The Director General

Maisons-Alfort, 22 January 2015

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on assessing the hazards of nicotine

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 22 January 2015 shall prevail.

On 28 May 2014, ANSES received a formal request from the French Directorates General for Competition, Consumer Affairs and Fraud Control; Labour; Health; and Risk Prevention, to conduct an expert appraisal on assessing the hazards of nicotine.

1. BACKGROUND AND PURPOSE OF THE REQUEST

The requirements for classification, labelling and packaging of liquid refills for electronic cigarettes are deduced from the acute toxicity values for nicotine (lethal dose 50 (LD_{50}) by the dermal route and LD_{50} by the oral route). Indeed, Section 3.1 of Annex I of Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, known as the "CLP Regulation", states that the acute toxicity estimate for the classification of a mixture is derived using:

- the LD_{50} or LC_{50} where available;
- the appropriate conversion value from Table 3.1.2 that relates to the results of a range test, or
- the appropriate conversion value from Table 3.1.2 that relates to a classification category.

Nicotine has a harmonised classification in Table 3.1 of Annex VI of the CLP Regulation as Acute tox. 1: H310 "Fatal in contact with skin", Acute tox. 3 (*): H301 "Toxic if swallowed", and Aquatic Chronic 2: H411 "Toxic to aquatic life with long-lasting effects". This current classification was derived from the former classification (Directive 67/548/EEC) using conversion values. Therefore, the harmonised classification for acute oral toxicity is indicated in this entry by the reference (*) in

the "Classification" column of Table 3.1, and must be regarded as a minimum classification (CLP, Annex VI, 1.2.1).

Therefore, up to now, the classification of liquid refills for electronic cigarettes has been performed using the appropriate conversion value, drawn from Table 3.1.2, that relates to a classification category. However, the CLP Regulation states that the classification of a mixture must be derived from the LD_{50} if these data are available. As the use of different classification methods makes it impossible to arrive at identical classifications in the case of liquid refills for electronic cigarettes, the classification of mixtures should be performed using the method preferred by the CLP Regulation.

ANSES's expert appraisal is in particular needed for the purposes of:

- conducting a review of the existing literature concerning acute toxicity data on nicotine for the dermal route and the oral route,
- identifying the most relevant LD_{50} value for nicotine by the oral route and by the dermal route,
- issuing an opinion on the advisability of submitting an application for revision of the harmonised classification of nicotine in accordance with Article 37 of the CLP Regulation.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 “Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)”.

The collective expert appraisal was undertaken by the Expert Committee (CES) on "Characterisation of substance hazards and toxicity reference values", on the basis of an initial report. Expert rapporteurs from the CES on "Characterisation of substance hazards and toxicity reference values" and the CES on "Assessment of chemical risks of consumer items and products" were appointed to investigate this matter. The methodological and scientific aspects of the work were presented to the CES on "Characterisation of substance hazards and toxicity reference values" on 10 July, 19 September and 9 October 2014. The work was adopted by the CES at its meeting on 9 October 2014.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.
The experts’ declarations of interests are made public via the ANSES website (www.anses.fr).
A conflict of interests was identified within the framework of this formal request. Accordingly, the expert concerned was asked to leave the meeting when this item was addressed, and therefore did not take part in the debates and decisions.

A literature review was carried out in order to obtain all available data relating to the LD_{50} for nicotine (consultation of international and national databases, summary reports, scientific publications). Additional data were also sought from the French competent authorities in the fields of plant protection products and medicinal products.
Concerning exposure data in humans, the reference standards and resources used by the poison control centres in the context of their emergency telephone hotline work were consulted.

3. ANALYSIS AND CONCLUSIONS OF THE CES

Oral route

The results available in animals on the acute toxicity of nicotine differ very widely. The eight LD_{50} values by the oral route range from 3.34 mg/kg to 188 mg/kg depending on the study and the species considered. Seven LD_{50} values out of eight came from studies conducted in rodents (rats and mice), the recommended species according to the current OECD guidelines.

These studies were performed on three different species: rats, mice and dogs, and are summarised in the table below.

