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PMSS for MR0000059-MR000061, MR0000133	Page 1 of 27
Computational Toxicology: Methodology	Version 1.0

Computational Approach to Assess the Cancer Risk from the Exposure to Chemicals Increased in THS2.2 Aerosol Compared to 3R4F Smoke.

Part 1: Methodology

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 2 of 27
Computational Toxicology: Methodology	Version 1.0

TABLE OF CONTENT

EXECUTIVE SUMMARY	3
ABBREVIATIONS	4
1 INTRODUCTION	6
2 METHODOLOGY	8
2.1 Computational toxicology for hazard identification	8
2.1.1 Endpoints	8
2.1.2 Protocol	11
2.1.3 Model validation	13
2.2 Hazard characterization.....	14
2.3 Differential chemical characterization of the aerosol for exposure assessment ...	15
2.3.1 Qualitative chemical characterization.....	15
2.3.2 Quantitative chemical characterization.....	16
2.3.3 Exposure assessment.....	16
2.4 Risk characterization.....	16
2.5 Reporting.....	17
2.6 Conclusions.....	18
3 BIBLIOGRAPHY.....	19
ANNEX 1.....	23

LIST OF TABLES

Table 1: <i>in vitro</i> and <i>in vivo</i> genetic toxicological assays	9
Table 2: Report content and accountability	18

LIST OF FIGURES

Figure 1: Risk assessment process overview	7
Figure 2: Current <i>in silico</i> components most relevant to genotoxicity	11
Figure 3: Protocol proposed at PMPSA for hazard identification	12

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 3 of 27
Computational Toxicology: Methodology	Version 1.0

EXECUTIVE SUMMARY

As part of the initial product characterization of the Tobacco Heating System (THS 2.2), non-targeted differential screening (NTDS) analyses of three THS 2.2 product variants (Regular: THSR, Menthol: THSM and High Menthol: THSH) have been performed to identify compounds which were potentially new, or significantly increased in THS 2.2 aerosol relative to 3R4F smoke. This document describes the risk assessment process applied to these chemicals to compare the carcinogenic potential of THS 2.2 aerosol with that of 3R4F smoke. The approach includes state of the art *in silico* toxicology methods for hazard identification and characterization, exposure assessment, and risk characterization.

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 4 of 27
Computational Toxicology: Methodology	Version 1.0

ABBREVIATIONS

AD	Applicability Domain
BHT	Butylated HydroxyToluene
BW	BodyWeight
CPV	Cancer Potency Value
DAI	Daily Aerosol Intake
DBV	Daily Breathed Volume
DC	Daily Consumption
DNA	DeoxyriboNucleic Acid
EMA	European Medicines Agency
GCxGC-TOFMS	Two-Dimensional Gas Chromatography with Time-Of-Flight Mass Spectrometry
GIST	Genetic Toxicity <i>In Silico</i>
HnB	Heat-not-burn
HPHC	Harmful and Potentially Harmful Constituent
HPV	High-Production-Volume
ICH	International Council for Harmonisation
ISO	International Organization for Standardization
IST	<i>In Silico</i> Toxicity
IUR	Inhalation Unit Risk
LC-HRMS	Liquid Chromatography - High Resolution Mass Spectrometry
3-MCPD	3-Monochloro-1,2-propanediol
NTDS	Non-Targeted Differential Screening
OECD	Organization for Economic Co-operation and Development
OEHHA	California Office of Environmental Health Hazard Assessment
OSF	Oral Slope Factor
PMDA	Japanese Pharmaceutical and Medical Devices Agency
QMRF	QSAR Model Reporting Format
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 5 of 27
Computational Toxicology: Methodology	Version 1.0

THS Tobacco Heating System
US EPA US Environmental Protection Agency
US FDA US Food and Drug Administration

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 6 of 27
Computational Toxicology: Methodology	Version 1.0

1 INTRODUCTION

‘Heat-not-burn’ tobacco products (HnB) are electronic devices that heat processed tobacco instead of combusting it. As part of the product characterization package, a non-targeted characterization of THS 2.2 aerosol chemical composition was performed. Through the chemical comparison of the THS2.2 aerosol with 3R4F cigarette smoke, this study identified several newly formed or increased compounds. There is a need to comprehensively assess the toxicological risks associated with these chemicals. However, for the vast majority of them, there is no, or very limited, toxicological data available.

Traditionally, the risk assessment of chemicals with limited toxicity data would require the generation of both experimental *in vitro* and *in vivo* data for hazard identification and hazard characterization. However, there are many reasons to move to an approach limiting such testing, especially the generation of *in vivo* data. First, conventional toxicity data generation is not particularly efficient for hazard assessment when a rapid understanding of potential toxicological consequences from exposure is needed. Second, conventional toxicology approaches can be challenging when a test item is difficult to obtain in sufficient amounts, or when conducting laboratory studies is challenging (e.g. monitoring the exposure to the test item, non-adapted test systems ...). Finally, conventional toxicity testing is slow and costly. Likewise, *in vivo* animal tests are constrained by time, ethical considerations, test material supply, and cost. More than time and financial burden, it is important to notice that new approaches have emerged, making this burden unnecessary. For instance, *in silico* methods are sufficient for the protection of public health

All these considerations lead to an increasing interest in applying computational methods to predict toxicological effects relevant to human health, as well as to support hazard and risk assessment activities. Indeed, *in silico* toxicity (IST) methods are much more agile than the usual battery of tests and *de facto* supports the 3R principle (replacement, refinement and reduction) relating to the use of animals in research ([Russell et al., 1959](#); [Ford, 2016](#)).

[Stanton and Kruszewski \(2016\)](#) quantified the benefits of using *in silico* and read-across methods and determined that the approach used across two voluntary high-production-volume (HPV) chemical programs for 261 chemicals prevented the use of 100,000 – 150,000 test animals and saved 50,000,000 to 70,000,000 US\$.

