JTI comment on the RIVM proposal for a new CLP classification of nicotine

According to Regulation (EC) No 1272/2008, (CLP Regulation) Annex VI, nicotine is classified as Toxic if swallowed (Acute Toxicity Category 3), Fatal in contact with skin (Acute Toxicity Category 1) and Toxic to aquatic life with long lasting effects (Aquatic Chronic 2).

Recently this classification was challenged and a new proposal for Harmonized Classification and Labelling was submitted to European Chemicals Agency (ECHA) by the Dutch National Institute for Public Health and the Environment (RIVM). In their proposal, the RIVM suggested to change acute oral toxicity Category 3 to acute oral toxicity Category 1 (Fatal if swallowed) and to add an additional classification, acute inhalation toxicity Category 2 (Fatal if inhaled).

Acute oral toxicity of nicotine

The proposed classification for acute oral toxicity was based on the lowest oral LD_{50} value (3.34 mg/kg bw in mice) found in the literature, a study published in 1969 that evaluated the toxicity of nicotine sulfate in mice and rats (Lazutka FA *et al*., 1969).

This study was not performed according to OECD guidelines and before the introduction of Good Laboratory Practice (GLP). It has a limited description of the experimental design and fails to provide information on animal strains, sex, age or number of animals per test group. Moreover, authors reported the LD_{50} values for both nicotine base and nicotine sulfate, however, only administration and applied doses of nicotine sulfate were described and no information was available for nicotine base. Applying the criteria documented by Klimisch H-J *et al.*, (1997) that are used to evaluate the inherent quality of scientific publications, results in a Klimisch score 3, indicating that the study from Lazutka FA *et al.,* is non-reliable (Klimisch scores rank from 1 (most reliable) to 4 (least-reliable)).

To support the relevance of the Lazutka FA *et al.,* (1969) study for the re-classification of nicotine, the RIVM also argued that mice are more appropriate for studying nicotine toxicity than rats. This rationale was primarily based on the high similarity of the human and the mouse Cytochrome P450 enzymes that are the principle enzymes in nicotine metabolism (CYP2A6 in the human and

CYP2A5 in the mouse). In contrast, the enzyme responsible for nicotine metabolism in rats is a member of the CYP2B family (Mwenifumbo JC & Tyndele RF, 2009). Although the Cytochrome P450 enzyme differs between mice and rats, the nicotine half-life in rats is 45-66 min (Kyerematen GA *et al*., 1988), which more closely resembles that of humans (120 min) (Benowitz N *et al.,* 1982, 2009). This is in contrast to the half-life documented for mice, 6-9 minutes (Peterson DR *et al.,* 1984; Siu EC &Tyndale RF, 2007) and as such, in terms of systemically available nicotine, the rat model is more relevant.

In support of this conclusion, and in contrast to Lazutka F A *et al*., (1969) more recent studies have demonstrated that mice are less sensitive to the acute effects of nicotine than rats, and therefore, needed a higher nicotine dose to achieve similar physiological responses e.g., the effective dose required to produce seizures in 50% (ED_{50}) of rats was 0.5 -1.0 mg/kg, while for mice, it was 2-6 mg/kg depending on strain (de Fiebre NC *et al.,* 2002; Miner LL and Collins AC, 1989).

A more relevant study for the classification of nicotine acute oral toxicity would be the study published by van den Heuvel *et al.,* (1990), which was conducted according to OECD Guideline 401. The LD_{50} was determined to be 68 mg/kg bw for male and 71 mg/kg bw for female rats, reconfirming the Category 3 classification (Toxic if swallowed). The publication specifies that the study was supported by the Commission of the European Communities and the UK Government, and was conducted under the patronage of the OECD. Additionally, another study (Yam J *et al.,* 1991) using the up-and-down method for acute oral toxicity testing was conducted according to OECD Guideline 425. The LD_{50} of nicotine was determined to be 70 mg/kg bw in females, which are reported to be equal or more sensitive than males. This result is in accordance with the LD_{50} determined with the classical LD_{50} method conducted by van den Heuvel *et al.,* (1990). Both of these studies, when evaluated, would be Klimisch score 2, indicating that they are reliable with restrictions.

In conclusion, the overall evidence supports the validity, reliability and relevance of the rat oral toxicity data. In contrast, the RIVM selected key study for the re-classification of nicotine is of limited reliability and relevance and may be further discounted by more recent human data (Bartschat S *et al.,* 2014; Eberlein CK *et al.,* 2014; Schipper EM *et al.,* 2014), which do not support the re-classification of nicotine as more harmful. Indeed, Mayer B (2014) concluded that the potential fatalities caused by the ingestion of small amounts of tobacco products or diluted nicotine-containing solutions are unjustified and should be revised in light of overwhelming data indicating that more than 0.5 g of oral nicotine is required for lethal nicotine intoxications in adult humans.

Acute inhalation toxicity of nicotine

With regard to acute inhalation toxicity of nicotine, the RIVM referred to a study published by Shao XM *et al*., (2012), which is well documented and conducted according to Environmental Protection Agency (EPA) Test Guidelines (EPA, 1998, 2002). An evaluation of this study results in a Klimisch score 2, indicating that it is reliable with restrictions. Unfortunately, as the exposure period was only 20 minutes, an extrapolation of the results was needed to convert the exposure to the standardized 4-hour period required under the CLP Regulation. The extrapolation resulted in an LC_{50} of 0.58 mg/L, indicating acute inhalation toxicity Category 2. It should be noted that there are guidance documents that would question the validity of extrapolations for exposure periods of less than 30 minutes (ECHA, 2014).

Ultimately, these results and final hazard classification could be misleading in light of the study from Syversen U *et al*., (1999). This publication documented the results of a 2-year rat inhalation study (exposure to nicotine 20 hours a day 5 days a week).The study utilized sixty eight Sprague Dawley rats (the same strain as those used by Shao XM *et al*., 2012). Exposure in this study, resulted in sustained plasma nicotine concentrations above 100 ng/ml, which exceeds those reported in the Shao XM *et al.,* (2012) publication (< 45 ng/ml). In fact, exposure concentration in the Syversen U *et al*., (1999) study was chosen to be 'without an effect on the well-being of the rats'.

Conclusion

JTI disagrees with the proposal of the RIVM to re-classify the acute oral and inhalation toxicity of nicotine. The key study that was selected by the RIVM for the reclassification of nicotine by the oral route (Lazutka FA *et al.,* 1969) should not be relied on due to the lack of quality, reliability and relevance as evaluated by Klimisch scoring. Furthermore and as explained above, it is our view that rats are the more relevant species for the determination of nicotine toxicity in humans than mice.

With regard to acute inhalation toxicity of nicotine, it is our view that a re-classification is not supported by the rationale suggested by the RIVM proposal due to the lack of validity of the data extrapolation, as well as other scientific evidence showing that rats can tolerate higher plasma nicotine levels without lethal effect.

In conclusion, JTI is of the opinion that the current CLP classification of nicotine is appropriate and that scientific evidence does not support its re-classification as suggested by the RIVM.

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