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# EU Classification of nicotine mixtures under CLP Regulation 1272/2008 (as amended and corrected)

## Bibra Proposal

30 June 2014

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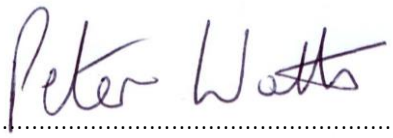
Signature: 

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# EU classification of nicotine mixtures under CLP Regulation 1272/2008 (as amended and corrected)

## Bibra Proposal

## INTRODUCTION

Bibra was asked for independent advice on the appropriate EU classification of mixtures containing nicotine, for acute toxicity by the oral and dermal exposure routes. The client asked that the classification be carried out according to current EU legislation as laid down in EU Regulation 1272/2008, as amended. In particular, the client asked about the concentration-related category transitions for nicotine mixtures (where the other components were not acutely toxic).

## KEY LEGISLATIVE REFERENCES

The overarching EU regulation for classification of substances and mixtures is EU Regulation 1272/2008[1]. Tables 3.1 and 3.2 of Annex VI of 1272/2008 set out the official EU classifications for numerous substances. This Regulation has been amended by five Adaptations to Technical Progress (Regulations EC 790/2009[2], EU 286/2011[3], EU 618/2012[4], EU 487/2013[5] and EU 944/2013[6]). A correction to Annex VI has also been published (Regulation EU 758/2013[7]). A consolidated version available on the ECHA website[8] takes into account 790/2009 and 286/2011, but not the third, fourth and fifth adaptations, or 758/2013.

[1] Regulation 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation 1907/2006. Official Journal of the European Union L353, 1-1355 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:en:PDF.

[2] Commission Regulation (EC) 790/2009 of 10 August 2009 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:235:0001:0439:en:PDF

[3] Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (Text with EEA relevance). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:083:0001:0053:en:PDF

[4] Commission Regulation (EU) No 618/2012 of 10 July 2012 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (Text with EEA relevance). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:179:0003:0010:EN:PDF

[5] Commission Regulation (EU) No 487/2013 of 8 May 2013 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (Text with EEA relevance). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:149:0001:0059:EN:PDF

[6] Commission Regulation (EU) No 944/2013 of 2 October 2013 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (Text with EEA relevance). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:261:0005:0022:EN:PDF#!

[7] Commission Regulation (EU) No 758/2013 of 7 August 2013 correcting Annex VI to Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (Text with EEA relevance). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:216:0001:0058:EN:PDF

[8] Consolidated version: Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance) as amended by Regulations EC 790/2009 and EU 286/2011. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2008R1272:20110419:EN:PDF

# HARMONISED ACUTE TOXICITY CLASSIFICATION OF NICOTINE (SUBSTANCE)

## *Acute oral toxicity*

EU experts on classification have reviewed the acute oral toxicity data on nicotine. Although the specific data that were reviewed are unknown to bibra, the experts agreed a classification as: Toxic if swallowed (T; R25). This implies a rat acute oral LD50 of between 25 and 200 mg/kg bw.

Under 1272/2008, this 67/548/EEC classification has been translated to its modern equivalent, which is: Toxic if swallowed. Acute Toxicity Category 3 (H301). This classification implies a rat acute oral LD50 value of between 50 and 300 mg/kg bw (i.e. slightly modified from 67/548/EEC criteria). Generically, this Category is assigned a "converted acute toxicity point estimate" (ATE) of 100 mg/kg bw (for use in the calculation of the ATE for classification of a mixture based on its components).

## *Acute dermal toxicity*

EU experts on classification have reviewed the acute dermal toxicity data on nicotine. Although the specific data that were reviewed are unknown to bibra, the experts agreed a classification as: Very toxic in contact with skin (T+; R27). This implies a rat or rabbit acute dermal LD50 of <50 mg/kg bw (24-hr contact time).

Under 1272/2008, this 67/548/EEC classification has been translated to its modern equivalent, which is: Fatal in contact with skin. Acute Toxicity Category 1 (H310). This classification implies a rat acute dermal LD50 value of 0-50 mg/kg bw (i.e. unchanged from 67/548/EEC criteria). Generically, this Category is assigned a "converted acute toxicity point estimate" (ATE) of 0.5 mg/kg bw (for used in the calculation of the ATE for classification of a mixture based on its components).

# ACUTE ORAL AND DERMAL TOXICITY VALUES FOR NICOTINE

## *Summary of acute oral lethal values*

In classification for acute toxicity, laboratory animal data (notably rat LD50s) are generally critical. For nicotine, reported rat oral LD50 values range from 50-188 mg/kg bw, with most between 50-83 mg/kg bw (DECOS, 2004; Gaines, 1960; Lazutka et al. 1969; Sine, 1993; Trochimowicz et al. 1994; Vernot et al. 1977; Yam et al. 1991). Mice may be slightly more sensitive, with most reported values lying between 16-60 mg/kg bw (DECOS, 2004; Trochimowicz et al. 1994; Vernot et al. 1977). A lower LD50 value (3.3 mg/kg bw) was reported in an early Eastern European study (Lazutka et al. 1969) of uncertain reliability.

