%)
		Alanine aminotransferase . increased	5 / 5 (1.0%)	5 / 5 (1.0%)	10 / 11 (1.0%)
		Aspartate aminotransferase . increased	2 / 2 (0.4%)	6 / 7 (1.2%)	8 / 9 (0.8%)
		Hepatic enzyme increased	2 / 2 (0.4%)	4 / 4 (0.8%)	6 / 6 (0.6%)
		Blood alkaline phosphatase . increased	1 / 1 (0.2%)	1 / 1 (0.2%)	2 / 2 (0.2%)
		Blood bilirubin increased	1 / 1 (0.2%)	1 / 1 (0.2%)	2 / 2 (0.2%)
		Bilirubin conjugated increased	0 / 0 (0.0%)	1 / 1 (0.2%)	1 / 1 (0.1%)
		Blood lactate dehydrogenase . increased	0 / 0 (0.0%)	1 / 1 (0.2%)	1 / 1 (0.1%)
		Liver function test abnormal	0 / 0 (0.0%)	1 / 1 (0.2%)	1 / 1 (0.1%)

Study	THS Variant	Adverse Events Preferred Term (PT)	THS 2.2 (n / events) (n %)	CC (n / events) (n %)	Overall Safety (n / events) (n %)
		Cholecystitis	1 / 1 (0.2%)	0 / 0 (0.0%)	1 / 1 (0.1%)
		Cholelithiasis	1 / 1 (0.2%)	0 / 0 (0.0%)	1 / 1 (0.1%)

Source: Clinical Study Report ZRHR-ERS-09-US, Appendix 5.2, Table 15.2.6.3 Summary of Adverse Events by System Organ Class and Preferred Term-Safety Population
Safety Time Period: Overall.
*52 subject included in the product test period in the beginning of the study are not listed here as no AEs related to liver function were reported.

4.2 Reporting of Changes in Liver Function Parameters in PMI Clinical Studies not reported as Adverse Events (normal to high shifts)

In PMI clinical studies the grading scheme CTCAE (in version 4.03 for the 8 PMI clinical studies) is used for grading of laboratory values (see **section 4.1** for details) and determining if an abnormal laboratory value has to be reported as AE or not. Important to note is that only abnormal laboratory values that are considered clinically relevant based on the judgement of the Investigator of the study are reported as AE. The rules applied in PMI clinical studies are laid out in **section 4.1**.

However, beyond the reporting and analysis of AEs, it is good pharmacovigilance practice to investigate shifts in safety parameters assessed in a study within a given study period as well as and across multiple studies, independent of whether these shifts are judged as clinically relevant or not. Such shifts in safety parameters can indicate trends for future safety signals and are therefore important to consider.

The evaluation of safety parameter shifts is part of the standard safety evaluation PMI performs beyond the sole reporting of AEs and PMI previously reported the following in Section 6.1.5.3.2 of PMIs MRTPA for IOQS the following normal to high shifts in the 8 clinical studies with regards to liver-function parameters.

In the 5-day Reduced Exposure studies (ZRHR-REXC-03-EU and ZRHR-REXC-04-JP) and in the single-use PK studies, no specific trends were observed in mean values of clinical chemistry parameters. Normal-to-high shifts (with high variability) were observed for:

- alanine-aminotransferase (ALT) was observed in all arms in both 5-day studies,
- total bilirubin and direct bilirubin in the THS arm of study ZRHR-REXC-03-EU and in the conventional cigarette and THS arms of study ZRHR-REXC-04-JP

In the 3-month Reduced Exposure Studies the following central tendencies were observed:

- In the two 3-month studies, mean changes from baseline in liver tests (ALT, AST, AP, total bilirubin, direct bilirubin and GGT) levels were small and showed no notable differences between study arms.
- a very slight increase in direct bilirubin in the THS and SA arms (more pronounced in the THS arm) in study ZRHM-REXA-08-US

Furthermore in the 3-month Studies – the following Normal-to-high Shifts were observed:

- Normal-to-high shifts included AST, ALT, GGT and total bilirubin in both studies ZRHM-REXA-08-US and ZRHM-REXA-07-JP
- There was no apparent trend in the normal-to-high shifts distribution between study arms, with the exception of total bilirubin in 3 subjects (who had no relevant medical history and no concomitant medication reported) in study ZRHM-REXA-07-JP in the THS and SA arms.