Method	LD_{50} (mg/kg)	Remarks	Reference
Oral, mice	3.34	Studies did not conform with the current OECD guidelines	Lazutka *et al.*, 1969
Oral, mice	24		Heubner, 1938
Oral, rats	52.5		Lazutka *et al.*, 1969
Oral, rats	50-60	Source study not available	Negherbon, 1959
Oral, rats	188	Studies did not conform with the current OECD guidelines	Ambrose *et al.*, 1946
Oral, rats	70 (males and females) 71 (females)	OECD 1981	Van den Heuvel *et al.*, 1990
Oral, rats	70 (females)	Up and Down procedure, ASTM (1987)	Yam, 1991 *et al.*, Lipnick *et al.*, 1995
Oral, dogs	9.2	Study did not conform with the current OECD guidelines	Franke *et al.*, 1932

Only two of these studies refer to the guidelines, or to validated experimental protocols. They were the study by Van den Heuvel *et al.* (1990), which complies with the 1981 OECD guidelines that have now been replaced, and the one by Yam *et al.* (1991), which conforms to the "Up and Down" procedure today advocated by the OECD (OECD, 2008) and the ASTM (1987). These studies, conducted in rats, both led to an LD_{50} of 70 mg/kg.

Apart from these two studies, the publications available on nicotine are all very old, and none conform to the guidelines. The European Commission's ToxRTool[1] software package was used to score these studies according to the Klimisch rating, in order to distinguish between them. They all obtained a score of 3, "Not reliable". This is mainly due to the lack of information and the few details given on the experimental protocols and methods used in these studies.

In total, there are five acute oral toxicity studies available in rats. There is a certain consistency since they all lead to LD_{50} values higher than 50 mg/kg, which would result in a classification in Category 3 for nicotine by the oral route. However, the two studies in mice lead to lower LD_{50} values, respectively 3.34 mg/kg and 24 mg/kg (Lazutka *et al.*, 1969; Heubner *et al.*, 1938). These results may suggest greater sensitivity in mice to the effects of nicotine, confirmed by the results of the study by Lazutka *et al.* (1969), which was conducted in parallel in rats and mice according to

[1] http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/archive-publications/toxrtool

the same experimental protocol. In spite of the more than doubtful quality of these two studies in mice (mainly because of identical mortality data between rats and mice, and the lack of information on the experimental protocol), this does raise the issue about choosing the species that seems most sensitive. This choice should also be compared with the value of 9.2 mg/kg obtained in dogs.

The data in humans were reported with the aim of helping with the final choice of LD_{50} values. These data are difficult to use because of the lack of information about the reported cases and the very wide disparity observed depending on the cases. There is indeed a great difference in sensitivity to nicotine, for example between non-smokers and smokers, with the latter rapidly developing a tolerance (Fattinger, 1997). There are also considerable inter-individual variations in the rate of nicotine absorption, as well as in the rate of elimination, particularly in conjunction with considerable genetic polymorphism in cytochrome P450 and glucuronidation activity (Benowitz *et al.*, 2009). In addition, besides the uncertainties surrounding the doses ingested in most cases of human exposure, the doses actually absorbed are certainly lower, mainly due to early vomiting or medical attention. The biological data needed to assess the internal dose are missing in most cases. Therefore, only taking into account the assessment of the exposure dose probably overestimates the dose to which humans can be exposed without lethal effect as it appears in the published cases.

In a poorly substantiated calculation, Mayer (2014) estimated the minimum lethal dose in humans at 0.5 to 1 g, i.e. 7 to 14 mg/kg. In adults, symptoms appear from an estimated ingestion of 21.25 mg (or approximately 0.3 mg/kg) of nicotine in a patient. Patients ingesting 1.5 g or 2 g have survived. In children, the first minor signs seem to appear from the ingestion of 0.2 mg/kg of nicotine. A half-cigarette (i.e. an estimated quantity of nicotine between 3 and 15 mg [0.25 to 1.25 mg/kg]) causes moderate symptoms, while two cigarettes (i.e. 12 to 60 mg of nicotine [1 to 5 mg/kg]) lead to severe symptoms.