[Reviews have reported estimated mean lethal acute oral doses in children and adults of about 10 mg (about 0.5 mg/kg bw) and about 30-60 mg (about 0.4-0.9 mg/kg bw), respectively (Arena, 1974; Gosselin, 1988; Lazutka et al. 1969). However, the scientific validity of these figures is unclear, and they do not seem to have played any role in the nicotine-classification deliberations of the EU expert group on harmonised classification.]

## *Summary of acute dermal lethal values*

In rats, acute dermal LD50 values of 140-285 mg/kg bw have been reported (Gaines, 1960; Trochimowicz et al. 1994), with rabbits (LD50 50 mg/kg bw) seemingly more sensitive (Trochimowicz

et al. 1994). In cats, doses of about 66-100 mg/kg bw caused clinical toxicity (vomiting, CNS effects and deaths (Travell, 1960).

## *Tabulated acute oral lethal studies*

| Species, Sex, Number | Brief study description (if available) | LD50 | Reference |
|---|---|---|---|
| Mouse, strain, sex and number not specified | LD50 study using nicotine base | 3.3 mg/kg bw | Lazutka et al. 1969 |
| Mouse, CF-1, male, number not specified | LD50 study using nicotine sulphate | 16 mg/kg bw | Vernot et al. 1977 |
| Mouse, strain, sex and number not specified | LD50 study | 24 mg/kg bw | DECOS, 2004 (cited as Ray91); Trochimowicz et al. 1994 |
| Mouse, strain, sex and number not specified | LD50 study | 50-60 mg/kg bw | Trochimowicz et al. 1994 |
| Rat, strain, sex and number not specified | LD50 study | 50 mg/kg bw | Sine, 1993 |
| Rat, strain, sex and number not specified | LD50 study | 50-60 mg/kg bw | Trochimowicz et al. 1994 |
| Rat, strain, sex and number not specified | LD50 study using nicotine base | 53 mg/kg bw | Lazutka et al. 1969 |
| Rat, Sprague-Dawley, male and female | LD50 estimated by fixed-dose procedure or the up-and-down method. In the fixed-dose procedure, groups of 5 males and 5 females were treated with one of four predetermined dose levels. In the up-and-down method, females were dosed, one at a time, starting with an estimate of the LD50 and adjusting the dose until 4 rats were treated. In both protocols, rats were observed for 14 days | 70-71 mg/kg bw | Yam et al. 1991 |
| Rat, Sprague- | LD50 study using nicotine | 75 mg/kg bw | Vernot et al. 1977 |

| Species, Sex, Number | Brief study description (if available) | LD50 | Reference |
|---|---|---|---|
| Dawley, male, number not specified | sulphate | | |
| Rat, Sherman, adult, female, 80/group | LD50 study using nicotine sulphate, rats observed for 4 days only | 83 mg/kg bw | Gaines, 1960 |
| Rat, strain, sex and number not specified | LD50 study | 188 mg/kg bw | DECOS, 2004 (cited as Ray91). |

## *Tabulated acute dermal lethal studies*

| Species, Sex, Number | Brief study description (if available) | LD50 | Reference |
|---|---|---|---|
| Rat, strain, sex and number not specified | LD50 study | 140 mg/kg bw | Trochimowicz et al. 1994 |
| Rat, Sherman, adult, female, 70/group | LD50 study on nicotine sulphate<br><br>[Note: rats were only observed for 5 days] | 285 mg/kg bw | Gaines, 1960 |
| Rat, Sprague-Dawley, 5 male and 5 female | A mixture of 18% nicotine and 82% of an ion-exchange resin applied at 2 g/kg bw to the covered skin for 24 hr, followed by rinsing with water<br><br>OECD Guideline study No. 402 | >360 mg/kg bw<br><br>[no deaths were seen] | Guerriero et al. 2001 |
| Rabbit, strain, sex and number not specified | LD50 study | 50 mg/kg bw | Trochimowicz et al. 1994 |
| Rabbit, strain, sex and number not specified | LD50 study | 140 mg/kg bw | UK PSD, 2008 |
| Cat, 21/group, sex not specified | Application of 200 mg nicotine or nicotine sulphate (providing approximately 66-100 mg nicotine/kg bw) to the uncovered skin. | The nicotine base produced overt CNS toxicity, vomiting, and 17/21 cats died in 21-195 | Travell, 1960 |

