CLH – Proposal for Harmonised Classification and Labelling of MITC for Carcinogenicity

Independent Opinion by [redacted], Toxicologist

Introduction

Metam is currently undergoing both the EU renewal of approval under Regulation (EC) 1107/2009 (PPP Regulation) and the harmonised classification procedure under the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation). The latter to determine, among others, whether Metam should be classified as carcinogenic, mutagenic, or reprotoxic (CMR). The RMS (Belgium) has proposed that MITC, the active metabolite of Metam, should be classified as Carc. 2 (H351). This document is an independent review of the carcinogenicity data on MITC to determine whether there is a justification, or not, that supports the opinion of the RMS.

Carcinogenicity Studies on MITC

There are four carcinogenicity studies on MITC, two where exposure was achieved via the drinking water, one in the mouse and the other in the rat. In the two other studies exposure was achieved via the inhalation route, one in the mouse and the other in the rat. The oral studies, both in mouse and in rat, are considered by all stakeholders not to show evidence for carcinogenic activity, so these will not be discussed further in this document.

A Combined Inhalation Chronic Toxicity and 24-Month Carcinogenicity Study of Methyl Isothiocyanate (MITC) in Rats – Kirkpatrick, 2015a

A whole-body combined chronic toxicity and 24-month carcinogenicity inhalation study was performed in rats using exposure levels of 0, 0.5, 5.0, and 20 ppm. The dRAR identifies this study as 'OECD 451 equivalent to EPA OCPP 870.4300'. In fact, the combined study is defined by OECD 453 not 451, the latter does not include an interim kill at 52 weeks. For neoplastic effects, both the RMS and the applicant agree that the NOAEL or NOAEC is 5 ppm.

The 20 ppm high concentration was considered to exceed the maximum tolerated dose (MTD; based on low mean body weight and cumulative body weight gain), but there were no MITC exposure-related early deaths and no negative effects on survival. The absence of an increased rate of mortality may have been associated with lower final bodyweights (at 20 ppm, -34% relative final bodyweight in males and -19.9% in females) and statistically significantly reduced food consumption. The food consumption data are not available in the dRAR document, but it does state that the food consumption values correlated with the body weight data so logically food consumption was reduced by more than 10%. **The bodyweight gain at termination was depressed by 46% in males and 27% in females**. In this case it is clear that the selected maximum dose level is significantly higher than that recommended in the OECD 451 test guideline, which indicates a maximum depression of body weight gain of approximately 10%.

Neoplastic effects were observed in the high dose group after both 52 and 104 weeks of exposure, but no such effects were observed at 0.5 or 5.0 ppm. However, non-neoplastic effects were observed at 0.5 and 5.0 ppm although the incidence levels were low. The RMS considers that '…*in the larynx, epithelial hyperplasia and squamous metaplasia occurred at meaningfully higher incidences from 0.5 ppm onwards, both in the ♂ and ♀. The incidences were dose-dependent or nearly, and amounted (♂ and ♀ grouped) to about 9/38/58/51% and 1/11/33/100% respectively on the doses of 0/0.5/5/20 ppm*." However, these effects were at lower levels of severity than as seen at 20 ppm and were not observed in all tissues of the respiratory tract. Furthermore, no neoplastic findings were observed at any dose level in the larynx, so the relevance of any dose related effects in the incidence of non-neoplastic findings in the larynx is limited.

In Table B.6.8.1.4.1/02-14 the RMS identifies what it considers to be evidence to justify that the irritant effects of MITC were dose-related and meaningfully higher at 0.5 ppm. For example, the RMS identifies an elevated incidence of hyperplasia in females at all three dose levels (23, 40, 51, 47 respectively), but no such increase was seen in males (47, 44, 59, 44 respectively), which suggests that the level seen in the female control group was exceptionally low. Furthermore, there is no correlation between the incidence of animals showing hyperplasia of the transitional epithelium and the incidence of neoplastic lesions, indicating that these observations contribute little to the question of classification. The same conclusion can be made for the other nasal tissues identified by the RMS as having meaningfully higher incidences; including metaplasia in Nasal level II squamous tissue, which was elevated in males at 0.5 ppm but not in females; hyperplasia in Nasal level III respiratory epithelium in females at 0.5 ppm but not in males, indeed in this case the incidence in the female control group appears to be exceptionally low. The same considerations apply to the other two nasal tissues identified by the RMS, degeneration of level IV olfactory epithelium and hyperplasia of level V respiratory epithelium. Furthermore, as the nasal levels increase from I to VI there is no consistent pattern of non-neoplastic findings at 0.5 ppm nor even at 5.0 ppm. These observations lead to the conclusion that a clear threshold exists for the induction of neoplastic findings and furthermore that there is no clear correlation between the non-neoplastic findings and the neoplastic findings.