The first regulation that formally included the use of *in silico* approaches to address information requirements for the purposes of hazard identification and risk assessment was REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) ([REACH, 2006](#)). This regulation, applies to chemicals manufactured or imported into the European Union where their import or use is not covered by other specified legislation. In addition, since 2014, with the implementation of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) M7 guideline ([ICH, 2014](#); [ICH, 2017](#)), regulatory authorities, such as the US Food and Drug Administration (US FDA), the Japanese

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PMSS for MR0000059-MR000061, MR0000133	Page 7 of 27
Computational Toxicology: Methodology	Version 1.0

Pharmaceutical and Medical Devices Agency (PMDA), and the European Medicines Agency (EMA) accept *in silico* assessments of the mutagenic potential of drug impurities.

Despite that, several issues have hindered the general acceptance and use of *in silico* methods on a larger scale. In particular, there remains a lack of generally accepted procedures for performing *in silico* assessments for the toxicological endpoints. Recently, a consortium of toxicologists, computational scientists, and regulatory scientists from across several industries addressed this shortcoming, and developed an IST protocol to support the implementation and foster greater acceptance of *in silico* approaches (Myatt et al., 2018, Hasselgren et al., 2019). This protocol is based on *in silico* predictions and/or available experimental data for a defined series of relevant toxicological effects or mechanisms.

The reliability of *in silico* predictions is determined alongside that of the experimental data. In addition, the approach includes the determination of the level of confidence in the overall assessment based on the relevance and reliability of the information retrieved.

In this document, we describe the risk assessment process that PMPSA intends to use to assess the carcinogenic risks associated with the chemicals found in higher concentrations in the THS 2.2 aerosols than in the smoke of the reference cigarette 3R4F (Figure 1). This process is inspired by the risk assessment for chemicals as described by Whaley et al. 2016.

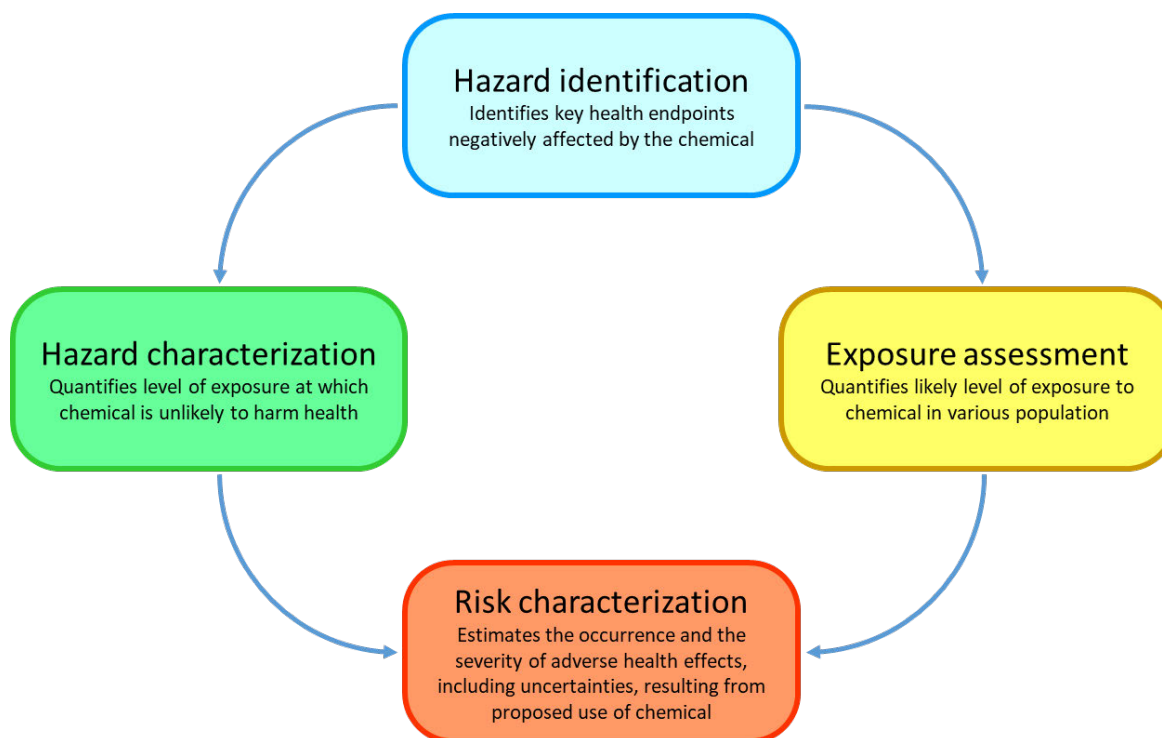


Figure 1: Risk assessment process overview

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 8 of 27
Computational Toxicology: Methodology	Version 1.0

This approach includes:

1. A **hazard identification** step, which includes a rigorous computational (*in silico*) genetic toxicology study that takes into account the latest recommendation defined in the IST protocol described by Myatt et al. (Myatt et al., 2018). This step will be applied to all chemicals and will identify those with genotoxic potential.
2. A **hazard characterization** step, which uses experimental data and quantitative structure-activity relationship (QSAR) models to predict points of departure for the final risk characterization. In addition, read-across (OECD, 2014) can be used, whereby the hazard of a target substance can be predicted from experimental data from structural analogues (source substances).
3. An **exposure assessment** step where information derived from NTDS will allow estimation of the total exposure for consumers.
4. A **risk characterization** step where data generated in previous steps will be used to assess the overall carcinogenic risk associated with genotoxic chemicals.

2 METHODOLOGY

2.1 Computational toxicology for hazard identification

2.1.1 Endpoints

Genetic toxicology concerns the effects induced by genetic alterations that may occur in somatic and/or germ cells following exposure to a chemical agent. Chemical agents can induce changes in DNA through direct or indirect interactions and the consequences of the genetic alterations may manifest as death and/or mutations in exposed cell populations. Genotoxicity testing for hazard identification and risk assessment is designed to characterize the ability of a chemical agent to induce genetic alterations (OECD, 2016). A comprehensive assessment of genotoxicity usually incorporates a battery of tests to evaluate mutagenicity, clastogenicity and aneugenicity. Table 1 list some *in vitro* and *in vivo* assays that are frequently used to assess genotoxicity, as well as annotation of the mechanism(s) each assay may identify.