| Species, Sex, Number | Brief study description (if available) | LD50 | Reference |
|---|---|---|---|
| | | min.<br><br>The sulphate caused milder effects and all 21 cats survived. | |
| Cat, 5 treated with free nicotine and 3 treated with nicotine sulphate, sex not specified | 2-10 ml "Nico-Fume Liquid" (containing 40% free nicotine) or 10 ml "Black Leaf 40" (containing 40% nicotine sulphate) was applied under cover to the clipped skin. In the free nicotine experiment, the skin of one cat was washed after 3 hours.<br><br>[Travell (1960) stated that the free nicotine doses causing death were 280-1500 mg/kg bw, and the nicotine sulphate dose was about 1100 mg/kg bw.] | Nicotine caused CNS effects and vomiting, loss of consciousness and death.<br><br>No effects were reported with the sulphate. | Faulkner, 1933 |

## SELECTION OF KEY LD50 VALUES FOR MIXTURE CLASSIFICATION

When multiple options are available for a rather simple and crude endpoint such as median lethality, selection of the most appropriate value for use in classification can be challenging.

According to Regulation 1272/2008 "The preferred test species for evaluation of acute toxicity by the oral and inhalation routes is the rat, while the rat or rabbit are preferred for evaluation of acute dermal toxicity". The original harmonised expert classification (under 67/548/EEC) for acute oral toxicity (Toxic if swallowed; T; R25) implies that the committee selected an acute oral LD50 of between 25 and 200 mg/kg bw as being key to classification. This indicates that the experts either dismissed or were unaware of three of the mouse studies. Under 1272/2008, the earlier 67/548/EEC classification has been translated to its modern equivalent (Toxic if swallowed; Acute Toxicity Category 3. H301), which is associated with an acute oral LD50 between 50-300 mg/kg bw. Without a detailed assessment of each LD50, it is not entirely clear which reports should be set aside. Nevertheless, the fact that all of the rat LD50 figures are 50 mg/kg bw or above supports the experts' choice of Category 3.

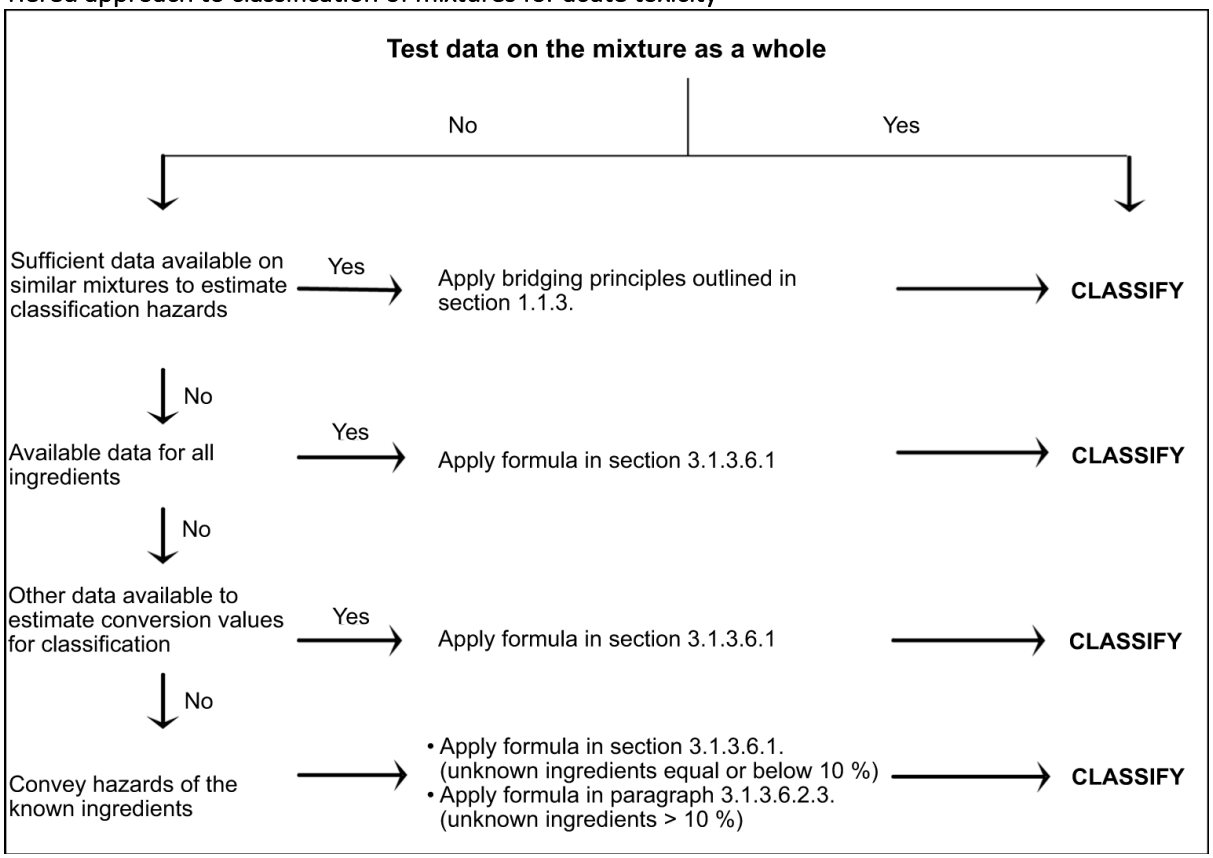
For the dermal classification, there seems to be a good case for the selection of the rabbit dermal LD50 of 50 mg/kg bw and a precautionary choice of assigning to the more toxic class (Category 1) when a value falls on the class boundary.