It has been reported in the literature that smoking cessation, even after short-term abstinence, seems to be associated with an increase in bilirubin concentration, which may be explained by uridine diphosphate glucuronosyltransferase (UGT) activity de-induction, as suggested by O'Malley (O'Malley 2014). UGT catalyzes the conjugation of bilirubin, the major metabolic pathway responsible for its disposition. Polycyclic aromatic hydrocarbons (PAH) are known to induce some isoforms of the UGT family (Zevin 1999). Suppression of exposure to PAH upon exclusive THS use or upon smoking abstinence may provide some explanations on the bilirubin increase.

In the recently completed 6 months Exposure Response Study (ZRHR-ERS-09-US) (submitted to FDA on June 8th 2018 – not yet published on the FDA website for PMIs MRTPA for IQOS) reporting of increases in liver parameters were highest in the CC group and lowest in the IQOS or dual-user group across all parameters. As in the studies reported above, temporary shifts in levels occurred in all groups.

Considering the findings described above, no particular safety signal emerges from the interpretation of liver-function parameter results in the studies of concern.

4.3 Point by Point Response

The authors stated that "*Following 5 days of exposure to IQOS, conventional cigarettes or smoking abstinence, plasma bilirubin was elevated in 8.8% of IQOS subjects compared with 0% of cigarette smokers and 2.6% in abstainers*." Furthermore, "*however, in the 5-day exposure study cited above, the rate of elevated bilirubin (>1.0 mg/ dL) in IQOS users was over three times higher than that*

observed with smoking abstinence (8.8% vs 2.6%), and the mean increase above baseline was 0.05 mg/dL with IQOS compared with −0.07 mg/dL with smoking abstinence. We can find no evidence in the literature that smoking cessation is associated with an increase in ALT."

These statements refer to the 5-Day Reduced Exposure Study conducted in Poland. Reported by the authors here is the change from baseline and refers to table 15.2.6.13 in the Appendix of the Clinical Study Report of ZRHR-REXC-03-EU part of PMIs MRTPA and posted on the FDA MRTP website.

At baseline levels of bilirubin in all three study groups (THS, CC and SA) were comparable (0.57-0.59mg/dl) with 94-96% of values reported as normal. Three subjects in the THS arm were reported with an elevated level for Bilirubin already at baseline compared with 2 subjects in the CC and 1 subject in the SA arm.

It is correct that at Day 6 levels of bilirubin were reported high in 8.8% or 7 subjects in the THS group and 2.6% or 1 subject in the SA group. However, important to note is that only 2 cases in the THS group arm were reported as clinically significant and were therefore reported as AEs (see **Table 2**). Furthermore, both cases were judged as not related to THS by the investigator.

Moreover, investigating all parameters which would belong to a standard laboratory liver panel (see above), the only parameter which was on a low level more increased in the THS group compared to the CC and SA groups was bilirubin. All other parameters (ALT, AST, GGT, LDH, AP) do not raise any concern and were comparable between all study groups.

In other words, the reporting of increases of bilirubin by the authors is done without providing any further context, i.e., considering a full liver-function standard safety panel (see above), clinical relevance, consideration of abnormal values already existing at baseline where no THS exposure has yet occurred, resolution of abnormal values over time or context to laboratory values observed in the other study groups over time. Such practice is medically invalid and misleading as it draws conclusion on one isolated parameter only, which in most medical diagnostics is not good practice.

Considering all data available, the only medically sound conclusion that can be drawn is that there is no sign of liver injury related to THS use.