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 9 of 27
Computational Toxicology: Methodology	Version 1.0

Table 1: *in vitro* and *in vivo* genetic toxicological assays

	OECD Test Guideline	Name	Endpoint
<i>In vitro</i>	471 (OECD, 1997a)	Bacterial reverse mutation test (Ames)	Gene mutation
	473 (OECD, 2016c)	In vitro mammalian chromosomal aberration test	Clastogenicity
	487 (OECD, 2016i)	In vitro mammalian cell micronucleus test	Clastogenicity/ Aneugenicity
	490 (OECD, 2016k)	In vitro mammalian cell gene mutation tests using thymidine kinase gene (MLA/TK6)	Gene mutation/ Clastogenicity
<i>In vivo</i>	474 (OECD, 2016d)	Mammalian erythrocyte micronucleus test	Clastogenicity/ Aneugenicity
	475 (OECD, 2016e)	Mammalian bone marrow chromosome aberration test	Clastogenicity
	478 (OECD, 2016)	Genetic toxicology: Rodent dominant lethal test	Chromosome aberration by clastogenicity / aneugenicity (gene mutations)
	483 (OECD, 2016h)	Mammalian spermatogonial chromosome aberration test	Clastogenicity
	489 (OECD, 2016j)	In vivo mammalian alkaline comet assay	DNA damage

Additional supporting information like the upregulation of DNA repair enzymes and related stress response pathways that focus on individual genes (e.g., Gadd45a ([Gentronix, 2018](#)), p53 ([Witt et al., 2017](#)), ATAD5 ([Fox et al., 2012](#)) or panels of genes ([Li et al., 2015](#)), or differential cytotoxicity using isogenic cell lines that have been knocked out for different DNA repair enzymes ([Yamamoto et al., 2011](#)) could be also used.

The maintenance of genome stability requires efficient mechanisms to repair double-stranded DNA breaks, which are mediated by the phosphorylation of multiple histone H2AX (γ -H2AX) molecules near the break site ([Nakamura et al., 2010](#)). Measuring cellular levels of γ -H2AX provides means to quantify the degree of DNA damage caused by an exposure. γ -H2AX levels can be measured using imaging-based methodologies that have been applied to various heated tobacco products to evaluate potential genotoxicity ([Gonzalez-Suarez et al., 2016](#); [Taylor et al., 2018](#)).

An important step in the hazard identification process is the assessment of the quality of any identified data as well as its relevance to any of the mechanistic assessments related to the major genotoxicity endpoints. A widely accepted methodology to assess data quality use the Klimisch scores ([Klimisch et al., 1997](#)). Such approach is currently used by ECHA in the Read-

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 10 of 27
Computational Toxicology: Methodology	Version 1.0

Across Assessment Framework (ECHA, 2017) and can be readily generated using the ToxRTool (European Commission, 2018). Klimisch scores rank data depending on how the experiment was conducted (and reported), and takes into consideration for example, whether the experiment was conducted according to Good Laboratory Practices (GLP), and whether details of the experiment are available for review. These scores provide a consistent and reproducible way to classify the reliability of the test results.

For chemicals with limited toxicity data, *in silico* methodologies can be used to generate predictions (Myatt et al., 2018). These include:

- Rule-based (or “expert”) systems that identify the presence of a structural moiety, also referred to as a structural alert, which may indicate genotoxic potential.
- Statistical QSAR models that use a variety of molecular descriptors such as structural fragments or physicochemical properties to predict activity.
- Read-across” (OECD, 2014) methodologies that utilizes experimental or computed properties, such as physicochemical properties, together with structural similarity and experimental data for structural analogs to extrapolate from source chemical(s) to (a) target (query) chemical(s) (OECD, 2014).

The types of *in silico* tools that can be developed for a specific endpoint are, to a great extent, driven by the availability (amount and quality) of experimental data for model development, as well as the degree to which the chemicals of interest exert their toxicity via a common mechanism. *In silico* tools are most easily developed for endpoints with a well understood and similar mode of action for which a large number of data points are available.

To identify potential genotoxic and carcinogenic hazards related to the increase of several chemicals, as determined through the chemical comparison of the THS2.2 aerosol with the smoke of the 3R4F, the following endpoints will be evaluated:

- Bacterial gene mutation
- Mammalian gene mutation
- Chromosome aberration *in vitro*
- Micronucleus *in vitro*
- Chromosome aberration *in vivo*
- Micronucleus *in vivo*
- Rodent carcinogenicity

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2.1.2 Protocol

Recently, Hasselgren et al. (Hasselgren et al., 2019) described a genetic toxicity *in silico* (GIST) protocol based on the IST approach published by Myatt et al. (Myatt et al., 2018) that outlines the process for determining the genotoxicity of a chemical agent, as well as the level of confidence of the assessment. The protocol covers the three major endpoints of genotoxicity and allows inclusion of both experimental data and *in silico* predictions as weight of evidence for genotoxicity. Figure 2 shows the evaluation process specifying the genotoxic effects and the endpoints that are applicable to the generation of *in silico* tools on the basis of data currently available in the public domain.