Rat oral LD50: >50 mg/kg bw.
Rabbit dermal LD50: 50 mg/kg bw.

## CLASSIFICATION OF NICOTINE MIXTURES

Mixtures should be classified in line with EC 1272/2008 (as amended). Guidance is given in section 3.1.3. ***Criteria for classification of mixtures as acutely toxic.*** This states that "*For mixtures, it is necessary to obtain or derive information that allows the criteria to be applied to the mixture for the purpose of classification*." Such information would include LD50 or ATE figures, for example. The approach to classification for acute toxicity is tiered, and is dependent upon the amount of information available for the mixture itself and for its ingredients.
A flow chart (Figure 3.1.1 in 1272/2008) outlines the process to be followed.

Tiered approach to classification of mixtures for acute toxicity




In this instance, "Test data on the mixture as a whole" are not available, nor are there "Sufficient data available on similar mixtures". However, there are "Available data for all ingredients", allowing classification by applying the formula in section 3.1.3.6.1.

Section 3.1.3.6. ***Classification of mixtures based on ingredients of the mixture (Additivity formula)*** provides guidance on such classification.

"3.1.3.6.1. Data available for all ingredients
In order to ensure that classification of the mixture is accurate, and that the calculation need only be performed once for all systems, sectors, and categories, the acute toxicity estimate (ATE) of ingredients shall be considered as follows:
(a) include ingredients with a known acute toxicity, which fall into any of the acute toxicity categories shown in Table 3.1.1;
(b) ignore ingredients that are presumed not acutely toxic (e.g., water, sugar);
(c) ignore ingredients if the oral limit test does not show acute toxicity at 2000 mg/kg bodyweight.

Ingredients that fall within the scope of this paragraph are considered to be ingredients with a known acute toxicity estimate (ATE).

The ATE of the mixture is determined by calculation from the ATE values for all relevant ingredients according to the following formula for Oral, Dermal or Inhalation Toxicity:

$(100/ATEmix) = \Sigma n\ (Ci/ATEi)$

where:
Ci = concentration of ingredient i (% w/w or % v/v)
i = the individual ingredient from 1 to n
n = the number of ingredients
ATEi = Acute Toxicity Estimate of ingredient i."

In the current exercise, bibra was told to assume that the non-nicotine ingredients of the mixtures are not acutely toxic, and nicotine is the only ingredient with a known acute toxicity.

## *Acute oral classification*

The boundary range for Categories 3 and 4 are 50-300 and 500-2000 mg/kg bw, respectively. This means that mixtures containing nicotine can be classified as follows:

| Nicotine concentration (%) | Estimated oral LD50 (mg/kg bw) | CLP Category |
|---|---|---|
| 100 | >50 | 3 |
| 16.6-100 | 50-300 | 3 |
| 2.5-<16.6 | 300-2000 | 4 |
| <2.5 | >2000 | Not classified |

## *Acute dermal classification*

The boundary range for Categories 1, 2, 3 and 4 are <50, 50-200, 200-1000 and 1000-2000 mg/kg bw, respectively. This means that mixtures containing nicotine can be classified as follows:

| Nicotine concentration (%) | Estimated dermal LD50 (mg/kg bw) | CLP Category |
|---|---|---|
| 100 | 50 | 1 |
| 25-100 | 50-200 | 2 |
| 5-<25 | 200-1000 | 3 |
| 2.5-<5 | >1000-2000 | 4 |
| <2.5 | >2000 | Not classified |

## NOTE

This bibra proposal focuses on the classification of mixtures, accepting the literature LD50 figures and the existing classification views of the harmonised experts. It did not attempt to critically evaluate the reliability of the actual LD50 figures. It is possible that a critical evaluation of the existing LD50 literature might lead to a more confident identification of the best LD50 figures to use in substance and mixture classification.

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