The authors continued that "*In another 5-day study, the mean increase in ALT was higher with IQOS than with conventional cigarettes or smoking abstinence (4.5, 2.9 and 1.6 IU/L, respectively).*"

These statements refer to the 5-Day Reduced Exposure Study conducted in Japan (ZRHR-REXC-04-JP). Reported by the authors here is the change from baseline for ALT and refers to table 15.2.6.13 in the Appendix of the Clinical Study Report of ZRHR-REXC-04-EU part of PMIs MRTPA and posted on the FDA MRTP website.

Considering the data reported in the study report for this study, there were 7 subjects with elevated ALT values reported at the end of the study in the THS group compared to 1 subject in this group at baseline. Two subjects in the SA group had elevated levels of ALT at the end of the study versus 3 subjects at baseline. No elevations of ALT were recorded in the CC group.

First, none of the values was rated as "abnormal – clinically relevant" and therefore none of these increased levels qualified as AE.

Second, the authors provide selective reporting of one laboratory parameter without providing any further context on the medical relevance, other laboratory parameters considered in a standard liver-function safety panel, and context to development of such laboratory values over time in the other study groups.

As an example, there were 8 cases of elevated bilirubin at the end of the study in the THS group of which 4 cases were already reported at baseline (prior to THS use). These 8 cases represent 10% of THS users. In the CC group, there were no cases of elevated bilirubin reported at baseline but 6 cases, representing 15% of subjects in the CC group, at the end of the study.

Again, considering all data available, the only medically sound conclusion that can be drawn is that there is no sign of liver injury related to THS use.

Another statement made by the authors was that "*In a 90-day study of exposure to mentholated IQOS, mentholated cigarettes or smoking abstinence, the only subject experiencing a grade 2 (moderate) increase in ALT was in the IQOS group.*"

It is correct that the clinical study report for the 3-month Reduced Exposure Study in Japan (ZRHM-REXA-07-JP) reports 1 case of grade 2 increase in ALT. This was however on Day 30 and not day 60 as reported by the authors.

To put this finding into perspective here are the cases of ALT increase reported in all groups:

First, reported as AEs are only 2 cases of ALT increase – one case in the THS arm on Day 30 and one in the SA arm on Day 6 of the study. Both AEs were judged as not related to study product.

At Baseline, 5 subjects had an increase in ALT in the THS group (grade 1) and 2 subjects in the CC arm (grade 1). None were rated as clinically significant and therefore not reported as AEs.

At Day 6, 12 (15%), 2 (4.8%) and 6 (15%) subjects in the THS, CC and SA study group, respectively had a grade 1 elevation of ALT.

At Day 30, the 12 observations in the THS group had resolved and only the one observation with the grade 2 increase in ALT in the THS group was reported. This case was also reported as AE. In the CC and SA arm 2 (4.8%) and 3 (7.5%) of subjects still experienced an increase in ALT (grade 1).

These low level changes in ALT in the different groups continued until the end of the study.

In summary, the change in grade 1 increases in ALT in all groups over time and the selective representation of one AE in the THS group at one specific time point, without mentioning the AE reported for the same parameter in the SA arm at another time point in the study and not considering any other of the liver-function parameters shows how invalid and misleading the reporting by the author is. To provide more context: Bilirubin, which was the parameter the authors focused on for the 5-Day Reduced Exposure study in the Poland study as an indicator of liver injury is, with variable mild elevations across all groups, otherwise normal in this study. All other parameters of the standard liver-function panel did not show any difference between study groups.

Finally, the authors reported that "*In another study, the rate of grade 1 (mild) increases in ALT after 60 days of exposure was highest with IQOS at 6.3% compared with 0% for conventional cigarettes and 2.6% with smoking abstinence"*.