An 18-Month Whole-Body Inhalation Carcinogenicity Study of Methyl Isothiocyanate (MITC) in Mice – Kirkpatrick, 2015b

An 18-month carcinogenicity whole-body inhalation study was performed in mice using MITC at exposure levels of 0, 1.0, 5.0, and 15 ppm. Treatment-related effects secondary to respiratory tract irritation were observed in the 5 and 15 ppm groups as evidenced by persistent lower mean body weight and body weight gain and lower food consumption. The 15 ppm high concentration was considered to exceed the maximum tolerated dose (MTD; based on low mean body weight and cumulative body weight gain), but there were no MITC exposure-related early deaths and no negative effects on survival. In fact, there was a positive effect on survival in both sexes, with a statistically significant trend, which was likely associated with lower final bodyweights (at 15 ppm, -21.4% relative mortality in males

and -16.2% in females) and statistically significantly reduced food consumption. The food consumption data are not available in the dRAR document but it does state that the food consumption values correlated with the body weight data so logically food consumption was reduced by more than 10%. **The bodyweight gain at termination was depressed by 69% in males and 49% in females**. In this case it is clear that the selected maximum dose level is significantly higher than that recommended in the OECD 451 test guideline, which indicates a depression of body weight gain of approximately 10%.

The NOEL was 1 ppm for effects outside of the respiratory tract. Since there were no statistically significant neoplastic findings in ♂ or ♀ and a single nasal neoplasm (benign papilloma) was found in only one MITC-exposed animal, there was insufficient evidence to indicate that MITC is carcinogenic in the CD-1 mouse. Although rare, spontaneous nasal neoplasms have been reported in mice. Toxicologically significant local effects were observed clinically in the eyes (opacity in 15 ppm group) and microscopically in the nasal tissues, olfactory bulbs, larynx, trachea and the cornea of the eyes. The NOEL for local effects was 1 ppm based on nasal lesions.

CLP Guidance on Classification for Carcinogenicity

The CLP guidance includes a definition of carcinogen in Annex I: 3.6.11, as follows (emphasis added):

"*Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens* ***unless there is strong evidence that the mechanism of tumour formation is not relevant for humans***." Furthermore, the guidance states that "*Substances are classified according to their potential to cause cancer in humans*."

And

"*Classification of a substance as a carcinogen requires expert judgement and consideration of many different factors (weight and strength of evidence) included in the hazard information on carcinogenicity."*

To determine strength of evidence the guidance on CLP references the definitions of IARC, for experimental animals, the definition of sufficient evidence includes "*sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;*

If we apply this definition to the data on MITC we can determine that the data do not meet the definition because:

- malignant tumours were observed in only one species (rat but not in mice),
- malignant tumours were observed in only one of four independent studies,
- there was no evidence of tumours at multiple sites,
- malignant tumours were only observed at a dose level that clearly exceeded the MTD because bodyweight gain was reduced by 2.7x to 4.6x the maximum level recommended by the OECD 451 test guideline, so it can be said that the study was not well-conducted.

The CLP guidance also includes the IARC definition of limited evidence for carcinogenicity in experimental animals, "*limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs*."

If we apply this definition to the data on MITC we can determine that the data can be said to meet the definition because, e.g.,

- the evidence for carcinogenicity is restricted to a single experiment,
- there are unresolved questions regarding the adequacy of the design, e.g., selection of upper dose level.

However, even limited evidence for carcinogenicity does not automatically lead to a classification for carcinogenicity, additional factors must be taken into consideration. One of the most important of these is the possibility of a confounding effect of excessive toxicity at test doses. It cannot be denied that the two studies that used the inhalation route of exposure included at least one dose level that greatly exceeded the maximum tolerated dose (MTD). The MTD, according to the guidance on CLP, is "…*the highest dose of the test agent during the bioassay that can be predicted not to alter the animal's normal longevity from effects other than carcinogenicity*." The maximum dose level used in both of the inhalation studies clearly impacted the longevity of the animals.

The guidance on CLP continues to say "*Excessive toxicity, for instance toxicity at doses exceeding the MTD, can affect the carcinogenic responses in bioassays. Such toxicity can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development as a secondary consequence unrelated to the intrinsic potential of the substance itself to cause tumours at lower less toxic doses*."

And (emphasis added),

"*Tumours occurring only at* ***excessive doses associated with severe toxicity generally have a more doubtful potential for carcinogenicity in humans****. In addition,* ***tumours occurring***

only at sites of contact *and/or only at excessive doses need to be carefully evaluated for human relevance for carcinogenic hazard*."