Accordingly, taking into account:

- the age and paucity of the data available in the literature,
- inter-species and inter-individual differences in sensitivity,
- human data showing the first signs of poisoning which can occur from 0.3 mg/kg in adults and 0.2 mg/kg in children,

the CES believes that the possibility of effects at low doses cannot be ruled out, especially among individuals who have not developed a tolerance. Consequently, and as a precaution, the lowest LD_{50}, obtained in mice, namely 3.34 mg/kg (Lazutka *et al.,* 1969), is selected, in spite of all the limitations presented by this study.

In order to respond to these uncertainties, the experts recommend conducting a study of the acute oral toxicity of nicotine in mice that meets the quality criteria currently recommended, in order to obtain valid values in the animal species considered to be the most sensitive.

Dermal route

The two acute toxicity values identified for the dermal route were 50 mg/kg and 140 mg/kg (cited in Quarterly Bulletin, Vol. 16, 1952, and Ben-Dyke *et al.*, 1970). These values come from studies in rabbits and rats respectively, two species recommended by the current OECD guidelines. As the source studies were not available, a qualitative assessment of the studies could not be carried out and none of the protocol points could be compared with the guidelines.

Therefore, in view of the unavailability of the source studies, the CES can neither confirm nor refute the current classification. Consequently, the CES suggests retaining this classification.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on "Characterisation of substance hazards and toxicity reference values" relating to the choice of a relevant LD_{50} value for nicotine concerning the dermal and oral routes. The value of 3.34 mg/kg for the oral route is selected, while for acute toxicity via the dermal route, since no data are available, the Agency selects the classification in Category 1, and therefore considers that the LD_{50} is below 50 mg/kg.

There is no report documenting the data and discussions that led to the existing classification, and no acute toxicity value is directly available from the regulations. In addition, nicotine has not yet been registered under the REACh Regulation.

Concerning the issue of classification of nicotine, the collective expert appraisal report concludes with a classification in Category 1 for each of the exposure routes. However, many limitations concerning acute oral toxicity were identified in the expert report. The LD_{50} values derived from these studies cannot therefore be used as such for the classification of mixtures. In this specific case, the CLP Regulation stipulates the use of conversion values, established from a range of experimental values or a classification category, as shown in the table below (Table 3.1.2 from Regulation (EC) 1272/2008 "CLP" - Annex I):

Exposure routes	**Classification category or experimentally obtained acute toxicity range estimate** (*see Note 1*)	**Converted Acute Toxicity point estimate** (*see Note 2*)
Oral (mg/kg bodyweight)	0 < Category 1 ≤ 5 5 < Category 2 ≤ 50 50 < Category 3 ≤ 300 300 < Category 4 ≤ 2000	0.5 10 100 500
Dermal (mg/kg bodyweight)	0 < Category 1 ≤ 50 50 < Category 2 ≤ 200 200 < Category 3 ≤ 1000 1000 < Category 4 ≤ 2000	5 70 300 1100

As a consequence, ANSES recommends that in view of the uncertainties associated with the source studies, the values of 0.5 mg/kg for the oral route and 5 mg/kg for the dermal route should be used for the classification of mixtures.

In addition, the classification entry 614-001-00-4 shown in Table 3.1 of Annex VI of the CLP Regulation comes from the conversion of the classifications mentioned in Annex I of Directive 67/548/EEC. The harmonised classification for acute oral toxicity is indicated in this entry by the reference (*) in the "Classification" column of Table 3.1, and must be regarded as a minimum classification (CLP, Annex VI, 1.2.1). It is applied if no other data or other information referred to in the first part of Annex I lead to it being classified in a more severe category compared to the minimum classification. Otherwise, classification in the most severe category must then be applied. This means that, unlike the other entries in Annex VI of the CLP Regulation, a different classification may be applied for oral acute toxicity, without it being necessary to modify its harmonised classification entry.

The review of the available data leads ANSES to conclude that a more severe classification than that of Annex VI for acute oral toxicity is justified.

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KEYWORDS

Nicotine, LD_{50}, acute toxicity, oral, dermal