The clinical study report for the 3-month Reduced Exposure study in the US (ZRHM-REXA-08-US) reports indeed that at Day 60, 5 subjects (6.3%) in the THS group compared with no subjects in the CC group and 1 subject (2.6%) in the SA group experienced a grade 1 increase in ALT. However, as these cases were all grade 1, they were not considered clinically relevant by the Investigator. Furthermore the authors did not report that at Day 91 of the study the number of subjects with an increase in ALT was 3.8% in the THS arm, 4.9% in the CC arm and 2.6% in the SA arm at Day 91.

5 DISCUSSION

First, to interpret the results from non-clinical and clinical studies and draw medically relevant conclusions the entire set of results have to be assessed and the study objectives and designs need to be accounted for. Stating the objectives of studies reported on as well as ensuring that results are comparable based on the study design is an important part of sound scientific evaluation.

It is correct that increased liver weight (relative to body weight), increased ALT activity, and vacuolization of hepatocytes have been observed in a 90-day inhalation study on THS performed on rats ((Wong et al. 2016)). These changes are most likely due to nicotine as discussed in detail in a 28-day rat inhalation study on nicotine and nicotine salts (Phillips et al. 2015), are often seen in xenobiotic agent exposures and are adaptive in nature. They are accompanied by metabolic changes but without histopathological degenerative changes (i.e. necrosis).

However, for the effects reported in our non-clinical studies the following need to be considered:

The exposure concentrations used for THS in the reported non-clinical studies were at the maximum tolerable level for rats (50 µg/L nicotine in test atmosphere). They therefore correspond to

approximately more than 100 HeatSticks per day, extrapolated to human exposure. In contrast, the 3R4F-exposed rats were exposed to a lower test atmosphere nicotine concentration, i.e. 23 µg/L nicotine which corresponds to about half the number of cigarettes/day.

Furthermore, due to the irritancy of the 3R4F smoke, the minute volume of the rats was lower than that of the THS-exposed rats. The difference in recovered biomarkers of nicotine exposure in the urine is greater than a factor 3. (3-fold higher exposure in the THS exposed rats). This explains the higher nicotine-mediated effects on the liver upon THS exposure compared to 3R4F smoke exposure.

To clinically assess and diagnose hepatotoxicity in humans, physicians use standard laboratory safety panels in combination with other medical tests and monitor how the panel results evolve over time. Selective reporting of various single parameters within a standard laboratory safety panel at specific time points and across different studies cannot be used to provide medically relevant conclusions.

When the individually reported parameters are put in context of a standard laboratory panel to assess the health status of the liver, and evaluated over the course of the different studies, there is no indication of liver toxicity related to THS use.

Furthermore, in the recently concluded 6-month Exposure Response Study, reports of increased liver parameters were highest in the CC group and lowest in the THS or Dual-Use groups considering all liver parameters. Furthermore, clinically relevant changes in liver parameters, using AE data, were extremely limited and the incidence of AEs related to liver parameters was between 2 and 3% and comparable between all of the study groups with unfavorable changes in a single laboratory parameter emerging in no more than 2% of all subjects in the safety population.

6 CONCLUSION

Applying the rigorous scientific principles we have applied throughout the various steps of our assessment program and

1) Considering study designs and objectives to enable accurate comparison of results between different study groups at equal nicotine exposure concentrations
2) Putting transient, adaptive changes into context and differentiate them from structural, non-reversible histological changes
3) Considering a medically sound evaluation of a standard panel of liver-function laboratory parameters in context and not as single parameters at one given time point in one study
4) Considering transient changes in laboratory parameters in all study groups instead of focusing on the THS study groups only
5) Most importantly considering clinically relevant changes, as reported in Adverse Event data, compared to focusing on clinically not relevant increases in single parameters

The only science-based conclusion that can be reached is that there is no indication of hepatotoxicity through THS use.