In the mouse study, even though the MTD was exceeded by a large margin and marked local irritation to the tissues at the site of contact occurred, no malignancy was induced. In the rat study, the MTD was also exceeded by a large margin and marked local irritation at the site of contact occurred, but in this case malignancy did occur. However, even in the rat, the effects were highly localised to specific levels in the nasal passages, such that malignancy was not observed in all nasal tissues, and irritancy was highly variable along the respiratory airways. This result profile is described by the guidance on CLP as supporting a classification in Category 2 or no classification. So, in this case it is critical to evaluate the weight of evidence to arrive at a conclusion.

Mode of Action and Relevance to Humans

One mode of action that it very important in the evaluation of chemicals for carcinogenicity is that of genotoxicity, because carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action; genotoxic or non-genotoxic. The genotoxicity potential of Metam sodium (the parent compound of MITC) is considered to be negative by the applicant, but in the opinion of the RMS it is positive. As a genetic toxicologist with >30 years of experience my evaluation of the data leads me to a conclusion that overall Metam sodium is not genotoxic. Therefore, the possibility that MITC has a genotoxic mode of action (MoA) for carcinogenicity is highly unlikely, especially as no carcinogenic potential was demonstrated in oral studies in the rat or mouse with MITC and the genotoxicity dataset for MITC includes negative *in vivo* micronucleus and Comet assays. Furthermore, a probable MoA has been described by the applicant that may explain both the equivocal findings for Metam sodium in the genotoxicity studies and the observation of malignant tumours in the rat nasal epithelium for MITC. This MoA involves glutathione depletion caused by excessive exposure to MITC, hypoxia caused by local irritation to the respiratory tissues, and nutrient depletion as a result of reduced food consumption. The possible MoA reduces the relevance of the carcinogenicity studies to man because it can hardly be considered likely that exposure to MITC could cause such effects in humans.

Meaningful Increases

It should be noted that the RMS introduces in their review a qualitative evaluation of quantitative data, that is not clearly objective or scientific. There are many examples in the dRAR where the RMS indicates that effects or incidences were "meaningfully higher", but without any definition of what is meant by the word meaningful. This terminology has been applied by the RMS whenever there were incidences of findings that were not statistically significant, either as an individual value or as part of a trend analysis but appeared subjectively as if they followed a trend or were simply higher than the control value. Furthermore, often the HCD data were ignored, and 'meaningful' values were in fact well within the range of the HCD. In other cases, low concurrent control values (e.g., control values below the HCD) were ignored as a probable explanation for the 'meaningful' increases. This could be considered to be a rather unscientific approach to the evaluation of scientific data.

Conclusion

The evaluation of the scientific data for the carcinogenicity of MITC shows that MITC can include malignant tumours in the nasal and laryngeal tissues of rats following exposure via the inhalation route. However, no similar effect is induced in the tissues of mice under the same exposure regime, nor does MITC induce tumours in any tissue following oral exposure in rats or mice. The tumours observed in the nasal and laryngeal tissues of rats are clearly linked to the marked local irritant properties of MITC and a clearly defined threshold was observed. In addition, the dose level where tumours were induced (20 ppm) exceeded the MTD by a significant margin, and therefore the validity and relevance of the data at 20 ppm in the rat is clearly compromised and therefore should add less weight to the weight of evidence argument for classification. Any scientific evaluation of the data should follow the classic accepted rules of the use of statistics and HCD to judge the significance of findings. Vague and undefined terminology such as 'meaningfully increased' to describe findings should be avoided because it ascribes a weight or value to data that may not be deserved.

In conclusion, a reasonable and balanced weight of evidence evaluation of the data available for the carcinogenicity potential of MITC, that follows the advice of the CLP guidance document, should arrive at a conclusion that the effects observed in the single study in the rat were secondary to local irritancy. There is insufficient evidence to identify a carcinogenic mode of action, one that may be distinct to the known and published secondary modes of action related to toxicity. Furthermore, MITC has been shown to be not genotoxic *in vivo*. On this basis, it may be concluded with confidence that there is no justification to propose a classification of Carc. 2, H351 for MITC.

References

Kirkpatrick, D. T. (2015a) A Combined Inhalation Chronic Toxicity and 24-Month Carcinogenicity Study of Methyl Isothiocyanate (MITC) in Rats

Kirkpatrick, D.T. (2015b) An 18-Month Whole-Body Inhalation Carcinogenicity Study of Methyl Isothiocyanate (MITC) in Mice

ECHA Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0, July 2017

Note. [redacted] is an independent toxicologist working for a regulatory consultancy company. The time expended reviewing the relevant documents and preparing this opinion were compensated by the applicant via EPPA. However, the opinion expressed in this document is mine alone and is based on my toxicological experience and knowledge. The applicant and EPPA had no influence on the expression of my opinion.