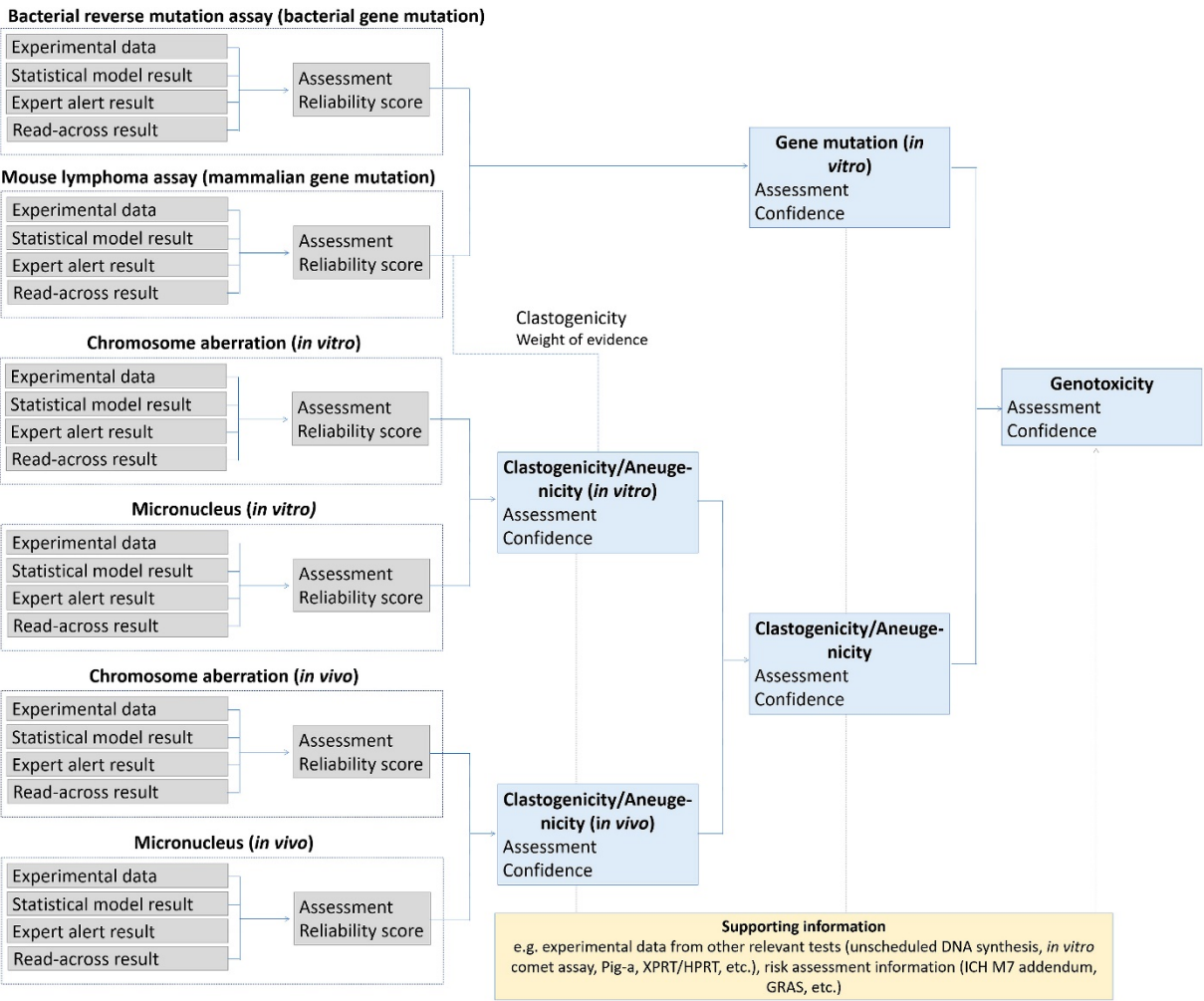


Figure 2: Current *in silico* components most relevant to genotoxicity

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 12 of 27
Computational Toxicology: Methodology	Version 1.0

To identify the hazards associated with the chemicals that were found to be higher in THS 2.2 aerosol than in 3R4F smoke, the GIST protocol described by Hasselgren et al. ([Hasselgren et al., 2019](#)) will be applied. In particular, experimental data will be retrieved from available databases and a reliability score will be calculated applying the methodology described in Myatt et al. ([Myatt et al., 2018](#)). In absence of available experimental data, read-across will be performed, if applicable. Similarly, *in silico* predictions will be integrated in the overall hazard identification and a full explanation of the computational basis for each prediction will be provided. In particular, this may include:

- Probabilistic information of the prediction from a statistical model (i.e., probability of being positive),
- How the predictions will be interpreted,
- Model training set information,
- Information on external validation testing and applicability domain of the models.

All the information, from both experimental and *in silico* methods, will be combined together in accordance with the published GIST protocol ([Hasselgren et al., 2019](#)) to obtain a final assessment of the genotoxic/carcinogenic potential of each chemical and the level of confidence linked to the prediction ([Figure 3](#)).

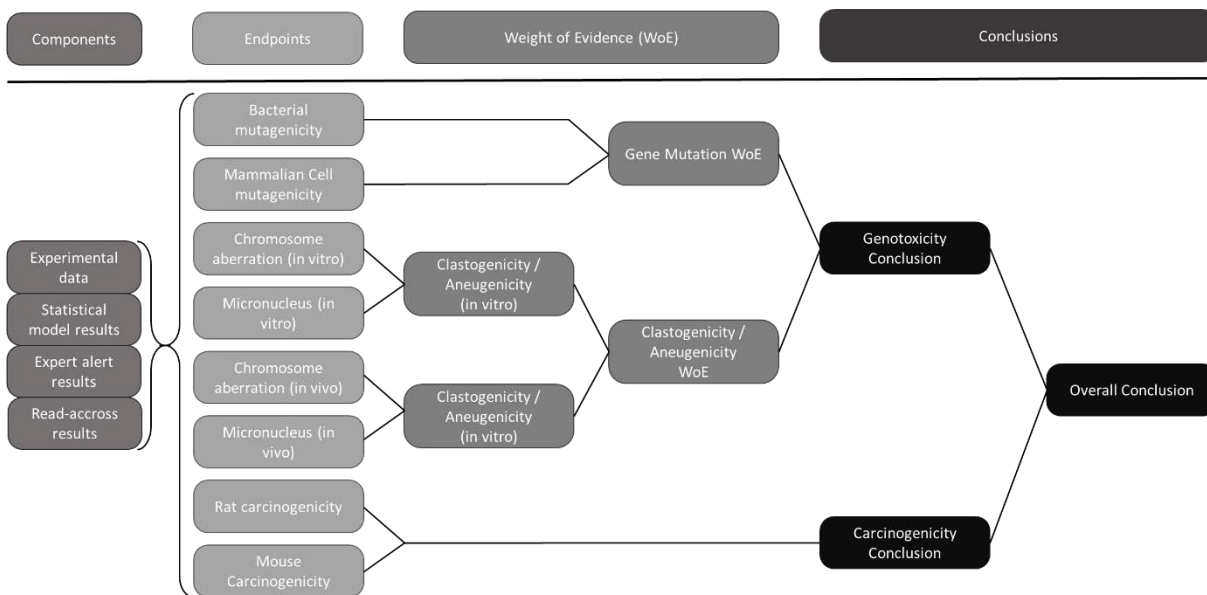


Figure 3: Protocol proposed at PMPSA for hazard identification

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 13 of 27
Computational Toxicology: Methodology	Version 1.0

2.1.3 Model validation

2.1.3.1 Training set

The application of *in silico* tools for hazard identification will involve an expert review of both the models and the predictions. It is important to determine that the models were built according to accepted criteria ([Myatt et al., 2016](#)), and using relevant training datasets. The data set used for endpoint training will dictate what can be predicted. In this context, and to ensure the best possible predictive power, the most contemporary data evaluation criteria will be taken into account.