7 REFERENCES AND RELATED DOCUMENTS

1. Alexander, D. J., C. J. Collins, D. W. Coombs, I. S. Gilkison, C. J. Hardy, G. Healey, G. Karantabias, N. Johnson, A. Karlsson, J. D. Kilgour, and P. McDonald. 2008. 'Association of Inhalation Toxicologists (AIT) working party recommendation for standard delivered dose calculation and expression in non-clinical aerosol inhalation toxicology studies with pharmaceuticals', *Inhal Toxicol*, 20: 1179-89.
2. Balakrishnan, A., and V. P. Menon. 2007. 'Protective effect of hesperidin on nicotine induced toxicity in rats', *Indian J Exp Biol*, 45: 194-202.
3. Chun L, Moazed F, Matthay M Calfee C and Gotts J (2018) Possible hepatotoxicity of IQOS. Tobacco Control, doi:10.1136/ tobaccocontrol-2018-054320. (PMID: 30131373).
4. Ennulat, D., D. Walker, F. Clemo, M. Magid-Slav, D. Ledieu, M. Graham, S. Botts, and L. Boone. 2010. 'Effects of hepatic drug-metabolizing enzyme induction on clinical pathology parameters in animals and man', *Toxicol Pathol*, 38: 810-28.
5. FDA. 2005. "Guidance for Industry
6. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER)." In.: FDA Maryland.
7. Hall, A. P., C. R. Elcombe, J. R. Foster, T. Harada, W. Kaufmann, A. Knippel, K. Kuttler, D. E. Malarkey, R. R. Maronpot, A. Nishikawa, T. Nolte, A. Schulte, V. Strauss, and M. J. York. 2012. 'Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop', *Toxicol Pathol*, 40: 971-94.
8. Kalpana, C., A. R. Sudheer, K. N. Rajasekharan, and V. P. Menon. 2007. 'Comparative effects of curcumin and its synthetic analogue on tissue lipid peroxidation and antioxidant status during nicotine-induced toxicity', *Singapore Med J*, 48: 124-30.
9. Lo Sasso, G., B. Titz, C. Nury, S. Boue, B. Phillips, V. Belcastro, T. Schneider, S. Dijon, K. Baumer, D. Peric, R. Dulize, A. Elamin, E. Guedj, A. Buettner, P. Leroy, S. Kleinhans, G. Vuillaume, E. Veljkovic, N. V. Ivanov, F. Martin, P. Vanscheeuwijck, M. C. Peitsch, and J. Hoeng. 2016. 'Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe -/- mice--a systems toxicology analysis', *Inhal Toxicol*, 28: 226-40.
10. Nayak, N. C., S. A. Sathar, S. Mughal, S. Duttagupta, M. Mathur, and P. Chopra. 1996. 'The nature and significance of liver cell vacuolation following hepatocellular injury--an analysis based on observations on rats rendered tolerant to hepatotoxic damage', *Virchows Arch*, 428: 353-65.
11. OECD. 2009. *Test No. 413: Subchronic Inhalation Toxicity: 90-day Study* (OECD Publishing).
12. Oviedo, A., S. Lebrun, U. Kogel, J. Ho, W. T. Tan, B. Titz, P. Leroy, G. Vuillaume, M. Bera, F. Martin, G. Rodrigo, M. Esposito, R. Dempsey, N. V. Ivanov, J. Hoeng, M. C. Peitsch, and P. Vanscheeuwijck. 2016. 'Evaluation of the Tobacco Heating System 2.2. Part 6: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of a mentholated version compared with mentholated and non-mentholated cigarette smoke', *Regul Toxicol Pharmacol*, 81 Suppl 2: S93-s122.