2.1.3.2 External validation

Best practices for validation of QSAR *in silico* models have been documented ([Myatt et al., 2016](#)), and models built using these best practices will be preferred. The Organization for Economic Co-operation and Development (OECD) has published a series of validation principles for *in silico* models ([OECD, 2004](#); [OECD, 2007](#)) and valid statistical or expert rule-based *in silico* methods. Such QSAR methods have:

- A defined endpoint,
- An unambiguous algorithm,
- A defined domain of applicability,
- Appropriate measures of goodness-of-fit, robustness and predictivity,
- A mechanistic interpretation, if possible.

Any *in silico* model will include documentation that supports an assessment of the model's scientific validity, including the toxicological effect or mechanism being predicted, version number, type of methodology, training set size and content, as well as any predictive performance information. Validation performance is documented in report formats such as the QSAR Model Reporting Format (QMRF) ([Triebe et al., 2017](#)). The level of adherence to the OECD principles and the performance statistics need to be appropriate for the purpose of the assessment.

2.1.3.3 Applicability domain

The applicability domain (AD) represents a region of the chemical space encompassing both the model descriptors and modeled response. It allows the estimate of the prediction uncertainty of the compound on the basis of its similarity to the training compounds involved in the model development. As described in the [Section 2.2](#), the *in silico* prediction will be only accepted when the test compound is sufficiently similar to the training set compounds of the QSAR model, often considering the significance of descriptors ([Netzeva et al., 2005](#); [Carrió et al., 2014](#); [Patlewicz et al., 2016](#)). This AD analysis may be performed automatically by some

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PMSS for MR0000059-MR000061, MR0000133	Page 14 of 27
Computational Toxicology: Methodology	Version 1.0

software to determine whether the training set compounds share similar chemical and/or biological properties with the test chemical.

2.2 Hazard characterization

Human, animal, *in vitro*, *in silico*, and mechanistic data are synthesized to produce toxicity values that are key inputs to risk-based decision making.

For data-rich substances, toxicity values can refer to available exposure thresholds published by regulatory agencies, reported in the literature or even produced in-house. Among them, Inhalation Unit Risk (IUR) values published by the California Office of Environmental Health Hazard Assessment (OEHHA) or the US Environmental Protection Agency (US EPA) are considered to evaluate cancer risk. They will be selected upon availability, whichever is the more conservative. IUR values refer to the increased cancer risk associated with lifetime exposure to 1 µg of substance per m³ air inhaled (EPA, 2005). In the absence of IUR values, the oral slope factor (OSF) from the US EPA or the cancer potency value (CPV) from the OEHHA can be used, depending on availability, to estimate cancer risk from systemic exposure. The CPV is an OEHHA-specific OSF and should not be confused with the cancer potency of the chemical as defined in Section 2.4 of this document. OSF or CPV refer to the increased cancer risk associated with lifetime oral exposure to 1 mg of substance per kg of bodyweight and per day (EPA 2005). Therefore, considering the daily breathed volume (DBV) in m³ and the bodyweight (BW) in kg,

$$IUR = \frac{OSF \times DBV}{BW \times 1000}$$

Bide and co-authors (Bide et al., 2000) reviewed the relationship between BW (kg) and respiratory minute volume (V(m), l.min⁻¹) and determined that $V(m)=0.499 \times BW^{0.809}$. It represents 15.5 l.min⁻¹ for a 70 kg person, which corresponds to 22.3 m³/day. For a 60 kg person, V(m)=13.7 l.min⁻¹, which corresponds to 19.7 m³/day. The European Chemical Agency (ECHA) recommends 20 m³ for DBV as standard values for dose calculations for humans exposed as consumers and via the environment (ECHA, 2012). Therefore, 20 m³ will be used for DBV in this assessment.

For data-poor substances, toxicity values can be derived from available exposure limits of structural analogs and metabolites, or predicted using a compendium of QSAR models. For instance, the Conditional Toxicity Value (CTV) predictor (Wignall et al. 2018) has been used to predict quantitative toxicity values, and the predicted thresholds have been used in a risk assessment process. As recommended by the OECD, the application of validated QSAR models for prediction of new data points requires a well-defined AD (Section 2.1.3.3). For instance, the developers of the CTV predictor mention in their publication (Wignall et al., 2018) that the global domain of applicability (or the chemical diversity represented by the

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 15 of 27
Computational Toxicology: Methodology	Version 1.0

model) was assessed by comparison with the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) data set based on the distribution of pairwise Euclidean distances (descriptors were first standardized so that the range in the data set was 0–1), with the following Z-score cutoffs: $Z < 3$ (less restrictive) or $Z < 1$ (more restrictive). The Z-score, for an entire population, is defined as follows:

$$z = \frac{(x - \mu)}{\sigma}$$

By calculating the Z-score, it allows to determine how far the predicted data point is from the mean and thus how well the predictive model performs. In other terms, the Z-score measures exactly how many standard deviations (σ) above or below the mean (μ) a data point is. In the CTV models, the AD refers to the Z-score and therefore to the number of σ the predicted data point is above (positive AD) or below (negative AD) the mean from the dataset used to build the model. It is also specified that typical AD cutoffs are below 3σ for a less restrictive domain and below 1σ for a more restrictive domain. A negative value indicates the chemical is within the applicability domain. In our assessment, three QSAR models have been chosen in order to derive toxicity thresholds, the CTV OSF, the CTV CPV, and the CTV IUR. For a given chemical,

- Among the 3 predicted values, both the value with the lowest AD and the most conservative value will be considered for further risk assessment, the latest representing therefore a worst case approach whereas the first being scientifically more relevant.
- Predicted values with negative AD will be preferred.
- Any predicted value with an AD value above 3 will be disregarded.
- Any predicted value with a positive AD value below 3 will be considered only if at least 2 from 3 predicted values have an AD value below 3.

2.3 Differential chemical characterization of the aerosol for exposure assessment

2.3.1 Qualitative chemical characterization

During the initial product characterization of THS 2.2, a non-targeted analysis of the THS2.2 aerosol was performed to identify compounds that were either new or increased relative to 3R4F smoke. Two different analytical platforms, GCxGC-TOFMS and LC-HRM-MS, were used, and a set of complementary analytical methods were applied. Aerosols for all test items were generated using the ISO intense smoking regime (ISO, 2018). Non-targeted differential screening (NTDS) using GCxGC-TOFMS and LC-HRM-MS represents a key methodology to not only comprehensively characterize the chemical composition of aerosols derived from

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 16 of 27
Computational Toxicology: Methodology	Version 1.0

different test items, but also to determine significant differences between complex mixtures based on semi-quantitative concentrations.

2.3.2 Quantitative chemical characterization

Semi quantitative analysis was performed on the basis of NTDS spectra. The mean semi-quantitative concentrations of the three variants Regular, Menthol and High Menthol were used as approximate exposure to each aerosol constituent. In total, 80 compounds were found to be new or at higher concentrations in THS 2.2 aerosol compared with the 3R4F cigarette smoke ([Annex 1](#)).

2.3.3 Exposure assessment

The exposure is assessed on the basis of the daily aerosol intake (DAI) and the yield of each characterized chemical compound.

The DAI will be determined on the basis of the daily consumption (DC), as follows:

$$DAI_i = Puff\ volume \times Puff\ number_i \times DC_i$$

i referring to the ith product (THS 2.2 or 3R4F).

2.4 Risk characterization

The cancer risk associated with the consumption of such products can be evaluated by determining cancer potencies and mean lifetime cancer risk. This methodology has been previously used to perform quantitative product risk assessment on tobacco smoke, HnB or electronic cigarette emissions ([Baumung et al., 2016](#); [Lachenmeier et al., 2018](#); [Pack et al., 2018](#); [Slob et al., 2020](#); [Stephens, 2017](#); [Xie et al., 2012](#)).

Cancer potencies of emissions from either THS 2.2 or 3R4F will be determined as described by Stephens ([Stephens, 2017](#)) with slight modifications. Briefly, constituents analyzed in THS 2.2 or 3R4F emissions will be selected according to following criteria:

- Known (IARC 1), probably (IARC 2A) or possibly (IARC 2B) carcinogenic to human, according to the International Agency for Research on Cancer (IARC),
- Compounds eliciting a genotoxic activity (mutagenicity, clastogenicity, aneugenicity) in one of the listed assay ([Table 1](#)),
- Compounds showing structural alert for genotoxicity or carcinogenicity.

Emphasis will be particularly put on substances that are increased in the aerosol of THS 2.2 in comparison to the smoke of 3R4F.

IUR will then be considered for each selected constituent.

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 17 of 27
Computational Toxicology: Methodology	Version 1.0

In case IUR values cannot be found for the chemical of interest, available IUR from structural analogs or metabolites will be used as surrogate to estimate its cancer potency. Cancer potencies will then be translated to mean lifetime cancer risk, considering a DBV of 20 m³.

The calculations will be performed according to the below formula, i referring to the ith product (THS 2.2 or 3R4F) and j to the jth compound:

$$Cancer\ Potency_i = \sum_{j=1}^n IUR_j C_{i,j}$$

$$Lifetime\ Cancer\ Risk_i = \frac{DAI_i}{DBV} \times Cancer\ Potency_i$$

2.5 Reporting

As summarized in [Table 2](#), the study outcome will be reported in the following way:

Cancer potency and lifetime cancer risk will be reported for each group of compounds and segmented according to the quality and the reliability of data. They will then be aggregated altogether to determine the mean lifetime cancer risk. This should allow to gain a better understanding of the contribution of each compound to the overall lifetime cancer risk in relation with the prediction reliability.

The following documents will be provided:

- Computational toxicology study reports from third-parties including:
 - Literature search
 - Read across for poor data compounds
 - QSAR prediction (Leadscope)
 - Expert knowledge-based prediction (Derek Nexus)
 - Reliability and confidence scoring
- Risk assessment report from PMI including:
 - IUR selection
 - Cancer potency determination
 - For each individual compound selected, evaluation of lifetime cancer risk
 - Evaluation of the mean lifetime cancer risk
 - Determination of the potential impact to health

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 18 of 27
Computational Toxicology: Methodology	Version 1.0

Table 2: Report content and accountability

Accountability	Deliveries
Third-parties	Literature search
	Read-across
	QSAR prediction
	Expert knowledge-based prediction
	Reliability scoring
	Confidence scoring
PMI	IUR selection from available databases
	IUR prediction using CTV
	IUR prediction using read-across
	Cancer potency determination
	Lifetime cancer risk for individual compounds
	Mean lifetime cancer risk
	Potential impact to health

2.6 Conclusions

With the development of innovative HnB products, a need to comprehensively assess the toxicological risks associated with this technology arises. Unfortunately, for the vast majority of the chemicals composing their aerosol, there is no, or very limited, toxicological data available. The risk assessment process we developed is not only based on conventional toxicity testing but also on computer-assisted methodologies combining read-across, QSAR, and Expert knowledge-based prediction. We believe that this approach is practical and robust to predict potential cancer risk associated with the use of HnB products.

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 19 of 27
Computational Toxicology: Methodology	Version 1.0

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 20 of 27
Computational Toxicology: Methodology	Version 1.0

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 21 of 27
Computational Toxicology: Methodology	Version 1.0

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 22 of 27
Computational Toxicology: Methodology	Version 1.0

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 23 of 27
Computational Toxicology: Methodology	Version 1.0

ANNEX 1

List of all identified substances, showing their CAS number, chemical name, the maximum concentration measured by GC/MS or LC/MS in any of the THSR, THSM or THSH and the maximum fold increase over the 3R4F calculated in any of the six analysis (THSR, THSM or THSH, using GC/MS or LC/MS).