13. Phillips, B., M. Esposito, J. Verbeeck, S. Boue, A. Iskandar, G. Vuillaume, P. Leroy, S. Krishnan, U. Kogel, A. Utan, W. K. Schlage, M. Bera, E. Veljkovic, J. Hoeng, M. C. Peitsch, and P. Vanscheeuwijck. 2015. 'Toxicity of aerosols of nicotine and pyruvic acid (separate and combined) in Sprague-Dawley rats in a 28-day OECD 412 inhalation study and assessment of systems toxicology', *Inhal Toxicol*, 27: 405-31.
14. Phillips, B., B. Titz, U. Kogel, D. Sharma, P. Leroy, Y. Xiang, G. Vuillaume, S. Lebrun, D. Sciuscio, J. Ho, C. Nury, E. Guedj, A. Elamin, M. Esposito, S. Krishnan, W. K. Schlage, E. Veljkovic, N. V. Ivanov, F. Martin, M. C. Peitsch, J. Hoeng, and P. Vanscheeuwijck. 2017. 'Toxicity of the main electronic cigarette components, propylene glycol, glycerin, and nicotine, in Sprague-Dawley rats in a 90-day OECD inhalation study complemented by molecular endpoints', *Food Chem Toxicol*.
15. Phillips, B., E. Veljkovic, S. Boue, W. K. Schlage, G. Vuillaume, F. Martin, B. Titz, P. Leroy, A. Buettner, A. Elamin, A. Oviedo, M. Cabanski, H. De Leon, E. Guedj, T. Schneider, M. Talikka, N. V. Ivanov, P. Vanscheeuwijck, M. C. Peitsch, and J. Hoeng. 2016. 'An 8-Month Systems Toxicology Inhalation/Cessation Study in Apoe-/- Mice to Investigate Cardiovascular and Respiratory Exposure Effects of a Candidate Modified Risk Tobacco Product, THS 2.2, Compared With Conventional Cigarettes', *Toxicol Sci*, 149: 411-32.
16. Phillips, B. W., W. K. Schlage, B. Titz, U. Kogel, D. Sciuscio, F. Martin, P. Leroy, G. Vuillaume, S. Krishnan, T. Lee, E. Veljkovic, A. Elamin, C. Merg, N. V. Ivanov, M. C. Peitsch, J. Hoeng, and P. Vanscheeuwijck. 2018. 'A 90-day OECD TG 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of the aerosol from the carbon heated tobacco product version 1.2 (CHTP1.2) compared with cigarette smoke. I. Inhalation exposure, clinical pathology and histopathology', *Food Chem Toxicol*.
17. Wong, E. T., U. Kogel, E. Veljkovic, F. Martin, Y. Xiang, S. Boue, G. Vuillaume, P. Leroy, E. Guedj, G. Rodrigo, N. V. Ivanov, J. Hoeng, M. C. Peitsch, and P. Vanscheeuwijck. 2016. 'Evaluation of the Tobacco Heating System 2.2. Part 4: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects compared with cigarette smoke', *Regul Toxicol Pharmacol*, 81 Suppl 2: S59-s81.
18. FDA Guidance for Industry “Drug-Induced Liver Injury: Premarketing Clinical Evaluation” (2009); https://permanent.access.gpo.gov/LPS112256/LPS112256_UCM072278.pdf

8 ABBREVIATIONS

3R4F	:	Research Cigarette of the University of Kentucky
AE	:	Adverse Event
ALT	:	Alanine Aminotransferase
AP	:	Alkaline Phosphatase
AST	:	Aspartate Transaminase
CC	:	Conventional Cigarette
CHTP	:	Carbon-Tip Heated Tobacco Product
CO	:	Carbon Monoxide
CTCAE	:	Common Terminology Criteria for Adverse Events and Common Toxicity Criteria
FDA	:	Food and Drug Administration
GGT	:	Gamma-Glutamyltransferase
IP	:	Investigational Product
LDH	:	Lactate Dehydrogenase
MTD	:	Maximum Tolerable Dose
MRTP(A)	:	Modified Risk Tobacco Product (Application)
NRT	:	Nicotine Replacement Therapy
OECD	:	
PAH	:	Polycyclic Aromatic Hydrocarbons
PK	:	Pharmacokinetic
PMI, R&D	:	Philip Morris International, Research and Development
THS	:	Tobacco Heating System (marketed as IQOS)
SA	:	Smoking Abstinence
UGT	:	Uridine Diphosphate Glucuronosyltransferase
UPN	:	Upper Limit of Normal Range