CAS number	Chemical name	THS 2.2 mean conc. [µg/item]	Fold increase over 3R4F
------------	---------------	------------------------------	-------------------------

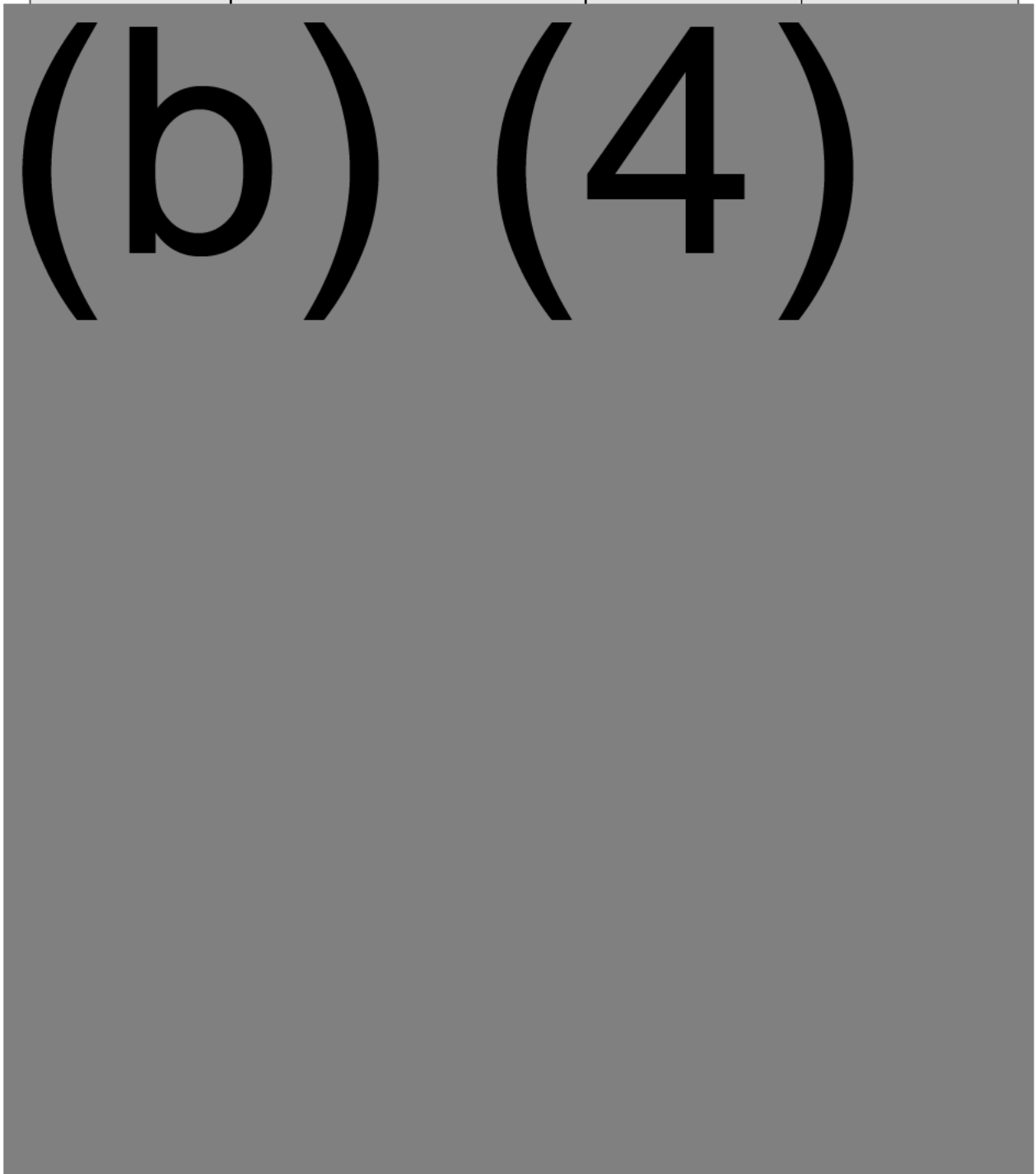
(b) (4)

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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 24 of 27
Computational Toxicology: Methodology	Version 1.0

CAS number	Chemical name	THS 2.2 mean conc. [µg/item]	Fold increase over 3R4F
------------	---------------	------------------------------	-------------------------

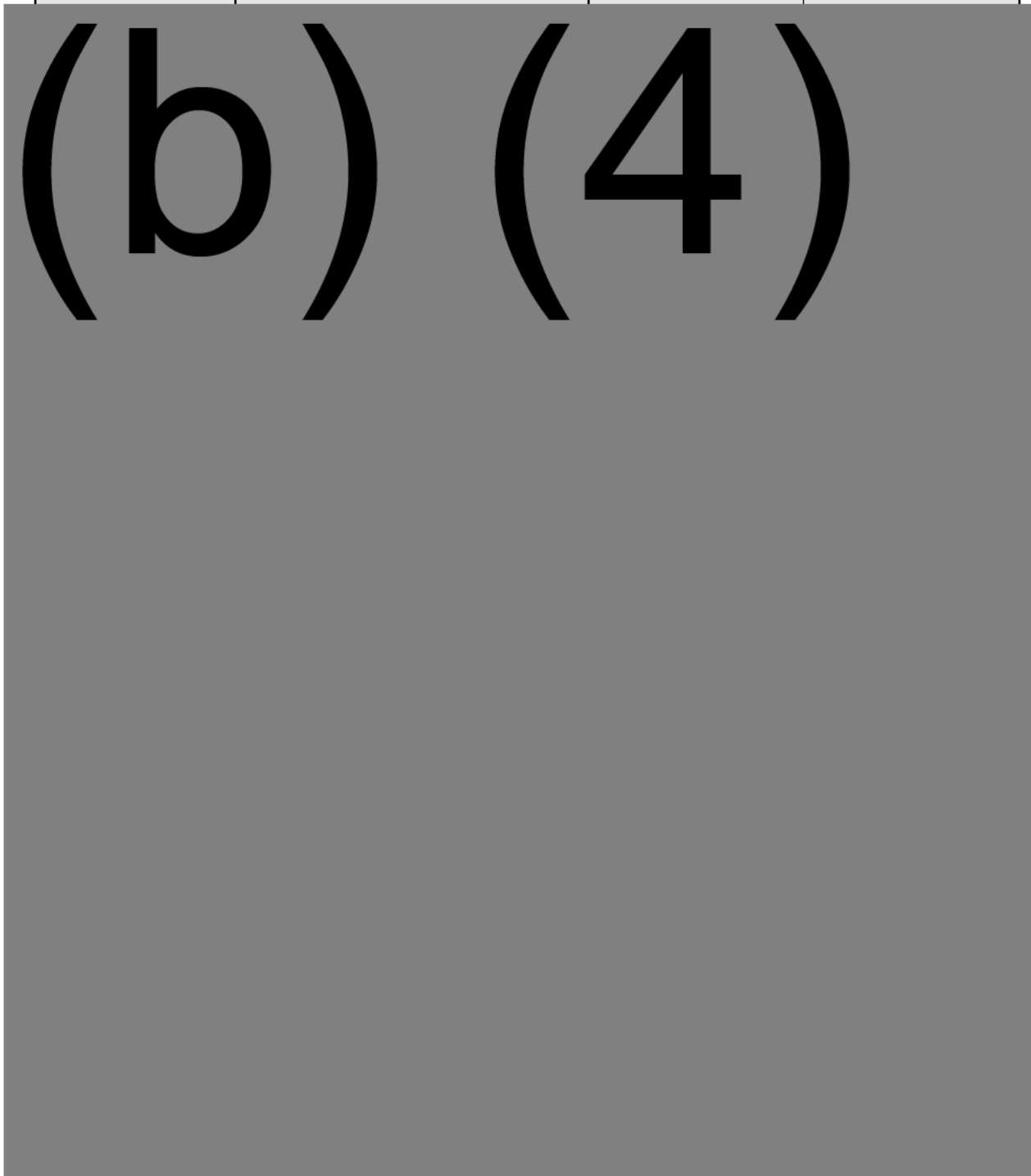


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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 25 of 27
Computational Toxicology: Methodology	Version 1.0

CAS number	Chemical name	THS 2.2 mean conc. [µg/item]	Fold increase over 3R4F
------------	---------------	------------------------------	-------------------------

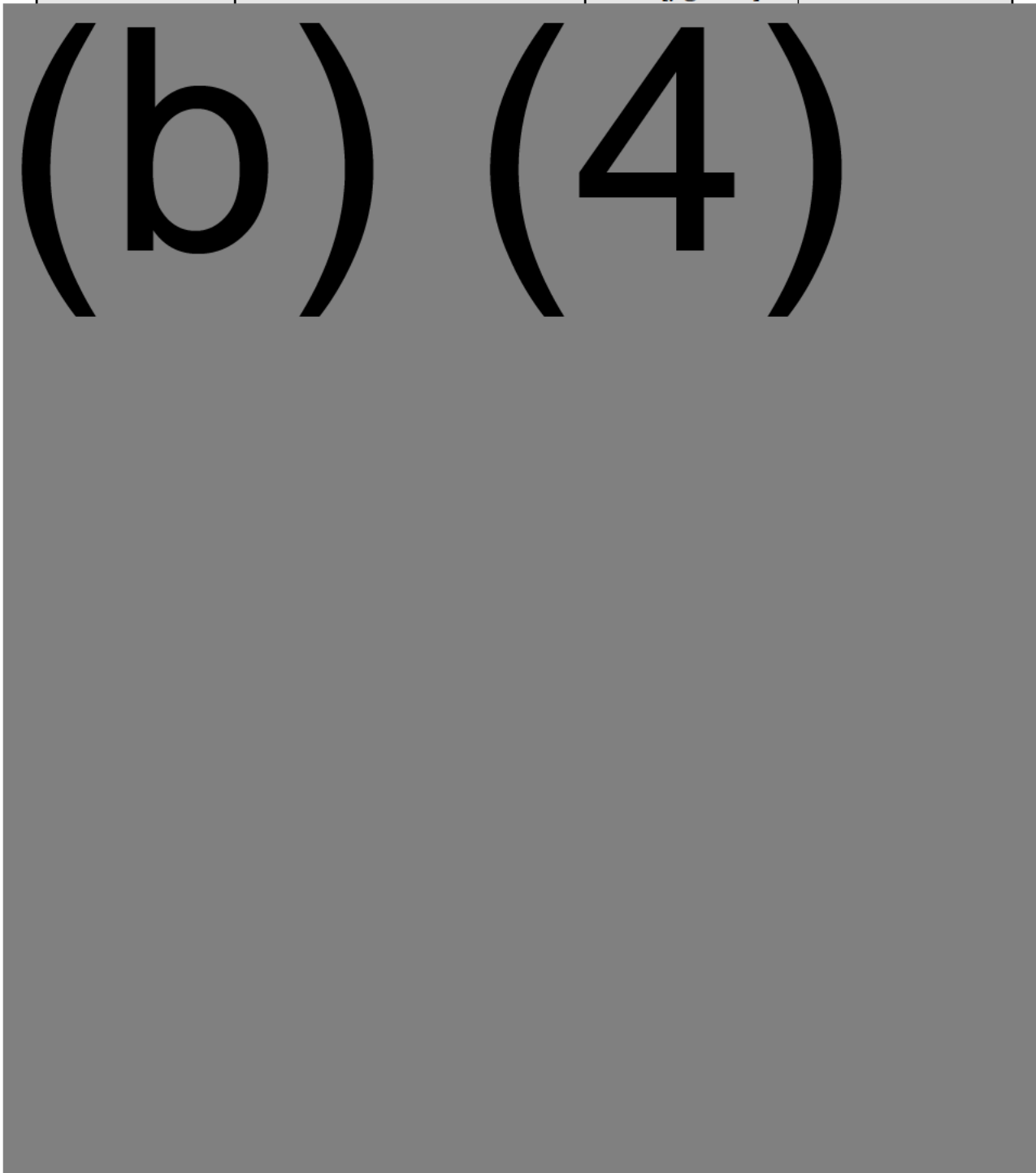


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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 26 of 27
Computational Toxicology: Methodology	Version 1.0

CAS number	Chemical name	THS 2.2 mean conc. [µg/item]	Fold increase over 3R4F
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Philip Morris Products S.A.	Confidential
PMSS for MR0000059-MR000061, MR0000133	Page 27 of 27
Computational Toxicology: Methodology	Version 1.0

CAS number	Chemical name	THS 2.2 mean conc. [µg/item]	Fold increase over 3R4F
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(b) (4)

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